

## TESTING HISTORICAL HYPOTHESES OF MORPHOLOGICAL CHANGE: BIOMECHANICAL DECOUPLING IN LORICARIOID CATFISHES

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**Abstract.**—The “decoupling hypothesis” has been proposed as a mechanistic basis for the evolution of novel structure and function. Decoupling derives from the release of functional constraints via loss of linkages and/or repetition of individual elements as redundant design components, followed by specialization of one or more elements. Examples of apomorphic decoupling have been suggested for several groups of organisms, however there have been few empirical tests of explicit statements concerning functional and morphological consequences of decoupling. Using the loricarioid catfishes, we tested one particular consequence of decoupling, the prediction that clades possessing decoupled systems having increased biomechanical complexity will exhibit greater morphological variability of associated structures than outgroups having no such decoupled systems. Morphometric procedures based on interlandmark distances were used to quantify morphological variance at three levels of design at successive nodes in the loricarioid cladogram. Additional landmark-based procedures were used to localize major patterns of shape change between clades. We report significantly greater within-group morphometric variance at all three morphological levels in those lineages associated with decoupling events, confirming our predictions under the decoupling hypothesis. Two of 12 comparisons, however, yielded significant variance effects where none were predicted. Localization of the major patterns of shape change suggests that disassociation between morphological and functional evolution may contribute to the lack of fit between variance predictions and decoupling in these two comparisons.

**Key words.**—Catfishes, decoupling, evolutionary morphology, functional design, Loricarioidea, morphometrics.

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Two central tenets emerging from historical analyses of the evolution of morphology are that intrinsic aspects of organismal design often exhibit regular historical patterns of form and function, and that hypotheses about how these general patterns are generated may be tested within an explicit phylogenetic framework (Lauder 1990). The search for general patterns of form and function is by no means new, and has a long and distinguished history in biology. Such concepts as Cuvier's principle of correlation of parts, Geoffroy's concept of unity of plan and composition, von Baer's law of development, Agassiz's threefold parallelism, expansion of the Meckel-Serres law and its extension in Haeckel's biogenetic law all deal at some level with large-scale patterns of organismal design. While it has been argued and recently become widely accepted that the comparisons among organisms should utilize a phylogenetic approach (Lauder 1981, 1982; Huey 1987; Brooks and McLennan 1991; Harvey and Pagel 1991; Miles and Dunham 1993), and while protocols for testing historical hypotheses have been suggested (Stiassny and Jensen 1987; Lauder and Liem 1989; Farrell et al. 1991; Lynch 1971; Garland et al. 1992), few empirical tests of intrinsic design features of organisms have been performed (see Emerson 1988 for one notable exception).

The repetition of individual elements as redundant design components, followed by specialization of one or more elements as a mechanistic basis for the evolution of novel structure/function has been suggested as a general pattern at both the molecular and organismal levels of organization (Ohno 1970; Lauder 1982, 1990; Raff et al. 1987). This con-

cept of decoupling of design elements is analogous to the release of developmental constraints imposed by high genetic correlations, or tight developmental interactions, both of which may constrain avenues for evolutionary change (Atchley and Hall 1991; Arnold 1992; McKittrick 1993). One consequence of decoupled design elements is increased potential for structural/functional diversification in descendant taxa. Examples of apomorphic decoupling of structural components have now been documented in many clades: (1) duplication of jaw opening mechanical systems in ray-finned fishes, involving release of the plesiomorphic functional constraint imposed by the original unimodal system (Lauder 1980, 1982; Lauder and Shaffer 1993); (2) duplication and rearrangement of muscle elements in the feeding mechanism of cichlid fishes, involving release of design constraints on the mechanics of prey manipulation and transport by the pharyngeal jaws (Liem 1973); and (3) decoupling of pharyngeal scrapers from the larynx in pigeons and songbirds, involving release of constraints on feeding and ventilation (Zweers 1985). Conversely, apomorphic coupling of structural components associated with decreased constructional variability and increased mechanical constraint has also been documented, such as that observed in the derived fusion of epioracoid cartilages in frogs (Emerson 1983, 1984). In these examples, however, explicit statements concerning the functional and morphological consequences of decoupling have not been tested in a rigorous historical framework, with the exception of the work by Emerson (1983, 1984), which remains the most complete test to date. In this paper, we provide an empirical test of the decoupling hypothesis in a clade of catfishes. Specifically, we evaluate the prediction that clades possessing decoupled elements will exhibit increased struc-

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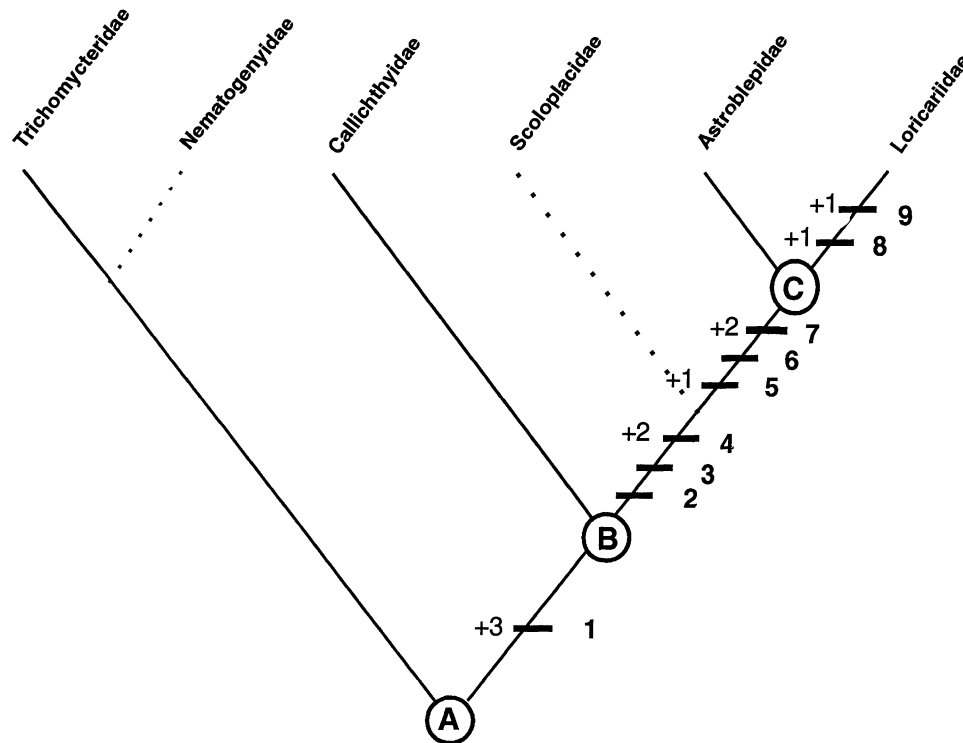


FIG. 1. Phylogeny of the loricarioid catfishes. Dashed lines denote taxa not included in the morphometric analyses. Decoupling events, represented by nine biomechanical apomorphies and the associated increase in numbers of biomechanical pathways, are mapped onto the cladogram and numbered as in Table 1. At this level of analysis there is no homoplasy in these characters. Nodes A, B, and C indicate three of four phylogenetic levels analyzed in this study. Each node involves a pair-wise comparison of sister taxa, and the basal node (not shown) is analyzed relative to outgroup, nonloricarioid taxa.

tural diversity compared to sister clades with coupled systems.

## BACKGROUND

### *Catfishes and Mechanical Design*

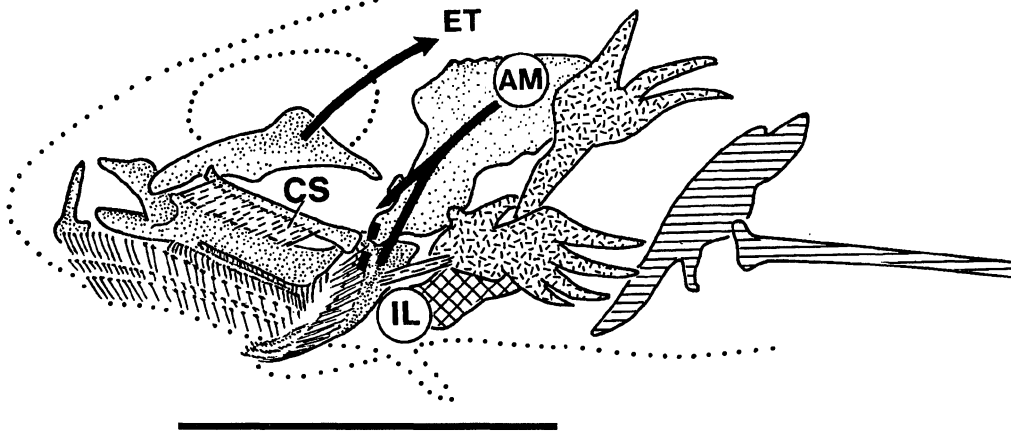
In a previous paper (Schaefer and Lauder 1986), we demonstrated that the loricarioid catfishes are a clade showing a pattern of progressive increase in the mechanical complexity of structures associated with feeding. Loricarioids comprise a monophyletic group of approximately 1000 species of catfishes in six families (Fig. 1). A well-supported phylogeny for the group is available (Schaefer 1990; de Pinna 1992), although within each major clade the relationships among taxa at lower phylogenetic levels are poorly known. Basal loricarioids (trichomycterids, nematogenyids) share with other catfishes and ostariophysan fishes the plesiomorphic condition for cranial structures and their mechanical linkages. The lower jaw adductor musculature is relatively undifferentiated, upper jaws are coupled to the skull, and the lower jaws and associated hyoid musculature are bilaterally coupled (Fig. 2, top). Further, the two mechanical pathways for lower jaw depression, both representing coupled mechanical systems also present in basal teleost fishes (Lauder 1985; Lauder and Shaffer 1993), are unmodified in primitive loricarioids: (1) the mandibulohyoid ligament linking the hyoid and mandible; and (2) the interoperculo-mandibular ligament linking the opercular series with the mandible. The functional design

represented by basal loricarioids is therefore relatively unmodified from the basic biomechanical design shared with outgroup actinopterygian (ray-finned fish) taxa (Lauder 1985). Relative to more derived loricarioids (Fig. 2, bottom), the mechanical design represented in basal loricarioids is one of fewer independent elements, is more mechanically constrained, and consequently exhibits fewer mechanical degrees of freedom.

We have previously described the historical sequence of changes in the morphology and mechanical design of the feeding mechanism in loricarioids (Schaefer and Lauder 1986) and outline the major features in Table 1. The distribution on the cladogram of apomorphic features and their mechanical consequences allows specification of the historical sequence of structural/functional change and particular hierarchical levels at which individual specializations have occurred (Fig. 1). The major pattern that emerged from that analysis is one of progressively increasing biomechanical complexity within the Loricarioidea (Fig. 2).

We further defined these morphological innovations in terms of their biomechanical consequences (Table 1). The upper jaws have become decoupled from the cranium, releasing the plesiomorphic constraint of tight attachment and immobility (Fig. 1: 1, 2, 5); the lower jaw has become decoupled from the opercular series, thereby releasing one of two plesiomorphic biomechanical pathways for lower jaw depression (Fig. 1: 4); the lower jaws and hyoid musculature have become decoupled from their plesiomorphic bilaterally

*Henonemus*  
(Trichomycteridae)



*Ancistrus*  
(Loricariidae)

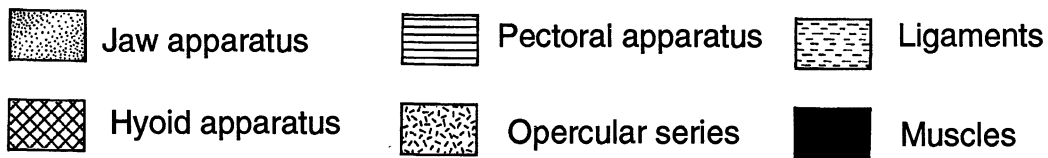
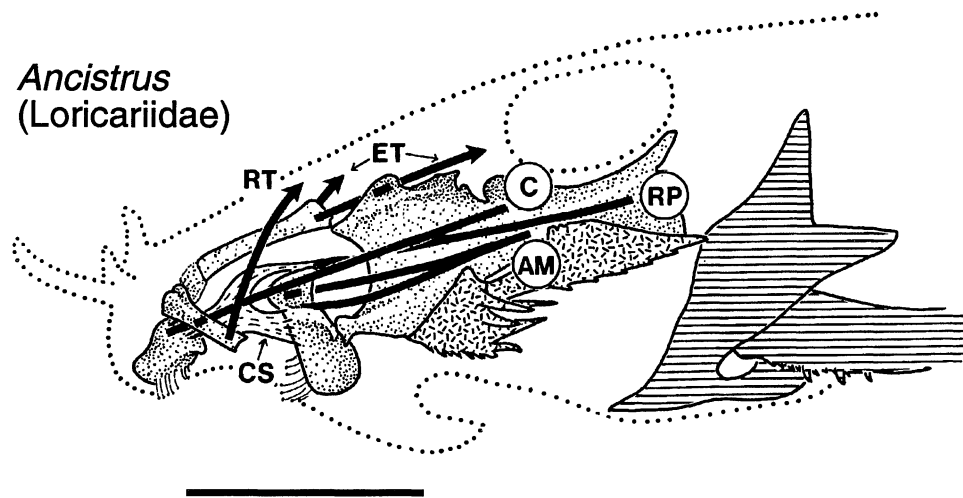


FIG. 2. Range of loricarioid morphological complexity. Top, *Henonemus*, family Trichomycteridae; bottom, *Ancistrus*, family Loricariidae. Note the increase in complexity of jaw musculature, as reflected in the increased number of jaw muscle divisions and the increase in number of anterior jaw bones (further discussion in text). Abbreviations as in Table 1; scale bar is 5 mm.

constrained midline attachments (Fig. 1: 3, 6, 7); a new, redundant linkage between hyoid and mandible was acquired (Fig. 1: 8); and the adductor musculature has differentiated into separated elements with novel attachments and functions (Fig. 1: 9). The increase in myological complexity is illustrated in Figure 2, which schematically compares lateral jaw musculature in trichomycterids and loricariids, taxa representing ends of the spectrum of morphological complexity within the clade. While the osteological specializations exhibit an overall pattern of increase in number of independent linkages between structural components, subsequent rearrangements of the musculature, with novel attachments to these decoupled elements, also show an overall increase in

the number of biomechanical pathways mediating function in the design of the loricarioid feeding mechanism.

*Decoupling and Its Consequences*

Our premise is that decoupling of primitively constrained, tightly integrated biomechanical systems is a general pattern in the evolution of morphology and an important mechanism facilitating morphological and functional innovation. Apomorphic increase in the number of pathways mediating major aspects of function and behavior, the first-order result of decoupling events, must be causally related to subsequent diversification of morphology and behavior (Emerson 1988;

TABLE 1. Predicted effects of biomechanical transformations on morphometric variance and their associated morphological bases. Events correspond with numbered character states in Figure 1. A variance effect is defined as greater ingroup generalized variance compared to its sister group. Levels 1–3 refer to morphological levels of analysis (see text, Fig. 3). Abbreviations: A, Astroblepidae; L, Loricariidae; C, Callichthyidae; T, Trichomycteridae; ET, extensor tentaculi; HHI, hyohyoideus inferioris; RT, retractor tentaculi; AM, adductor mandibulae; GH, geniohyoideus; RP, retractor palatini; Rpmx, retractor premaxillae; SH, sternohyoideus.

Node	Sister pair	Event	Morphological basis	Change in # biomechanical pathways <sup>a</sup>	Predicted effect <sup>b</sup>
Basal	T + C + A + L vs. other siluroids	none	none	none	none
A	C + A + L vs. T	1. changes in upper jaw osteology and myology	loss tight linkage between upper jaw and cranium	+3; upper jaw: ET, RT, AM	Levels 1, 2
B	A + L vs. C	2. changes in upper jaw myology and arthrology	RT muscle insertion shift onto maxilla; loss pterygo-ethmoid ligament	none	none
		3. changes in hyoid musculature	morphological changes in HHI, separate insertions on hyoid arch	none	none
		4. decoupling of mandible from opercular linkage	loss of interoperculomandibular ligament	+2; lower jaw: bilateral AM	Levels 2, 3
		5. changes in premaxilla and adductor musculature	novel AM division (Rpmx) with direct insertion onto premaxillae	+1; upper jaw: RP	Levels 1, 2
		6. separation of intermandibular joint	loss of tight attachment between mandibles at midline	none <sup>c</sup>	Level 3
		7. changes in hyoid musculature	bilateral separation of GH, with distinct insertions onto mandible	+2; lower jaw: bilateral GH	Level 3
C	L vs. A	8. novel mandibular-hyoid linkage	tight attachment of intermandibular cartilage to hyoid	+1; lower jaw: SH	Level 3
		9. changes in adductor muscle complex	novel AM subdivision with indirect insertion onto premaxillae (RP)	+1; upper jaw: RP	Levels 1–3

<sup>a</sup> Musculo-skeletal system(s) involved in the decoupling of biomechanical pathways. Bilaterally symmetrical pathways counted separately when bones affected are not firmly united at the midline.

<sup>b</sup> Morphological level at which decoupling hypothesis predicts increased variance. "None" indicates no predicted increase in variance for any of the three morphological levels of analysis.

<sup>c</sup> No change in number of biomechanical pathways, yet increase in number of independent elements resulting from loss of tight midline attachment of mandibles.

Lauder and Shaffer 1993). For example, frogs with bilaterally decoupled pectoral girdles exhibit greater range in girdle shape (Lynch 1971; Trueb 1973) and a correlation between degree of fusion and degree of change in girdle morphology and orientation (Emerson 1988). The loricarioid catfishes offer yet another example of decoupled morphologies and the phylogenetic framework with which to test the generality of the decoupling hypothesis.

We previously (Schaefer and Lauder 1986, p. 505) outlined a set of predictions on the relationship between numbers of biomechanical pathways and aspects of morphology and function in the feeding mechanism within this clade and a general protocol for testing those predictions. In general, we predict that clades possessing decoupled biomechanical systems and fewer constrained linkages will exhibit greater morphological variability of associated structures compared with sister-clades and outgroups having no such decoupled systems (Schaefer and Lauder 1986, Lauder and Liem 1989); specifics of the predictions are discussed below. Increased numbers of mechanical linkage systems in the jaws increase the possibility for changes and for new connections and functions to be acquired.

In this study, we test this hypothesis using generalized morphometric variance as a proxy for morphological variability. We apply a statistical test at three levels of morphological design at specific levels in the cladogram corresponding to particular decoupling events in the history of loricarioid morphological evolution (Fig. 1).

## METHODS

Our choice of exemplar taxa within loricarioid families was designed to evenly sample the major subfamilial taxonomic groups, and therefore the major morphological "baupläne" within families, without over representation of any one taxonomic category (specimens examined are listed in Appendix I). Scoloplacids (four species of miniature catfishes) and nematogenyids (one species) were not included in the analysis due to limited comparative material. Their absence from our comparisons does not affect our ability to test the generality of the decoupling hypothesis. However, absence of scoloplacids does preclude us from fully resolving the association between decoupling and morphological change at that particular level of the cladogram. Approximately 25 specimens of eight species were sampled within each family (Table 2); generic-level representation was variable among families. A diversity of siluroid taxa were selected to represent a nonloricarioid outgroup. Because of the specimen-invasive nature of data acquisition, the extent that our sampling objectives could be met was a function of specimen availability in museum collections. The unit of analysis was the individual specimen. True random taxon sampling representing identical numbers of species within taxon categories was not possible due to limitations on preserved museum material available for dissection. Thus, we note that our study is not based on a random statistical sample of individuals in each clade (and hence is not strictly repeatable

TABLE 2. Numbers of specimens (individuals) and taxa examined.

Group	Family	Number of genera	Number of species	Total specimens
Nonloricarioid siluroids	Ariidae	2	2	5
	Doradidae	2	2	7
	Mochokidae	3	4	5
	Pimelodidae	2	2	4
	Sisoridae	3	3	5
Total		12	13	26
Loricarioids	Trichomycteridae	5	8	25
	Callichthyidae	4	8	26
	Astroblepidae	1	8	25
	Loricariidae	8	8	25

in a statistical sampling sense), but within the constraints of available material we endeavored to obtain specimens from all major subgroups within each family.

### Morphometrics

We performed a series of morphometric analyses based on homologous interlandmark distances designed to quantify multivariate variance in morphological aspects of particular relevance to the key biomechanical predictions outlined below. Additional landmark-based procedures were used to localize the major patterns of shape change between comparisons at each node (described below). All data were acquired as landmark coordinates defining homologous points on structural elements of the major functional components of the feeding mechanism. Landmarks were chosen to quantify major patterns of morphometric variation among families at three progressively less-inclusive levels of morphological design (Fig. 3a–c, respectively): level 1, hyoid and mandibular arch with two points on the pectoral girdle, as seen in ventral view (eight landmarks, 16 variables); level 2, the mandibular arch, focusing on the suspensorium of the mandible (seven landmarks, 13 variables); level 3, mandible (11 landmarks, 22 variables). Following data acquisition at level 1, the mandibular arch was dissected from the right side of the specimen to permit data acquisition at levels 2 and 3. Landmarks were taken as pixel coordinates and variables were taken as linear interlandmark distances (landmarks and variables shown schematically in Fig. 3; described in Appendix II) on catfish specimens cleared with trypsin and differentially stained for cartilage and bone (Taylor and Van Dyke 1985). Although our system of landmark designations was based on positional homology and was designed to sample relevant structures associated with biomechanical evolution, our choice of interlandmark distance variables was designed to quantify shape, without regard to a priori notions of the potential effects of biomechanical evolution on those structures. Although other landmarks homologous across taxa could have been added to this analysis, the 26 homologous landmarks chosen provide thorough coverage of cranial morphology relevant to testing the decoupling hypothesis.

Loricarioids are remarkably variable for body size among and within taxa. Adults may range in size from as small as 11 mm standard length (SL) and 6 mm head length (HL) in some scoloplacids and trichomycterids, to as much as 1500 mm SL and 300 mm HL in some loricariids. Although the

evolution of body size has undoubtedly played a major role in the history of these fishes, we are interested in comparing the magnitudes of morphometric variation between sister taxa that are largely or entirely free of variation due to overall size. As discussed by Rohlf and Bookstein (1987), there has been no general agreement on methods for adjusting for the effects of size in morphometric analyses. In choosing a particular method for removing the effects of size on our estimates of morphological variation, we require a method that produces general size vectors that are parallel among groups, akin to adjustment for unequal slopes in the analysis of covariance. The shear technique (Humphries et al. 1981; Rohlf and Bookstein 1987) and the Burnaby method (Burnaby 1966) are two popular multivariate methods for size adjustment, but which have no explicit procedures for dealing with nonparallel size vectors among groups (Rohlf and Bookstein 1987, p. 365). Our preliminary analyses of these data strongly suggested the presence of among-groups allometric heterogeneity. We therefore used a more general method for size adjustment based on geometric shape coordinates as defined by Bookstein (1991). A new set of size-adjusted shape coordinates was generated from the original landmark coordinates using the program UNIGRAPH by Les Marcus (all software cited here is available via the morphometrics archive at <http://life.bio.sunysb.edu/morph/morph.html>). For each specimen, the following landmark pairs at each morphological level were selected as baseline endpoints and assigned coordinates (0, 0) and (1, 0) respectively: level 1: 1, 4; level 2: 9, 15; level 3: 16, 25. Shape coordinates were then computed for all specimens by rotation and scaling of the landmarks relative to the unit baseline. Shape coordinates were thus adjusted for size for each individual specimen and were then used to compute “size-free” interlandmark distances as the input to our variance analyses.

### Multivariate Variance

To test the predicted relationship between biomechanical complexity and morphological variability, we compared relative multivariate variance between pairs of sister taxa at each node in the cladogram (Fig. 1), including a basal comparison between loricarioids and a generalized sister group comprised of representative nonloricarioid siluroids chosen from a number of siluroid families representing diverse morphologies, since there is no general agreement on the sister group of the loricarioids. There is also no general agreement on methods for estimating multivariate variance (a good general discussion is given by Van Valen 1978). The determinant of the variance-covariance matrix is the usual scalar measure of variation in more than one dimension and is equivalent to the product of the eigenvalues (Dempster 1969). This parameter suffers the limitation of being small when most correlations are small and vanishes when one or more eigenvalues approach zero (Van Valen 1974). A better measure of multivariate variation is the total variance, generalized as the sum of the eigenvalues or trace of the variance-covariance matrix (Ashton and Rowell 1975; Van Valen 1978). At each node in the cladogram, in addition to the basal node (Fig. 1), we partitioned the total data into relevant sister-taxon pairs and computed total variance as the trace of the within-groups

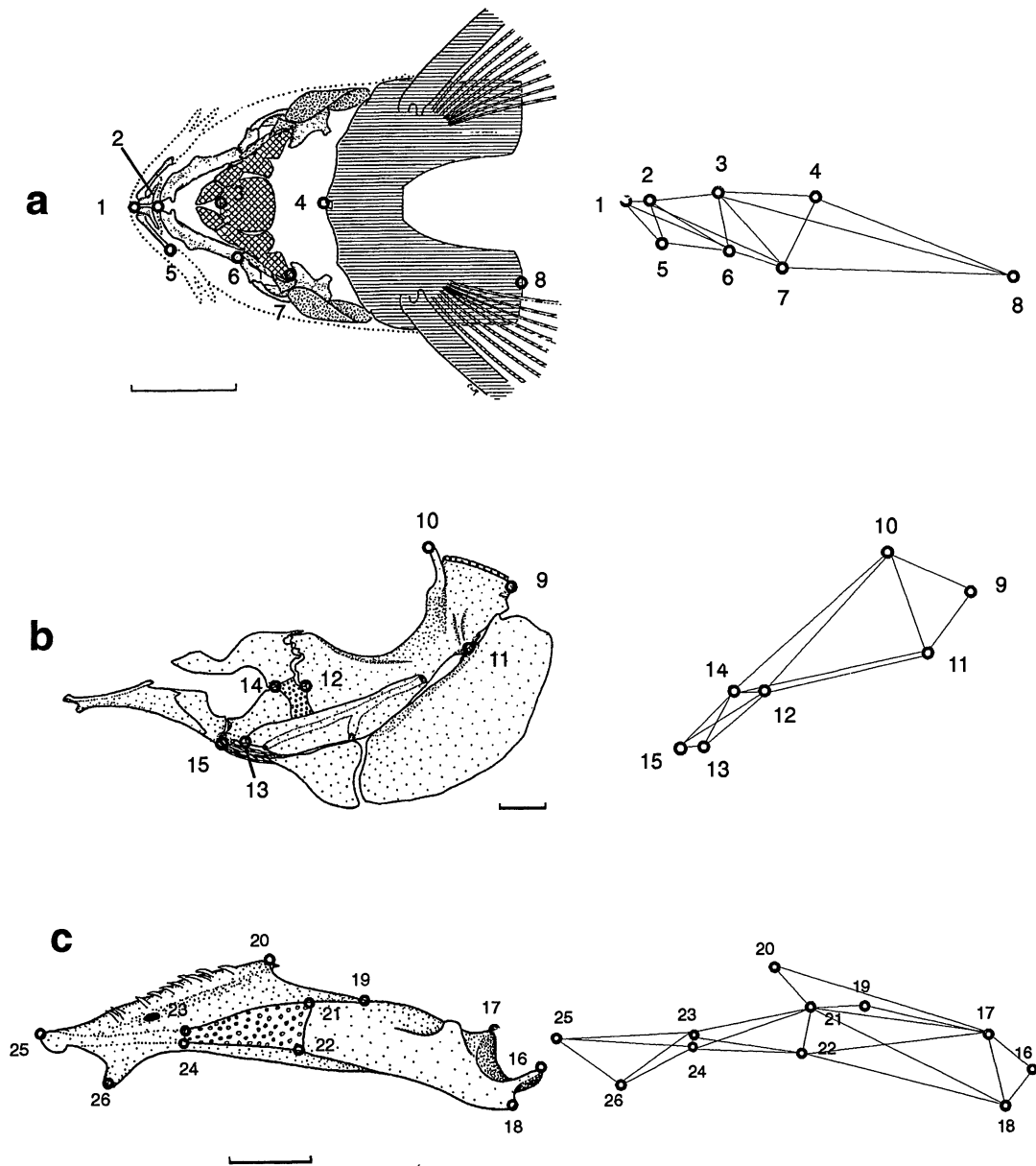


FIG. 3. Morphological levels used in this analysis, homologous landmarks, and interlandmark distance measures illustrated relative to a generalized loricarioid (callichthyid). The diagrams to the right illustrate the distances calculated at each level: (a) level 1, inclusive of the pectoral complex, hyoid and mandibular arches; (b) level 2, inclusive of the mandibular arch (jaw suspensorium plus opercular series); (c) level 3, inclusive of the mandible only. Landmarks and variables described in more detail in Appendix II; scale is 1 mm.

variance-covariance matrix based on “size-free” interlandmark distances as defined above.

*Morphometric Dispersion*

As an auxiliary measure of morphological variability, we computed the average within-group Euclidean distance among landmarks for each sister-clade comparison as above. Computation and weighting adjustments to correct for sample size followed Foote (1991, p. 470–471). This parameter addresses morphological variability in a slightly different way than that revealed by examining multivariate variance. The former examines the extent that sister clades differ for av-

erage within-group morphological distance among individuals. The latter examines overall multivariate morphometric variance between groups, is roughly proportional to the squared perimeter of the morphospace hypervolume occupied, and takes into account both total scatter as well as dispersion among points.

*Hypothesis Tests*

The magnitude of the difference between sister-taxon pairs for multivariate variance as well as morphometric dispersion was computed as the ratio of the relative generalized multivariate variance and average within-group Euclidean dis-

TABLE 3. Total within-groups multivariate variance, *F*-values and significance results, based on size-adjusted shape coordinates. Bootstrap resampling results based on 5000 replicates.  $N_i/N_j$  = # observations in sister pair;  $V_i, V_j$  = total within-group multivariate variance of sister pair;  $F = V_i/V_j$ ;  $meanF$  = mean of bootstrap replicated *F* estimates;  $sdF$  = standard deviation of bootstrapped *F*.

Node	Level	$N_i/N_j$	$V_i$	$V_j$	<i>F</i>	<i>P</i> <sup>1</sup>	Bootstrap results			
							$meanF$	$sdF$	limit <sup>2</sup>	limit <sup>3</sup>
Basal	1	101/26	.540	1.391	0.388	ns	1.120	.400	0.336	0.088
	2		.134	.087	1.551	**	1.003	.114	1.226	1.297
	3		.201	.115	1.748	**	0.973	.255	1.473	1.631
A	1	76/25	.529	.308	1.718	**	1.022	.179	1.373	1.484
	2		.135	.089	1.521	**	1.008	.105	1.214	1.279
	3		.122	.240	0.507	ns	0.968	.280	0.419	0.246
B	1	50/26	.452	.115	3.921	**	1.018	.164	1.338	1.440
	2		.125	.094	1.331	**	1.006	.095	1.191	1.250
	3		.076	.033	2.334	**	0.853	.175	1.196	1.305
C	1	25/25	.170	.105	1.623	**	1.021	.213	1.439	1.571
	2		.046	.020	2.346	**	1.011	.115	1.235	1.306
	3		.110	.013	8.744	**	0.972	.363	1.674	1.909

<sup>1</sup> \*\* = *P* < 0.01.

<sup>2</sup> Upper limit of 95% confidence interval of bootstrapped *F*.

<sup>3</sup> Upper limit of 99% confidence interval of bootstrapped *F*.

tance, respectively. Significance of the magnitude of the ratios was tested using a bootstrap resampling procedure based on randomized sampling with replacement and partitioning of the data into two groups of size equivalent to the number of observations in each sister-taxon pair. One-tailed *P*-values were obtained by comparing the observed ratio to the distribution of 5000 empirical ratios obtained from the randomization procedure under the null hypothesis of ratio 1.

The specific changes in the biomechanics of the loricarioid skull that were used to generate predictions about patterns of morphological variation are listed in Table 1. These changes are based on our previous analysis of loricarioid skull functional morphology (Schaefer and Lauder 1986), and reflect phylogenetic novelties in skull mechanics at four phylogenetic levels (Fig. 1): (1) comparisons of loricarioids as a whole to outgroup, nonloricarioid catfishes (basal node); (2) comparisons of callichthyids + astrolepids + loricariids

to their sister group, the trichomycterids (node A); (3) comparisons of astrolepids + loricariids to their (functional) sister group, the callichthyids (node B); and (4) comparisons of loricariids to their sister group, the astrolepids (node C). Comparisons at each of these four phylogenetic levels were made for the three morphological levels of analysis discussed above yielding, 12 total tests, the results of which are shown in Tables 3 and 4. Note that we have not performed sequential Bonferonni corrections for the statistical tests presented in Tables 3 and 4. Each test at each level constitutes its own independent a priori hypothesis. Our analysis is not equivalent to conducting multiple ANOVAs on a single morphometric data set, which would require adjustment for multiple comparisons (Rice 1989). In addition, we observed that the table-wise *P*-values obtained in these analyses were all less than 0.01 and that most were in fact near 0.001.

For each phylogenetic comparison of sister clades, the

TABLE 4. Average within-groups Euclidean distance, *F*-values and significance results, based on size-adjusted interlandmark distances. Bootstrap resampling results based on 5000 replicates.  $N_i/N_j$  = # observations in sister pair;  $W_i, W_j$  = average within-group Euclidean distance scores for sister pair;  $F = W_i/W_j$ ;  $meanF$  = mean of bootstrap replicated *F* estimates;  $sdF$  = standard deviation of bootstrapped *F*.

Node	Level	$N_i/N_j$	$W_i$	$W_j$	<i>F</i>	<i>P</i> <sup>1</sup>	Bootstrap results			
							$meanF$	$sdF$	limit <sup>2</sup>	limit <sup>3</sup>
Basal	1	101/26	.753	.292	2.580	ns	3.955	.547	5.027	5.366
	2		.387	.081	4.795	**	3.904	.236	4.367	4.513
	3		.449	.086	5.250	**	3.920	.477	4.855	5.151
A	1	76/25	.695	.176	3.950	**	3.054	.250	3.544	3.699
	2		.364	.096	3.798	**	3.046	.176	3.391	3.500
	3		.337	.152	2.215	ns	3.089	.408	3.889	4.146
B	1	50/26	.553	.151	3.657	**	1.934	.155	2.238	2.334
	2		.295	.122	2.408	**	1.926	.098	2.118	2.179
	3		.227	.081	2.804	**	1.938	.207	2.344	2.472
C	1	25/25	.270	.211	1.280	**	1.008	.099	1.202	1.263
	2		.143	.096	1.486	**	1.001	.072	1.142	1.187
	3		.217	.075	2.918	**	1.018	.196	1.402	1.524

<sup>1</sup> \*\* = *P* < 0.01.

<sup>2</sup> Upper limit of 95% confidence interval of bootstrapped *F*.

<sup>3</sup> Upper limit of 99% confidence interval of bootstrapped *F*.

analysis of skull mechanical linkages generated predictions as to the morphological level at which increased variance would or would not be expected under the decoupling hypothesis. For example, Table 1 shows that at the basal node (where loricarioids are compared to outgroup catfishes), no changes in mechanical linkages within the skull exist, and we predict no change in morphological variance within the ingroup. In contrast, callichthyids + astroblepids + loricariids share novelties in upper jaw mechanics involving the loss of the primitively tight linkage between the maxilla and the neurocranium as well as the insertion of three muscles on the upper jaw (Table 1). Given the general hypothesis presented above, that increased mechanical decoupling of structural components should be associated with an increase in the possible arrangements and diversification of those components, we expect increased morphological variance in descendent clades at this level. Specifically, for this comparison at node A, we predict that the ingroup clade will show increased morphological variance at morphological levels 1 and 2, which are most closely associated with the upper jaw function, but not at level 3, which characterizes lower jaw (mandible) structure. This prediction is given in the right-hand column of Table 1.

Similar predictions were generated for each of the four phylogenetic levels based on an analysis of loricarioid skull functional morphology. As shown in Table 1, many mechanical changes in the skull occurred at some phylogenetic levels, and we treat all mechanical linkage changes together when generating predictions for that phylogenetic level. Thus, for node B (Table 1), six discrete changes in mechanical linkage systems in the skull occur in the ingroup taxa (astroblepids + loricariids) as compared to the sister-group clade (callichthyids). While some observed novelties in skull mechanics predict no change at any of the three morphological levels (e.g., events 2 and 3, Table 1), others such as events 4, 6, and 7 predict changes at morphological level 3. Considering all six mechanical changes, predictions for increased variance are made for all three morphological levels at this node.

There are four important issues associated with our predictions of phylogenetic changes in variance. First, note that we do not suggest that a unit amount of variance is associated with each novel mechanical skull linkage. Rather, we make the simpler and less specific prediction that increased variance is expected for descendant clades where changes in mechanical linkage systems in the skull have occurred. Second, we base the predictions we make on the occurrence of discrete changes in the *mechanical linkage systems* (or biomechanical pathways) within the skull, and *not* on general morphological changes. Our identification of mechanical linkages is founded on the relatively large body of data on the structure and function of the skull in teleost fishes that has been accumulated over the last 20 years (Alexander 1967; Liem 1970, 1980; Nyberg 1971; Lauder 1981, 1983; Schaefer and Lauder 1986; Westneat 1990, 1994; reviewed in Lauder 1985) supplemented by our own study of catfish cranial morphology. In essence, by tracking changes in mechanical linkages in the skull we are estimating the "mechanical degrees of freedom" of the structural components of the skull, and the number of *potential* pathways for controlling bone movement

during respiration and feeding. The changes that we identify in jaw mechanical couplings are anatomically based, and despite recent progress in evaluating the kinematic consequences of changes in fish skull and jaw morphology (Westneat 1990, 1994), we still have little idea of the specific biomechanical effect of these changes on feeding behavior. Such data await experimental analyses of living taxa. Third, our morphometric measures used to test the predictions do not in all cases precisely track functional regions of the skull predicted to vary. Thus, we may be too conservative in our predictions and miss changes in variance that did occur as a consequence of mechanical linkage change, as a result of inadequately capturing the morphology of the relevant skull region. Fourth, descendant taxa "carry with them" ancestral changes in linkages as a result of retaining plesiomorphic characters, and variance may, in a sense, accumulate phylogenetically. This could lead us to find increased variance in descendant clades as a result of changes in mechanical linkage systems that occurred earlier. For this clade of catfishes, however, biomechanical changes in the skull occurred at each successive node. A finding of increased variance in a clade such as the Loricariidae, for example, could be due to changes predicted at node C, or to effects of linkage changes at node B (Fig. 1). We minimize this problem by restricting comparisons to individual sister-pairs and thereby examine the effects of biomechanical linkage evolution on morphological change following divergence from their shared common ancestor. In the Discussion we explore further some potential hierarchical effects on variance in this clade.

#### *Localization of Shape Change*

To interpret the relationships between our predictions and empirical results, we used the method of thin-plate splines (Bookstein 1991) to describe patterns of shape change, modeled as deformations between landmarks for each morphological level at each node of the cladogram. Detailed description of the method is given in Bookstein (1991). Shape deformations are decomposed into both uniform and non-uniform components. The uniform component describes shape change that is geometrically uniform over the form, while the nonuniform component reveals the extent that shape change is localized in different regions of the form. We used the TPSPLINE program (version 9/14/94; Rohlf and Bookstein 1990) for the analysis and visualization of localized shape change, based on the size-adjusted shape coordinate data described above. At each node, the composite average set of shape coordinates was computed for each sister taxon and its first-level outgroup. The total nonaffine component of shape deformation describes the overall pattern of landmark displacements required to transform one form into another, while the partial warps derived from each pair-wise comparison describe patterns of deformation at progressively decreasing scale.

#### RESULTS

In general, the distance-based morphometric data confirm our predictions relative to the decoupling hypotheses. Ten of the twelve total comparisons precisely matched our a priori



predictions under the decoupling hypothesis; we found no significant departure between observed and expected results (chi square = 0.889,  $P = 0.35$ ). We observed significantly greater within-group morphometric variance at all three morphological levels in those lineages with decoupling events in their respective cladogenetic histories. These results were identical for both average total multivariate variance (Table 3) and total Euclidean distance analyses (Table 4). The Pearson correlation coefficients between sister clades for total multivariate variance and average Euclidean distance were significant (ten correlations ranged from  $r = 0.65$ – $0.95$ ;  $P$  ranged from 0.021–0.001). However, there was negligible correlation (not significantly different from zero,  $P$  ranged from 0.27–0.59) between either total multivariate variance or average Euclidean distance and increase in numbers of biomechanical pathways (Fig. 1;  $r = -0.17$  to  $-0.36$ ). While the former indicates the close match between outcomes based on total variance and Euclidean distance, the latter result indicates no basis for expecting that individual decoupling events contribute equal amounts to the overall change in morphometric variance during lineage diversification.

Our results matched the prediction of increased morphological variability in clades associated with increase in numbers of biomechanical pathways in 10 of 12 comparisons for multivariate variance. Two of those 12 comparisons, both at the basal node, did not fit our predictions under the decoupling hypothesis: (1) basal node, level 2; (2) basal node, level 3. Both comparisons yielded significantly greater total variance and average within-group Euclidean distance in sister taxa where none was expected. We discuss each of these separately.

As predicted, we observed no significant variance effect for basal node level 1 comparisons (Tables 3 and 4). Based solely on the decoupling hypothesis, due to the absence of associated biomechanical change at that level of the cladogram we predicted no significant difference in morphological variability between sister pairs at the basal node (Table 1, basal node). Our choice of a composite nonloricarioid outgroup, comprised of representatives from a number of silurid families, provides for a more conservative test of the hypothesis at the basal node than would be obtained by comparing loricarioids to their exact sister group, had the latter been known and specified. Loricarioids, on average, were significantly more variable than nonloricarioid catfishes with respect to suspensorial shape (level 2;  $F = 1.551$ ,  $P < 0.01$ ) as well as for mandible shape (level 3;  $F = 1.748$ ,  $P < 0.01$ ), suggesting that factors other than those associated with biomechanical complexity may be responsible for this variance effect where none was expected. We also did not expect a significant variance effect at node A, level 3 where morphological change of biomechanical relevance associated with this clade (Table 1) does not involve modification to the mandible. Here, however, our prediction of no variance effect was borne out. Thus, the match between observed and predicted variance effects involved both significant and nonsignificant results, the only exceptions being 2 of 3 basal node comparisons. Below, we discuss these two departures from prediction, in addition to those comparisons where our predictions were upheld, in terms of details of the overall patterns of shape change at each level of the cladogram.

## DISCUSSION

The primary goal of this paper is to analyze patterns of morphological change in a monophyletic clade to test a specific historical hypothesis. The hypothesis we test is a purely structural one at the phenotypic level: decoupling of primitively linked structural components in the catfish feeding mechanism is predicted to be associated with increased morphological diversity in descendent clades. To this end, our hypothesis is tested by analyzing structural disparity, rather than the number of speciation events. "Disparity" has been used by Wills et al. (1994) as an appropriate term in discussions of structural diversification to avoid conflation with "diversity," a term that connotes the extent of speciation. We make no claims about the effect of biomechanical decoupling of structural components on subsequent speciation rates (although such claims can also be tested historically; see Sanderson and Donoghue 1994); rather, we aim only to test the relevant structural hypothesis. The results of our tests show that in 10 of 12 planned comparisons at three different morphological levels, decoupling of primitively linked components of jaw morphologies is associated with increased morphological disparity. By comparing sister clades at each node on the loricarioid cladogram (Fig. 1), we conducted a set of hierarchically nested comparisons that cannot be considered strictly independent, but which nonetheless test the decoupling hypothesis at each node by comparing structural diversity in paired lineages that share a common ancestor and common origin. We interpret the result of our tests as showing strong support for a causal historical relationship between decoupling and structural diversity in this clade. Such results have been previously suggested for ray-finned fishes (Lauder and Liem 1989), but an explicit test of this hypothesis has not been available. To date, perhaps the most direct test of the historical structural effect in a clade with a primitive morphological innovation has been that of Emerson (1988), who showed that fusion of the epicoracoid cartilages in frogs (eight phylogenetically independent cases of which were analyzed) resulted in a predictable historical pattern of shape change in descendant taxa. Our results complement and extend Emerson's analysis by directly examining the extent of morphological variation in descendant clades, and by providing a case study in which decoupling (rather than coupling) has occurred. Despite a number of caveats that must be kept in mind when evaluating such historical tests, the evolutionary diversification in loricarioid catfish feeding morphology appears to be due, at least in part, to the increased mechanical and structural flexibility permitted by a successive phylogenetic reduction in the constraints on constructional flexibility. For example, the loricariid + astroblepid clade (Fig. 1) shares several structural novelties including the loss of the primitively tight linkage between the right and left lower jaw bones, and an increase in the number of distinct muscle divisions that attach to moveable jaw bones (Table 1). These structural changes are not found in outgroup clades. Mechanically, these novelties would be predicted to allow an increased variety of movement patterns by the upper and lower jaws, and a concomitant increase in structural variation associated with new movement patterns. This prediction is supported by our test results (Table 3).

One issue that could potentially affect our results is a consequence of the hierarchical nesting of our sister-clade tests. If each family contributes a fixed amount of variance, then comparative tests toward the base of the cladogram might of necessity find that ingroup clades (which will tend to include more families than outgroups at this level; Fig. 1 and Table 1) should possess significantly higher variance than outgroup clades. This result was not found. The level 1 comparison at the basal node actually provided the lowest observed *F*-ratio for any of our comparisons (Table 3) due to the low variance value for the ingroup. Alternatively, if variance propagates up the phylogeny due to a steady accumulation of new biomechanical linkages, then one would predict that the ratios for ingroup to outgroup taxa should also increase. However, our actual results show that variance frequently decreases, as the sequence of values for successively more terminal outgroups at level 1 shows (Table 3): 1.39, 0.31, 0.11, 0.10. Also, we note that our terminal comparison (at node C, Fig. 1) compares two families that we have sampled equivalently. For this comparison our multivariate ratio for level 3 was the highest obtained for any of our comparisons (8.7) due largely to the reduced variance shown at this level by the Astroblepidae. These results suggest that it is unlikely for this analysis to have been confounded by broad trends in variance that arise purely as a consequence of the phylogenetic hierarchy.

#### *Evaluating Erroneous Predictions*

Two of 12 comparisons yielded significant variance effects where none were predicted. We can examine in detail the results of the morphometric analyses for insight into these departures from prediction using the thin-plate spline analytical method which has several advantages in its ability to describe shape change. For our purposes, the main advantage is in the decomposition of the nonaffine (nonuniform) component into successively lower scales of resolution, as revealed by examining the patterns of geometric deformation described by the partial warps. If we were to observe that a large component of shape change was localized in regions of form unassociated with the relevant biomechanical evolution at that node in the cladogram, then we have some basis for claiming a disassociation between morphological and functional evolution. However, localization of functionally significant shape variation is not a prerequisite for empirical support of the decoupling hypothesis in the remaining 10 comparisons where significant variance effects were observed. We do not claim that decoupling must necessarily result in shape variation of biomechanical relevance; instead we suggest that divergence of shape among taxa subject to greater functional constraints will be localized in anatomical regions not associated with mechanical effect.

*Suspensorium Shape Change.*—The total nonaffine shape difference in level 2 at the basal node is one component of shape change at this level in the cladogram. This component describes a contrast involving a ventral shearing of the posterior portion of the suspensorium, an anteroventral lengthening between landmarks 10 and 12, and deepening of the anterior portion of the quadrate between landmarks 13 and 14 (Fig. 4a). The outgroup-trichomycterid comparison (Fig.

4b), relative to the outgroup-CAL comparison (Fig. 4c), both can be regarded as components of shape change at that node, demonstrating that each sister clade contributes different localized shape change to the total deformation (Fig. 4a). The trichomycterid component contributes most to the anterior dorsoventral stretch of the quadrate, while the CAL component contributes most strongly to the posterior shear of the suspensorium.

We can make no case for any particular functional relevance to these shape contrasts between loricarioids overall and outgroup, nonloricarioid siluroids. No major biomechanical component of cranial evolution has been identified at the basal loricarioid diversification. However, eye size and position differ dramatically among catfishes and the effect of these factors on shape and position of the underlying suspensorial bones have been noted previously (e.g., Schaefer 1991; Arratia 1992). The observed shape contrast involving deformation of the hyomandibula, in particular evolution of the anterior lamina of that bone (Arratia 1992, p. 79), may have a large component due to evolution in eye size and position, changes not associated with the functional morphology of the underlying adductor musculature. Further, the overall contrast at this particular node is a composite of individual, lineage-specific components. While the localization and description of shape change is not a direct test of the decoupling hypothesis, we note here the presence of significant within-group variance in suspensorium shape coupled with a large degree of lineage-specific heterogeneity in shape deformation apparently unrelated to major biomechanical evolution.

*Mandibular Shape Change.*—The total nonaffine component of shape difference in Level 3 at the basal node describes a pattern of shape deformation localized in three separate regions of the mandible (Fig. 5a). The largest contribution to the total deformation (partial warp 2, bending energy 0.3720, 37.5% of total) can be described as a shortening of the relative length of the Meckel's cartilage and compaction of the synchondral articulation (landmarks 21–24) between dentary and anguloarticular bone components of the mandible. An additional, lesser contribution (partial warp 1, bending energy = 0.2461, 24.8% of total) involves a lengthening and dorsoposterior shear of the coronoid region (landmarks 19, 20) relative to the mesial synchondral joint between anguloarticular and dentary bones (landmarks 21–24). A third minor localized shape deformation involves a dorsoventral stretching of the anterior dentary between landmarks 25 and 26 (partial warp 6, bending energy = 0.0225, 2.3% of total). We can decompose this overall deformation into that attributable to the individual sister taxa. The outgroup-trichomycterid comparison (Fig. 5b) reveals a large contribution to both the compaction of the synchondral articulation and the dorsoposterior shear of the coronoid region revealed by examining the total deformation at this node (Fig. 5a). However, this contrast involves an enhanced dorsoposterior displacement and an anteroposterior shortening of the coronoid not revealed by examining the overall deformation. The outgroup-CAL comparison (Fig. 5c) depicts an overall pattern of deformation quite similar to the total deformation in the mandible at this node.

Unlike the pattern of deformation described for the sus-

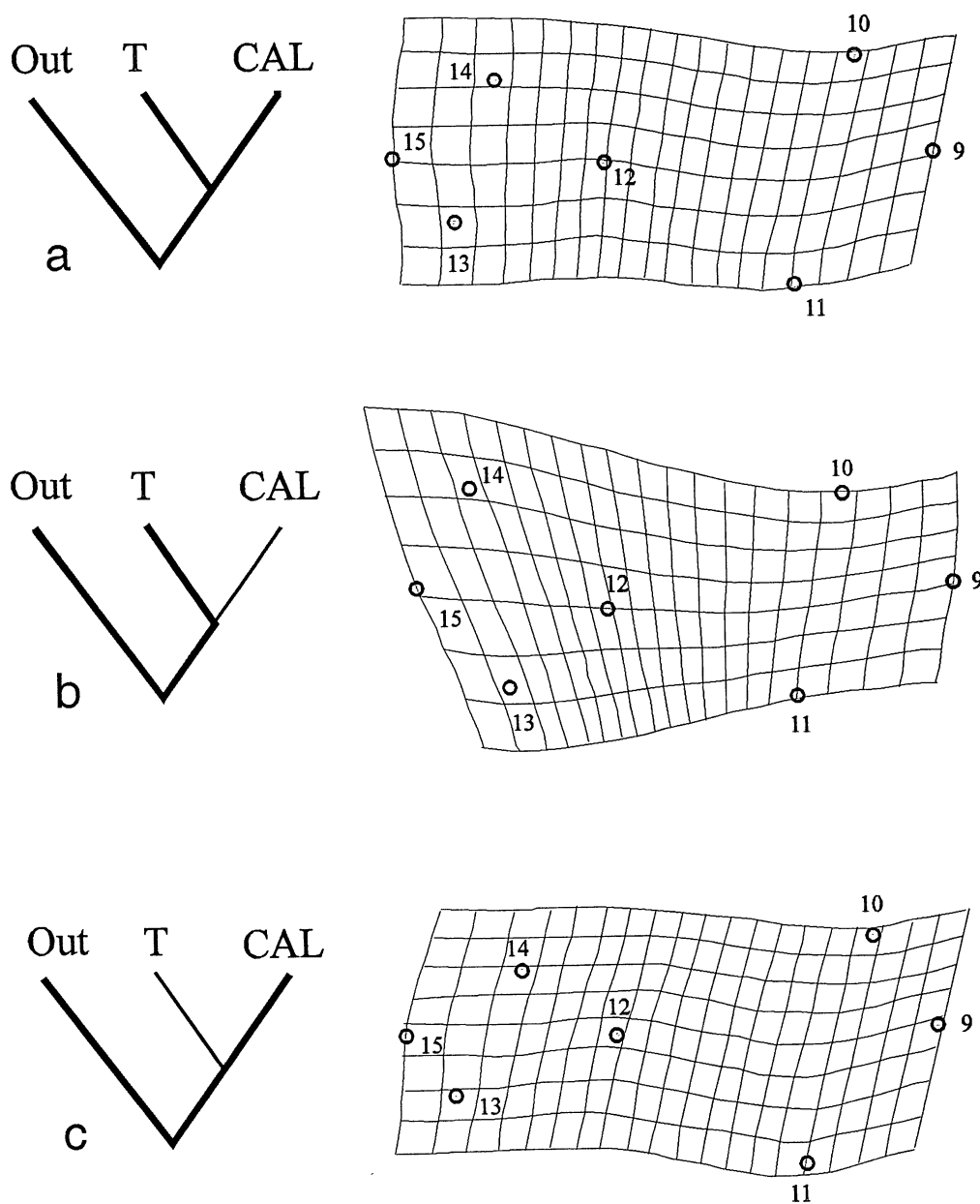


FIG. 4. Total nonaffine shape deformation in the jaw suspensorium between sister pairs at the basal node. This figure shows the pattern of morphological change for one of the two incorrect predictions at the basal node (Table 3, level 2). Landmarks numbered as in Figure 3b: (a) nonloricarioid siluroids to loricarioids (total bending energy [be] = 0.0649); (b) nonloricarioids to trichomycterids (total be = 0.1633); (c) nonloricarioids to callichthyids + astroblepids + loricariids (total be = 0.0622).

ensorium, the total deformation in the mandible at the basal node involves a lesser degree of within-lineage heterogeneity in terms of localized shape change. We cannot make a similar claim for mandibular shape change unassociated with bio-mechanical evolution. We suspect that the compaction of the synchondral joint is mechanically related to a shortening of the lever arm and strengthening of the articulation between mandible elements, yielding increased force transmission to the mandibular tooth-bearing surface during feeding. Such a morphological change would suggest that feeding behavior in loricarioids involves decreased rapidity of mandibular depression, but increased force application to the prey relative

to the outgroup catfish clades examined here. This change may be related to scraping of food from substrate surfaces, and would represent a significant change from velocity-dominated mandibular depression mechanisms in outgroup-suction feeding fishes. The expansion of the coronoid region may have a similar interpretation in terms of mechanical advantage due to increased insertion area and moment arm for an expanded adductor muscle and its derivatives. Lengthening of the distance between points 19 and 20 (Fig. 5) could increase the adductor moment arm, decreasing the velocity of jaw adduction but increasing the force of the bite. Expansion of the anterior mandibular depth (Fig 5: 25 to 26)

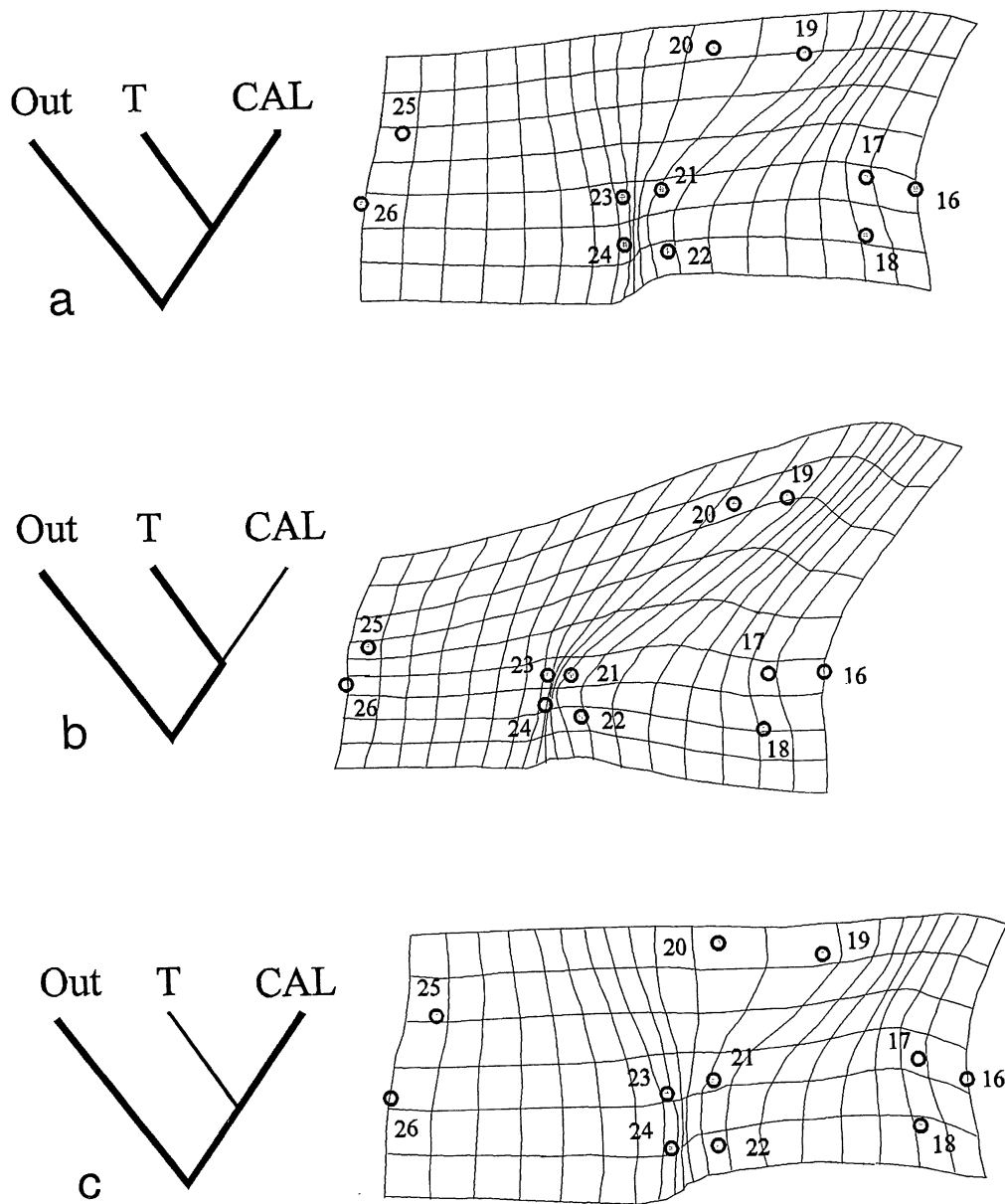


FIG. 5. Total nonaffine shape deformation in the mandible between sister pairs at the basal node. This figure shows the pattern of morphological change for one of the two incorrect predictions at the basal node (Table 3, level 3). Landmarks numbered as in Figure 3c: (a) nonloricarioid siluroids to loricarioids (total bending energy [be] = 0.9922); (b) nonloricarioid siluroids to trichomycterids (total be = 1.6424); (c) nonloricarioid siluroids to callichthyids + astroblepids + loricariids (CAL) (total be = 0.9846).

is also consistent with greater application of force to the mandible, as a deeper mandible is better able to resist bending moments imposed when the lower jaw is applied to the substrate. In sum, the pattern of deformation illustrated in Figure 5 is consistent with the transition from a primitive suction feeding mechanism to a feeding system involving proportionally greater amounts of scraping, and hence the generation of increased mandibular resistive forces. The fact that such morphological changes were not predicted from our measurement of biomechanical decoupling events emphasizes that changes in feeding behavior and functional morphology may also arise from proportional changes in skull bones and muscles. These results also suggest that at the

origin of the Loricarioidea, key features involved with origin of a loricarioid-style feeding mechanism were changes in the shape of lower jaw morphology, and that novel biomechanical linkages evolved later in concert with increased versatility and variability in the jaw and skull.

#### *Decoupling and Morphology*

We have documented a significant association between morphometric variance and biomechanical complexity in the loricarioid catfishes. Sister clades possessing increased numbers of biomechanical pathways mediating function in the feeding mechanism of these fishes exhibit significantly great-

er within-group variability for these structures compared to outgroups having experienced no such biomechanical evolution. An important general hypothesis regarding the evolutionary consequence of decoupling has been supported in this case study, confirming our predictions (Schaefer and Lauder 1986) as well as those of Emerson (1988).

The general relevance of our findings involves empirical support for the interpretation that release of design constraints and duplication of functional pathways is causally related to subsequent historical diversification. We suggest that the comparative historical approach, and methods for testing historical hypotheses utilized here, offer one means for placing the study of functional design, particularly the analysis of functional constraints, in a hypothetico-deductive framework. While it has been recognized that limits on the diversity of phenotypes resulting from constrained development have phylogenetic pattern (Alberch 1980; Wake 1982; Maynard Smith et al. 1985; Fink 1988), most proposals of developmental constraint are largely descriptive and lack an explicit, testable causal mechanism (but see Atchley and Hall 1991). Mechanistic hypotheses are not tested simply by observing that the range of variation in a structure is less than the range of theoretically possible variants in an organism or taxon.

To further explore the historical basis for the association between changes in mechanical linkages and morphological variation in the loricarioids, we might attempt to integrate intrinsic analyses of design with experimental studies of function and behavior. This integration of functional and behavioral data could address in more detail the mechanistic consequences of decoupling in the loricarioid feeding mechanism. For example, we might quantify variance in motor patterns in relevant jaw muscles, variations in modulation of pressure changes during feeding, or the range of bone movements during different behaviors. Asymmetry of muscle function between right and left sides could also be measured. Comparisons of functional and behavioral variables using the methods described here would allow this study, based on morphology alone, to be extended to examine historical relationships among other types of organismal traits such as function and behavior. For example, to what extent do changes in the number of mechanical linkages relate to changes in variability of muscle function? Or, does osteological variation correlate (historically) with variability in motor output to feeding musculature? While such studies will be substantially more complex than this one in requiring functional data on living organisms from numerous taxa, they will allow variability in both structure and function to be analyzed in a historical context. Such studies are currently rare, and yet would provide considerable insight into patterns of historical change in form and function.

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#### APPENDIX I

List of material examined. Symbolic codes for institutional collections follow Leviton et al. (1985).

- ARIIDAE: *Ariopsis felis*, ANSP 145667 (3: 49.0–49.5 mm SL); *Bagre bagre*, ANSP 121328, 2, 82.2, 83.0 mm SL.
- DORADIDAE: *Acanthodoras cataphractus*, ANSP 161507 (3: 52.7–57.4 mm SL); *Anduzedoras microstomus*, ANSP 161518 (4: 31.2–48.5 mm SL).
- MOCHOKIDAE: *Brachysynodontis batensoda*, USNM 230106 (1: 105.1 mm SL); *Chiloglanis carnosus* PARATYPE, MCZ 50541 (1: 62.3 mm SL); *Chiloglanis micropogon*, USNM 303409 (1: 29.0 mm SL); *Chiloglanis polypogon*, USNM 304263 (1: 27.7 mm SL); *Hemisynodontis membranaceus*, USNM 313584 (1: 78.4 mm SL).
- PIMELODIDAE: *Brachyrhamdia imitator*, ANSP 139850 (2: 30.8–37.8 mm SL); *Pimelodella chagresi*, ANSP 99936 (2: 38.8–40.3 mm SL).
- SISORIDAE: *Euchiloglanis hodgarti*, ANSP 159668 (2: 34.5–44.0 mm SL); *Glyptosternum platypogonoides*, ANSP 59198 (2: 43.5–51.5 mm SL); *Glyptothorax laosensis*, ANSP 76834 (1: 35.0 mm SL).

- TRICHOMYCTERIDAE: *Acanthopoma bondi*, ANSP 139636 (3: 21.5–32.1 mm SL); *Acanthopoma bondi*, ANSP 136018 (1: 20.2 mm SL); *Ochmacanthus alternus*, ANSP 135920 (3: 35.9–40.2 mm SL); *Ochmacanthus orinoco*, ANSP 163018 (3: 26.3–30.2 mm SL); *Plectrochilus* sp., ANSP 167710 (3: 47.4–55.0 mm SL); *Plectrochilus* sp., ANSP 131344 (2: 55.1, 65.3 mm SL); *Trichomycterus striatum*, ANSP 104215 (2: 50.8, 58.2 mm SL); *Trichomycterus amazonicus*, ANSP 160591 (2: 38.2, 39.8 mm SL); *Trichomycterus dispar*, ANSP 21355 (3: 72.0–88.2 mm SL); *Vandellia* sp., ANSP 162672 (3: 42.7–49.6 mm SL).
- CALLICHTHYIDAE: *Callichthys callichthys*, ANSP 130428 (3: 29.1–44.1 mm SL); *Corydoras bondi*, ANSP 168189 (4: 18.5–22.4 mm SL); *Corydoras metae*, ANSP 134686 (3: 28.4–33.6 mm SL); *Corydoras* sp., ANSP 139635 (3: 22.9–30.4 mm SL); *Corydoras* sp. A, ANSP 149060 (3: 31.6–34.2 mm SL); *Corydoras* sp. C, ANSP 139633 (3: 20.2–25.2 mm SL); *Dianema longibarbis*, USNM 284636 (1: 58.6 mm SL); *Hoplosternum thoracatum*, ANSP 133138 (3: 50.2–63.8 mm SL); *Hoplosternum thoracatum* ANSP 162691 (3: 50.1–57.4 mm SL).

ASTROBLEPIDAE: *Astroblepus boulengeri*, FMNH DJS81-119 (2: 34.1, 66.2 mm SL); *Astroblepus formosus*, ANSP 71650 (4: 28.5–32.0 mm SL); *Astroblepus grivalvii*, FMNH 96628 (1: 39.3 mm SL); *Astroblepus longifilis*, ANSP 104208 (4: 40.6–66.2 mm SL); *Astroblepus longifilis*, FMNH 70017 (2: 64.0, 64.7 mm SL); *Astroblepus orientalis*, ANSP 168821 (3: 33.3–49.0 mm SL); *Astroblepus simonsii*, FMNH 84655 (1: 51.0 mm SL); *Astroblepus* sp., ANSP 126190 (2: 55.1, 56.1 mm SL); *Astroblepus* sp., USNM 273600 (2: 39.1–41.3 mm SL); *Astroblepus* sp., USNM 302652 (4: 28.3–57.5 mm SL); *Astroblepus* sp., USNM 302678 (1: 35.3 mm SL).

LORICARIIDAE: *Chaetostoma* sp., ANSP 138975 (4: 41.9–62.4 mm SL); *Dentectus barbarmatus*, ANSP 131631 (4: 69.4–95.7 mm SL); *Hemiancistrus* sp., ANSP 162174 (4: 55.0–66.9 mm SL); *Hemiodontichthys acipenserinus*, ANSP 138866 (4: 79.3–103.2 mm SL); *Hypoptopoma joberti*, UMMZ 204339 (2: 83.5, 84.5 mm SL); *Hypoptopoma joberti*, ANSP 134438 (1: 48.0 mm SL); *Hypostomus* sp., ANSP 160623 (2: 49.2, 53.1 mm SL); *Pseudancistrus* sp., ANSP 160600 (2: 61.3, 69.6 mm SL); *Pseudohemiodon* sp., ANSP 162337 (2: 87.5, 88.5 mm SL).

## APPENDIX II

Landmarks used in the morphometric analyses (Fig. 3). Variables listed below represent straight-line interlandmark distances.

*Level one:* Ventral view, right side: pectoral; hyoid; mandibular, and palatal arches. 1, anteromedial tip of premaxilla at tooth row; 2, anteromedial tip of mandible at tooth row; 3, anterior most point on urohyal at midline; 4, anteromedial margin of cleithrum; 5, lateral tip of maxilla; 6, ventrolateral process of mandible (anguloarticular bone) for insertion of mandibulohyoid ligament; 7, dorsolateral process of anterohyal for insertion of mandibulohyoid ligament; 8, tip of posterior cleithral process.

Variables: 1-2, 2-3, 3-4, 4-8, 7-8, 6-7, 5-6, 1-5, 1-6, 2-5, 2-7, 3-6, 2-6, 3-7, 3-8, 4-7.

*Level two:* Lateral view, right side of mandibular arch: 9, posterior limit of hyomandibular articular condyle; 10, dorsal tip of hyomandibular dorsal process; 11, anterior limit of hyomandibular opercular condyle; 12, anterodorsal limit of hyomandibular symplectic synchondrosis; 13, anterior limit of preopercular-quadrata suture; 14, posterodorsal limit of quadrata (in tricomycetids, this is represented by a slender dorsal process, while in other loricarioids, the process is absent and the landmark is identified as the posterodorsal extremal limit of the quadrata); 15, anterior tip of quadrata mandibular condyle.

Variables: 9-10, 10-11, 10-14, 9-11, 11-12, 12-14, 11-14, 10-12, 12-13, 14-15, 12-15, 13-14, 13-15.

*Level three:* Medial view, right side of mandible: 16, posterior tip of anguloarticular-interoperculomandibular process; 17, dorsomedial limit of mandibuloquadrata articular condyle; 18, ventromedial limit of mandibuloquadrata articular condyle; 19, dorsal tip of anguloarticular coronoid process; 20, dorsal tip of dentary coronoid process (in loricariids, the dentary coronoid is developed into a large, concave process; the landmark is identified as the posterior limit of the concavity where it emerges from the main body of the dentary); 21, posterodorsal limit of the anguloarticular buttress with Meckel's cartilage; 22, posteroventral limit of the anguloarticular buttress with Meckel's cartilage; 23, anterodorsal limit of the dentary buttress with Meckel's cartilage; 24, anteroventral limit of the dentary buttress with Meckel's cartilage; 25, anterodorsal tip of dentary; 26, anteroventral tip of dentary.

Variables: 16-17, 16-18, 17-18, 17-19, 19-21, 17-20, 17-21, 17-22, 18-21, 18-22, 20-21, 21-22, 22-23, 21-23, 22-24, 21-24, 23-24, 24-26, 24-25, 23-26, 25-26, 23-25.