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COMPARATIVE ANALYSIS OF MORPHOLOGICAL DIVERSITY: DOES DISPARITY ACCUMULATE AT THE SAME RATE IN TWO LINEAGES OF CENTRARCHID FISHES?

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Abstract.—Evolutionary lineages differ with regard to the variety of forms they exhibit. We investigated whether comparisons of morphological diversity can be used to identify differences in ecological diversity in two sister clades of centrarchid fishes. Species in the *Lepomis* clade (sunfishes) feed on a wider range of prey items than species in the *Micropterus* clade (black basses). We quantified disparity in morphology of the feeding apparatus as within-clade variance on principal components and found that *Lepomis* exhibits 4.4 and 7.4 times more variance than *Micropterus* on the first two principal components. However, lineages are expected to diversify morphologically and ecologically given enough time, and this pattern could have arisen due to differences in the amount of time each clade has had to accumulate variance. Despite being sister groups, the age of the most recent common ancestor of *Lepomis* is approximately 14.6 million years ago and its lineages have a total length of 86.4 million years while the age of the most recent common ancestor of *Micropterus* is only about 8.4 million years ago, and it has a total branch length of 42.9 million years. We used the Brownian motion model of character evolution to test the hypothesis that time of independent evolution of each clade's lineages accounts for differences in morphological disparity and determined that the rates of evolution of the first two principal components are 4.4 and 7.7 times greater in *Lepomis*. Thus, time and phylogeny do not account for the differences in morphological disparity observed in *Lepomis* and *Micropterus*, and other diversity-promoting mechanisms should be investigated.

Key words.—Brownian motion, functional morphology, *Lepomis*, *Micropterus*, rate of morphological evolution.

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One of the most intriguing patterns in nature is that morphological diversity is not equally distributed among lineages. Some groups, such as lampreys and flamingos, exhibit relatively little variation in body and head shape (Gatesy and Middleton 1997; Potter and Gill 2003, respectively), while other groups, such as teleost fishes and Hawaiian honeycreepers, display a spectacular variety of forms. Many factors are thought to lead to this inequity in morphological diversity, ranging from potentially strong effects of community interactions (Hutchinson 1959; Schluter 1998), opportunities provided by the invasion of novel habitats (Losos et al. 1997; Baldwin and Sanderson 1998), and a variety of intrinsic factors that may constrain or promote the capacity of any given body plan to diversify (Vermeij 1973; Lauder 1990; Middleton and Gatesy 2000; Alfaro et al. 2004). Species richness is one facet of diversity, but a thorough characterization must account for the variety of species as well as their number. Thus, understanding the processes that influence morphological diversification is fundamental to understanding biodiversity.

In recent years, evolutionary biologists have quantified the diversity of forms within taxa as morphological variation, or disparity (reviewed by Foote 1997). Disparity is some metric of the amount of morphospace occupied by a group and is often measured as within-group variance (Foote 1997). Although comparisons of this metric can be used to investigate the distribution of morphological diversity at some point in time, they may be of limited utility to test hypotheses about the mechanisms responsible. Because lineages are expected to diversify morphologically and ecologically given enough time, the hypothesis that time of independent evolution explains differences in extant diversity must be falsified before other mechanistic hypotheses can be invoked.

In this study, we ask whether differences in diet diversity in two sister clades of the North American freshwater fish radiation, Centrarchidae (Teleostei), are reflected in disparity of characters of the feeding mechanism. We then ask whether time and phylogenetic history of each clade's component lineages account for differences in disparity or if, instead, differences in the rate of morphological evolution are implicated.

We focus on *Lepomis* (sunfishes) and *Micropterus* (black basses), which are each monophyletic and sister taxa with strong phylogenetic support (Near et al. 2004; Fig. 1). However, despite having evolved independently for the same amount of time, we will show that *Lepomis* species feed on a wider range of prey items than *Micropterus* species. *Lepomis* includes species that feed predominantly on gastropods and others that feed heavily on crayfish and fish, while the remaining *Lepomis* species eat varying proportions of aquatic immature insects, terrestrial insects, microcrustacea, crayfish, and small fish (Etnier and Starnes 1993). In contrast, *Micropterus* species feed on the same suite of prey items, including crayfish, fish, and aquatic immature insects (Etnier and Starnes 1993). These taxonomic categories of prey items impose different functional demands on fish predators for their capture and processing, varying in size, elusiveness, and hardness. Because a fish's ability to meet these demands is largely a function of its morphology, we predict that the greater diet diversity of *Lepomis* will be associated with greater disparity of the feeding apparatus when compared to *Micropterus*.

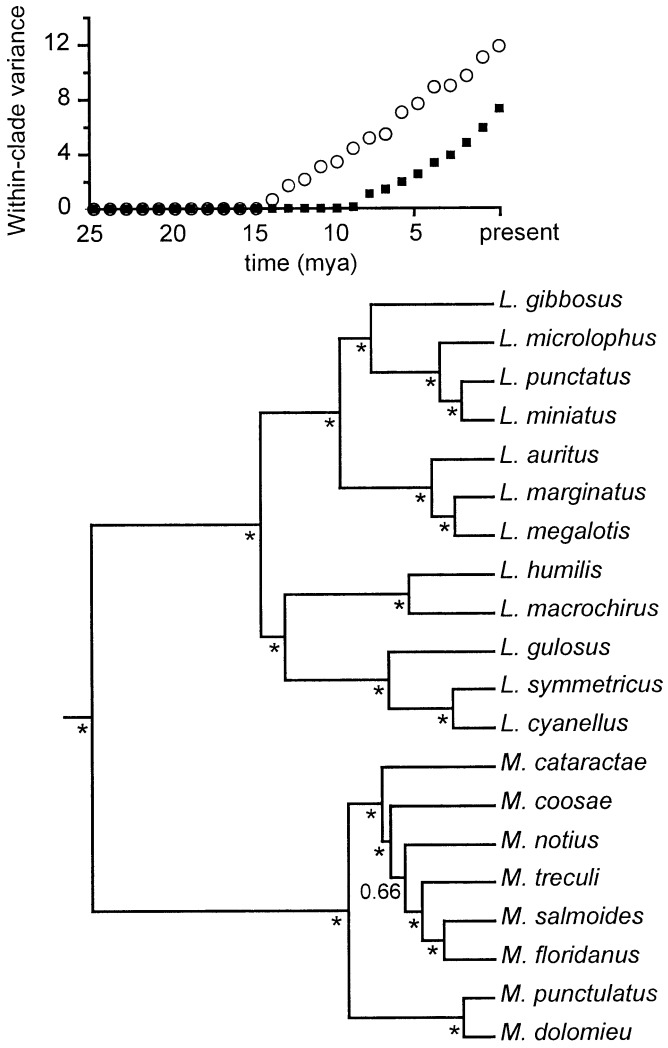

FIG. 1. The relationship between phylogeny, rate of morphological evolution, and within-clade variance under Brownian motion character evolution. The phylogeny is presented as a chronogram with branch lengths in millions of years, and asterisks indicate nodes supported by Bayesian posterior probabilities greater than 0.95 (Near et al. 2005a). Scale for branch lengths is represented on the x-axis of the variance through time plot. Expected within-clade morphological variance for *Lepomis* (open circles) and *Micropterus* (filled squares) at each point in time is the mean of 500 replicates of Brownian motion character evolution in which the rate parameter, σ^2, was set equal to one for both clades. Simulations of species' values were carried out in Brownie (B. O'Meara et al., unpubl. ms.), using the ''simulatetipvaluesmanytimes.m'' function. Given the phylogeny with branch lengths in absolute time and the same rate of morphological evolution in both clades, *Lepomis* is expected to exhibit greater within-clade variance than *Micropterus*.

Attempts to implicate diversity-promoting mechanisms to explain differences in morphological diversity require more than comparison of within-clade morphological variance. Because morphological variance is expected to increase partly as a function of time, comparisons among clades of different ages are confounded with time. Previous attempts to control for time have involved limiting comparisons to sister clades (Brooks and McLennan 1993) or to clades of approximately the same age (Warheit et al. 1999; Losos and Miles 2002). However, such comparisons may fail to adequately control for time in two ways. First, morphological variance in a clade cannot begin to increase until the time of the first lineage-splitting event in the group's history. Second, even though groups are the same age, their component lineages might have evolved independently for different amounts of time. In the Centrarchidae, the age of the most recent common ancestor (MRCA) of the 12 extant *Lepomis* species is estimated to be 14.6 million years ago, and the total branch length within this clade is estimated to be 86.4 million years; the MRCA of the eight extant *Micropterus* species is estimated to be 8.4 million years ago and total clade branch length is 42.9 million years (Fig. 1). As a consequence of age differences between crown group MRCAs and total branch lengths, simple comparisons of morphological variance between these sister groups will not account for differences in time of independent evolution.

To control for these confounding influences, we used the Brownian motion model of continuous character evolution, which models character change as a random walk along each lineage of a phylogeny. The model's single parameter, σ^2, is the time-independent variance of the normal distribution from which character displacement at each step in the random walk is sampled (Felsenstein 1985). Because character change in each lineage is assumed to be independent, variance among lineages is expected to be an increasing function of time and the parameter σ^2 (Martins 1994). In this way, the parameter σ^2 can also be thought of as the rate at which variance accumulates within a clade or the rate of morphological evolution within a clade (Garland 1992). Under the assumption that this rate parameter is constant throughout the history of a clade, it can be estimated and used to compare groups as a time-independent estimate of morphological diversity (Garland 1992; Hutcheon and Garland 2004).

According to the Brownian model, a clade with an older MRCA and greater total branch length is expected to exhibit greater character variance even when the rates of morphological evolution do not differ. This can be illustrated in the comparison of *Lepomis* and *Micropterus*. Allowing a character to evolve according to a Brownian motion process with the same rate parameter for both groups leads to the expectation over many replicates of evolution that character variance in *Lepomis* is higher than in *Micropterus* (Fig. 1). This example emphasizes that the difference in time of independent evolution between these clades may provide sufficient explanation for variance differences; therefore, tests for equivalence of rates are necessary to assess this hypothesis.

In this study we integrate a well-resolved phylogenetic hypothesis, robust molecular inferred age estimates, information on diet compiled from numerous studies, and data on species' mean character values to test the association of diet diversity and morphological disparity in the extant lineages of *Lepomis* and *Micropterus* and to investigate whether differences in disparity are associated with differences in rates of morphological evolution. The results of this study will demonstrate whether disparity meaningfully reflects diet diversity when disparity is based on characters that have predictable consequences on feeding performance and whether differences in disparity require a mechanistic explanation other than time and phylogeny.

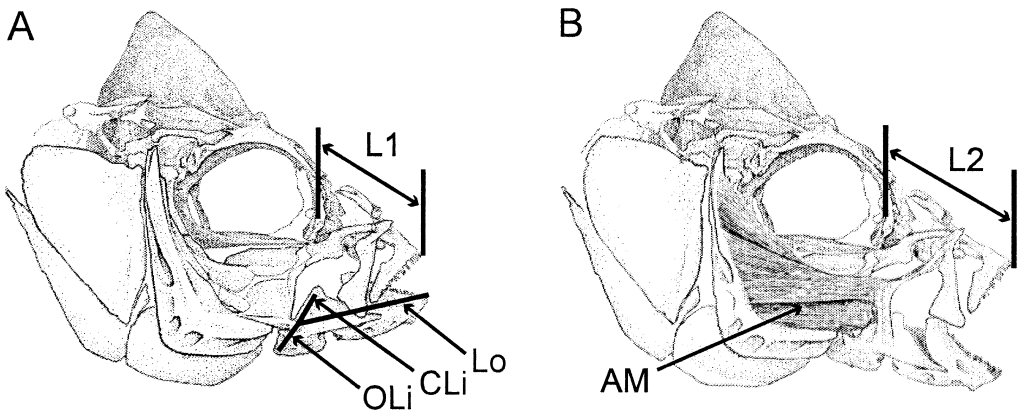

FIG. 2. Oral jaws characters illustrated on an open- (A) and closed-mouth (B) bluegill, *Lepomis macrochirus,* skull. Upper jaw protrusion is the difference between L2 and L1. The lower jaw opening in-lever (OLi), closing in-lever (CLi), and out-lever (Lo) are illustrated in (A). The adductor mandibulae (AM) is illustrated in (B).

MATERIALS AND METHODS

Diet Diversity

To more rigorously investigate the claim that *Lepomis* species have greater diet diversity than *Micropterus* species, we synthesized published data on each species' diet. We compiled data from studies that quantified taxonomic composition of diets for individuals of typical adult body size. Methods used to quantify diet composition differed among studies—some used percent of individuals within the sample whose gut contained a particular taxonomic category, others used the percent of the total number of prey items contributed by each category, and others used the percent of the total volume of prey items contributed by each category. For each study, we averaged over localities when multiple populations were sampled and ranked the five most common prey items provided that each prey item made at least a 10% contribution to the diet (see Appendix 1 available online only at http://dx.doi.org/10.1554/04-588.1.sl). To account for methodological differences across studies, we described each species' diet as the most common prey category from each study and those prey categories that ranked in the top five in more than one study. When a species was represented by only one diet study, we described that species' diet as the most common diet items given in that study according to the aforementioned criteria.

Morphological Measurements

We measured characters whose variation has predictable consequences for a fish's ability to meet the functional requirements of prey capture and processing. Previous research on the functional morphology of the feeding apparatus of centrarchid fishes provided the basis for expectations of how variation in morphology affects feeding performance on various prey types. The maximum sized prey that a fish can capture as well as the size that maximizes energy return are limited by the area of the predator's gape (Werner 1977). We measured gape as the area of the ellipse, whose major and minor axes are gape width and height. At maximum opening, we measured gape width as the distance between the coronoid processes of the left and right articular bones and height as the distance between the tooth plates on the premaxilla and dentary. Many aspects of feeding performance and energetics will scale with body size, so we also collected data on the maximum total length attained by each species (Page and Burr 1991).

The ability of a fish predator to capture elusive prey is limited by the speed of mouth opening and closing, which is a function of the capacity of muscles to generate force and velocity and the lower jaw to transfer force and velocity to mouth opening and closing (Wainwright and Shaw 1999). The adductor mandibulae (AM) muscle actuates mouth closing by direct attachments to the lower and upper jaw (Lauder 1985; Fig. 2). The AM was dissected from formalin-preserved specimens and stored in 70% ethanol. We measured AM mass as an indication of its force producing capacity (Richard and Wainwright 1995; Wainwright et al. 2004).

The capacity of the lower jaw to transfer motion and force from muscles and linkage systems to mouth opening and closing is reflected in its lever arms: the mouth opening in-lever was measured as the distance between attachment of the interoperculo-mandibular ligament on the lower jaw and the quadrate-articular joint (i.e., the point of rotation of the lower jaw); the mouth closing in-lever was measured as the distance between the point of attachment of the AM and the point of rotation of the lower jaw; and the out-lever for both opening and closing was the distance between the rotation point and the anterior tip of the mandible (Barel 1983; Richard and Wainwright 1995; Fig. 2). The mechanical advantage of opening and closing are defined as the ratio of respective in-levers to out-lever and reflect a trade-off between transmission of force and velocity to the anterior tip of the lower jaw; smaller ratios will transfer more velocity per unit input velocity, whereas larger ratios transmit more force.

The capacity to capture elusive prey will also be affected by the ability of a fish to get close enough to entrain the prey in a suction-induced flow of water into the mouth. The extent of premaxillary (i.e., upper jaw) protrusion will affect the distance between predator and prey and potentially the success of capture (Waltzek and Wainwright 2003). We measured protrusion as anterior translation of the premaxillary tip during mouth opening (Fig. 2).

Following capture, centrarchid fishes process prey in their pharyngeal jaw apparatus—a system of modified branchial arches immediately anterior to the esophagous—by movements of the upper and lower tooth plates attached to these bones. The levator posterior (LP) muscle provides the primary force for adduction (Wainwright 1989; Galis and Drucker 1996); we measured LP mass as an indication of force production in this muscle, and thus capacity to crush hard prey (Wainwright 1988; Wainwright et al. 2004).

Specimens and Sampling

All measurements were made on at least three preserved specimens of each species and means per species were used to estimate species' character values. Specimens from the following species were borrowed from museum collections: *L. cyanellus L. humilis*, *L. megalotis*, *L. miniatus*, *L. symmetricus*, *M. cataractae*, *M. coosae*, *M. dolomieu*, *M. floridanus*, *M. notius*, *M. punctulatus*, and *M. treculi* (see Appendix 2 available online only at http://dx.doi.org/10.1554/04-588.1.s2). Specimens of the remaining species were collected in Florida, fixed in 10% formalin, and stored in 70% ethanol (see Appendix 2 available online). After the AM and LP muscles had been dissected out, specimens were cleared using trypsin and double-stained using an Alcian-blue cartilage stain and alizarin red bone stain (Taylor 1967). This method permitted clear identification of the relevant landmarks on the specimens (see above). All lower jaw lever arm measurements were made under a dissecting microscope using an ocular micrometer.

Body Size Corrections

We corrected for between-species differences in character values that are due to differences in body size by regression of log-transformed species' character values against log-transformed standard length (SL). To make all characters dimensionally similar, we took the cube root of AM and LP masses and the square root of gape area. We then obtained size-corrected species' means using a method employed by Blomberg et al. (2003). Briefly, because regressions that involve species as datapoints violate the assumption of independence of errors (Felsenstein 1985; Garland et al. 1992) and to protect against grade shifts, which could bias estimation of the allometric exponent (Nunn and Barton 2000), we used regressions of standardized contrasts, obtained using CAIC (Purvis and Rambaut 1995), to estimate allometric slope. This slope was then imposed on the regression of species' character values against standard length, the intercept was fit using the least-squares method, and we obtained residuals.

We derived sets of size-corrected character values using two different methods. First, we regressed each character against body size and obtained residuals for *Lepomis* and *Micropterus* separately. This analysis provided size-corrected character values for each species, representing the deviations from the clade-specific allometric relationship. However, these character values were not appropriate for the principal components analysis (see below) because the allometric relationships differed between clades. Therefore, we also conducted regressions and obtained residuals for all *Lepomis* and *Micropterus* species pooled together. These size-corrected values were then used in our principal components analysis.

Comparisons of Morphological Variance

To test whether the clade with the greater diet diversity also exhibited higher morphological disparity, we compared variances of principal components scores calculated for *Lepomis* and *Micropterus*. We carried out a principal components analysis on the correlation matrix of species' maximum total length and the set of size-corrected character values obtained by regression of all species' values pooled together (see above). We calculated within-clade variance on each principal component and compared *Lepomis* and *Micropterus* using an *F*-test. We also compared within-clade variation using Levene's test, which has been shown to perform better when underlying data do not fit a normal distribution (Conover et al. 1981; Schultz 1983). To reveal single characters whose variance differed significantly between clades, we calculated univariate variances for each character and clade. For this analysis, we used the set of size-corrected character values obtained by separate, within-clade regressions (see above), and compared within-clade variances for each character using *F*-tests and Levene's tests. All tests were one-tailed, and we assessed significance of variance differences using the sequential Bonferroni correction for multiple comparisons (Rice 1989). Although degrees of freedom for both tests are likely inflated due to nonindependence of species' character values, we applied them because we were interested in comparing these results to those of rates comparisons, which we view as a phylogenetically correct comparison of trait variance.

Phylogenetic Analysis and Estimation of Divergence Times

Phylogenetic relationships of all 32 recognized centrarchid species were analyzed using aligned DNA sequences from Near et al. (2005a). This dataset was comprised of seven gene regions, including three mitochondrial DNA genes (ND2, 16S rRNA, and three tRNAs) and four nuclear genes (S7 ribosomal protein intron 1, calmodulin intron 4, rhodopsin, and *Tmo4C4*). A partitioned mixed-model Bayesian analysis was used to estimate both the phylogenetic tree and branch lengths. Details concerning the Bayesian analysis, including the specific nucleotide substitution models used and assessment of node support, are provided in Near et al. (2005a).

Cross-validation of fossil age estimates resulted in the identification of six consistent fossil calibration points (Near et al. 2003, 2005a,b), and these were used to convert branch lengths from substitutions per site to absolute age in millions of years. We corrected for the observed among lineage rate heterogeneity using penalized likelihood as implemented in the computer program r8s (Sanderson 2002, 2003). A single fossil calibration point was treated as a fixed minimal age estimate and the remaining five fossil dates were treated as minimal age constraints. Cross-validation of the smoothing parameter value using fossil-based model cross-validation followed the protocol outlined in Near and Sanderson (2004).

Testing the Appropriateness of the Brownian Motion Model of Character Evolution

Because estimates and comparisons of rates of morphological evolution are based on the assumption that the characters investigated reflect Brownian motion evolution, we tested whether the model could be rejected for any character. We used the computer program Continuous (Pagel 1997, 1999) to test three predictions of the Brownian model: (1) time of shared evolution is proportional to covariance between species' character values; (2) the variance of character change on each branch of the phylogeny is proportional to branch length; and (3) the Brownian rate parameter, σ^2, is constant throughout the history of the clade. The fit of a character to these predictions can be assessed by estimation of the parameters, λ, κ, and δ, respectively, and the maximum likelihood estimates of these parameters scale the branch lengths of the phylogeny to best fit Brownian motion character evolution (Pagel 1997, 1999). Thus, we rejected the model for a character if the likelihood ratio test for any parameter rejected the null hypothesis that the parameter value is equal to one. We tested the fit of the Brownian motion model separately within each clade. We assessed the significance of the likelihood ratio test statistics using alpha levels adjusted for multiple comparisons by the sequential Bonferroni method (Rice 1989). These significance levels were adjusted separately for each parameter and separately for principal components and the set of individual characters.

We also diagnosed the fit of the Brownian model to the data using the Pearson correlation between the absolute value of standardized contrasts (equal to the magnitude of the difference in character values between two nodes divided by the square root of the branch length separating those nodes) and their standard deviations, each of which is equal to the square root of the branch length for the contrast (Garland et al. 1992). Under the assumption that a character evolves in a Brownian way, standardized contrasts should exhibit no correlation with branch length (Hutcheon and Garland 2004).

Comparisons of Rates of Morphological Evolution

When we could not reject the Brownian model of evolution for a character, we tested for equivalence in rates of morphological evolution to test the hypothesis that time of independent evolution of each clade's component lineages accounts for differences in character variance. We employed two methods. First, we used a *t*-test to compare the central tendencies of the absolute values of standardized independent contrasts, which provide independent estimates of the rate at each node in *Lepomis* and *Micropterus* (Garland 1992). Second, we implemented a computer program, Brownie (B. O'Meara, C. Ané, M. Sanderson, and P. Wainwright, unpubl. ms.), to estimate and compare the rate parameter, σ^2, in *Lepomis* and *Micropterus* using a maximum-likelihood approach. Under the Brownian motion model of character evolution, the maximum-likelihood estimator of the rate parameter and its likelihood score are functions of the vector of species' character values, the ancestral value of the character, the number of taxa in the clade, and the branch length covariance matrix, whose diagonal elements are the time to the MRCA of the clade and whose off-diagonal element, t_{ij}, is the time of shared evolution for tip nodes *i* and *j* (B. O'Meara et al., unpubl. ms.). Thus, the input for the program was a vector of species' character values as well as the covariance matrix based on the *Lepomis* and *Micropterus* chronogram (Fig. 1). The hypothesis that rates do not differ between clades was tested using a likelihood-ratio test, in which the null model is that rates are equal in the two groups (i.e., one rate parameter) and the full model is that rates are different in the two groups (i.e., two rate parameters). We obtained *P*-values for the likelihood ratio test statistic by comparison with a χ^2 distribution with one degree of freedom; however, this test is nonconservative for comparisons involving 25 or fewer taxa (B. O'Meara et al., unpubl. ms.). Therefore, we also obtained *P*-values using a parametric bootstrapping procedure implemented in Brownie. Here, species' character values were simulated 1000 times given the *Lepomis* and *Micropterus* branch length covariance matrix and a one rate (i.e., equal rates) model, a null distribution of likelihood-ratio test statistics was generated, and a *P*-value was obtained by comparison of the observed likelihood-ratio test statistic with this distribution (B. O'Meara et al., unpubl. ms.). Sequential Bonferroni corrections were applied separately to principal components and univariate character rates comparisons (Rice 1989).

RESULTS

Diet Diversity

Our synthesis of species' diet composition confirms that *Lepomis* species feed on a wider range of prey items than *Micropterus* species (Fig. 3, Table 1). With the exception of terrestrial vertebrates, which occurred only in the diet of one population of *M. salmoides* (Hodgson et al. 1997), the diet items of *Micropterus* species are a subset of those of *Lepomis* species. Both clades contain species that include hemipterans, odonates, ephemeropterans, terrestrial insects, fish, and crayfish. The *Lepomis* clade contains the warmouth, *L. gulosus*, whose diet resembles *Micropterus* species in that it feeds heavily on crayfish and fish. Additionally, the diet of green sunfish, *L. cyanellus*, is made up largely of crayfish, a category that dominates nearly all *Micropterus* species. Broad categories that occur in *Lepomis* species' diets that make no substantial contribution to *Micropterus* species include microcrustacea, numerous taxa of aquatic immature insects, and gastropods (Fig. 3, Table 1).

Comparisons of Morphological Variance

The results of the principal components analysis are shown in Figure 4. We retained only PC 1 and PC 2, which combined to account for 74% (50% and 24%, respectively) of the total variation among all species in size and trophic characters. The variance within *Lepomis* is significantly higher than within *Micropterus* on both PC 1 ($F = 4.39$, $P = 0.030$; Levene's statistic = 6.21, $P = 0.023$) and PC 2 ($F = 7.37$, $P = 0.007$; Levene's statistic = 9.72, $P = 0.006$). PC 1 separates *Lepomis* and *Micropterus* species into distinct clusters in morphospace (Fig. 4). The characters that load strongly on PC 1 are those that differ most between *Lepomis* and *Micropterus*: maximum total length ($r = 0.37$), gape ($r = 0.41$), upper jaw protrusion

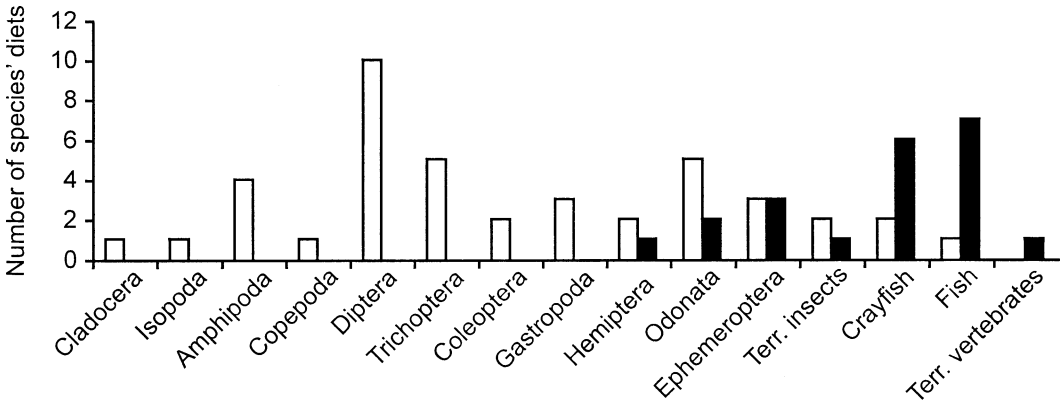

FIG. 3. Histogram illustrating the number of *Lepomis* (open bars) and *Micropterus* (filled bars) species that feed on each prey category. The collective diet of *Micropterus* species is nearly a subset of that of *Lepomis* species.

($r = -0.41$), lower jaw out-lever ($r = 0.45$), and LP mass ($r = -0.43$). Principal component 2 correlates strongly with the remaining characters: AM mass ($r = 0.58$) and lower jaw closing ($r = 0.50$) and opening in-lever ($r = 0.49$). We chose not to retain additional principal components because their eigenvalues were less than one (no single axis accounted for more than 10% of the total variation) and all functional characters correlated strongly with one of the first two principal components.

Variance comparisons of single characters revealed that *Lepomis* exhibits greater variance in all characters measured, ranging from nearly 11 times (LP mass) to 1.3 times (maximum total length) greater than *Micropterus*. However, significant variance differences were discovered only in gape ($F = 9.16$, $P = 0.003$; Levene's statistic = 7.35, $P = 0.014$; Fig. 5), AM mass ($F = 7.02$, $P = 0.008$; Levene's statistic = 5.05, $P = 0.037$), and LP mass ($F = 10.95$, $P = 0.002$; Levene's statistic = 6.72, $P = 0.018$). Additionally, the mechanical properties of the lower jaw, closing (Cl) and opening (O_p) lever ratios, show nonsignificant variance differences even though variance is greater in *Lepomis* for both characters ($F_{\text{Cl-LR}} = 3.55$, $P = 0.052$; Levene's statistic$_{\text{Cl-LR}} = 3.22$, $P = 0.09$; $F_{\text{Op-LR}} = 2.49$, $P = 0.118$; Levene's statistic$_{\text{Op-LR}} = 0.86$, $P = 0.37$).

Fit of the Brownian Model to Functional Characters

We did not find sufficient evidence to definitively reject the Brownian motion model for any character. Tests of PC 1 in *Micropterus* indicate that the κ parameter, which assesses whether variance of character change is proportional to time, is significantly greater than one (κ[MLE] = 3.0, $P = 0.012$; Table 2). This result indicates that long branches accumulate greater character change proportional to their length than short branches (Pagel 1997, 1999). However, the correlation between standardized contrasts and their standard deviations for PC 1 in *Micropterus* was nonsignificant ($r^2 = 0.19$, $P = 0.32$), and, in contradiction to the former result, this diagnostic provides no evidence that long branches exhibit greater proportional changes than shorter branches. Therefore, we concluded that in combination these tests do not provide strong evidence for lack of fit of the Brownian model for PC 1.

In addition, tests of PC 2 in *Lepomis* reveal that the λ parameter, which assesses whether character covariance between taxa reflects phylogenetic relatedness, is less than one with marginal significance (λ[MLE] = 0.0, $P = 0.025$; Table 2). This result indicates that the distribution of PC 2 scores in *Lepomis* is independent of phylogeny (Freckleton et al. 2002) and seems to be driven by the evolution of AM mass and lower jaw closing in-lever in *Lepomis* ($\lambda[\text{MLE}]_{\text{AM}} = 0.0$, $P = 0.005$; $\lambda[\text{MLE}]_{\text{CLi}} = 0.0$, $P = 0.011$), with which PC 2 is strongly correlated. Because violation of the Brownian prediction for this parameter is supported by marginal *P*-values and no other parameter provided evidence for violation of Brownian evolution of these characters, we did not reject the Brownian motion model and proceeded with rates comparisons between *Lepomis* and *Micropterus*. However, results of this test promote a cautious interpretation of rates comparisons involving these characters, as there is some evidence that they have not evolved in a Brownian way. If these results are viewed as violation of the Brownian model—that the distribution of character values is independent of phylogeny—then estimates of within-clade variance are not confounded by time and phylogeny and the *F*-test or Levene's tests are appropriate for comparison of morphological diversity.

Comparisons of Rates of Morphological Evolution

Both principal components have evolved at a greater rate in *Lepomis* relative to *Micropterus*, and this result is consistent using both the standardized contrast and likelihood methods. PC 1 has evolved 4.4 times more rapidly in *Lepomis*, and we rejected the hypothesis that rates of PC 1 evolution are equal (P[standardized contrasts] = 0.028; $P[\chi^2] = 0.038$; P[bootstrap] = 0.050; Fig. 6). PC 2 has evolved 7.7 times more rapidly in *Lepomis*, and this difference was also significant (P[standardized contrasts] = 0.028; $P[\chi^2] = 0.005$; P[bootstrap] = 0.006; Fig. 6).

Even though estimates of rates of evolution are greater in *Lepomis* for all univariate characters, we could not reject the hypothesis that rates are equal in the two clades for any single character (Fig. 6). These rates range from 6.3 times (AM mass) to 1.1 times (lower jaw opening in-lever) greater in *Lepomis*, but none of these differences are significant after adjusting alpha levels according to the sequential Bonferroni correction

TABLE 1. Species' diets and means for all characters measured. Maximum total length (max. TL) was used to represent adult sizes, and standard length (SL) is the mean size of the specimens from which measurements were made. Cl-LR, lower jaw closing lever ratio; Op-LR, lower jaw opening lever ratio; UJ-Pro, upper jaw protrusion distance; CLi, length of lower jaw closing in-lever; OLi, length of lower jaw opening in lever; Lo, length of lower jaw out-lever; AM, adductor mandibulae mass; LP, levator posterior mass. All linear measurements are given in millimeters, areal measurements in squared millimeters, and masses in grams.

Clade	Species	Primary diet items	Max. TL	Cl-LR	Op-LR	SL	Gape	UJ-Pro	CLi	OLi	Lo	AM	LP
Lepomis	*auritus*	Diptera, Trichoptera	240	0.217	0.287	95.0	218.6	2.9	2.417	3.194	11.112	0.0903	0.0027
	cyanellus	crayfish, Coleoptera, Diptera, Odonata	310	0.187	0.253	95.7	572.4	2.3	2.611	3.528	13.933	0.1169	0.0046
	gibbosus	Gastropoda, Coleoptera, Diptera, Trichoptera	400	0.258	0.276	97.7	331.7	2.7	2.806	3.000	10.882	0.1308	0.0264
	gulosus	crayfish, fish, Odonata	310	0.205	0.239	87.7	781.7	2.9	3.109	3.627	15.200	0.0947	0.0030
	humilis	Diptera, Hemiptera, Trichoptera	150	0.278	0.268	81.0	366.9	1.7	3.028	2.917	10.882	0.0798	0.0010
	macrochirus	Amphipoda, Cladocera, Diptera, Ephemeroptera, terrestrial insects	410	0.200	0.242	92.7	268.1	3.2	2.222	2.695	11.112	0.0750	0.0016
	marginatus	Amphipoda, Copepoda, Gastropoda, Diptera	120	0.180	0.273	61.0	115.4	1.8	1.333	2.028	7.427	0.0226	0.0006
	megalotis	Ephemeroptera, Odonata, Trichoptera, terrestrial insects	240	0.289	0.332	96.7	383.8	2.8	3.167	3.628	10.940	0.1431	0.0046
	microlophus	Gastropoda, Diptera	250	0.230	0.234	93.3	278.5	3.1	2.750	2.806	11.976	0.0610	0.0206
	miniatus	Amphipoda, Isopoda, Diptera, Trichoptera	200	0.210	0.262	101.3	318.6	2.0	2.694	3.361	12.839	0.1409	0.0093
	punctatus	Amphipoda, Diptera, Ephemeroptera, Odonata	200	0.189	0.212	97.3	314.6	2.0	2.889	3.250	15.315	0.1521	0.0063
	symmetricus	Diptera, Hemiptera, Odonata	93	0.206	0.253	61.7	209.5	1.8	1.722	2.111	8.349	0.0298	0.0023
Micropterus	*cataractae*	crayfish, fish, Ephemeroptera	390	0.164	0.138	74.0	400.9	1.0	2.583	2.167	15.718	0.0466	0.0002
	coosae	crayfish, fish, Ephemeroptera	470	0.173	0.170	105.7	743.4	1.8	3.028	2.972	17.503	0.1292	0.0009
	dolomieu	crayfish, fish, Ephemeroptera, Odonata	690	0.223	0.187	104.7	685.0	1.7	3.512	2.936	15.718	0.1259	0.0008
	floridanus	fish	970	0.188	0.163	103.3	919.1	2.1	3.389	2.944	18.022	0.1271	0.0009
	notius	crayfish, fish	360	0.179	0.153	98.3	839.5	1.8	3.139	2.694	17.561	0.1131	0.0005
	punctulatus	crayfish, fish, terrestrial insects	610	0.177	0.177	85.0	593.0	1.4	2.476	2.476	13.991	0.0749	0.0004
	salmoides	crayfish, fish, terrestrial vertebrates, Ephemeroptera, Hemiptera	970	0.202	0.199	99.3	846.7	2.0	3.339	3.282	16.525	0.0927	0.0005
	treculi	insufficient data	400	0.174	0.159	100.7	686.2	1.6	2.889	2.639	16.582	0.1307	0.0004

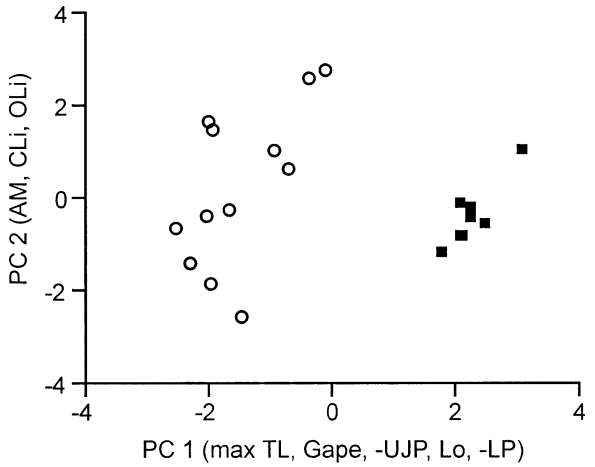

FIG. 4. Scatterplot of *Lepomis* (open circles) and *Micropterus* (filled squares) species' scores on principal components (PC) 1 and 2. These two axes represent 74% of the total variation in the correlation matrix. Character abbreviations in parentheses on axis labels indicate loadings greater than 0.35 in magnitude. Negative signs preceding character abbreviations indicate negative loadings. Character abbreviations are the same as in Table 1. Variance within *Lepomis* is 4.4 and 7.4 times greater than *Micropterus* on PC 1 and PC 2, respectively.

for multiple comparisons. However, characters that exhibit nearly a four-fold greater rate in *Lepomis*—which includes gape, upper jaw protrusion, lower jaw out-lever, AM mass, and LP mass—have *P*-values near or less than the uncorrected significance level of 0.05. Additionally, the rate of evolution of the lower jaw closing and opening lever ratios were greater in *Lepomis*, but these differences were also not significant.

DISCUSSION

Our analyses demonstrate that the rate of morphological evolution is a more appropriate metric than disparity for comparisons of morphological diversity among clades. Although comparisons of within-group morphological variance can be useful for examination of patterns of diversity at some point in time, variance comparisons may confound two distinctly different causes of trait variance—time and the rate of evolution of the trait. Determining whether variance differences between clades are due to differences in the amount of time the lineages have had to accumulate variance or to differences in the rate of evolution of the trait has major implications for understanding why morphological diversity is distributed among lineages as it is. Our results indicate that differences in time of independent evolution between our focal clades do not explain differences in disparity; thus, some other mechanism should be investigated to explain the elevated rate of evolution of the *Lepomis* feeding mechanism relative to *Micropterus*. Furthermore, we propose that estimates of rates of morphological evolution have broader applicability than estimates of variance. Because the rate of morphological evolution represents a time- and phylogeny-independent measure of morphological diversity, rates can be used to compare morphological diversity in any pair of clades (Garland 1992; Hutcheon and Garland 2004).

Morphological Disparity in Centrarchids

Greater diet diversity in *Lepomis* relative to *Micropterus* is associated with greater disparity in functional characters of the feeding apparatus. *Lepomis* species collectively include a greater taxonomic variety of prey items in their diets than *Micropterus* species (Fig. 3, Table 1). Concomitant with

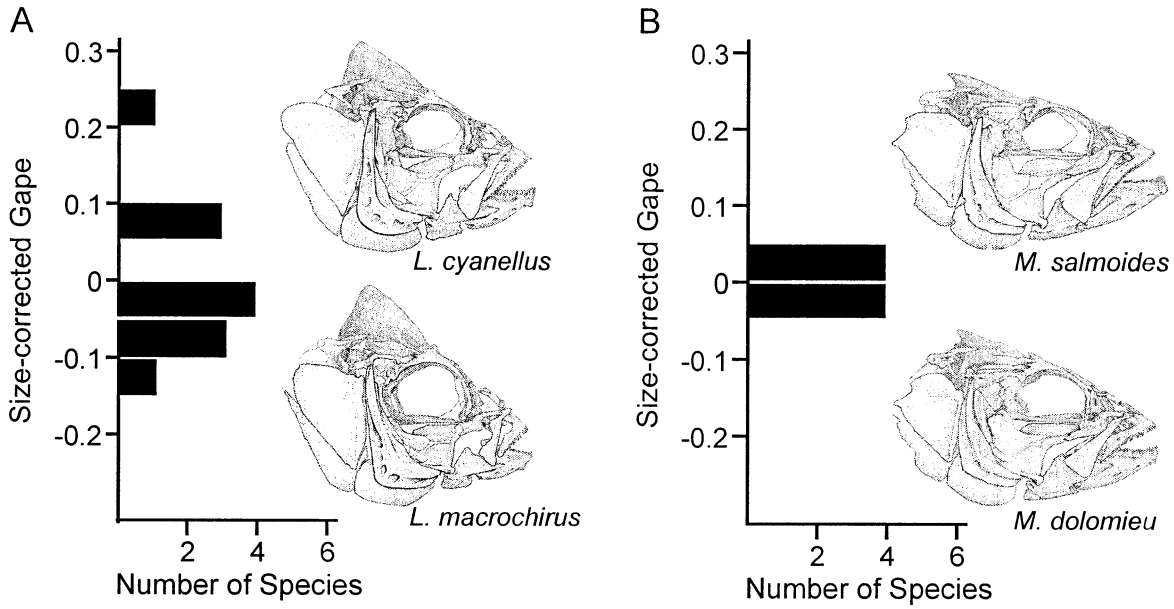

FIG. 5. Distributions of size-corrected gape in *Lepomis* (A) and *Micropterus* (B), and drawings representing extremes in the range of variation exhibited by each group. Within *Lepomis*, the green sunfish (A, top), *L. cyanellus*, has one of the largest gape areas and bluegill (A, bottom), *L. macrochirus*, has the smallest. The largemouth bass (B, top), *M. salmoides*, and smallmouth bass (B, bottom), *M. dolomieu*, represent these extremes in *Micropterus*. *Lepomis* exhibits 9.2 times more variance in mouth gape than *Micropterus*.

TABLE 2. Maximum-likelihood estimates, likelihood-ratio test statistics, and *P*-values obtained from the computer program Continuous (Pagel 1997, 1999) for λ, κ, and δ for principal components (PC) 1 and 2. These parameters describe how well the Brownian motion model fits species' character values given the *Lepomis* and *Micropterus* phylogeny, the likelihood-ratio tests assess whether the Brownian model can be rejected for any character, and *P*-values were obtained by comparison to the χ^2 distribution with one degree of freedom.

		H_0: λ = 1 (given κ = δ = 1)			H_0: κ = 1 (given λ = δ = 1)			H_0: δ = 1 (given λ = κ = 1)		
Clade	Character	λ(MLE)	ln LR	*P*-value	κ(MLE)	ln LR	*P*-value	δ(MLE)	ln LR	*P*-value
Lepomis	PC 1	0.40	1.48	0.085	0.18	0.43	0.356	3.00	1.00	0.158
	PC 2	0.00	2.50	0.025*	0.93	0.00	0.932	3.00	1.64	0.070
Micropterus	PC 1	1.00	0.00	1.000	3.00	3.13	0.012*	1.62	0.06	0.737
	PC 2	0.00	0.55	0.294	1.59	0.26	0.467	3.00	0.44	0.346

* Significant after correction for multiple comparisons by the sequential Bonferroni method.

greater taxonomic variety is greater variety of functional requirements for prey capture and processing. *Micropterus* species' diet items represent only a subset of the range of functional demands imposed on *Lepomis* species. In general, the diets of *Micropterus* species are dominated by large, elusive prey such as fish and crayfish, while the diets of *Lepomis* species include prey that vary more extensively in size and elusiveness as well as hardness. Variance differences between *Lepomis* and *Micropterus* on PC 1 and PC 2 imply that, for the characters examined in this study, comparison of trophic morphological diversity is informative with regard to differences in diet diversity (Figs. 3, 4). This result is consistent with the hypothesis that trophic morphology has evolved in association with diet and that as *Lepomis* lineages diverged to fill a variety of diet niches, its trophic morphology evolved concurrently. Although we did not test this hypothesis directly, this conclusion is bolstered by the a priori expectation that each character affects a fish's capacity to meet the functional demands imposed by different prey categories.

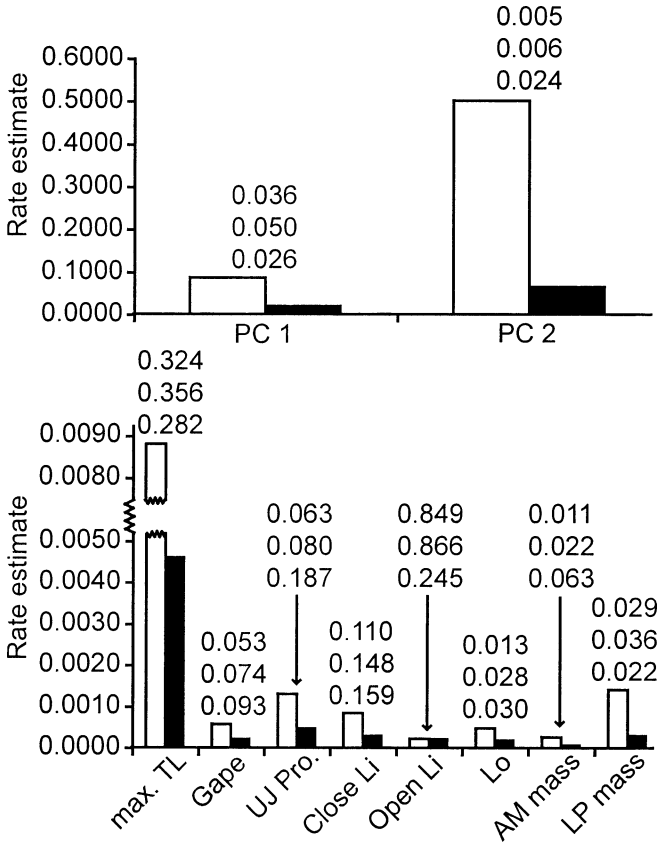

FIG. 6. Comparisons of the rate of morphological evolution in *Lepomis* (open bars) and *Micropterus* (filled bars). Rate estimates are represented by the heights of the bars. Numbers above bars give the *P*-value based on the χ^2 distribution (top), *P*-value based on the parametric bootstrapping procedure (middle), and *P*-value based on a *t*-test involving the distribution of standardized contrasts (bottom). Character abbreviations are the same as in Table 1.

Our investigation of single characters revealed that *Lepomis* exhibits greater variance in gape (Fig. 5), AM mass, and LP mass. Diet analysis showed that *Micropterus* species feed primarily on prey items that fall in the large extreme of the range of prey sizes included in *Lepomis* species' diets. The greater variance in gape within *Lepomis* reflects the greater range of prey sizes found in *Lepomis* diets. Because the AM is the primary muscle involved in mouth closing and because the power required to close the mouth at a given velocity will vary with mouth size, variation in AM likely reflects variation in prey size and elusiveness. Finally, inclusion of gastropods in the diets of *Lepomis* species (*L. gibbosus*, *L. marginatus*, and *L. microlophus*) imposes functional demands not experienced by any *Micropterus* species. While *L. marginatus* appears to ingest small snails whole, *L. gibbosus* and *L. microlophus* are known to crush snail shells in their pharyngeal jaws (Lauder 1983; Mittlebach 1984), which must be capable of delivering enough force to overcome the resistance imposed by the calcified shell. Inclusion of hard prey in *Lepomis* species' diets is associated with greater variance in the primary pharyngeal jaw adductor muscle, the LP. It should be noted that classification of the dollar sunfish, *L. marginatus*, as a molluscivore should be treated with some skepticism. Diet data for this species was taken from a sample of one population, the study contains no report regarding whether snail shells were found crushed or whole (McLane 1955), and this species lacks the enlarged LP muscle found in the other two molluscivores.

Rates of Morphological Evolution in Centrarchids

We used three methods to test the hypothesis that rates are equal in *Lepomis* and *Micropterus*: (1) standard likelihood-ratio tests using a χ^2 distribution; (2) likelihood-ratio tests involving a null distribution based on parametric bootstrapping; and (3) comparison of the central tendencies of the distributions of standardized contrasts. Although the likelihood-ratio tests and standardized contrasts approach provide similar results in our analysis, we point out the following

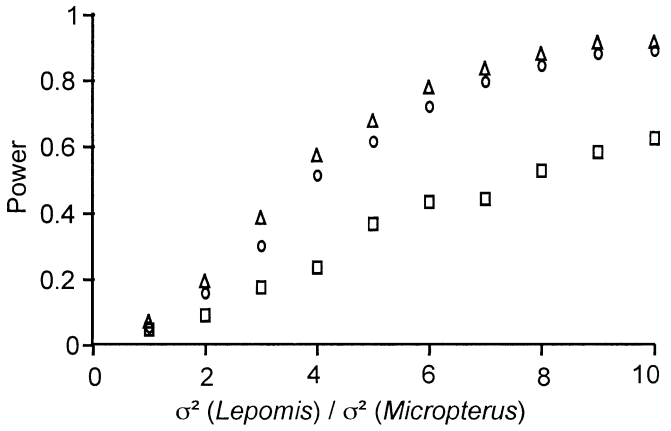

FIG. 7. Comparison of statistical power between 1 standard likelihood-ratio tests using the χ^2 distribution (triangles), 2 likelihood-ratio tests involving null distributions based on parametric bootstrapping (circles), and 3 *t*-tests involving standardized contrasts (squares). The x-axis represents the ratio of the rate in *Lepomis* to the rate in *Micropterus*. At each value of the x-axis, Brownian motion character evolution was simulated 500 times on the *Lepomis* and *Micropterus* phylogeny (see Fig. 1) using Brownie's ''simulatetipvaluesmanytimes.m'' function (O'Meara et al., unpubl. ms.), and these simulated species character values were used as input for the three methods. The y-axis represents the proportion of simulations that returned a significant rate difference ($\alpha = 0.05$). Also note that at equal rates (i.e., $x = 1$), the likelihood-ratio test using the χ^2 distribution commits too many Type I errors (7% of simulations returned significant results); however, the likelihood-ratio test involving parametric bootstrapping and the standardized contrasts approach both have appropriate Type I error rates (5% of simulations returned significant results for both methods).

considerations for choosing between them. First, given the *Lepomis* and *Micropterus* phylogeny and branch lengths, the two likelihood-ratio tests exhibit greater power. Based on simulations of Brownian motion evolution, we found that both likelihood methods have higher probabilities of returning a significant result than the standardized contrasts approach for rate differences of two- to 10-fold (Fig. 7; comparison of power of these methods under broader conditions is presented in B. O'Meara et al., unpubl. ms.). Second, the likelihood-ratio test involving the χ^2-test is nonconservative for the comparison of *Lepomis* and *Micropterus* ($\alpha = 0.07$), but the likelihood-ratio test involving parametric bootstrapping and the standardized contrasts approach exhibit appropriate Type I error rates ($\alpha = 0.05$ for both tests). Third, although the three tests are about equally simple to use, requiring implementation of computer programs that call for similar inputs (i.e., phylogeny with branch lengths and character values for tip taxa), some researchers might prefer the standardized contrasts approach because of familiarity with and frequency of use of independent contrasts in comparative analyses. Finally, because all methods are based on the Brownian motion model of character evolution, it should be noted that all three tests make the same assumptions and test the same hypothesis: that the rate parameter is equal in the focal clades.

An additional method that tests this hypothesis involves comparison of the *F*-statistic or Levene's test statistic obtained from species' character values to a distribution of test statistics obtained by computer simulation of character evolution on the phylogeny given one rate of evolution (Garland et al. 1993). An advantage of this method is that it allows tests of the hypothesis under additional models of character evolution, not just Brownian motion. Although such analysis is beyond the scope of this study, investigation into the best-fitting model of evolution for functional characters is an important avenue of further research.

Time of independent evolution does not account for differences between *Lepomis* and *Micropterus* in trophic morphological diversity. In addition to having had more time to accumulate morphological variation, *Lepomis* has also experienced higher rates of PC 1 and PC 2 evolution. Comparisons of rates of evolution of single characters add to this conclusion. Although univariate differences in character variance are not associated with significant differences in rates of evolution, rates are estimated to be higher in *Lepomis* (Fig. 6). In fact, a majority of the characters measured (AM mass, LP mass, gape area, upper jaw protrusion, and lower jaw outlever) exhibit rate differences associated with *P*-values below or near the nominal α of 0.05. The absence of significance in these rate differences is in part an effect of limited statistical power to detect differences at significance levels corrected for multiple comparisons. In our comparison of *Lepomis* and *Micropterus*, power is a function of the structure of the tree and the number of extant lineages. Therefore, we are unable to control power to detect rate differences of a given magnitude. These considerations might promote a more liberal interpretation of rates comparisons associated with *P*-values near 0.05 (Moran 2003; Nakagawa 2004), in which case, differences in rates of evolution of these characters can be taken to support the claim that the rate of evolution of the feeding apparatus is elevated in *Lepomis* relative to *Micropterus*. Rather than time and phylogeny alone, this rate difference must also be invoked to explain differences in extant diversity.

What mechanisms are responsible for the elevated rate of morphological evolution in *Lepomis* relative to *Micropterus*? One possible explanation is that time to sympatry (sensu Barraclough and Vogler 2000) is lower in *Lepomis* than in *Micropterus*. In the course of synthesizing diet data, we uncovered a tendency for more *Lepomis* species to coexist in localities sampled (See Appendix 1 available online), and all sister species pairs in *Lepomis*, except *L. miniatus* and *L. punctatus*, exhibit near complete range overlap (Lee et al. 1980; Warren 1992). In contrast, any given *Micropterus* species will exhibit sympatry with only *M. punctulatus* and *M. salmoides* (Lee et al. 1980; Near et al. 2003). If the ability of congeneric species to coexist is limited by diet and morphological similarity, then reduced time to sympatry could increase the rate of morphological evolution. However, it is unclear whether reduced time to sympatry causes a higher rate of evolution of the trophic apparatus or if a higher rate of evolution allows reduced time to sympatry. Diversity-promoting mechanisms such as these remain to be thoroughly investigated.

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