

Genetic Basis of Adaptive Shape Differences in the Cichlid Head

R. C. ALBERTSON, J. T. STREELMAN, AND T. D. KOCHER

From the Hubbard Center for Genome Studies and the Department of Zoology, University of New Hampshire, 35 Colovos Road, Durham, NH 03824 (Albertson, Streelman, and Kocher). R. C. Albertson is currently at the Department of Cytokine Biology, The Forsyth Institute, 140 Fenway, Boston, MA 02115.

Address correspondence to R. C. Albertson at the address above, or e-mail: calbertson@forsyth.org.

Abstract

East African cichlids exhibit an extraordinary level of morphological diversity. Key to their success has been a dramatic radiation in trophic biology, which has occurred rapidly and repeatedly in different lakes. In this report we take the first step in understanding the genetic basis of differences in cichlid oral jaw design. We estimate the effective number of genetic factors that control differences in the cichlid head through a comprehensive morphological assessment of two Lake Malawi cichlid species and their F_1 and F_2 hybrid progeny. We estimate that between one and 11 factors underlie shape difference of individual bony elements. We show that many of the skeletal differences in the head and oral jaw apparatus are inherited together, suggesting a degree of pleiotropy in the genetic architecture of this character complex. Moreover, we find that cosegregation of shape differences in different elements corresponds to developmental, rather than functional, units.

There is mounting evidence that selection on trophic morphology plays a strong role in the speciation of certain groups of vertebrates. This trend has been documented in Galapagos finches (Grant and Grant 1997; Sato et al. 2001), North American salamanders (Adams and Rohlf 2000), arctic charr (Skulason et al. 1989, 1996), three-spine stickleback (McPhail 1992, 1994), whitefish (Schluter and McPhail 1993), and cichlids on two continents (Albertson et al. 1999; Martin and Bermingham 1998; McKaye et al. 1998; Schliwen et al. 1994). These and other studies (e.g., Echelle and Kornfield 1984; Nagelkerke et al. 1994; Tichy and Seegers 1999) suggest that selection on trophic morphology, if not causative, at least closely accompanies divergence. Unfortunately, the genetic basis of such morphological differences is poorly understood. To paraphrase a question posed by Orr and Coyne (1992), how many genes underlie the adaptive differences between species?

East African cichlids are a textbook example of adaptive morphological radiation (Futuyma 1986). The rapidity and extent of morphological divergence in this group is unparalleled among vertebrates. The most dramatic changes seem to involve the oral jaw apparatus. Freed from the constraints of prey-processing, the oral jaws have evolved highly specialized modes of food collection (Liem 1973), setting the stage for the stunning radiation in trophic biology we see in each of the three East African Great Lakes (Victoria, Tanganyika, and Malawi). Molecular and geological studies suggest that these radiations are extremely recent

(Greenwood 1974; Kocher et al. 1995; Meyer 1993; Owen et al. 1990), and remarkably similar trophic morphologies have evolved convergently in different lakes (Kocher et al. 1993).

This report takes the first step in elucidating the genetic architecture of the cichlid oral jaw apparatus. We first evaluate differences in the craniofacial skeleton between two cichlid species that employ different modes of feeding. Our morphometric analysis includes four skeletal elements that constitute the oral jaws (articular, dentary, maxilla, and premaxilla), two bony elements associated with the oral jaw apparatus (suspensorium and neurocranium), and oral jaw dentition. We then quantify morphological variance in hybrid generations, and, employing the Castle-Wright estimator, biometrically estimate the number of genetic factors that underlie differences in shape. Finally, we infer the genetic correlation among skeletal elements by identifying structural units inherited together in the F_2 . Our results provide insight into the total number of determinants that underlie shape differences in this adaptively significant character complex.

Materials and Methods

Species

Labeotropheus fuelleborni (LF) and *Metriaclima zebra* (MZ) are two rock-dwelling species from Lake Malawi that shared a common ancestor less than one million years ago (Meyer 1993). Although both species forage on algae, they employ

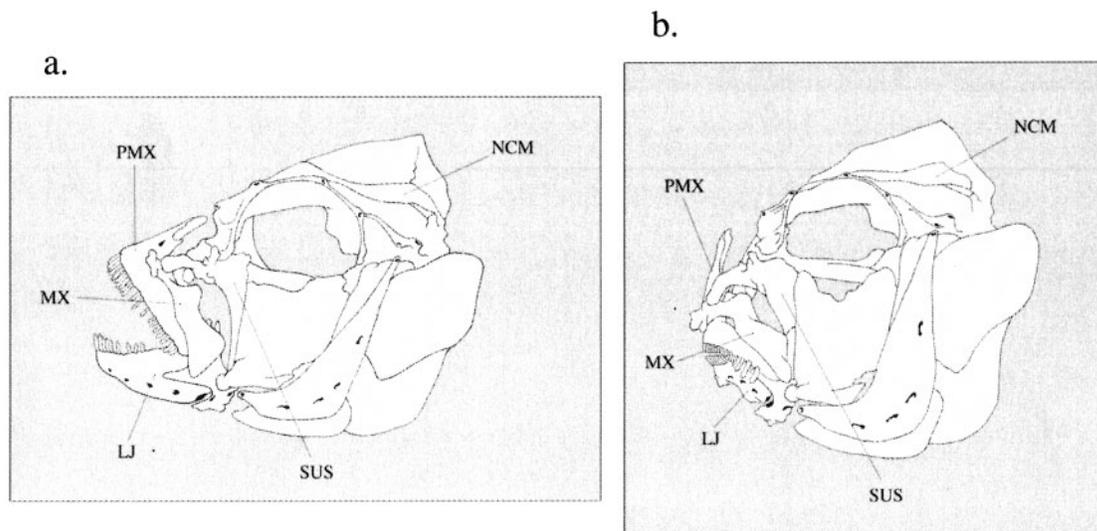


Figure 1. Craniofacial skeletal morphology of *Metriaclima zebra* (a) and *Labeotropheus fuelleborni* (b). LJ, lower jaw; MX, maxilla; NCM, neurocranium; SUS, suspensorium; and PMX, premaxilla.

different modes of feeding (Ribbink 1990), occupy different microhabitats (Ribbink et al. 1983), and are characterized by very different oral jaw morphologies (Albertson and Kocher 2001; see Figure 1). MZ has a moderately sloped head, a large horizontally directed vomer, and a terminal, isognathous mouth (Stauffer et al. 1997). It feeds on diatoms and loose algae by brushing these items from algal beds or by sucking them from the water column (McKaye and Marsh 1983; Reinthal 1990; Ribbink et al. 1983). LF has a large fleshy snout, a vertically directed vomer, and a robust inferior-subterminal mouth. The orientation of its mouth allows LF to bite attached algae from rocks while swimming nearly parallel to the substrate (Ribbink et al. 1983).

Preparation of Specimens

Parental specimens used in this study were lab-reared F₁ animals generated from wild-caught stock. Hybridization between the species occurs readily under no-choice conditions (Crapon de Caprona and Fritsch 1984; Loiselle 1971; McElroy and Kornfield 1993). We obtained hybrids by crossing MZ females with LF males in a 500-gallon pool. Second-generation hybrids were generated from four parental animals: two sires and two dams. One hundred sixty specimens were used in this study, 20 of each parental species, 20 F₁ hybrids, and 100 F₂ hybrids. MZ, LF, and their hybrids reach sexual maturity by 10 to 12 months. Animals were examined no earlier than 12 months of age, and more typically at 18 months. Animals were sacrificed with MS-222 in accordance with a protocol approved by the University of New Hampshire Animal Care and Use Committee (ACUC). Specimens were then prepared for morphometric analysis with dermestid beetles, which cleaned and disarticulated skeletal elements of the head. We collected each of the four

elements that make up the oral jaws (the dentary, articular, premaxilla, and maxilla), as well as the neurocranium and suspensorium. Images of individual skeletal elements were captured with a SPOT digital camera (Diagnostic Instruments, Inc.) mounted on a Zeiss SV11 dissecting scope. Images were imported into NIH Image (version 2.1), and landmark positions were scored as (x, y) coordinates.

Morphometric Technique: Oral Jaw Morphology

We assessed differences in oral jaw morphology by means of landmark-based morphometrics. Landmarks used in this study are described in Albertson and Kocher (2001) and Albertson (2002). Superimposition of landmark data was performed with a Procrustes generalized least squares fit (GLSF) algorithm (Gower 1975; Rohlf and Slice 1990) in Morphometrika 7.0 (Walker 1999). A least-squares approach superimposes configurations so that the sum of squared distances between corresponding landmarks is minimized. This is achieved by scaling, translating, and rotating specimens with respect to a mean consensus configuration.

Thin-plate spline (TPS) analysis was performed in Morphometrika 7.0 (Walker 1999). The TPS technique rigorously implements D'Arcy Thompson's concept of Cartesian grid deformations (Thompson 1917). A thorough description of the technique may be found in Bookstein (1989, 1991). In short, TPS models the form of an infinitely thin metal plate that is constrained at some combination of points but is otherwise free to adopt the target form in a way that minimizes bending energy. In morphometrics this interpolation is applied to a Cartesian coordinate system in which deformation grids are constructed from two landmark configurations (Bookstein 1991). The total deformation of the thin-plate spline can be decomposed into geometrically

orthogonal components based on scale (Rohlf and Marcus 1993; Yarooh 1996). These components (partial warps) can be localized to describe precisely what aspects of shape are different. Partial-warp scores are the shape variables used in all subsequent analyses.

TPS analysis was performed on all animals (LF, MZ, F₁, and F₂). Thus, all specimens were evaluated, and partial-warp scores were calculated, relative to the same mean consensus configuration. A principal component analysis (PCA) was performed in Morphometrika 7.0 (Walker 1999) on partial-warp scores (including the uniform component) of parental animals to identify the major axis of variation that distinguished LF from MZ. Principal component scores were then calculated for hybrid animals by multiplying hybrid partial-warp scores by the parental eigenvectors in the space of partial warps. Thus, segregation of hybrid progeny was assessed in the dimension that distinguished parental species.

Assessing Tooth Morphology

F₂ dentition is a continuum between the fully bicuspid dentition of MZ and the fully tricuspid dentition of LF. Fourteen teeth in the first row of both the upper and lower jaw (seven on either side of the mandibular symphysis) were given a score between 2 and 3 (in 0.25 intervals) for 20 F₁ and 100 F₂ individuals. Tooth scores were meant to evaluate the relative height of the third cusp. A score of 2 was given to fully bicuspid dentition (MZ), while a score of 3 was given to fully tricuspid dentition, with even cusp heights (LF). The average tooth score for each element was used for subsequent analyses.

Calculation of Effective Number of Factors

The number of genetic factors that underlie morphological differences was estimated by applying the Castle-Wright estimator (Lynch and Walsh 1998) to PC1 or tooth scores:

$$\hat{n}_e = \frac{(\bar{X}_{LF} - \bar{X}_{MZ})^2 - \sigma_{X_{LF}}^2 - \sigma_{X_{MZ}}^2}{8(\sigma_{F_2}^2 - \sigma_{F_1}^2)}$$

where n_e is the effective number of genetic factors, X_{LF} and X_{MZ} are the parental means, $\sigma_{X_{LF}}^2$ and $\sigma_{X_{MZ}}^2$ are the variances of the parental means, and $\sigma_{F_1}^2$ and $\sigma_{F_2}^2$ are the variances of the hybrid means.

The Castle-Wright method assumes that loci are unlinked, alleles are of equal effect, genes with positive and negative influence are fixed in alternate lines, and, most critically, alleles have an additive effect on phenotype. Violations of one or more of these assumptions will generally lead to an underestimate of the number of effective factors (Zeng 1992; Zeng et al. 1990).

Analysis of line crosses enables one to estimate the relative contribution of additive, dominance, and epistatic effects on the inheritance of the trait, or traits, in question. We used weighted least squares regression to compare observed and expected means and SEs of P₁, P₂, F₁, and F₂. This approach enables one to estimate the parameters of an

additive (A) and additive-dominance (AD) model of gene action. Formally called a joint-scaling test, this approach can be found in detail in Lynch and Walsh (1998). Briefly, we estimated the expected mean phenotype of the F₂ (μ_0), the composite additive effect (α_i^c) for the A model, as well as the composite dominance effect (δ_i^c) for the AD model.

For the A model we tested the null hypothesis of purely additive gene action with a χ^2 test statistic with df equal to the number of lines minus the number of estimated parameters. Next, we evaluated the AD model to test for any contribution of dominance. The difference between the test statistics, χ^2_A and χ^2_{AD} is equivalent to a likelihood ratio test statistic and is denoted L, with df equal to the difference between the df in the A and AD models. The likelihood ratio test statistic provides a test for the hypothesis that dominance explains a significant proportion of the variance.

Epistasis could not be evaluated by means of a joint-scaling test, because there were not enough line means available (e.g., no backcross lines). But we could evaluate epistasis with a simple *t* test:

$$\Delta = z_{F_2} - \left(\frac{z_{P_1} + z_{P_2}}{4} \right) + \left(\frac{z_{F_1}}{2} \right)$$

In the absence of epistasis, the expected value of Δ is zero because at each locus the F₂ should be 25% P1P1, 50% P1P2, and 25% P2P2. The variance of the test statistic is as follows:

$$\sigma_{\Delta}^2 = \sigma_{F_2}^2 + \left(\frac{\sigma_{F_1}^2}{4} \right) + \left(\frac{\sigma_{P_1}^2 + \sigma_{P_2}^2}{16} \right)$$

Under the assumption that the sampling distribution of Δ is normal, the ratio:

$$\frac{|\Delta|}{\sqrt{\sigma_{\Delta}^2}}$$

provides the *t* test for epistasis. If the ratio is greater than 1.96, the null hypothesis of no epistasis can be rejected at the 95% confidence level (Lynch and Walsh 1998).

Finally, to identify skeletal elements that are inherited together in the F₂, we performed a Pearson correlation analysis on PC1 scores for each structure.

Results

Skeletal Morphology

Detailed descriptions of the differences in skeletal morphology between LF and MZ are presented in Albertson and Kocher (2001). Major aspects of shape difference reflect differences in feeding performance (see Figure 2). Morphological adaptations of LF are consistent with a biting mode of feeding, and include (1) a short, robust, U-shaped oral jaw to optimize biting surface area; (2) a relatively high articular process, suggesting greater force transmission of the adductor mandibulae when biting; (3) a robust maxilla; (4) a long ascending arm of the premaxilla; (5) an obtuse angle formed by the two arms of the premaxilla; (6) an expanded preorbital region of the skull; and (7) a down-turned vomer,

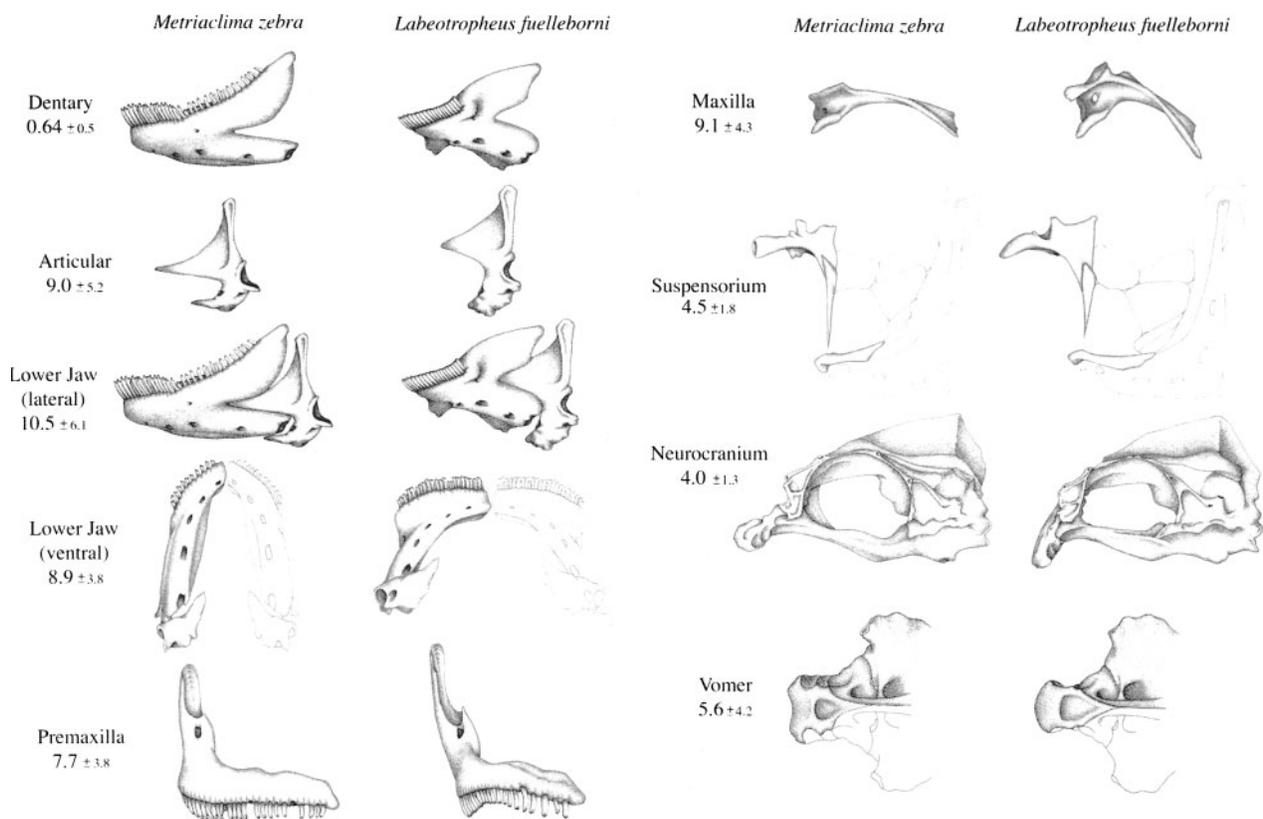


Figure 2. Number of genetic factors that underlie geometric shape differences between MZ and LF.

similar to that in species with an inferior-subterminal mouth. Alternatively, MZ jaw morphology is characteristic of a species that employs a suction mode of feeding. Salient aspects of shape include (1) a longer, narrower lower jaw; (2) a short articular process, suggesting a more rapid jaw closing motion (greater rate of angular rotation); (3) a thin maxilla; (4) a long dentigerous arm of the premaxilla; (5) an acute angle formed between the arms of the premaxilla; and (6) a swollen, horizontally directed vomer, similar to that in other suction feeding species. F_1 hybrid morphology is typically intermediate between LF and MZ (Albertson and Kocher 2001), implying a generally additive mode of action of alleles responsible for shape differences.

Tooth Morphology

As with oral jaw morphology, differences in oral jaw dentition reflect different modes of food collection (see Figure 3). LF has a row of closely spaced tricuspid teeth on both the upper and lower jaws. Like that of other species that crop filamentous algae from the substrate, LF dentition resembles the cutting edge of shearing scissors. MZ has a row of intermittently spaced bicuspid teeth on both jaws. Cusp height is uneven in MZ, with the dominant cusp facing the mandibular symphysis. MZ dentition resembles the teeth of a comb and is similar to that in other species that feed on loose algae and diatoms. Both species have a posterior row

(or rows) of smaller tricuspid teeth. F_1 tooth morphology is roughly intermediate between LF and MZ. F_1 hybrids have three cusps, none of which are the same height. The middle cusp is large and resembles the dominant cusp in MZ. The “second” cusp is of intermediate height. The “third” cusp is the smallest and faces the mandibular symphysis. F_2 dentition resembles that of the F_1 , but with dramatic variation in the height of the third cusp. Some F_2 teeth are truly tricuspid, like those of LF, whereas others are truly bicuspid, as in MZ. This variation is found both between and within F_2 individuals.

PCA of Partial-Warp Scores

Results of the PCA are presented in Figure 4. The PC axis that separates the parental species (in all cases this is PC1) accounts for 81% of the variance between MZ and LF for the lower jaw in the lateral view, 94% of the difference for the lower jaw in the ventral view, 90% for that of both the maxilla and premaxilla, 71% for that of the suspensorium, and 69% and 65% for that of the neurocranium and vomer, respectively. The lower values associated with the skull are not altogether unexpected, as neurocranial characters are known to show high levels of intraspecific variation in cichlids (Reinthal 1990). It is also important to note that shape differences in the neurocranium are restricted to the

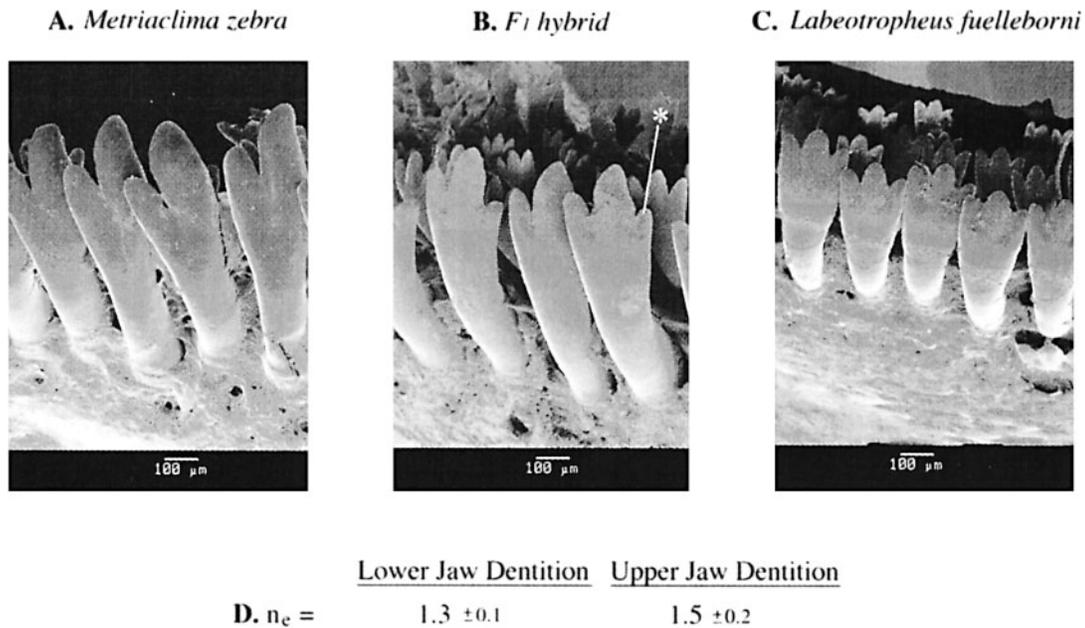


Figure 3. (A–C) SEM images of lower jaw dentition in MZ, F_1 , and LF; the asterisk is the third cusp in hybrid animals that tends to vary in size. (D) Estimated number of factors that underlie upper and lower jaw cuspidness.

anterior (ethmoidal) region of the skull (Albertson and Kocher 2001).

Depending on the element, parental species are separated along the PC axis by 5 to 13 environmental SD units (because all F_1 animals should be genetically identical, environmental SD units are taken as the F_1 SD for each structure). For every skeletal element, the F_1 and F_2 distribution falls between the parental species, suggesting an additive mode of inheritance. F_2 morphology also exhibits much greater variance relative to the F_1 . For several elements parental morphology is regenerated in the F_2 (Figure 4).

Inheritance

We find no evidence to reject the additive model of gene action for either oral jaw morphology or dentition (Table 1). In all cases the chi-square statistic is not sufficient to reject the additive model (A). We therefore accept the null hypothesis of no difference between observed and expected means. For each element, the test statistic for the additive-dominance (AD) model is also not significant, so there is no statistical support for any contribution of dominance in the data. Finally, for every skeletal element we accept the null hypothesis of no epistasis. In all, these data suggest that the assumption of additive gene action for the Castle-Wright estimator is appropriate in our study.

Castle-Wright Estimator

Shape differences between LF and MZ for each bony element are determined by fewer than 11 factors (Figures 3 and 4). Difference in the premaxilla, maxilla, lower jaw in the

ventral view, and lower jaw in the lateral view is controlled by 7.7, 9.1, 8.9, and 10.5 factors, respectively. Shape of the articular is controlled by 9 factors, whereas difference in the dentary is controlled by only 1 factor. We estimate that 4.5 factors determine shape difference in the suspensorium, 4 affect the neurocranium in the lateral view, and 5.6 affect the vomerine process in the ventral view. Cusp number in both the upper and lower jaw seems to be determined by a single factor. With the notable exception of the dentary and tooth shape, most bony elements appear to be controlled by a similar number of loci (4–10).

There does not appear to be an obvious relationship between the size of an element, or the number of landmarks, and the estimated number of genetic factors that contribute to shape difference. The neurocranium and suspensorium were two of the largest structures examined in the analysis, with eight and six landmarks used to describe shape change, respectively. These structures also had two of the lowest estimates of any bony element: 4.0 and 4.5, respectively. The maxilla, on the other hand, was one of the smallest bony elements examined, with four landmarks used to capture shape. The maxilla also had the second largest value of n_e , 9.1.

The relationship between morphological disparity and number of loci is also noteworthy. When only bony elements are considered, the difference between parental means is significantly correlated with n_e ($r = .79$; $P = .012$). Since mean difference is built into the Castle-Wright estimator, we expect n_e to scale with mean shape difference. Interestingly, there does not appear to be a significant association between divergence in parental tooth shape (11–14 SD) and the estimated number of loci responsible for this difference ($n_e = 1.3$ –1.5).

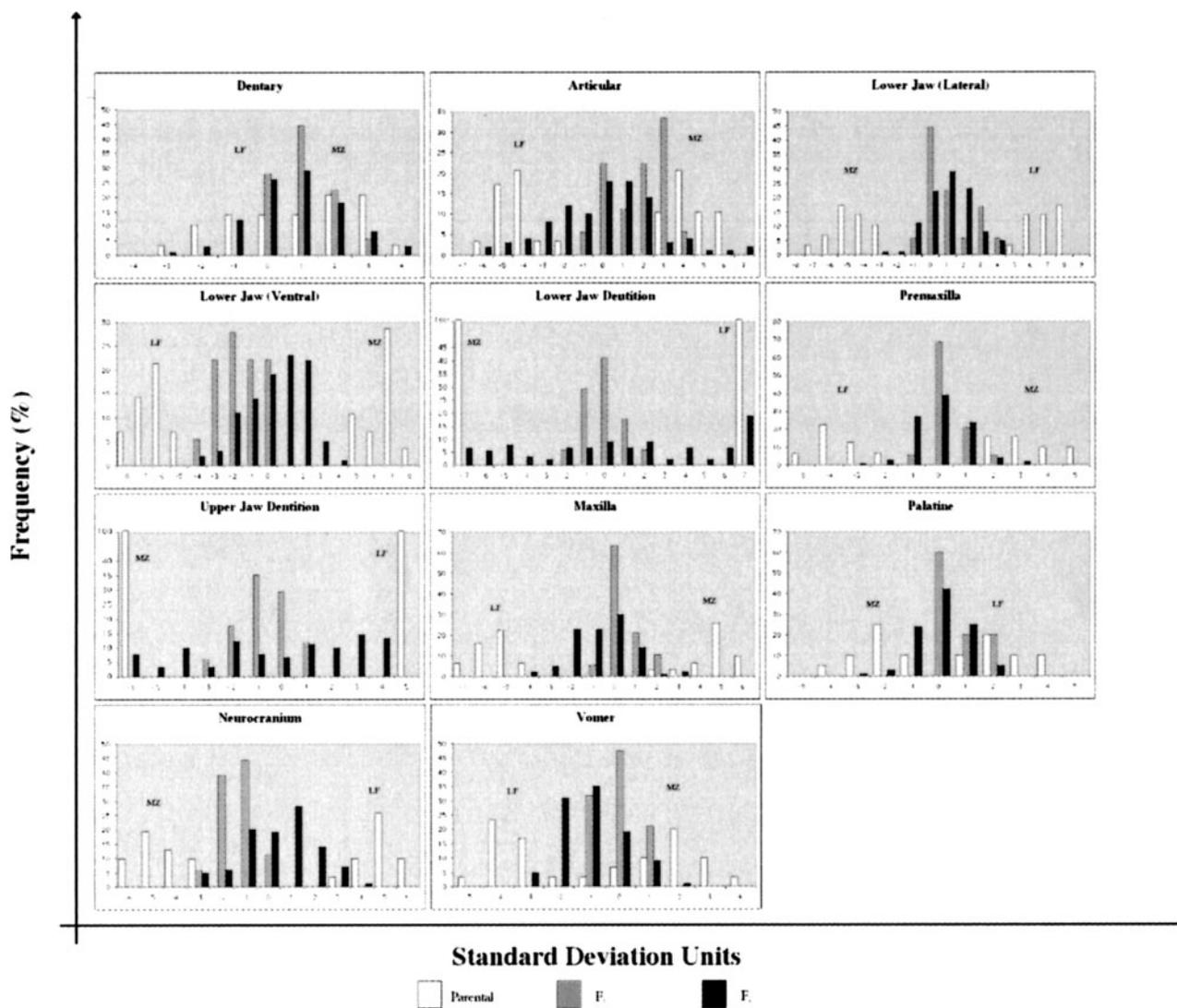


Figure 4. Distribution of craniofacial characters for parental species and both hybrid generations. Note the regeneration of parental morphology in the F₂ for several characters. The y axis is frequency (%) and the x axis is environmental SD units, taken as the SD of the F₁ generation for each element.

Correlation Among Characters in the F₂

The primary objective of our correlation analysis was to gain insight into the total number of determinants that underlie shape differences in the cichlid head. When each bony element is considered independently, the number of genetic determinants appears to be small (<11); however, the sum of the independent estimates for each structure is much larger (>50). Results from the correlation analysis show that the shape of many bony elements are inherited together. We therefore expect that some loci will affect shape differences in multiple structures, and the total number of determinants that distinguish LF and MZ will be less than the sum of the independent estimates.

Twenty-three of the 55 possible associations are statistically significant (Table 2). Many of the correlations

are conceptually intuitive, such as the strong, positive correlations between the upper and lower jaw dentition ($P < .001$), the lower jaw in the lateral and ventral view ($P < .001$), and the maxilla and premaxilla ($P < .01$). Interestingly, tooth shape is not associated with the shape of either the dentary or premaxilla (elements within which teeth develop), but is correlated with both the articular and suspensorium ($P < .05$). The two skeletal subunits of the lower jaw, the dentary and articular, are not correlated with one another, but the articular is correlated with virtually every other bony element in the head. Lateral and ventral views of the neurocranium are not correlated. The neurocranium does, however, show a strong positive correlation with the lower jaw in the lateral view ($P < .001$).

Table 1. Observed and expected means (X) and standard errors (SE) for oral jaw bony elements

| | Observed | | | Expected (A) | | | | | | Expected (AD) | | | | | | A χ^2 | AD Λ | Epistasis t | |
|---------------------|----------|---------|----------------|----------------|---------|---------|----------------|----------------|---------|---------------|----------------|----------------|---------|---------|----------------|------------|----------------------|----------------------|----------------------|
| | LF | MZ | F ₁ | F ₂ | LF | MZ | F ₁ | F ₂ | LF | MZ | F ₁ | F ₂ | LF | MZ | F ₁ | | | | F ₂ |
| | Dentary | | | | | | | | | | | | | | | | | | |
| X | -0.0414 | 0.0386 | 0.0063 | -0.0011 | -0.0375 | 0.0425 | 0.0025 | 0.0025 | -0.0414 | 0.0386 | 0.0063 | 0.0025 | -0.0414 | 0.0386 | 0.0063 | 0.0025 | 0.0025 | | |
| SE | 0.0307 | 0.0218 | 0.0207 | 0.0374 | 0.0249 | 0.0198 | 0.0130 | 0.0130 | 0.0302 | 0.0216 | 0.0200 | 0.0130 | 0.0302 | 0.0216 | 0.0200 | 0.0131 | 0.091 ^{n/s} | 0.082 ^{n/s} | 0.09 ^{n/s} |
| Articular | | | | | | | | | | | | | | | | | | | |
| X | -0.0577 | 0.0539 | 0.0184 | -0.0033 | -0.0476 | 0.0640 | 0.0082 | 0.0082 | -0.0577 | 0.0539 | 0.0184 | 0.0082 | -0.0577 | 0.0539 | 0.0184 | 0.0082 | 0.0082 | | |
| SE | 0.0127 | 0.0125 | 0.0170 | 0.0331 | 0.0096 | 0.0116 | 0.0071 | 0.0071 | 0.0100 | 0.0125 | 0.0165 | 0.0071 | 0.0100 | 0.0125 | 0.0165 | 0.0090 | 1.763 ^{n/s} | 1.643 ^{n/s} | 0.334 ^{n/s} |
| Lower jaw (lateral) | | | | | | | | | | | | | | | | | | | |
| X | 0.1170 | -0.1092 | -0.0059 | 0.0026 | 0.1121 | -0.1141 | -0.0010 | -0.0010 | 0.1170 | -0.1092 | -0.0059 | -0.0010 | 0.1170 | -0.1092 | -0.0059 | -0.0010 | -0.0010 | | |
| SE | 0.0194 | 0.0201 | 0.0183 | 0.0254 | 0.0181 | 0.0187 | 0.0120 | 0.0120 | 0.0200 | 0.0200 | 0.0387 | 0.0120 | 0.0200 | 0.0387 | 0.0200 | 0.0200 | 0.214 ^{n/s} | 0.194 ^{n/s} | 0.127 ^{n/s} |
| Lower jaw (ventral) | | | | | | | | | | | | | | | | | | | |
| X | -0.1531 | 0.1531 | -0.0329 | 0.0074 | -0.1695 | 0.1367 | -0.0164 | -0.0164 | -0.1531 | 0.1531 | -0.0329 | -0.0164 | -0.1531 | 0.1531 | -0.0329 | -0.0164 | -0.0164 | | |
| SE | 0.0245 | 0.0224 | 0.0271 | 0.0398 | 0.0220 | 0.0205 | 0.0133 | 0.0133 | 0.0243 | 0.0222 | 0.0257 | 0.0133 | 0.0243 | 0.0222 | 0.0257 | 0.0148 | 1.713 ^{n/s} | 1.355 ^{n/s} | 0.556 ^{n/s} |
| Lower jaw dentition | | | | | | | | | | | | | | | | | | | |
| X | 3.0000 | 2.0000 | 2.4383 | 2.5620 | 2.9692 | 1.9692 | 2.4692 | 2.4692 | 3.0000 | 2.0000 | 2.4383 | 2.4692 | 3.0000 | 2.0000 | 2.4383 | 2.4692 | 2.4692 | | |
| SE | 0.0672 | 0.0672 | 0.0672 | 0.3156 | 0.0608 | 0.0608 | 0.0387 | 0.0387 | 0.0671 | 0.0671 | 0.0671 | 0.0387 | 0.0671 | 0.0671 | 0.0671 | 0.0412 | 0.719 ^{n/s} | 0.632 ^{n/s} | 0.292 ^{n/s} |
| Premaxilla | | | | | | | | | | | | | | | | | | | |
| X | -0.1077 | 0.1010 | 0.0066 | -0.0016 | -0.1027 | 0.1059 | 0.0016 | 0.0016 | -0.1077 | 0.1010 | 0.0066 | 0.0016 | -0.1077 | 0.1010 | 0.0066 | 0.0016 | 0.0016 | | |
| SE | 0.0257 | 0.0350 | 0.0173 | 0.0294 | 0.0224 | 0.0262 | 0.0123 | 0.0123 | 0.0245 | 0.0332 | 0.0173 | 0.0123 | 0.0245 | 0.0332 | 0.0173 | 0.0141 | 0.152 ^{n/s} | 0.140 ^{n/s} | 0.098 ^{n/s} |
| Upper jaw dentition | | | | | | | | | | | | | | | | | | | |
| X | 3.0000 | 2.0000 | 2.4618 | 2.6113 | 2.9809 | 1.9809 | 2.4809 | 2.4809 | 3.0000 | 2.0000 | 2.4618 | 2.4809 | 3.0000 | 2.0000 | 2.4618 | 2.4809 | 2.4809 | | |
| SE | 0.0966 | 0.0966 | 0.0966 | 0.3063 | 0.0877 | 0.0877 | 0.0548 | 0.0548 | 0.0964 | 0.0964 | 0.0954 | 0.0548 | 0.0964 | 0.0964 | 0.0954 | 0.0583 | 0.299 ^{n/s} | 0.117 ^{n/s} | 0.418 ^{n/s} |
| Maxilla | | | | | | | | | | | | | | | | | | | |
| X | 0.0792 | -0.0743 | 0.0124 | -0.0030 | 0.0842 | -0.0693 | 0.0075 | 0.0075 | 0.0792 | -0.0743 | 0.0124 | 0.0075 | 0.0792 | -0.0743 | 0.0124 | 0.0075 | 0.0075 | | |
| E | 0.0165 | 0.0134 | 0.0096 | 0.0203 | 0.0132 | 0.0117 | 0.0067 | 0.0067 | 0.0162 | 0.0133 | 0.0093 | 0.0067 | 0.0162 | 0.0133 | 0.0093 | 0.0067 | 0.760 ^{n/s} | 0.495 ^{n/s} | 0.484 ^{n/s} |
| Palatine | | | | | | | | | | | | | | | | | | | |
| X | 0.0430 | -0.0645 | 0.0162 | -0.0008 | 0.0564 | -0.0511 | 0.0027 | 0.0027 | 0.0430 | -0.0645 | 0.0162 | 0.0027 | 0.0430 | -0.0645 | 0.0162 | 0.0027 | 0.0027 | | |
| SE | 0.0239 | 0.0230 | 0.0151 | 0.0209 | 0.0101 | 0.0145 | 0.0078 | 0.0078 | 0.0107 | 0.0170 | 0.0142 | 0.0078 | 0.0107 | 0.0170 | 0.0142 | 0.0083 | 2.980 ^{n/s} | 2.952 ^{n/s} | 0.153 ^{n/s} |
| Neurocranium | | | | | | | | | | | | | | | | | | | |
| X | 0.0488 | -0.0458 | -0.0144 | 0.0003 | 0.0409 | -0.0538 | -0.0064 | -0.0064 | 0.0488 | -0.0458 | -0.0144 | -0.0064 | 0.0488 | -0.0458 | -0.0144 | -0.0064 | -0.0064 | | |
| SE | 0.0093 | 0.0108 | 0.0071 | 0.0163 | 0.0082 | 0.0090 | 0.0048 | 0.0048 | 0.0092 | 0.0107 | 0.0069 | 0.0048 | 0.0092 | 0.0107 | 0.0069 | 0.0048 | 2.561 ^{n/s} | 2.547 ^{n/s} | 0.118 ^{n/s} |
| Vomer | | | | | | | | | | | | | | | | | | | |
| X | -0.0514 | 0.0514 | 0.0155 | -0.0037 | -0.0437 | 0.0592 | 0.0078 | 0.0078 | -0.0514 | 0.0514 | 0.0155 | 0.0078 | -0.0514 | 0.0514 | 0.0155 | 0.0078 | 0.0078 | | |
| SE | 0.0189 | 0.0203 | 0.0146 | 0.0207 | 0.0162 | 0.0169 | 0.0090 | 0.0090 | 0.0185 | 0.0198 | 0.0138 | 0.0090 | 0.0185 | 0.0198 | 0.0138 | 0.0090 | 0.903 ^{n/s} | 0.595 ^{n/s} | 0.498 ^{n/s} |

In all cases the chi-squared statistic is not significant (n/s) for the additive model (A). We therefore accept the null hypothesis of no difference between observed and expected means. Thus, an additive model of inheritance appears to sufficiently explain the data. Furthermore, in all instances, the test statistic Λ for the additive-dominance (AD) model is not significant, suggesting that dominance does not account for a significant portion of the variance. Finally, a simple t test for epistasis reveals no evidence for epistasis at the 95% confidence level.

Table 2. Pearson correlation between oral jaw characters

| | DNT | ART | LJL | LJV | LJD | PMX | UJD | MX | SUS | NCM | VMR |
|-----|---------|--------|---------|--------|---------|--------|-------|------|------|------|------|
| DNT | 1.00 | | | | | | | | | | |
| ART | 0.02 | 1.00 | | | | | | | | | |
| LJL | 0.62*** | 0.31** | 1.00 | | | | | | | | |
| LJV | 0.46*** | 0.12 | 0.61*** | 1.00 | | | | | | | |
| LJD | 0.00 | 0.21* | 0.11 | 0.05 | 1.00 | | | | | | |
| PMX | 0.11 | 0.21* | 0.22* | 0.20* | 0.05 | 1.00 | | | | | |
| UJD | 0.03 | 0.23* | 0.11 | 0.03 | 0.95*** | 0.03 | 1.00 | | | | |
| MX | 0.00 | 0.20* | 0.07 | 0.29** | 0.04 | 0.29** | 0.01 | 1.00 | | | |
| SUS | 0.09 | 0.22* | 0.29** | 0.16 | 0.21* | 0.12 | 0.20* | 0.15 | 1.00 | | |
| NCM | 0.30** | 0.21* | 0.47*** | 0.25* | 0.12 | 0.22* | 0.18 | 0.14 | 0.04 | 1.00 | |
| VMR | 0.01 | 0.29** | 0.12 | 0.12 | 0.08 | 0.09 | 0.10 | 0.11 | 0.12 | 0.12 | 1.00 |

Twenty-three of the 55 possible associations are significant. DNT, dentary; ART, articular; LJL, lower jaw in the lateral view; LJV, lower jaw in the ventral view; LJD, lower jaw dentition; PMX, premaxilla; UJD, upper jaw dentition; MX, maxilla; SUS, palatine region of the suspensorium; NCM, neurocranium; and VM, vomer.

* $P < .05$.

** $P < .01$.

*** $P < \text{Bonferroni correction}$.

Discussion

Genetic Basis of Adaptation

The Castle-Wright estimator has been employed to evaluate the genetic basis of adaptation in several evolutionary model systems, including stickleback (Hatfield 1997) and *Bicyclus* (Wijngaarden and Brakefield 2000). Although several assumptions underlie this estimator, recent modeling (Otto and Jones 2000), as well as quantitative trait locus (QTL) analyses (Peichel et al. 2001; Westerbergh and Doebley 2002), suggests that the method performs quite well. In all cases the estimator is taken as a minimum number of genetic factors. When the actual number of genetic factors is small (≤ 20 ; Otto and Jones 2000), or when the assumptions are met (Westerbergh and Doebley 2002), the difference between various approaches (e.g., Castle-Wright versus QTL) is quite small.

We used the Castle-Wright estimator to take the first steps in understanding the genetic basis of differences in oral jaw morphology among cichlid fishes. During the early radiation of Lake Malawi's cichlids, an important functional divergence probably occurred between the three basic modes of feeding: biting, sucking, and ram-feeding (Albertson et al. 1999; Greenwood 1974; Liem 1991)—a trend observed in many other groups of fishes (McPhail 1992, 1994; Schluter and McPhail 1993; Skulason et al. 1989, 1996). We examined MZ and LF because they are members of a monophyletic clade that lie on opposite ends of the biting-sucking continuum.

We find that the number of factors that underlie shape differences along this continuum is relatively small. For example, interspecific differences in dental cuspidness are determined by approximately one gene. This is supported by the Castle-Wright estimator, as well as the roughly trimodal distribution of tooth shape observed in the F_2 . Dentition features, prominently in discussions of adaptive radiation in cichlid fishes (Greenwood 1974; Futuyma 1986; Ribbink et al. 1983; Ruber et al. 1999), because it tracks extremely well with feeding performance, making it a good indicator of

trophic niche. Moreover, differences in tooth shape have been detected both between sister taxa (Ruber et al. 1999), and among different populations of the same species (Streelman JT, 2001, unpublished data). The observation that major differences in tooth shape (i.e., cusp number) may be controlled by as little as one gene suggests that this character has the potential to respond to selection extremely quickly.

Other notable observations include the estimated number of factors for the dentary and articular. Collectively, these two bones constitute the lower jaw. Our estimates suggest that the dentary and articular have very different capacities for morphological change. The dentary is controlled by one factor, suggesting that it, like tooth shape, has the potential to quickly respond to changes in the environment. On the other hand, the articular seems to be under the control of several loci, suggesting that morphological divergence in this character may require coordinated change at multiple loci.

The skull is a large and dynamic structure. Unlike the pharyngeal skeleton, which develops entirely from cranial neural crest cells, the neurocranium is derived from both neural crest and paraxial mesoderm. Functionally, the skull is associated with the oral jaw apparatus by way of the ethmoidal region of the skull; the pharyngeal jaws, by way of the pharyngeal apophysis; and the epaxial musculature, by way of the supraoccipital crest. Given the developmental and functional complexity of this structure, it is noteworthy that differences in skull shape between MZ and LF may be explained by as few as four genetic factors. The ascertainment that shape difference is limited to the anterior-most region of the neurocranium (Albertson and Kocher, 2001) may help in explaining this observation.

Phenotypic Correlations

Many of the associations (or lack thereof) among elements in Table 2 make sense in the context of recent molecular and

developmental discoveries in other model organisms. For example, several genes in mouse have been characterized as affecting tooth development. Although at least two (*dlx1* and *dlx2*) affect upper and lower jaw dentition independently, most result in tooth defects on both the upper and lower jaws (i.e., *msx1*, *msx2*, *pax9*, *fgf8*, and *pitx2*; Lin et al. 1999; Peters et al. 1998; Qui et al. 1997; Satokata and Maas 1994; Trumpp et al. 1999). Therefore, it is not surprising that we find tooth shape in the upper and lower jaws to be highly correlated ($r = .95$; $P < .001$). We expect that the same locus (loci) will affect cusp number in the upper and lower jaw of cichlids.

Tooth shape is not inherited with the bone within which teeth develop (dentary and premaxilla), but it is correlated with both the articular and suspensorium. These results suggest that the same loci may have a pleiotropic effect on tooth shape and the shape of the articular and suspensorium. These two elements develop from cartilaginous precursors within the first pharyngeal arch. Both tooth morphogenesis and chondrogenesis involve antagonistic signaling between FGFs (fibroblast growth factors) and BMPs (bone morphogenetic proteins; Peters and Balling 1999). For example, BMP4-soaked beads implanted into Meckel's cartilage explants induce exogenous cartilage formation (Semba et al. 2000), and mouse mutants lacking an FGF receptor will develop longer vertebrae (Crossley and Martin 1995). Similarly, Noggin (a BMP4 antagonist) beads implanted in developing incisors are responsible for a transformation of tooth identity (Tucker et al. 1998). Thus, FGFs, as well as BMPs, may be good candidates for the genetic determinants of size and shape in both teeth and bone that develop from cartilaginous precursors.

The articular and suspensorium are correlated with one another. A significant phenotypic correlation suggests that a common set of loci underlies form in these two elements. Thus, selection on one element will have an effect on the other. Developmentally, the articular and suspensorium are derived from the dorsal and ventral cartilaginous subunits of the first pharyngeal arch. Several zebrafish mutants are known (i.e., *suc*, *sbe*, *stn*, and *hoo*) that disrupt the development of both these elements (Piotrowski et al. 1996; Schilling et al. 1996).

The maxilla and premaxilla are correlated with one another. Both of these elements are dermal bone (bone that develops without a cartilaginous ascendant) that likely originates from mid-brain neural crest cells (Köntges and Lumsden 1996). Maxillary and premaxillary osteocytes also precipitate at approximately the same time (Albertson RC, 2000). Because most existing zebrafish mutants are lethal by 5 days postfertilization, which is approximately a day before dermal bones appear, the developmental players involved in this process remain largely a mystery.

The lower jaw in the lateral view is highly correlated with the lower jaw in the ventral view, suggesting that a common set of loci affects both jaw length and jaw width. The lower jaw in the lateral view is also highly correlated with the neurocranium. The lower jaw and the skull are not adjacent to one another, and it seems difficult to imagine that this association is a result of steric or functional interactions. The cartilaginous precursors of both the lower jaw and neuro-

cranium are among the first head structures to be seen in the developing teleost embryo, and a multitude of zebrafish mutants have been described (i.e., *low*, *fla*, *bab*, *vgo*, *dol*, and *cyc*) that affect both the jaw and the anterior region of the skull (Kimmel et al. 2001; Piotrowski et al. 1996; Schilling et al. 1996).

The dentary is not correlated with the articular ($r = .02$). Functionally, the dentary fuses to the articular early in development to form the functioning lower jaw. Developmentally, however, these two elements are quite distinct. The dentary is a dermal bone that originates from mid-brain neural crest cells. The articular is endochondral and develops from both mid- and hindbrain cranial neural crest (Köntges and Lumsden 1996). The articular also appears 4 days earlier than the dentary in cichlid development (Albertson RC, 2000). Like the masseteric and alveolar regions of the mouse mandible (Cheverud 2001), the articular and dentary clearly show that different developmental units are inherited separately.

Conclusion

Our results lend support for the hypothesis that differences in the cichlid oral jaw apparatus are controlled by relatively few genes, and that pleiotropy figures prominently in the genetic architecture of the cichlid head. Moreover, we find that patterns of phenotypic correlation correspond to developmental rather than functional units. A number of genes involved in craniofacial development have been characterized in model organisms, most of which seem to have been conserved over vertebrate evolution. It remains to be seen whether these same genes are also implicated in fine-scale adaptive variation among species.

The breadth of diversity that characterizes lacustrine cichlid assemblages makes them ideal systems within which to study adaptive radiation and the evolution of feeding mechanisms. Important future directions should include a more comprehensive dissection of the genetic and developmental architecture of the cichlid head. This knowledge will help identify the fundamental units upon which natural selection acts, as well as better facilitate an understanding of how the oral jaw apparatus responds to selection. A formal test of integration, whether morphological, as in Liem (1980) and Zelditch (1987), genetic, as in Cheverud (1982, 2001) and Leamy et al. (1999), or developmental, as in Mezey et al. (2000), would go a long way toward this goal. Also, given the estimated number of genes identified here, it appears feasible to conduct an experiment to map loci underlying these quantitative traits. An experiment with approximately 200 F₂ should have the power to detect most, if not all, of the genetic determinants of shape difference between MZ and LF (Beavis 1997).

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