

GENETIC ARCHITECTURE SETS LIMITS ON TRANSGRESSIVE SEGREGATION IN HYBRID CICHLID FISHES

R. CRAIG ALBERTSON^{1,2} AND THOMAS D. KOCHER³

¹*Department of Cytokine Biology, The Forsyth Institute and Department of Oral and Developmental Biology, Harvard School of Dental Medicine, Boston, Massachusetts 02115*

²*E-mail: calbertson@forsyth.org*

³*Hubbard Center for Genome Studies, University of New Hampshire, Durham, New Hampshire 03824*

Abstract.—The role of hybridization in the evolution of animal species is poorly understood. Transgressive segregation is a mechanism through which hybridization can generate diversity and ultimately lead to speciation. In this report we investigated the capacity of hybridization to generate novel (transgressive) phenotypes in the taxonomically diverse cichlid fishes. We generated a large F₂ hybrid population by crossing two closely related cichlid species from Lake Malawi in Africa with differently shaped heads. Our morphometric analysis focused on two traits with different selective histories. The cichlid lower jaw (mandible) has evolved in response to strong directional selection, and does not segregate beyond the parental phenotype. The cichlid neurocranium (skull) has likely diverged in response to forces other than consistent directional selection (e.g., stabilizing selection), and exhibits marked transgressive segregation in our F₂ population. We show that the genetic architecture of the cichlid jaw limits transgression, whereas the genetic basis of skull shape is permissive of transgressive segregation. These data suggest that natural selection, acting through the genome, will limit the degree of diversity that may be achieved via hybridization. Results are discussed in the context of the broader question of how phenotypic diversity may be achieved in rapidly evolving systems.

Key words.—Adaptive radiation, cichlids, jaw, skull, transgressive segregation.

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Cichlid fishes (Teleostei, Perciforms) have the remarkable ability to radiate in very short periods of time, making them an excellent system to study the mechanisms that create biodiversity in vertebrates. Hybridization has recently been offered as a means through which rapid diversification can be achieved in this system (Seehausen 2004), and a hybrid origin has been posited for a number of cichlid species (Ruber et al. 2001; Salzburger et al. 2002; Smith et al. 2003). In this report we investigate whether the genetic diversity of cichlids is organized in such a way that hybridization can generate phenotypic diversity.

Transgressive segregation is the appearance of hybrid individuals that exhibit extreme or novel phenotypes relative to parental lines (DeVicente and Tanksley 1993). This phenomenon can facilitate speciation if the transgressive phenotype is heritable and confers an adaptive advantage on the hybrid population (Lewontin and Birch 1966). Marker-based genetic experiments suggest that transgressive segregation typically results from the recombination of complementary alleles at different loci within hybrid populations, leading to phenotypic values that exceed those of either parental line (Rieseberg et al. 1999). An important requirement for transgressive segregation is the presence of quantitative trait loci (QTL) with antagonistic effects (i.e., allelic effects opposite to the direction of mean phenotypic differences) in the parental population (Rieseberg et al. 2003). A population diverging via stabilizing selection or genetic drift, or with a history of varying selective regimes, is expected to possess more alleles with antagonistic effects, whereas a population evolving under consistent directional selection will accumulate alleles with consistent directional effects (Orr 1998; Rieseberg et al. 2003). If two populations are fixed for alleles of opposite effect, transgressive segregation in their hybrids becomes improbable from a genomic perspective (Rieseberg

et al. 2003). Thus, directional selection and hybridization represent antagonistic forces with respect to biodiversity. On one hand, selection acts to fix functional alleles. On the other, transgressive segregation requires antagonistic variation at the same functional loci. By this logic we hypothesize that traits that have evolved in response to consistent directional selection will not exhibit transgressive segregation in hybrid populations, whereas those that have diverged in response to other forces will.

We explore the effects of hybridization on two traits with different selective histories. Genetic studies recently demonstrated that the cichlid feeding apparatus has undergone a history of strong directional selection, whereas the skull has most likely diverged in response to other forces such as stabilizing selection (Albertson et al. 2003). Using the same F₂ mapping population, we observed transgressive segregation for the shape of the cichlid skull but not the mandible. We argue that the genetic architecture of the mandible imposes limits on transgressive segregation. Implications of this study are twofold. First, we present evidence that hybridization has the potential to generate novel phenotypes in this group, and thus may be a contributing factor in their diversification and success. However, we also demonstrate that natural selection has set limits on transgressive segregation in cichlid hybrids. Thus, while hybridization may contribute to cichlid diversity, its effects will be limited to specific traits (i.e., those that have not diverged in response to consistent directional selection), and to specific times in their adaptive history (i.e., before lines have become fixed for the direction of QTL effects).

METHODS

Study Species

We examined the neurocranium and lower jaw of two Lake Malawi, Africa, cichlid species. *Labeotropheus fuelleborni*

(hereafter referred to as LF) and *Metriaclima zebra* (MZ) are two rock-dwelling species from Lake Malawi. MZ has a moderately sloped head, terminally oriented mouth, and large horizontally directed vomer (Stauffer et al. 1997). MZ feeds on diatoms and loose algae by either brushing these items from algae-beds, or sucking them from the water column (McKaye and Marsh 1983; Ribbink et al. 1983; Reinthal 1990). LF has a large fleshy snout, robust inferior-subterminal mouth, and vertically directed vomer. The orientation of its mouth allows LF to crop firmly attached algae from rocks while swimming parallel to the substrate (Ribbink et al. 1983).

Parental specimens used in this study were laboratory-reared F₁ animals generated from wild-caught stock. Hybridization was achieved by crossing male LF with female MZ in 500-gallon pools. All animals were reared in a common environment, fed a common food source, and raised to at least 18 months in age (well beyond sexual maturity). Specific information regarding the breeding and maintenance of cichlid stocks is described by Albertson and Kocher (2001). Animals were sacrificed with MS222 in accord with a protocol approved by the University of New Hampshire Animal Care and Use Committee. Specimens were then prepared for morphometric analysis using dermestid beetles, which cleaned tissue from the head.

One hundred and fifty-five animals were used in this study, including 15 MZ, 15 LF, 20 F₁ hybrids, and 100 F₂ hybrids. We also examined five specimens of *Pseudotropheus trophops* "red cheek" (PT), a species with an intermediate oral jaw design to see where they fell in the morpho-space defined by LF, MZ, and their hybrid progeny.

Morphometric Technique

Differences in the neurocranium were assessed via landmark-based morphometrics in Morphometrika 7.0 (Walker 1999). First, superimposition of landmark data was achieved using a Generalized Procrustes Analysis (GPA) (Gower 1975; Rohlf and Slice 1990), which superimposed landmark configurations so that the sum of squared distances between corresponding landmarks was minimized by scaling, translating, and rotating specimens with respect to a mean consensus configuration. We then performed a thin-plate spline (TPS) procedure. TPS models the form of an infinitely thin metal plate that is constrained at some combination of points (i.e., landmarks) but is otherwise free to adopt a 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). These components (partial warps) can be localized to describe precisely what aspects of shape are different. A principal component analysis (PCA) was then performed on partial warp scores, formally referred to as relative warp analysis (RWA) (Bookstein 1996), to identify the major axes of shape variation.

RESULTS

Segregation of Jaw Shape

F₁ and F₂ hybrid jaw shape was generally intermediate relative to LF and MZ along the first principal component axis (PC1), which accounted for approximately 50% of the total variation (see Fig. 1A). Shape variation along this axis pertained largely to the length and height of the lower jaw (note vectors on the jaw in Fig. 1A). PT jaw shape was intermediate to LF and MZ along PC1, and plotted within the F₂ distribution. Principal component 2 (PC2) accounted for 20% of the phenotypic variance. Hybrid variance did not exceed that of parental lines along this axis. Shape variation along PC2 was subtle and distributed over the entire structure (note vectors).

Segregation of Neurocranium Shape

Hybrid skull shape was intermediate relative to MZ and LF along PC2, which accounted for approximately 30% of the phenotypic variance (Fig. 1B). Shape variation along PC2 was limited to the anterior (ethmoidal) region of the skull (note vectors on the skull in Figure 1B). F₂ hybrids were transgressive relative to MZ and LF along PC1, which accounted for 40% of the phenotypic variance. Shape change along PC1 was limited to the posterior (otical) region of the skull (note vectors). MZ and LF showed little difference in their position along PC1, but F₂ hybrids had a distribution that was clearly beyond the range of either parent.

Transgressive segregation in the shape of the skull affected two functionally relevant traits. Variation along PC1 described the height of the supraoccipital crest (SOC, Fig. 1B), and the depth of the pharyngeal apophysis (PA, Fig. 1B). The supraoccipital crest provides the surface area where the epaxial musculature inserts, and the pharyngeal apophysis is a synovial joint that articulates with the upper pharyngeal jaw. The shape of these regions of the skull is intimately linked with feeding performance in cichlids and other bony fishes (Greenwood 1978; Liem 1974; Lauder 1979; Liem 1980; Barel 1983; Carroll et al. 2004). LF and MZ are similar in terms of the height of the supraoccipital crest and the depth of the pharyngeal apophysis, but F₂ hybrids are transgressive in these two characters. Interestingly, PT individuals also group within the two dimensional description of F₂ skull shape. Thus, not only is hybrid morphology transgressive, but it also reproduces a naturally occurring phenotype.

DISCUSSION

Implications of Hybridization

There is some debate as to whether or not hybridization can facilitate speciation. On one hand, intermediacy is expected to place hybrids at a disadvantage in the presence of parental lines (Mayr 1963; Schluter 2000). Alternatively, it has been argued that hybridization is a vital source of genetic variation and phenotypic novelty (Lewontin and Birch 1966; Rieseberg et al. 1999). Recently, a hybrid swarm theory of adaptive radiation was formalized (Seehausen 2004). Building on the work of other investigators (Stebbins 1959; Lewontin and Birch 1966; Templeton 1981), Seehausen (2004)

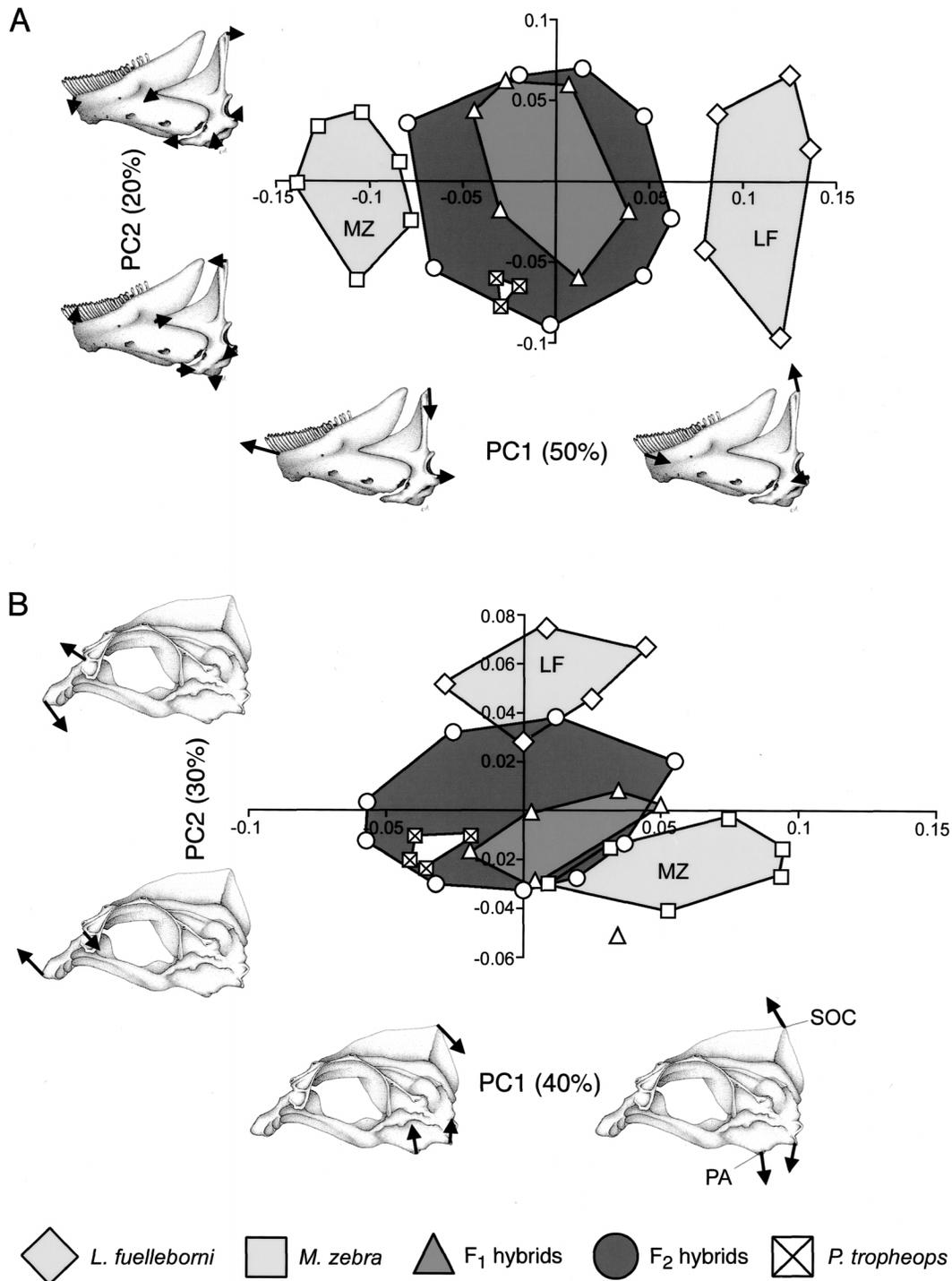


FIG. 1. Segregation of jaw and skull shape: shaded areas represent the two-dimensional variance for each sample. Illustrations of jaw and skull shapes represent the mean consensus configuration ($x = 0, y = 0$). Vectors indicate the direction and magnitude of difference from the consensus configuration. In all cases vector lengths represent 150% of the actual magnitude of morphometric difference. (A) Jaw shape. MZ and LF are differentiated along principal component axis 1 (PC1), which accounts for 50% and the phenotypic variance. Shape variation along this axis pertains to the length and height of the lower jaw. F₁ and F₂ hybrids, and PT are intermediate relative to MZ and LF along PC1. PC2 explains 20% of lower jaw shape variance, which is distributed over the entire element. Hybrid populations are not transgressive along PC2. (B) Skull shape: PC1 explains 40% of the phenotypic variance and is limited to the posterior (otical) region of the skull. Transgressive segregation is observed in the F₂ population along PC1. PC2 explains 30% of the phenotypic variance and describes variation in the shape of the anterior (preorbital) region of the neurocranium. MZ and LF are distinguished along PC2. PT individuals plot within the two-dimensional F₂ distribution. The supraoccipital crest is labeled SOC, and the pharyngeal apophysis, PA.

noted that hybridization will often occur under the same ecological conditions as adaptive radiations (i.e., environmental instability). He postulates that if parental populations brought together under specific environmental conditions contain adequate variation at functional loci, and if the environment contains a sufficient number of underutilized niches, then hybridization could facilitate the initial bursts of evolution that often precede adaptive radiations (Seehausen 2004). The hybrid swarm theory is supported by instances of hybridization in various adaptive radiations including Darwin's finches (Grant and Grant 1992) and African cichlids (Ruber et al. 2001; Smith et al. 2003), and may explain why younger radiations are often more species rich than older ones (Seehausen 2004). As species numbers increase, resources become scarce, and natural selection will stabilize species numbers by fixing alleles at functional loci (Seehausen 2004), thus eliminating the genomic prerequisites for speciation via hybridization.

Genetic studies on the cichlid head offer some empirical support for the hybrid swarm theory of adaptive radiation (Albertson et al. 2003). Four loci were identified that had a quantitative effect on the shape of the skull. Of these, two exhibited consistent allelic effects (i.e., in the same direction), and two exhibited antagonistic effects (i.e., in the opposite direction). Thus, antagonistic alleles for skull shape have accumulated in both LF and MZ populations, and it is for this trait that we observed dramatic transgressive segregation among hybrids (see Fig. 1B). On the other hand, out of 22 loci that affect the shape of the lower jaw, only one showed antagonistic allelic effects. The accumulation of alleles with consistent effects on the shape of the lower jaw is likely due to a history of consistent directional selection on cichlid jaw shape (Albertson et al. 2003). As predicted, transgressive segregation was not observed for the shape of the lower jaw (see Fig. 1A).

It is important to note that the accumulation of antagonistic alleles for skull shape does not discount a role for natural selection in the evolution of the cichlid neurocranium; it merely suggests that consistent directional selection was less prominent in the evolutionary history of the skull compared to the oral jaws. Indeed, given the diverse functional demands imposed on the skull by eye size, brain structure, and feeding performance (Barel 1983; Huber et al. 1997; Carroll et al. 2004), it is likely that the neurocranium has been shaped by stabilizing selection, and that its final form represents a compromise among various functional and developmental constraints. Regardless of the origin, the genetic architecture of the skull and jaw differ in such a way that one is permissive of transgressive segregation, and the other is not.

Summation and Synthesis

There has been a recent surge in research aimed at understanding the role of hybridization in the explosive evolution of certain East African cichlid lineages (McElroy and Kornfield 1993; Sturmbauer 1998; Ruber et al. 2001; Salzburger et al. 2002; Sturmbauer et al. 2001; Smith et al. 2003). We bring a genomic perspective to bear on the question of the role hybridization has played in the evolution of this system. Although the data presented above demonstrate that

hybridization has the potential to generate novel phenotypes, quantitative genetic data suggest that its effects on traits typically associated with the dramatic radiation of this group (i.e., the oral jaw apparatus) will be limited.

The evolution of Lake Malawi cichlids is characterized by a sequence of divergence in marchohabitat, feeding morphology, and male reproductive traits, respectively (Danley and Kocher 2001). Fixation of alleles with consistent directional effects on jaw shape (Albertson et al. 2003), as well as divergent selection and parallel evolution of male nuptial colors (Allender et al. 2003), supports a strong role for selection at each stage of the radiation. Thus, directional selection has likely acted quickly to sort functional alleles among cichlid species, thereby limiting the necessary genetic variation for transgressive segregation. It is therefore unlikely that hybridization has played a consistent role throughout the diversification of this group. Instead, our data are consistent with the hybrid swarm theory of adaptive radiation in cichlids, in which the diversifying effects of hybridization (though potent) were limited to the early stages of the radiation (i.e., before lines became fixed for the traits listed above).

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