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RESEARCH ARTICLE

Hybridization Promotes Evolvability in African Cichlids: Connections Between Transgressive Segregation and Phenotypic Integration

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Abstract Hybridization is a potential source of novel variation through (1) transgressive segregation, and (2) changes in the patterns and strength of phenotypic integration. We investigated the capacity of hybridization to generate novel phenotypic variation in African cichlids by examining a large F2 population generated by hybridizing two Lake Malawi cichlid species with differently shaped heads. Our morphometric analysis focused on the lateral and ventral views of the head. While the lateral view exhibited marked transgressive segregation, the ventral view showed a limited ability for transgression, indicating a difference in the genetic architecture and selective history between alternate views of the head. Moreover, hybrids showed a marked reduction in integration, with a lower degree of integration observed in transgressive individuals. In all, these data offer novel insights into how hybridization can promote evolvability, and provide a possible explanation for how broad phenotypic diversity may be achieved in rapidly evolving groups.

Keywords Adaptive radiation · Variation · Constraint

Introduction

Hybridization is widely accepted to play a major role in evolutionary divergence among plant species (Ehrlich and Wilson 1991). Zoologists, on the other hand, have been more reluctant to accept a role for hybridization in promoting diversification. While there are examples from animals that explicitly demonstrate that hybridization has played a role in speciation through the formation of polyploids (Sites et al. 1990; Vrijenhoek et al. 1989; Bullini and Nascetti 1990; Anderson and Evans 2009), gene flow is generally thought to present an obstacle to specialization, raising doubts about more general roles for hybridization in promoting animal diversification (Dobzhansky 1940; Mayr 1942; Via 2009). The occurrence of hybrid zones is generally thought to be linked to environmental typology whereby hybrid genotypes are favoured in intermediate habitats (Grant and Grant 1994). When hybrids have moderately high fitness the chance of introgression increases, which can in turn lead to a higher degree of genetic variation in recipient populations. Occasionally these conditions can provide a burst of variation referred to as a hybrid swarm (Gilliard 1959; Stebbins 1959; Mayr 1963; Potts and Reid 1985; Seehausen 2004).

The novel combinations of alleles that result from hybridization can increase evolvability through various phenotypic effects. For example, transgressive segregation, whereby hybrids exhibit extreme or novel phenotypes relative to parental lines, can result from hybridization (Ackermann et al. 2005, 2006). These new phenotypes can facilitate speciation if they are heritable and confer a fitness advantage on the hybrid population (Lewontin and Birch 1966). At the genetic level transgressive segregation typically occurs due to the recombination of complementary alleles at different loci within hybrid populations (Rieseberg et al. 1999a). An important requirement for transgressive segregation therefore is the presence of alleles with antagonistic phenotypic effects within the parental populations. Alternatively, if two populations are

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fixed for alleles of opposite effect (e.g., via directional selection), transgressive segregation in their hybrids becomes unlikely from a genetic perspective (Rieseberg et al. 2003b; Albertson and Kocher 2005). Based on this information, it can be reasonably deduced that traits that exhibit transgressive phenotypes possess relatively high levels of genetic variation, compared to traits where transgression does not occur. Moreover, a lack of genetic variation is consistent with directional selection acting on traits, whereas relatively more genetic variation is consistent with divergence due to other processes of evolution including genetic drift or stabilizing selection (Orr 1998; Rieseberg et al. 2003a; Albertson et al. 2003; Albertson and Kocher 2005). In this way, the occurrence of transgressive segregation can be used as an indirect measure of both the genetic architecture and selective history of a trait.

Integration refers to the relationship among traits, and the manifestation of phenotypic covariation/correlation is attributable to underlying functional, developmental, and/or genetic organization (Berg 1960; Clausen and Heisey 1960; Maynard Smith et al. 1985; Cheverud 1988; Schlichting 1989; Schlichting and Pigliucci 1998; Murren 2002; Armbruster et al. 2004; Zelditch et al. 2009). Hybridization has been shown to have significant effects on the correlation structure among phenotypic traits (Grant and Grant 1979; Clausen and Heisey 1960; Murren 2002; Ackermann et al. 2006; Grant and Grant 1994). By conforming to fitness trade-offs among traits, or even being demonstrably antagonistic to the direction of selection (Futuyma 2010), phenotypic correlations can imply evolutionary constraint. Thus, by altering the correlation structure (i.e., integration) among traits, hybridization may act to ‘break’ evolutionary constraints and open new ecological opportunities. Mounting evidence suggests that phenotypic integration is based on multiple, interacting alleles (Pavlicev et al. 2009), but the genetic basis for changes in patterns or levels of integration remains unclear. Moreover, given that adaptive patterns of integration can differ across divergent habitats (Parsons and Robinson 2006), it is thought that integration is evolvable, but the selective regimes acting on this trait as well as its connection to other phenomena that influence evolvability (e.g., transgressive segregation) also remains unclear. For example, it is possible that integration is under directional selection, lacks the requisite genetic variation, and will be invariant with respect to hybridization. Alternatively, because intermediate levels of integration are thought to be optimal for promoting evolvability (Wagner and Altenberg 1996), it is equally likely that integration is under stabilizing selection. In this scenario, the accumulation of positive and negative alleles that underlie phenotypic integration within a population would make this trait susceptible to transgressive segregation. Thus, identifying connections between transgressive phenotypes and integration can lead to a deeper understanding of the genetic basis and selective history of phenotypic integration, as well as a more comprehensive understanding of how hybridization effects evolvability.

In this study we investigate the phenotypic effects of hybridization within an F2 population generated by crossing two phenotypically distinct cichlid species from Lake Malawi. Specifically, we assess the extent to which hybridization has the potential to increase phenotypic evolvability within this group by investigating (1) whether transgressive segregation occurs in craniofacial morphology, (2) whether levels of integration change between the F2s and parental lines, (3) whether a relationship exists between the occurrence of transgressive phenotypes and levels of phenotypic integration, and (4) whether the phenotypic variation generated by a hybridization event matches the major trajectory of divergence for the lake Malawi cichlid radiation as a whole.

Methods

Study Species

We examined the lateral and ventral views of craniofacial shape in two Lake Malawi, Africa, cichlid species. These were *Labeotropheus fuelleborni* (hereafter referred to as LF) and *Tropheops* ‘red cheek’ (TRC), which are two specialized benthic foragers that possess shortened jaws relative to other Lake Malawi cichlids. However, there are major differences in foraging mode and jaw width between these two species. LF has a particularly wide mouth which it uses to scrape firmly attached algae from the surface of rocks, while TRC has a narrow jaw which it uses to ‘pluck’ strands of filamentous algae (Ribbink et al. 1983).

Specimens of parental species used in this study were lab-reared F1 animals generated from wild-caught stock. Hybridization was achieved by crossing a single male TRC with a single female LF in a 40 gallon aquarium. All animals were reared in a common environment, fed common flake-food diets, and reared to at least 1 year of age, which is well beyond the age of sexual maturity for these species. Animals were sacrificed with MS222 in accordance with Syracuse University Institutional Animal Care and Use protocols. Specimens were preserved in 70% ethanol, and minor dissections were performed to expose functionally salient anatomical structures in both the lateral and ventral view. A total of three hundred and sixty nine specimens were used in this study, including 31 LF, 40 TRC, and 298 F2 hybrids.

Morphometrics

Variation in the lateral and ventral views of the head was quantified using a geometric morphometric approach. A total of 16, and 10 landmarks were respectively collected on the lateral and ventral views of the head (Fig. 1). To reduce the effects of size and orientation we performed a Generalized Procrustes Analysis (GPA) for each of the lateral and ventral datasets (Rohlf and Slice 1990). This process superimposes landmark configurations to minimize the sum of squared distances between corresponding landmark configurations by scaling, rotating, and translating specimens. To remove the potential effects of allometry from the data we then performed a multiple regression of shape on ln geometric centroid size to generate allometry-free landmark data sets based on residuals from this analysis. Landmark data was collected using TPSdig2, GPA was performed using Coordgen6h, and multiple regression was done using Standard6 (all available at: http://www.life.bio.sunysb.edu/morph/).

To generate data suitable for further statistical analysis we then performed a thin-plate spline (TPS) procedure to generate partial warp scores for lateral and ventral datasets. 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 (partial warps) based on scale (Rohlf

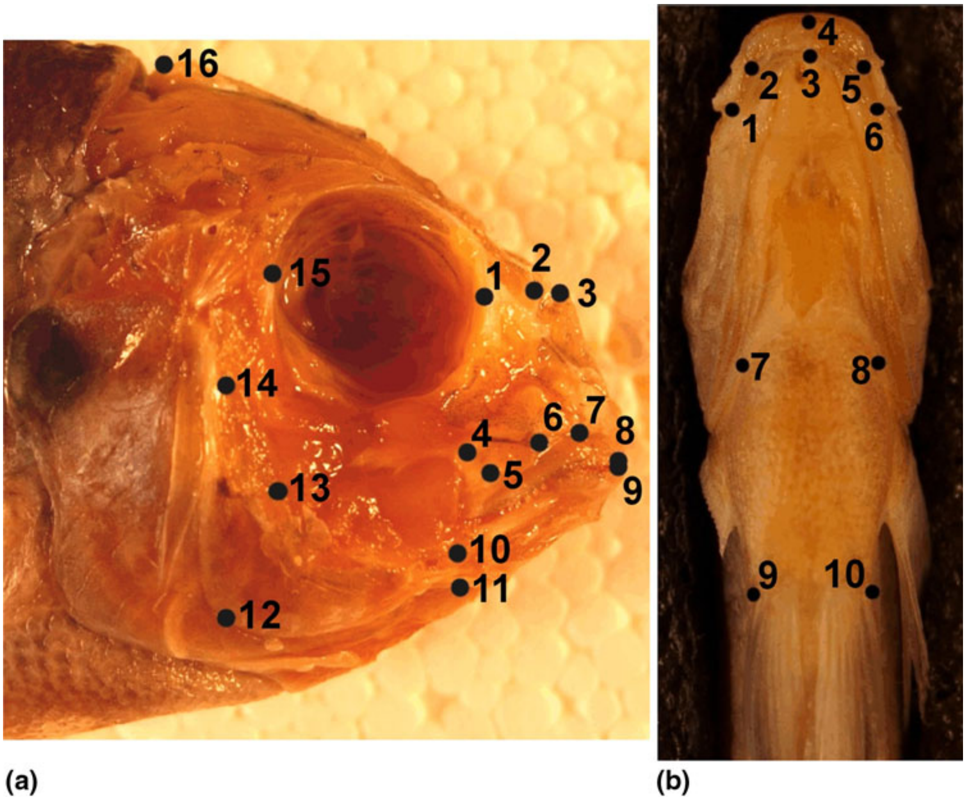

Fig. 1 The landmarks used to quantify shape in the lateral and ventral view of cichlids. For **a** *1* The most anterio-ventral point of the eye socket; *2* Joint between the nasal bone and the neurocranium; *3* Posterio-dorsal tip of the ascending process of the premaxilla; *4* Insertion of the A2 division of the *adductor mandibulae* on the articular process of the mandible; *5* Maxillary-articular joint (lower point of rotation of the maxilla); *6* Insertion of the A1 division of the *adductor mandibulae* on the maxilla; *7* Maxillary-palatine joint (*upper* rotation point of the maxilla); *8* Tip of the anterior-most tooth on the premaxilla; *9* Tip of the anterior-most tooth on the dentary; *10* Articular-quadrate joint (*lower* jaw joint); *11* Insertion of the interopercular ligament on the retroarticular process (point at which mouth opening forces are applied); *12* Posterio-ventral corner of the preopercular; *13* Most dorsal point on the origin of the A2 division of the *adductor mandibulae* jaw closing muscle on the preopercular; *14* Most posterio-ventral point of the eye socket; *15* Most ventral point on the origin of the A1 division of the *adductor mandibulae* jaw closing muscle on the preopercular; *16* Dorsal-most tip of the supraoccipital crest on the neurocranium. For **b** *1,6* Articulation points of the mandible; *2,5* Most posterior points of the tooth-bearing cusp of the mandible; *3* Most posterior point of the midline of the mandible; *4* Most anterior point of the midline of the mandible; *7,8* Posterior extent of medial branchiostegal rays; *9,10* Insertion points of the pelvic fins

and Marcus 1993). TPS was performed using PCAgen (available at: http://www.life.bio.sunysb.edu/morph/).

Identifying Transgressive Phenotypes

Our approach for identifying transgressive phenotypes was conservative and based on measures of Procrustes distance. Procrustes distances are standardized measures in shape space that indicate how far an individual is from a consensus shape. To identify transgressive phenotypes we first calculated the average Procrustes distance for each of the LF and TRC parental lines, and then found the Procrustes distance between these averages. The Procrustes distance of each individual F2 to the average shape of each parental species was then determined. We deemed F2 individuals to be transgressive when they exhibited a Procrustes distance to both LF and TRC averages that exceeded the Procrustes distance between these averages.

Integration Within and Between Lateral and Ventral Views

We tested for differences in integration among the LF, TRC, and F2 hybrid samples. To determine whether integration differed within either the lateral and ventral views we calculated the variance of eigenvalues from PCA models based on our previously generated partial warp scores. Eigenvalues are a scalar value used to represent the amount of variation each eigenvector accounts for in a given PCA (Manley 1994; Zelditch et al. 2004). If covariation among traits is high, the first few PCs present large eigenvalues relative to later ones, and the variance of eigenvalues is high. If covariation is low, PCs have similar eigenvalues and variance among them is low (Cheverud et al. 1989; Herrera et al. 2002). We used a procedure that bootstrapped the differences in eigenvalue variance 1,000 times by sampling with replacement from rows of our partial warp scores.

To determine relationships between lateral and ventral views we used a Procrustes matrix superimposition. This method is a type of matrix correlation that allows for tests of association using raw untransformed data, even when the number of variables differs (Peres-Neto and Jackson 2001). Using the partial warp scores of the ventral and lateral data sets the concordance between these two matrices was determined and tested based on a goodness-of-fit measure. The sum of the squared residuals between eigenvector matrices provides a goodness-of-fit statistic (m12) that ranges between 0 and 1, and identifies the optimal superimposition that can be used as a metric of concordance. Small values of m12 correspond to small residual variation and, hence, a high concordance of matrices.

Additionally, the Procrustes matrix superimposition provides residual variation for each specimen. This was useful for determining whether identified transgressive individuals differed from non-transgressive individuals in their degree of integration. For this analysis we performed a superimposition on all F2 specimens, followed by ANOVAs using transgressive and non-transgressive as a grouping variable.

Comparing the Primary Axis of Variation in F2s to Malawi

Using the lateral view of craniofacial shape a previous study identified a primary axis of divergence for Lake Malawi cichlids that mainly involved a reciprocal lengthening and shortening of the preorbital jaw region (Cooper et al. 2010). This axis was also common to the cichlid radiations of both Tanganyika and Victoria (Cooper et al. 2010). In line with the hybrid swarm theory of cichlid adaptive radiations (Seehausen 2004) we tested whether our hybrid population shared this important morphological trajectory. We therefore conducted pairwise tests to determine whether shape space orientations, as defined by the first PC axis, were different among the Malawi radiation, F2s, and within F2s the transgressive and non transgressive individuals. Specifically, we used the program SpaceAngle to determine if the observed angle between two shape spaces differed from those calculated from random sub-divisions of either dataset. A bootstrapping procedure (900 sets) was used to define the 95% CI for the angles calculated from re-sampling each of the two original datasets being compared. If the observed angle fell within either of the two 95% CIs, then the orientations of the two original shape spaces were not considered significantly different.

Results

Transgressive segregation was prevalent for shape in the lateral view, while relatively rare in the ventral view. Over 45.6% of F2s were transgressive in the lateral view indicating the presence of high levels of segregating genetic variation in the F2. Alternatively, for the ventral view only 4.0% of the F2s were transgressive, which suggests that low levels of genetic variation exists within the parental species for this trait.

We also found that hybridization lowers the degree of integration among traits. Specifically, while levels of integration did not differ between parental lines for either the lateral or ventral views, our analysis of eigenvalues revealed that integration within each of the lateral and ventral views was significantly lower in the F2s relative to either parental line (Table 1). Notably, the average magnitude of this reduction in a standardized metric of integration was much greater in the lateral view (over 300% greater) than in the ventral view. Thus, similar to the results presented above, it is the lateral view of the skull that is most affected by hybridization.

Significant levels of integration were found between the lateral and ventral views for F2 hybrids, as well as LF, and TRC parental lines (all $P < 0.001$). However, our goodness of fit metric indicated that this integration was substantially weaker for the F2s ($m12 = 0.88$) relative to both LF and TRC parental lines ($m12 = 0.62$, and 0.69 respectively). Moreover, the integration between lateral and ventral views was reduced to a greater extent in F2 animals that were transgressive with respect to craniofacial shape relative to those that were not (ANOVA, $F_1 = 46.22$, 60.88, respectively for lateral and ventral transgressives, both $P < 0.001$). Data from both groupings displayed homogeneity of variance (Levene's test both $P > 0.6$), and normality (Shapiro–Wilk test both $P > 0.1$).

It has been demonstrated that measures of integration can be influenced by variance artifacts, whereby increasing variance results in stronger degrees of integration (Hallgrimsson et al. 2009). However, shape variance, as measured by the Procrustes distance to the reference, was highest in our F2s for both the lateral and ventral views (Table 2), while integration in F2s was consistently lower. Therefore, while tests for the potential issue of variance artifacts are available (Young et al. 2010), we did not feel that this was necessary in our case.

Finally, the trajectory of the primary axis of variation in our F2 hybrids was not significantly different from the trajectory possessed by the Malawi radiation. This is notable given that both species used to generate the F2 population define one end of the primary axis of craniofacial variation among Malawi cichlids (Cooper et al. 2010). In all three cases (i.e., all F2, transgressive F2, and non-transgressive F2) the observed difference in trajectory did not exceed that of bootstrapped confidence intervals. These patterns suggest that the primary axis of variation across Lake Malawi cichlid species is remarkably conserved even within ecotypes, and that while hybridization is an effective means of increasing shape variation and decreasing trait correlations, it cannot significantly alter the primary axis of shape variation (Fig. 2).

Discussion

It has been proposed that the cichlid fishes of the East African rift lakes could represent a hybrid swarm due to their propensity to hybridize and their ability to rapidly diverge (Ruber et al. 2001; Salzburger et al. 2002; Danley and Kocher 2001; Seehausen 2004). Both phylogenetic and geological data have been used to support this assertion. From a phylogenetic perspective, many East African rift-lake cichlid lineages, especially with Lake Malawi, are best described as a polytomy (Joyce et al. 2011), which is a signature of a hybrid swarm (Seehausen 2004). Moreover, the geological history of the African rift valley suggests that large fluctuations in water levels may have promoted wide scale hybridization events by bringing previously allopatric populations together, leading to a breakdown in mate recognition and rampant hybridization (Cohen et al. 2007; Genner et al. 2010). Our findings extend this important body of results by showing that hybridization can increase evolvability by (1) providing new phenotypic variation previously unseen by selection, through transgressive segregation, and (2) by decreasing the degree of integration among phenotypic traits, possibly breaking evolutionary constraints. For the first time we also show (3) that there is a connection between the occurrence of transgressive phenotypes and levels of phenotypic integration. This adds substantially to our understanding of the evolutionary implications of hybridization. While it is unknown whether the hybrid phenotypes produced by our experiment would confer a fitness advantage in nature, the finding that their primary trajectory did not differ from that of the Malawi radiation itself, which spans multiple ecotypes, suggests that there could be a strong ecological relevance for the phenotypes produced.

Data presented here also contribute to the wider debate as to whether adaptive divergence can happen under conditions where hybridization can occur. On one hand, both

Table 1 Comparisons of levels of integration in the lateral and ventral views

Comparison	Ventral			Lateral		
	Standardized integration value	95% bootstrapped confidence interval	P	Standardized integration value	95% bootstrapped confidence interval	P
LF vs. TRC	0.32_{LF}, 0.33_{TRC}	−0.063, 0.050	0.896	0.28_{LF}, 0.25_{TRC}	−0.091, 0.010	0.140
TRC vs. F2	0.33_{TRC}, 0.29_{F2}	0.026, 0.111	<0.001	0.25_{TRC}, 0.16_{F2}	0.084, 0.156	<0.001
LF vs. F2	0.32_{LF}, 0.29_{F2}	0.0191, 0.101	0.002	0.28_{LF}, 0.16_{F2}	0.119, 0.196	<0.001

Integration is compared among F2 hybrids, LF and TRC parental lines. Confidence intervals are based on 1,000 bootstraps

Table 2 Variance of Procrustes distances within the F2 hybrids, LF and TRC parental lines

Group	Ventral	Lateral
F2	0.304	0.227
LF	0.295	0.150
TRC	0.225	0.219

Values are given for both the lateral and ventral views. All values have been multiplied by 1,000

field and laboratory studies suggest that hybrids can be at a disadvantage and exhibit reduced fitness (Schluter 1996, 2000; Neff 2004). Conversely, other work suggests that hybridization can generate new phenotypes that confer a fitness advantage (Lewontin and Birch 1966; Rieseberg et al. 1999b). We submit that the outcome of hybridization is largely influenced by ecological conditions over long-term temporal timescales. In this context fluctuating environments may play an important dual role. First they may increase the likelihood of hybridization events during more stressful periods when mates or habitats are limited (Grant and Grant 1994; Seehausen 2004). Second, during more ecologically relaxed periods when the number of niches has been expanded, novel phenotypic variation provided by hybridization may allow for underutilized resources to be exploited, leading to further niche expansion. These are precisely the conditions that were likely present for the ancestral members of the African Rift lake cichlids as lake levels fluctuated during previous drought periods (Cohen et al. 2007; Seehausen 2004). Similarly, hybridization in other adaptive radiations during stressful conditions and subsequent rapid divergence has been observed in contemporary timescales (Grant and Grant 1992, 1994). In this context our results, especially because the primary axes of variation are matched between hybrids and the Malawi radiation, provide direct support for the hybrid swarm theory of speciation.

The reduction of integration in hybrids, especially those displaying transgressive phenotypes, suggests a means by which evolutionary constraints can be averted. Prior studies have tested whether integration between traits can be broken through a process of artificial selection favouring a low degree of trait interaction with little success (Conner 2002). Perhaps the more radical reshuffling of genetic variation that occurs during hybridization events provides a more viable way to change the patterns and strength of integration. Although integration was reduced in both lateral and ventral views, there was a much more subtle loss of integration in the ventral view, the trait in which far fewer individuals exhibited transgressive phenotypes. This suggests that like changes in the shape of ventral traits, the promotion of integration of ventral morphology may also be the result of directional selection and involve the fixation of antagonistic alleles. It is likewise notable that integration was dramatically reduced in the lateral view, where the highest degree of transgressive segregation in shape was observed. These results suggest that different selective regimes are acting on the lateral and ventral views of the skull with respect to both shape and integration. This pattern makes sense with respect to the ecology of these two species. Both LF and TRC are specialized "biting" species adapted to feed on attached strands of filamentous

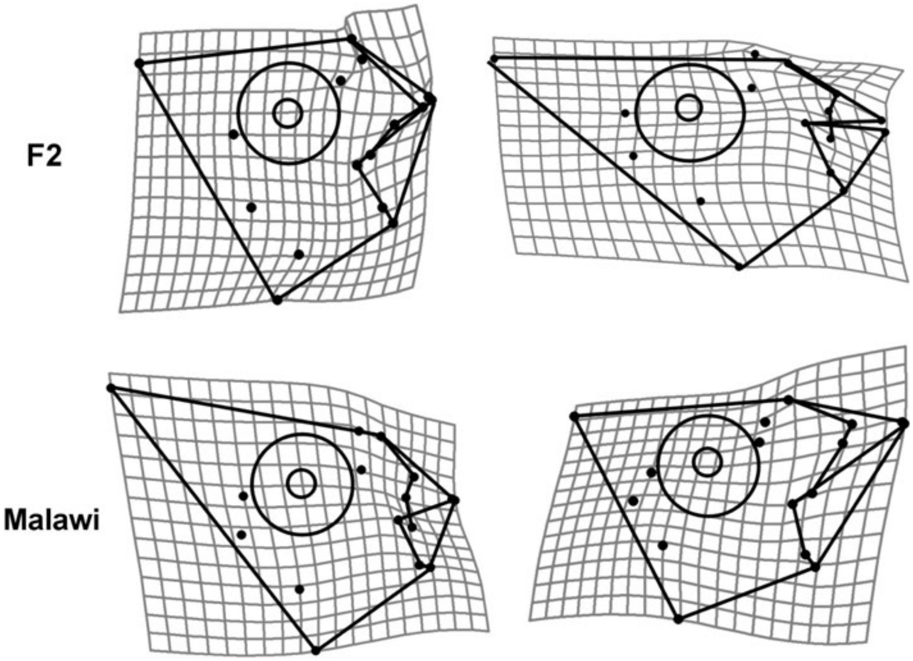

Fig. 2 Deformation grids depicting the shape changes occurring in the lateral view on PC1 for F2 hybrids, and for the Malawi radiation as a whole. The variation expressed for F2 hybrids has been magnified three times to accentuate phenotypic changes. Additional statistical tests reveal that the trajectories for the PC1 axes did not differ

algae (Ribbink et al. 1983), and this general mode of feeding is reflected in a short, steeply descending craniofacial profile that is characteristic of both species. The specific means by which LF and TRC collect algae, however, is markedly different. Whereas LF crops large mouthfuls of algae, TRC picks individual strands (Ribbink et al. 1983), and this divergence in feeding mode is reflected in very different ventral jaw morphologies. LF possesses a very wide jaw to accommodate its cropping mode of feeding, and TRC has a narrow, beak-shaped jaw, which is optimal for picking algae from the substrate. The common lateral jaw configuration of both LF and TRC likely reflects an early divergence between rock- and sand-dwelling species, an ecomorphological split that is primarily characterized by a difference in lateral head/jaw length (Cooper et al. 2010). Our data suggest that this trait may be under balancing selection and/or controlled by separate loci in LF and TRC. In either scenario, positive and negative alleles have accumulated in the parental populations, and have recombined in the F2 hybrids to produce extensive transgressive segregation in shape and integration. Ventral jaw morphology, on the other hand, exhibited a very different pattern consistent with directional selection acting on both shape and integration. LF and TRC feed on a common food source and exhibit largely overlapping distributions (Ribbink et al. 1983). Since differences in jaw width enable a degree of habitat partitioning (Ribbink et al. 1983; Albertson 2008), this difference in morphology is likely driven and maintained by directional selection, which will reduce genetic variation and is supported by the observation here that little to no transgressive segregation was observed in this view.

It should be noted that there are other possible scenarios, in addition to differing selective regimes, that could explain differences in the degree of transgressive segregation between the lateral and ventral views. For example, there may be a larger number of loci influencing shape in the lateral view, some of which may be under weaker selection and therefore possess greater levels of genetic variation. This effect may have been aided by the measurement of more total landmarks in the lateral view. Furthermore, as mentioned above, the short-jawed phenotypes in these species could be based on different alleles. Finally, the lateral view of the head could be more sensitive to environmental effects, such that variation in foraging behavior would increase shape variation present in the lateral but not ventral view. This last point is particularly relevant since the animals used in this experiment were not fed on their natural diets, but instead on flake food. Future experiments are currently underway using this cross to explore the effects of different diet treatments on the genotype-phenotype map as well as on patterns of phenotypic integration.

Although we have reported a link between the occurrence of transgressive segregation and levels of integration, it is important to note that our data cannot speak to whether a common genetic mechanism is acting on both shape and integration. On one hand, the relationship between these traits could be tied to a similar genetic basis (e.g., pleiotropy between shape and integration). Alternatively, this association could be driven by a common selective regime acting on different sets loci for shape and integration. Indeed, either scenario appears possible from a genetic perspective, as QTL mapping studies have identified both types of loci—those with an affect on both shape and integration, and others that specifically contribute to the promotion of statistical relationships among phenotypic traits without effecting mean values (Cheverud 2004; Pavlicev et al. 2008). Understanding the precise genetic basis of shape and integration in cichlids will provide a fruitful opportunity for future research.

While integration was substantially reduced for the lateral view in our hybrid F2s, their primary trajectory of divergence was statistically similar to that of the Malawi radiation. Since the direction of PC axes reflect the pattern of integration, these data suggests that hybridization may primarily affect the strength of integration not its patterns. This property of hybridization may have been important for allowing East African cichlids to evolve along a seemingly fixed trajectory (Young et al. 2009; Cooper et al. 2010). Hybridization could have been effective at loosening the degree of trait interactions allowing for the initial invasion of new niches where stronger relationships among particular traits, but not completely new patterns of integration, may have been favoured. In concordance with this, it is also possible that such decreases in the strength of integration could have had substantial functional consequences, perhaps enabling the evolution of new biting, or suctioning tactics. Using the current data set, we could test the ecomorphological significance of changes in integration by identifying the most and least integrated hybrids and testing for differences in functionally relevant traits.

The actual pattern of trait integration rather than its strength may have a deeper ancestral basis. This is suggested by comparisons across the Rift Lake cichlids where the same primary trajectory of divergence, an increasing and decreasing size of the preorbital region of the head, is shared among the Malawi, Victoria, and Tanganyika cichlid radiations (Cooper et al. 2010). While it is possible that the pattern of craniofacial integration, reflected in the trajectory of PC1, could have repeatedly evolved in situ for each cichlid radiation, work in other fish systems suggests that this phenotypic trajectory is prevalent at a much deeper phylogenetic scale. For example this pattern is present across other families of perciform fishes, in cases of divergence below the species level (Cooper and Westneat

2009), at the population level (Jastrebski and Robinson 2004), as well as in plastic responses to benthic and limnetic diets (Parsons and Robinson 2007). This suggests that some types of integration can be deeply rooted in phylogeny, creating a bias (though not necessarily a constraint) for adaptive evolution even in the face of hybridization.

Given that the species used in our experiment both had relatively short jaws it is remarkable that resultant hybrid phenotypes followed this long-jaw/short jaw axis of variation. The lengthening and shortening of the preorbital region on this axis matches a major functional and ecological split between biting and suction feeding across teleosts (Cooper et al. 2010). This preserved pattern of integration may be a case of the 'evolution of evolvability' (Pigliucci 2008) whereby patterns of phenotypic integration may have aligned with interactions favoured by selection. Modelling of G matrix evolution has shown that this scenario is at least plausible, resulting in an alignment of Gmax and selection (Pavlicev et al. 2009). Therefore, while hybridization may increase the variation available to selection, and even push phenotypes beyond parental means through transgressive segregation, in this study it has not served to create new phenotypic trajectories.

There has been a consistent interest in research aimed at understanding the role of hybridization in the explosive evolution of East African cichlids (McElroy and Kornfield 1993; Sturmbauer 1998; Ruber et al. 2001; Salzburger et al. 2002; Sturmbauer et al. 2001; Smith et al. 2003; Stelkens et al. 2009). Here we bring a new perspective on the question of what effects hybridization has played in determining major patterns of evolution in this system. The data presented above suggests that hybridization has the potential to generate novel phenotypes, but that this ability is dependent upon the selection regime the trait has been exposed to. In the case of the ventral view of the skull, it appears that hybridization would have little effect on the degree of shape variance. On the other hand hybridization increases variation in the lateral view, in a way that matches major evolutionary trends (Cooper et al. 2010). Additionally, hybridization consistently decreases the degree of phenotypic integration, which could open new opportunities for cichlid evolution within a favourable ecological context. Given the experimental tractability of cichlids, including the ability cross divergent species (Stelkens et al. 2009), and the accumulation of genetic and genomic resources (Loh et al. 2008), this system could stand as an important model to study the genetic basis of shape in concert with integration, and significantly advance our understanding of how hybridization has influenced the adaptive radiation and evolvability of vertebrates.

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