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Genetic dissection of adaptive form and function in rapidly speciating cichlid fishes

Frederico Henning,[1,2,*] Gonzalo Machado-Schiaffino,[1,*] Lukas Baumgarten,[1] and Axel Meyer[1,3]

[1]*Department of Biology, University of Konstanz, 78464 Konstanz, Germany*

[2]*Department of Genetics, CCS, Federal University of Rio de Janeiro, Ilha do Fundão, 21941-599 Rio de Janeiro, Brazil*

[3]*E-mail: axel.meyer@uni-konstanz.de*

Genes of major phenotypic effects and strong genetic correlations can facilitate adaptation, direct selective responses, and potentially lead to phenotypic convergence. However, the preponderance of this type of genetic architecture in repeatedly evolved adaptations remains unknown. Using hybrids between *Haplochromis chilotes* (thick-lipped) and *Pundamilia nyererei* (thin-lipped) we investigated the genetics underlying hypertrophied lips and elongated heads, traits that evolved repeatedly in cichlids. At least 25 loci of small-to-moderate and mainly additive effects were detected. Phenotypic variation in lip and head morphology was largely independent. Although several QTL overlapped for lip and head morphology traits, they were often of opposite effects. The distribution of effect signs suggests strong selection on lips. The fitness implications of several detected loci were demonstrated using a laboratory assay testing for the association between genotype and variation in foraging performance. The persistence of low fitness alleles in head morphology appears to be maintained through antagonistic pleiotropy/close linkage with positive-effect lip morphology alleles. Rather than being based on few major loci with strong positive genetic correlations, our results indicate that the evolution of the Lake Victoria thick-lipped ecomorph is the result of selection on numerous loci distributed throughout the genome.

KEY WORDS: Adaptation genetics, convergent evolution, foraging performance, Haplochromines, QTL mapping, RAD-seq.

The fit between organisms and their environments is one of the most striking outcomes of adaptive evolution. Fundamental aspects of the genetic basis of adaptation such as the number of loci and the extent of genetic independence between traits affect the direction of adaptive responses (Schluter 1996; Losos 2011) and the contribution of traits to speciation (Servedio et al. 2011; Flaxman et al. 2013; Feder et al. 2014). Their importance in shaping adaptive radiations remains widely debated (Orr 2005; Hendry 2013; Laland et al. 2014; Wray et al. 2014). Adaptation itself is difficult to demonstrate (Endler 1986) and most investigations focus on measurable proxies (e.g., morphology, coloration) rather than on primary targets of selection (Arnold 1983; Losos 2011).

The number of loci that typically underlie adaptations is a longstanding debate in adaptation genetics. Fisher's geometric model was widely successful in promoting the view that adaptations typically have highly polygenic genetic bases and led to a consensus, which was in line with Darwin's original emphasis on slow and gradual change (Orr 2005). As late as 1992 however, it was realized that there was actually scarce empirical support for this consensus (Orr and Coyne 1992). To stimulate the collection of relevant data Orr and Coyne articulated three criteria: (a) The study must be sufficiently powered; (b) the phenotypic differences must be of adaptive significance, and; (c) the trait must differ between natural populations or species.

There has been a surge of publications using both laboratory crosses and population-based association/divergence mapping in a variety of systems, which have shown mixed support for the notions of major genes versus minor genes as the typical genetic basis of adaptation (Hall et al. 2006; Chan et al. 2010; van't Hof et al. 2011; Ellegren et al. 2012; Greenwood et al. 2012; Nadeau et al. 2012; Greenwood et al. 2013; Kowalko et al. 2013; Linnen et al. 2013; Weber et al. 2013; Arnegard et al. 2014; Miller et al. 2014; Poelstra et al. 2014), including cichlids (Albertson et al. 2003b;

*These authors contributed equally to this work.

Streelman et al. 2003; Roberts et al. 2009; O'Quin et al. 2012, 2013). As pointed out recently by Rockman (2012), the increased attention given to genes of large effect is likely to reflect ascertainment bias in favor of more tractable traits, as well as technical aspects of genetic mapping that favor the discovery of major genes. Nevertheless, the incorporation of the effects of the time of recruitment, distance from adaptive optima, drift, and more recently gene flow (Orr 1998a, 2005; Dittmar et al. 2016 and references therein) to Fisher's geometric model leads to predictions that there are circumstances in which major genes are expected to form the bulk of the genetic bases of adaptations. Some of these predictions have recently received empirical support from studies of locally adapted *Mimulus* species (Ferris et al. 2016) and in experimental manipulations done in sticklebacks (Rogers et al. 2012).

While evolutionary convergence is frequently seen as an illustration of predictable solutions to similar pressures that are found by natural selection, it is also recognized that genetic correlations can direct or constrain evolutionary responses (Schluter 1996; Losos 2011). The extraordinary convergence of trophic morphologies, such as those found in cichlid fishes has led to the question of whether there are biases in the generation of phenotypic variation that direct adaptive evolution toward certain trajectories (Brakefield 2006), an issue that is a matter of current debate (Laland et al. 2014; Wray et al. 2014). Some potential sources of bias in the origin of variation include small mutational and genetic target sizes of the convergent phenotype (i.e., the number of loci that underlie traits) (Gompel and Prud'homme 2009), as well as genetic covariation in the case of multitrait phenotypes. Genetic correlations are the result of tight linkage or pleiotropy between loci underlying different traits (Lande 1984). Positive genetic correlations result from concordant effect signs, that is when a substitution of one allele at such a locus leads to an increase in the adaptive value of both traits and have the potential of facilitating the evolution of multitrait phenotypes. Negative correlations result from discordant effect signs in tightly linked, or pleiotropic loci (i.e., antagonistic pleiotropy) and might constrain adaptation. A mixture of concordant and discordant signs can neutralize the overall impact of shared loci (Gardner and Latta 2007). Convergent evolution could be seen as the product of biases in the origin of variation if for instance, the genetic architecture of repeatedly evolved adaptations is dominated by a few loci that have large and concordant effects on multiple subtraits.

A specialized morphology consisting of hypertrophied lips, narrow, and pointed heads (Fig. 1) is a striking example of convergent evolution that is replicated across several cichlid radiations (Kocher et al. 1993; Oliver and Arnegard 2010; Colombo et al. 2013; Manousaki et al. 2013; Burress 2014; Henning and Meyer 2014; Machado-Schiaffino et al. 2017). Thick-lipped ecomorphs are typically suction-feeders that forage for prey within rocky crevices (Video S1) (Keenleyside 1991). The repeated evolution of the thick-lipped ecomorph is thought to reflect parallel adaptation to several sources of selection associated with foraging in narrow rocky crevices, including accessing prey (Baumgarten et al. 2015; Machado-Schiaffino et al. 2017), generating sufficient suction power and detecting prey through sensorial specializations (Oliver and Arnegard 2010). Transcriptomic evidence has shown that hypertrophied lips in African and Neotropical cichlids share molecular-developmental mechanisms (Colombo et al. 2013). However, it is unknown whether these parallel patterns of gene expression are restricted to downstream effects or also include upstream genes because the genetic basis of this suite of traits has not been investigated.

Haplochromis (*Paralabidochromis) chilotes* and *Pundamilia nyererei* are rock-restricted species that are widely distributed in Lake Victoria (Witte and Van Oijen 1990; Seehausen and Bouton 1998). *Haplochromis chilotes* is a specialized insectivore that is characterized by thick, lobed lips. *P. nyererei* is more abundant and its diet consists of mainly zooplankton and a smaller proportion of insects that are obtained by picking, snapping and to a lesser extent pull-scraping (Seehausen and Bouton 1998). Previous work showed that differences in the performance of both species can be assessed experimentally by measuring the success of obtaining prey from angled substrates (Baumgarten et al. 2015). Natural populations of both species vary considerably regarding certain traits (e.g., coloration) but the between-species differences in lip and head morphology (hereafter "LM" and "HM") are consistent regardless of the populations sampled (Seehausen 1996). The maximum divergence time between Lake Victorian haplochromines is generally accepted to be 15–100 thousand years (Johnson et al. 1996; Keller et al. 2013; Brawand et al. 2014). Consistent with their recent divergence, *P. nyererei* and *H. chilotes* can be crossed in the laboratory and generate fertile F_1 hybrids (Stelkens et al. 2010).

Here, we genetically dissect the divergent trophic morphologies of *H. chilotes* and *P. nyererei* and ask whether the repeated evolution of thick-lipped cichlids is likely to have been facilitated by the presence of major genes and strong, positive genetic correlations (Laland et al. 2014; Seehausen et al. 2014; Wray et al. 2014). To validate the fitness effects of the variation in morphological traits and the detected QTL, we developed an assay that yields a continuous measurement of foraging performance and is suitable for genetic mapping. Specifically, we (a) describe the positions and effects of loci influencing between-species variation in morphology and foraging performance; (b) test if the variation in head and lip morphologies is genetically independent; and (c) test the adaptive significance of both morphological variation and the detected genetic loci.

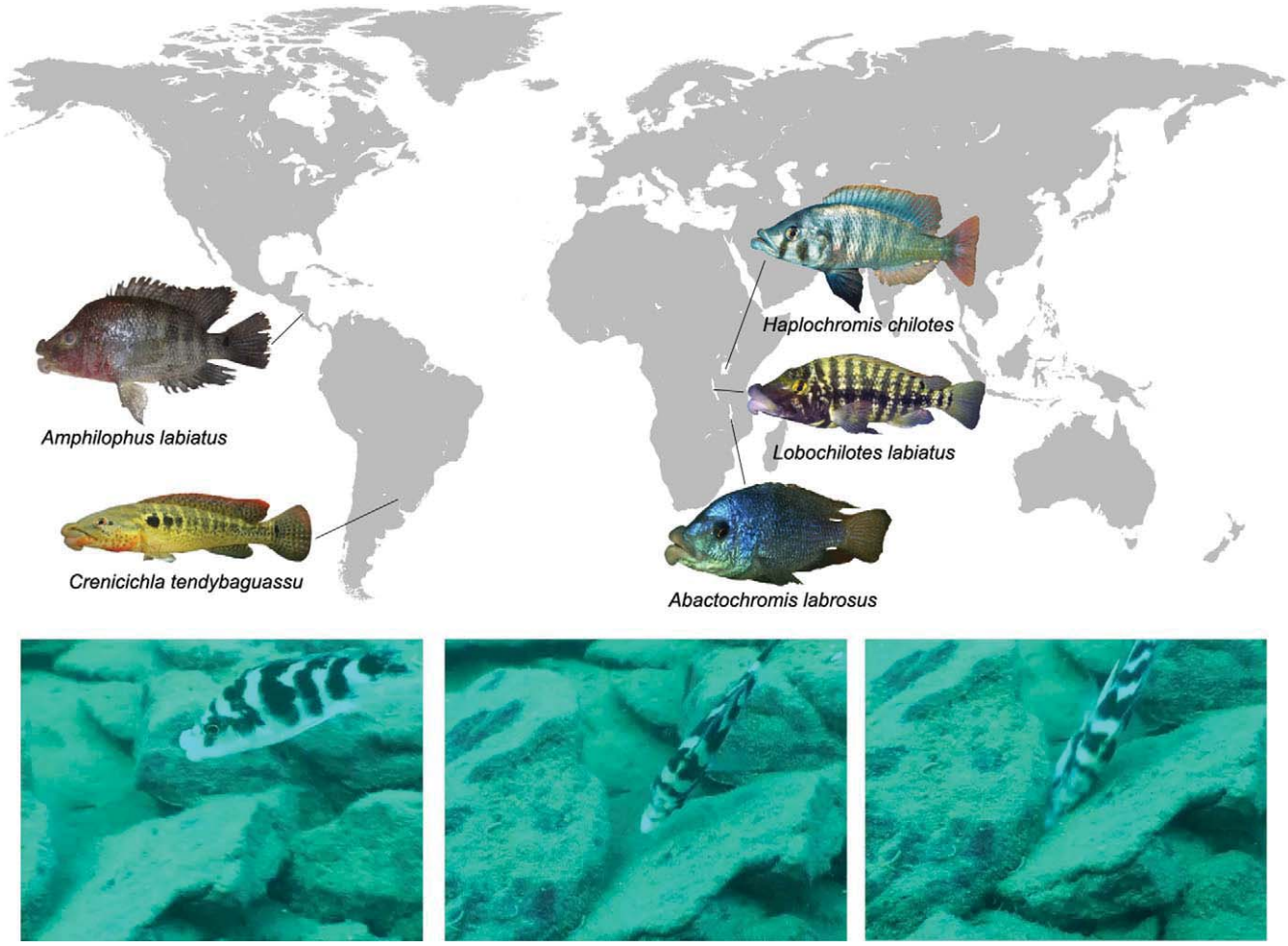

Figure 1. **Convergent evolution and function of hypertrophic lips. Representative species are shown from the cichlid radiations of the African great lakes, Central America, and South America. Photographs were kindly provided by Erwin Schraml, Ad Konings, and Oliver Lucanus. In the image sequence on the bottom, an individual *Placidochromis milomo* (representative of the lake Malawi radiation) is seen searching for prey (left), targeting a rocky crevice (center), and accessing the prey (right).**

Materials and Methods

EXPERIMENTAL CROSSES

The genetic mapping panel was obtained by hybridizing a *H. chilotes* male and a *P. nyererei* female. Laboratory stocks were established using specimens obtained from commercial breeders and have been maintained by full-sibling mating in the Animal Research Facility (University of Konstanz) for over 10 years. Species selection was based on the specialized morphology of *H. chilotes* and previous reports that it can be hybridized with *P. nyererei* (Stelkens et al. 2010). Large between-species differences regarding morphology and foraging performance are preserved in captivity and there are no indications that trait values were affected by the breeding scheme that was used during stock maintenance (Baumgarten et al. 2015). Interspecific F_1 hybrids were obtained by housing a male *H. chilotes* with three female *P. nyererei* in a 360l tank. After a few weeks, one mouthbrooding female was spotted, transferred to a 360l tank and kept there until the larvae were free-swimming ($\approx$15 days). Four F_1 hybrids (one male and three females) reached reproductive maturity and were fully viable and fertile. The F_2 generation was obtained by intercrossing the three F_1 females with the F_1 male multiple times. A total of 22 broods were isolated with an average brood size of 18 F_2 fish that reached maturity. Larvae were raised in 1/6, 1/3, or 1/2 compartments of a 360l tank to minimize the effects of density and decrease the variance of body size. *H. chilotes is* commonly referred to as *Paralabidochromis chilotes* in the recent literature. Here, we opted to follow the "generic classification of the haplochromines" (van Oijen 1996) to avoid confusion until a taxonomic revision is carried out.

MOLECULAR METHODS

Genomic DNA was extracted from pectoral fin samples from 291 F_2s, four F_1s and both parental individuals (*H. chilotes* male

and *P. nyererei* female) using the Zymo genomic DNA extraction kit. Genomic DNA was treated with RNAse A. The parentals were included in three different libraries to increase coverage and guarantee sufficient confidence in assigning homozygous genotypes. Double digest RADseq libraries were prepared following Peterson et al. (2012). Briefly, 1 μg of Genomic DNA per sample was double-digested using the restriction enzymes PstI and MspI (New England BioLabs) for 3 hours at 37°C. P1 and P2 adapters were ligated to the digested DNA using T4 ligase for 30 minutes at room temperature. A total of 300 individually barcoded samples were pooled in six libraries. Size selection for each library was performed using the Pippin Prep (Sage Science, Beverly, MA) with a selected size-range of 325–400 bp. Genomic libraries were single-end sequenced (100 bp length) in six lanes on an Illumina HiSeq 2000.

ddRAD MARKER SELECTION

Raw sequence reads were trimmed to a length of 100 bp–-the last base was trimmed based on the drop in FastQC scores—and demultiplexed using Stacks (Catchen et al. 2011). Only high sequencing quality reads, with correct barcodes and unambiguous RAD site were retained. Demultiplexed reads were aligned to the *P. nyererei* reference genome using GSNAP (Wu and Watanabe 2005). We required unique alignments allowing for a maximum of two mismatches and no terminal alignments. The ref_map.pl parameters in Stacks were set as default except for the following parameters: minimum depth coverage to report a stack (–m 5) and upper bound for epsilon (–bound_high 0.05, to reduce the probability of false-homozygotes). The genotypes for each marker were exported using the F_2 design in the genotypes program in Stacks, requiring that both parentals were homozygous for different alleles and that at least 150 F_2 individuals were genotyped per marker (-*r* 150) with a minimum coverage of 20 reads per individual (–m 20) and allowing for automatic corrections (–c). The variable sites were uniformly distributed across the entire read lengths.

LINKAGE MAP ESTIMATION

A total of 1687 markers passed the quality filters and were used for linkage map construction using the maximum likelihood algorithm implemented in JoinMap v.4 software (Van Ooijen 2006) following guidelines for quality control (Van Ooijen 2006; Broman and Sen 2009) and the same procedures and thresholds that were thoroughly described elsewhere (Henning et al. 2014). Briefly, individuals were excluded from linkage map construction if they had $> 30\%$ missing genotypes ($n = 38$) and loci were excluded if they were under severe segregation distortion (P-value < 0.01, $n = 143$) or had $>20\%$ missing genotypes ($n = 434$). The grouping of markers was determined based on an independence LOD threshold of five and orders were optimized by (a) comparing maps obtained using the maximum likelihood and regression algorithms implemented in JoinMap (Henning et al. 2014); (b) visually inspecting graphical genotypes; and (c) analyzing improbable genotypes as given by JoinMap. The cross-link of all markers were inspected using the plot.rf function in R/qtl and the recombination frequency per individual and per library was inspected using the countXO function to detect error-prone individuals or sequencing library batch effects (Broman and Sen 2009). Finally, the congruence between the genetic map and the *P. nyererei* draft genome (P_nyererei_broad_scaffolds_v1) was analyzed.

The number of markers in RAD datasets normally exceeds the number of observed crossovers. Furthermore, unique placements in the absence of observed crossovers can sometimes be the result of missing data alone (Henning et al. 2014). All of this results in marker redundancy (markers that map to the exact same genetic location and cannot be distinguished based on observed crossovers) and incorrect orders and distances. Redundancy was eliminated by combining all the markers that mapped to identical positions and/or could not be placed with confidence owing to missing data. These concatenated markers were named with the prefix "c" followed by two digits indicating the LG and two digits identifying the order within each LG. This approach allowed us to (i) increase computational efficiency and eliminate the need of random marker elimination or "jiggling" in the QTL mapping software; (ii) reduce the effects of stochastic placement dependent on missing data (Henning et al. 2014); and (iii) reduce the amount of overall missing data, since the combined markers consisted in the sum of the total genotypic observations from the linked markers.

MORPHOLOGICAL TRAIT MEASUREMENTS

Standard photographs were taken from the lateral and dorsal view of 15 fish from each of the parental populations, the F_1 hybrids and 291 F_2s at 12–15 months of age. Fish were anaesthetized with MS-222 (Sigma) and standardized photographs were taken from the dorsal and lateral views. Measurements from standardized photographs were performed using ImageJ software. A combination of linear and geometric morphometric measurements were employed to assess morphological variation associated with hypertrophied lips and head shape (Fig. S1).

Morphological traits values were obtained for between 284 ("Lip PC") and 291 ("LA") F_2s. The following linear measurements were considered: lip area ("LA"); upper lip area ("ULA"); lower lip area ("LLA"); lip length ("LL'); head length ("HL"); and head angle ("HA") (Fig. S1). Geometric morphometrics was carried out to measure head shape ("HS") and lip shape ("LS"). Eight landmarks and eight semi-landmarks were placed on the dorsal view of the fish. The landmarks were: (A1), (A2), (A8), and (A7) posterior and anterior extreme of the right and left orbit, (A3) and (A6) right and left starting point of the upper lip, (A4) tip of the upper lip, (A5) tip of the snout at the base

of the upper lip. Semi-landmarks were placed on the outlines of the snout and the upper lip (Fig. S1). HS and LS were measured by placing equally spaced semi-landmarks on each side of the dorsal view (A9–A16 for LS, A17–A26 for HS), in relation to landmarks A2, A4, A5, and A7. Each landmark was digitized in tpsDig version 2.16 (Rohlf 2010a). Relative warps analysis was performed to remove all nonshape variation in tpsRelw version 1.49 (Rohlf 2010b). The positions of the semi-landmarks were moved along an estimated curve between the neighboring points to minimize squared distances between the adjusted positions and the corresponding points in the consensus. Statistical analysis of each shape was performed with classifier variables (species, sex) and with SL as a covariate in MorphoJ version 1.05f. Allometric effects were detracted from the shape using a pooled regression within each species with the Procrustes coordinates as dependent variables and SL as independent variable.

The relationships of all traits with standard length were tested using linear models in R. Those traits where the relationship with size was significant were size-corrected by obtaining the residuals from a regression of each measurement on standard length. Of all the traits we investigated, only HS varied between sexes, which might reflect female adaptations for mouthbrooding. However, correction was deemed unnecessary because sex-corrected phenotypic values resulted in identical QTL mapping results, presumably because the relationship with sex does not interact statistically with species assignment. This makes biological sense since both species are mouthbrooders. Area measurements are the residuals of the regression on body area. Normality was tested using the Shapiro–Wilks test (shapiro.test function in R) and all traits were visually inspected using qqnorm and hist functions in R and the suggested quality control procedures of R/qtl (Broman and Sen 2009). All traits were normally distributed and were scaled to units of F_2 phenotypic standard deviations. The signs of the phenotypic values of HA was reversed (multiplied by –1) for the QTL mapping analysis so that higher trait values were always present in *H. chilotes* to reflect the predicted adaptive direction (see the "Signatures of Natural Selection" section below).

The traits were grouped in the following two categories (Fig. S1): lip morphology ("LM") and head morphology ("HM"). LM comprises measurements of lip area ("LA," "ULA," "LLA") and lip length ("LL"). HM includes measurements of head length ("HL"), head angle ("HA"), and head shape ("HS"). "LIP PC" consists of the first principal component from a PCA of a series of lip traits (Fig. S1). LIP PC was not included in any trait category because the measurement includes lip shape (LS) and is not independent from head shape thus rendering the analysis of genetic correlations uninterpretable. Measurement-correlated traits are independently affected by measurement error and/or capture different aspects of the phenotypic variation in complex traits. Because slightly different aspects of the traits are measured, the analysis of measurement-correlated traits allows the detection of a greater number of QTL that underlie biologically relevant traits that are too complex to be represented by a single measurement. Discussing the extent of the shared genetic basis in these traits is biologically meaningless, but including them in the analysis is methodologically relevant because it creates an internal control of phenotyping, QTL mapping and QTL colocalization analysis (e.g., these traits should be correlated, share a significant amount of QTL and have similar QTL effect distributions).

FORAGING PERFORMANCE

We previously developed a laboratory assay to measure performance using a series of discrete angles (Baumgarten et al. 2015). In the present study, the acrylic glass structures were designed to yield a continuous measurement that is suitable for QTL mapping: the minimum angle that each fish (both parental and F_2s) can forage on. The acrylic structures consist of an angle of 60° at the base that gradually reduces with height (15 cm), finishing with an angle of 15° (Fig. 2C and Fig. S2). Small equally sized pieces of mosquito larvae were placed at regular intervals of 3 mm along the inner vertical axis and were fixed by drying at 50°C for 5 minutes. The experimental tanks (60 × 120 × 50 cm) were divided into four compartments using Plexiglas dividers. The experimental fish were starved for two days prior to the experiment and were transferred into the experimental compartments 24 hours before the beginning of each trial for acclimation. Nonexperimental fish were maintained in the background compartment to maintain social interactions and improve the acclimation of the experimental fish.

Foraging performance was measured in 10 individuals of each of the parental species (*H. chilotes* and *P. nyererei*) and 162 F_2 individuals. Phenotypic values are the residuals of the linear regression of the maximum foraging height on standard length. Due to the feasibility of collecting foraging data, the sample size is reduced compared to the morphological traits, which invariably leads to a less precise estimation of QTL effects. Nevertheless, care was taken to avoid bias and to ensure that the full spectrum of variation in HM and LM was represented in the foraging data. A video showing examples of the foraging trials is available in the supplementary files (Video S2).

PHENOTYPIC CORRELATIONS IN F2 HYBRIDS

This analysis aimed at (a) testing the contributions of the different morphological components to foraging performance, and; (b) investigating the degree to which LM and HM segregate independently. The overall level of phenotypic correlation in the F_2 recombinant population was measured using Spearman's rho. The significance of the following correlations were tested: (a) between morphological (LM, LIP PC, and HM) and foraging traits and; (b) between LM and HM. F_2 correlations emerge due to genetic correlations (when traits share QTL with concordant

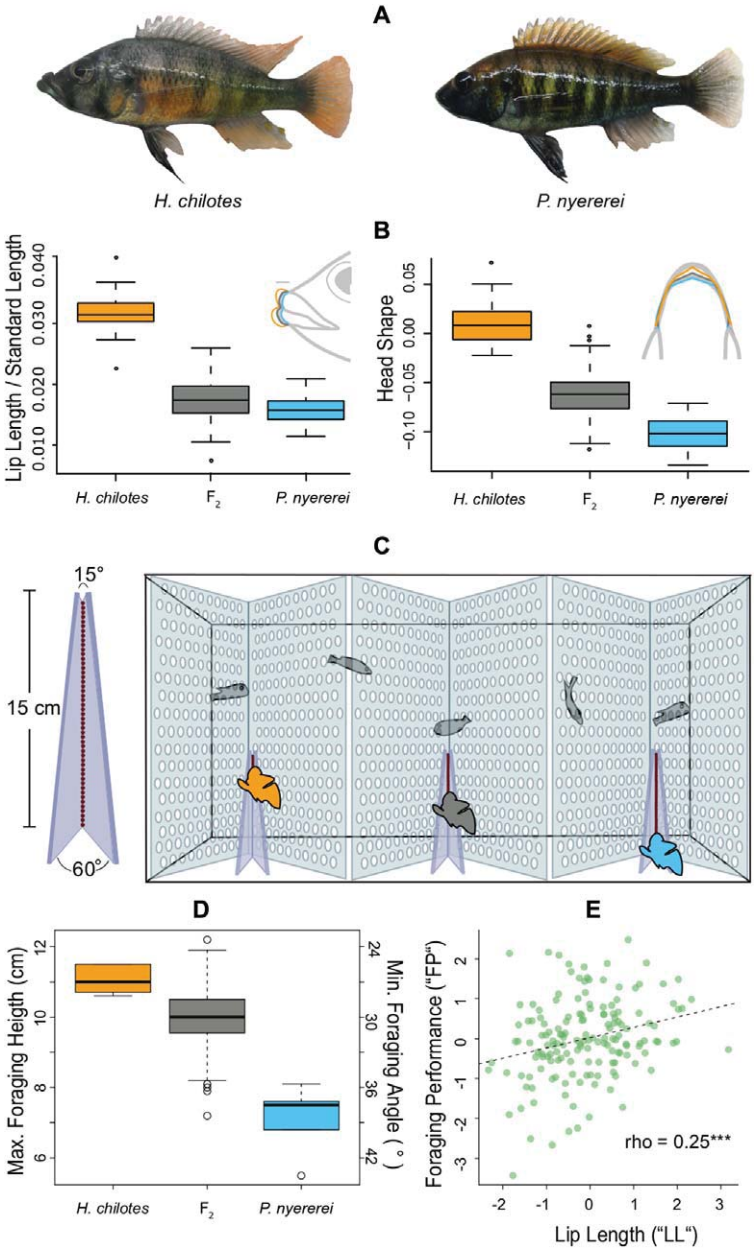

Figure 2. **Foraging performance and morphological traits are correlated and segregate in F_2 hybrids. (A) Male specimens of both species used in the experiment. Photographs are of individuals from the parental populations anesthetized with MS-222 (Sigma), which leads to the darkening of melanic pigmentation patterns. (B) Distribution of phenotypic values in representative traits in the parental and F_2 populations. (C) The acrylic device (left) and the experimental setting (right) developed to measure foraging performance. D) Differences between the parental and F_2 populations in foraging performance. (E) The correlation between foraging performance and lip length. Spearman's correlation coefficient is shown.**

signs through linkage or pleiotropy) but also environmental correlations (e.g., when one trait is the functional consequence of another trait or covaries due to similar plastic responses).

QUANTITATIVE TRAIT LOCI (QTL) MAPPING

QTL mapping was performed for all traits using interval mapping (IM) and composite interval mapping (CIM) followed by a final evaluation using multiple interval mapping (MIM). In comparison to IM and CIM (or multiple QTL mapping – MQM), MIM has higher detection power, leads to more precise parameter estimates and allows for the simultaneous evaluation of interactions between detected QTL (Kao et al. 1999). However, QTL models based exclusively on MIM searches can be subject to overparameterization as sample sizes decrease. To overcome these

limitations, we took the following steps. The initial MIM model included all the QTL identified by IM and composite interval mapping, CIM using chromosome-wide LOD thresholds derived from 500 permutations and *P*-value cutoff of 0.05 as inclusion criteria. IM was performed in R/qtl (Broman and Sen 2009) and WinQTL Cartographer v2.5 (available at http://statgen.ncsu.edu/qtlcart/). CIM and MIM analysis were conducted in WinQTL Cartographer v2.5. The positions of all QTL main effects included in the initial MIM models were optimized and the significance of each QTL main effect was tested. Nonsignificant QTL based on the BIC criteria and those with LOD scores below 2.5 were excluded and model optimization proceeded as previously described (Silva et al. 2012). We aimed at the discovery of the maximum number of QTL and several observations suggest that the procedure we employed appropriately controlled for false-discovery: (a) many of the suggestive QTL identified in the IM and CIM searches were eliminated from the initial model using the BIC model selection criteria; (b) the amount of genetic variance explained does not suggest overparametrization, and; (c) the estimated QTL positions and effects are consistent across phenotypically correlated traits.

QTL were considered to colocalize when their 1-LOD intervals overlapped. Individual QTL effects, the total amount of phenotypic variance and the estimates of genetic variance (i.e., broad sense heritability) were obtained from the variance decomposition tables produced for the final MIM models in WinQTL Cartographer. Epistatic and dominant effects were grouped and analyzed as nonadditive because the current implementation of MIM in WinQTL Cartographer only models epistatic interactions among QTL with significant main effects. Interactions that are unaccounted for in the QTL model will resemble dominant effects.

SIGNATURES OF NATURAL SELECTION

The distribution of effect signs was tested using the QTL sign test (QTLST) (Orr 1998b) using a custom R function written by Muir et al. (2014). The sign test was only applied to Lip PC and trait categories LM, HM because it only has power to reject the null hypothesis when the number of detected QTL > 6. No individual trait other than ULA had as many detected QTL with significant additive effects. Additive effects (in units of F_2 standard deviation) were pooled in each trait category (Albertson et al. 2003a) and in the event of shared QTL, the effect with highest LOD support was selected for testing. Inclusion of the smallest colocalized effects led to congruent results. All tests were conducted in R version 3.1.1 (Team 2014). In addition, the genome-wide additive effect estimates derived from interval mapping were used to compare the mean effects of adaptive and nonadaptive traits. The mean effects of LM, HM, and FP were calculated from 10 cM windows and were compared to the estimates for traits that also differ between the parentals but are likely nonadaptive (body depth, anal fin base, and caudal peduncle length).

The designation of "positive" or "negative" allelic effects is based on the direction of adaptation for foraging in crevices (i.e., adaptive and maladaptive, respectively). "Positive" effects are those that facilitate foraging in crevices. For most traits, the positive allelic effect increases the trait value, because hypertrophied lips and elongated heads are present in *H. chilotes*. The effects are reversed in the case of head angle (HA) because narrower and pointier heads facilitate foraging in crevices. To allow for the graphical comparison of the concordance of the effects of colocalizing QTL, the trait values of HA were reversed (multiplied by –1). Therefore, all alleles derived from *H. chilotes* are expected to increase trait values. Because we measured foraging performance in our mapping panel, the assignment of adaptive/maladaptive alleles was done directly by using the effect on foraging at the detected morphology QTL as a reference.

Results

LINKAGE MAP CONSTRUCTION AND GENOME ANCHORING

A saturated linkage map consisting of 1122 ddRAD markers distributed across 22 linkage groups with a total size of 1225.68 cM was obtained, in agreement with the expectation based on the known haploid chromosome number in Haplochromine cichlids (Thompson 1981; Poletto et al. 2010) (Table S1). Eliminating redundancy led to the final linkage map used for QTL mapping that had 752 uniquely placed markers. The median interval size is 0.97 cM, with 10 intervals larger than 10 cM and a single interval (17 cM) that is larger than 15 cM (Table S2 and Figs. S3–S4). All but nine marker placements were congruent with the current *P. nyererei* draft genome sequence (Table S2). Two of these showed evidence of allelic dropout and were excluded from further analysis. Other incongruent markers showed no indications of genotyping errors and could be indicative of structural variations, genome fragmentation, or misassembly. Comments highlighting the incongruences were added to Table S2.

The map of correspondence between our linkage map and the *P. nyererei* (Brawand et al. 2014) draft genome sequence shows a high level of congruency, which allowed for a high quality anchoring of the current scaffolds to our linkage map (Table S2). The comparison of genetic and physical distances did not point to the presence of large inversions segregating in our cross (i.e., no pairs of nonrecombining markers that are separated by large physical distances were found). The physical distance between redundant markers and adjacent uniquely placed markers ranged from 4 bp to 1.86 Mb (median = 83 Kb) and 7 Kb–6.36 Mb (median = 424 Kb), respectively. Therefore, marker redundancy is likely to have been caused by the close physical proximity of markers or small inversions in the parentals.

Table 1. Phenotypic correlation matrix of F_2 hybrids of all measured traits.

	LA	ULA	LLA	LL	LIPC	HA	HL	HS
ULA	0.579***							
LLA	0.545***	0.224***						
LL	0.497***	0.437***	0.298***					
LIPC	0.809***	0.716***	0.682***	0.729***				
HA	**–0.001**	**0.073**	**–0.059**	**-0.04**	–0.014			
HL	**0.149***	**0.055**	**0.066**	**0.108**	0.078	–0.302***		
HS	**0.129***	**–0.016**	**0.076**	**–0.042**	0.023	–0.241***	0.238***	
FP	**0.241****	**0.186***	**0.107**	**0.252*****	**0.266*****	**–0.087**	**0.13**	**0.075**

Lip area ("LA"), upper lip area ("ULA"), lower lip area ("LLA"), lip length ("LL"), lip principal component ("LIP PC"), head length ("HL"), head angle ("HA"), head shape ("HS"), and foraging performance ("FP"). The values of Spearman's rank correlation coefficient are given below the diagonal. Correlations between different trait groups are highlighted in bold.

***,**,and *** represent *P* values of < 0.05, <0.01, and <0.001, respectively.**

PHENOTYPIC VARIATION AND CORRELATIONS IN F2 HYBRIDS

All traits, including foraging performance differed between the parental populations and segregated in the F_2 mapping panel (Fig. 2, Fig. S5). Trait values that facilitate foraging were present in *H. chilotes* (longer lips and narrower heads). Several LM measurements (LL, ULA, and LA) and Lip PC were correlated with foraging performance with coefficients ranging between 0.19 and 0.27. HM traits showed no association with foraging performance, with the possible exception of HL, where a marginally nonsignificant relationship was found ($P = 0.08$, rho $= 0.13$). Variation in HM traits was also generally independent from variation in LM or LIPC traits (rho $=$ –0.08 to 0.15), with the exception of two comparisons involving LA that were significant. All correlation coefficients are shown in Table 1.

QTL MAPPING AND THE GENETIC ARCHITECTURE OF ADAPTATION

The underlying genetic architecture was found to include many loci of small effect and a few of moderate effect (Table S3) that are distributed across all but three LGs (Fig. 3A). The number of detected QTL ranged from four to 11. Some LGs have a high clumping of QTL underlying all traits, indicating that these LGs have a moderate effect on most traits investigated. Specifically, LG11, LG13, LG20, and LG23 are associated with several traits across their entire length (Fig. 3A and Fig. S5). The majority of the detected foraging performance QTL colocalized with QTL underlying morphological traits, particularly LM (Fig. 3B) and in some cases, there was colocalization of QTL that influence all trait groups (Fig. 3C). The largest effect QTL account for 12.6%, 7.1%, and 9.3% of the F_2 phenotypic variance in LM, HM, and foraging performance, respectively. The distribution of effect sizes we found suggests that our detection threshold is approximately 2% of F_2 phenotypic variance (Fig. 4C). In the final MIM QTL models, genetic effects on lip measurements are composed of mainly additive effects that explain on average 31% of the phenotypic variance (84% of the genetic variance). In contrast, additive effects accounted for only 22% phenotypic (64.95% genetic variance) in HM and 23% (67.24% of the genetic variance) in foraging performance traits (Table S4).

GENOME-WIDE SIGNATURES OF NATURAL SELECTION

Haplochromis chilotes alleles were biased in their effect signs: The proportion of the positive additive effects was 14/15 in LM, 10/13 in HM, 4/4 in foraging performance and 10/11 in LIP PC (Table S3). The QTL sign test rejected the null hypothesis in LM indicating an excess of positive effect *H. chilotes* alleles ($P <$ 0.05). In the case of the QTL with overlapping 1-LD intervals between HM and LM, all *H. chilotes* alleles are adaptive for LM but nearly half were negative for HM (Fig. 4). In contrast, all of the *H. chilotes* alleles at QTL unique to HM were adaptive. Furthermore, the inspection of genome-wide additive effect plots suggests that regions on different chromosomes or on the same chromosome but genetically distant from detected QTL also appear to have positive additive effect in the adaptive traits. As an example, the lip area trait (LA) mapped to at least five genomic regions (Fig. 5A). Not only are all of the additive effects positive in the detected QTL, but also in LGs where no QTL were detected, such as LGs 11–16 (Fig. 5B). This is also the case for the other analyzed traits (Fig. S7).

Discussion

We investigated the genetic basis of foraging performance, lip, and head morphology in a cross between two haplochromine cichlid species from one of the youngest and largest known cichlid radiations, *H. chilotes* and *P. nyererei* (Brawand et al. 2014). The first species combines a suite of morphological adaptations that

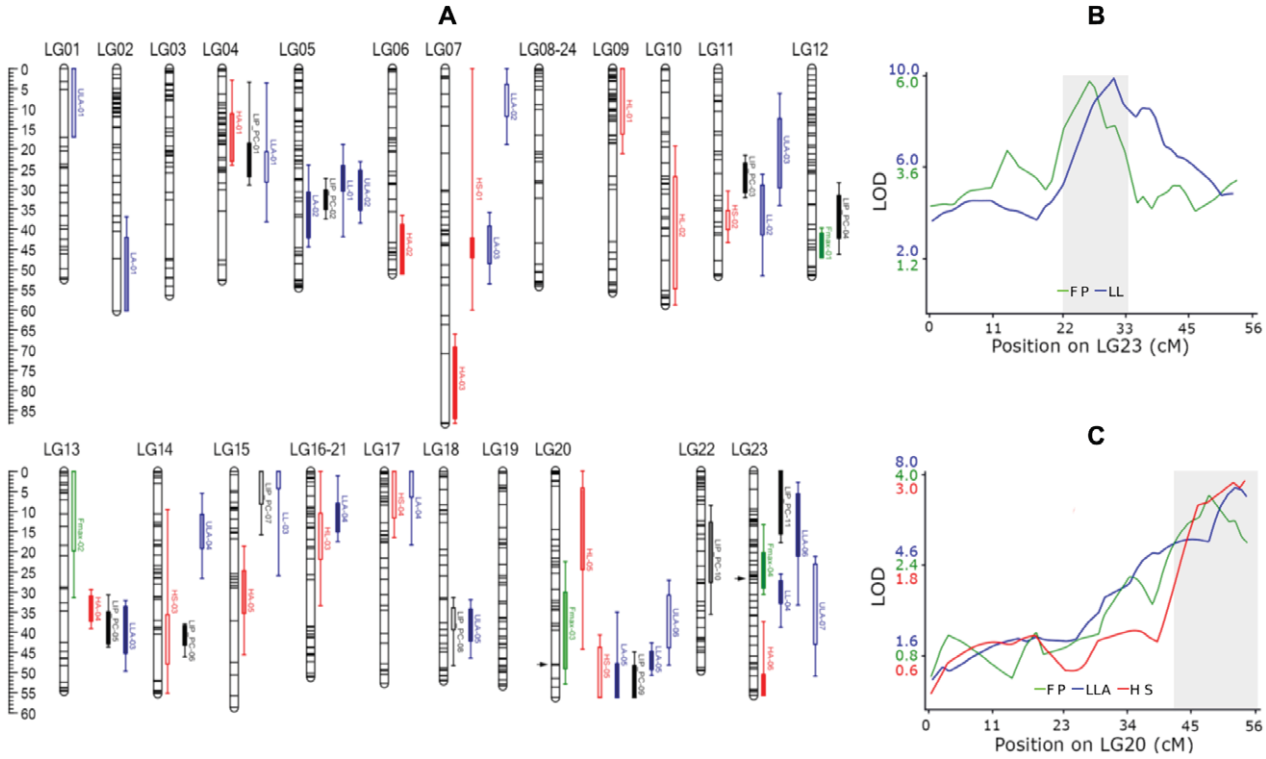

Figure 3. QTL map of foraging performance and associated morphological traits. (A) Distribution of all detected main effect QTL for all trait groups: foraging performance ("FP," green), lip morphology ("LM," blue), head morphology ("HM," red), and lip principal component ("LIP PC," black). The map distance in cM is given by the scales on the left. Thick and thin bars represent the 1- and 2-LOD intervals, respectively. (B–C) Overlapping LOD profiles of QTL for different trait groups (shown with arrowheads in A). To avoid redundancy, only the most highly supported QTL from each of the different trait groups ("LM," "HM," "LIP PC," and "FP") are shown. The overlap of the 1-LOD intervals in B and C is represented by the grey boxes.

are associated with foraging in rocky crevices for invertebrate larvae and that evolved in most cichlid adaptive radiations (Keenleyside 1991). Deciphering the contribution of traits to fitness can prove complicated even in model systems (Cook et al. 2012; Zeller et al. 2012). It is clear that natural selection has shaped morphology in many textbook examples of adaptation (e.g. Albertson et al. 2003a) however, the primary target of selection is foraging capacity, which certainly involves diverse classes of traits (e.g., metabolic, behavioral) in addition to morphology. This multifaceted aspect of adaptation might be expected to involve more complex interactions between loci (Huang et al. 2012) as well as a higher number of them (Arnegard et al. 2014). To paraphrase Arnold (1983), our results show that it is possible to measure adaptive significance directly also at the genetic level.

We found strong evidence for a role of hypertrophied lips in foraging success and that numerous loci were recruited since the divergence between these two species. These findings constitute strong support for the adaptive significance of hypertrophic lips and highlight the genome-wide effects of the response to natural selection of polygenic traits in recent adaptive radiations (Flaxman et al. 2013; Feder et al. 2014). The evolution of this multitrait phenotype does not appear to be dominated by positive genetic correlations or small genetic target sizes, which can bias phenotypic evolution. Rather, the genetics of these adaptive differences in trophic morphology is consistent with a model of mostly small effect loci, where only a few loci explain more than 5% of phenotypic variation and an increasing number of loci smaller effects. Phenotypic correlations between trait groups were generally low. Despite the detection of several colocalizing QTL, the effects were not always concordant revealing potential genetic trade-offs in the evolution of hypertrophied lips and pointed heads.

It is predicted that major genes with pleiotropic function might be particularly important in local adaptation in the presence of gene flow (Seehausen et al. 2014; Dittmar et al. 2016; Ferris et al. 2016). We found evidence for the existence of positive genetic associations (through either pleiotropy or tight linkage) and some evidence for clustering on LG23. However, none of these factors seem to explain a large amount of between-species differences. The genetic architecture of these traits is more aptly described as uncorrelated, consisting of small-to-moderate additive effects across numerous loci. However, this does not

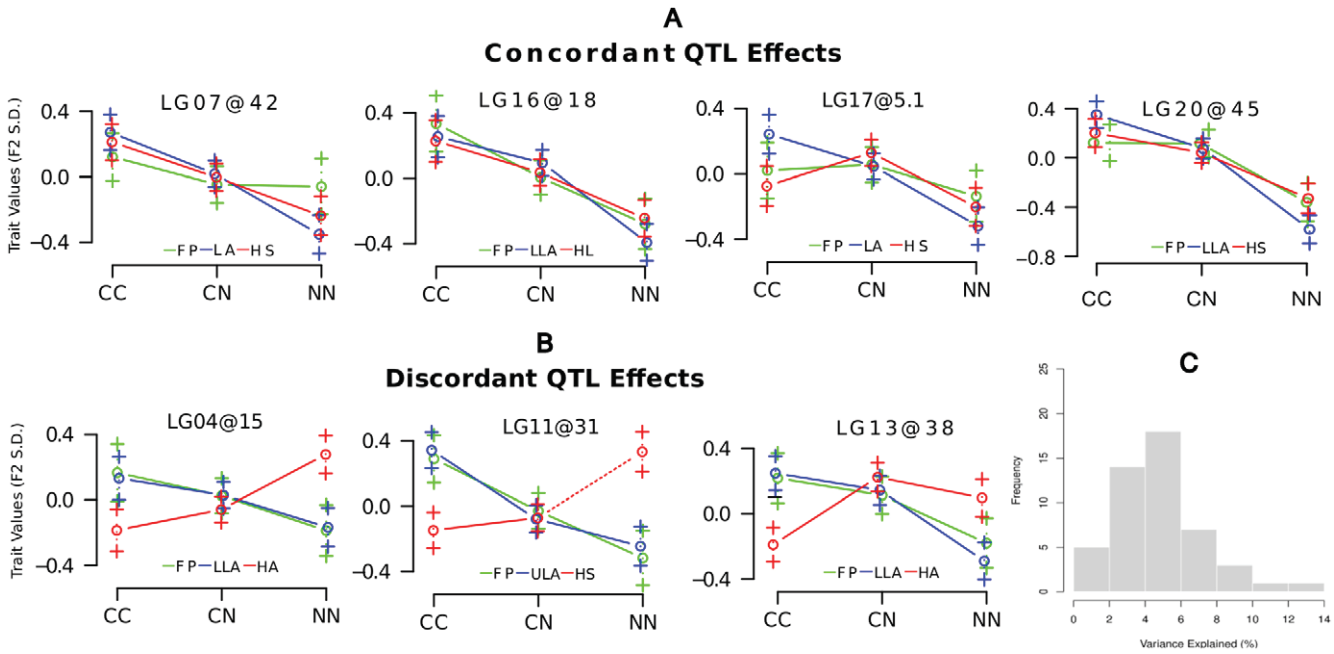

Figure 4. Direction and distribution of QTL effects. (A–B) Concordant and antagonistic allelic effects at colocalizing QTL for lip and head morphology (in units of F_2 standard deviation). Alleles inherited from *Haplochromis chilotes* and from *Pundamilia nyererei* are represented by "C" and "N," respectively. Slopes of opposite signs are indicative of antagonistic effects because all traits were polarized with regards to foraging in crevices. C alleles are expected to increase phenotypic values for all traits (see Methods). Intersecting effect slopes are more apparent in the comparison between homozygous genotypes (CC and NN) since nonadditive genetic variation can result in CN genotypes having phenotypic values above or below the expected under a purely additive model (e.g., HS at LG17 or HA at LG13). (C) Distribution of detected additive effects. Effect sizes are expressed in percentage of explained F_2 phenotypic variance.

rule out that though currently small, it is precisely the loci with positive correlation and larger effects that were important in the very early instances of divergence or under high levels of gene flow. This should be investigated with the comparison of the genome-wide pattern differentiation between *H. chilotes* and other sympatric haplochromines.

THE GENETIC ARCHITECTURE OF ADAPTATION IN THICK-LIPPED CICHLIDS

The genetic basis of lip measurements is composed of mainly additive effects across numerous loci that are scattered throughout the genome. A large number of QTL of small effect that individually explained up to 12% of F_2 phenotypic variation were detected on all but three LGs. Loci at LG11 and LG23 are associated with multiple traits in what appear to be multiple, closely linked QTL. The genetic basis of the morphological traits we analyzed is consistent with that of other adaptive trophic morphologies analyzed in cichlids (e.g., Albertson et al. 2003a; Parnell et al. 2012; Albertson et al. 2014) and also with what is thought to be the most common genetic architecture underlying quantitative phenotypes in general (Albert et al. 2008; Flint and Mackay 2009).

These conclusions are only strengthened by considering that our estimates of effect sizes and number of QTL are likely to be overestimates and underestimates, respectively. The actual genetic architecture underlying these traits is probably composed of many more undetected QTL with small effects (Flint and Mackay 2009). The development of next-generation sequencing technologies facilitated the use of forward-genetics on nonmodel organisms (Schneeberger 2014) and today a large number of studies meet Orr and Coyne's third criterion ("naturally occurring phenotypes"). However, considerable difficulties still exist for meeting Orr and Coyne's first criterion ("sufficient power") in QTL mapping using non-model organisms. The size of the F_2 panels is only a fraction of those used for genetic investigations in established models (Beavis 1994; Fishman et al. 2002; Laurie et al. 2004). The use of low sample sizes decreases the probability of detection of small effect QTL (i.e., increases the detection threshold), leads to biased estimates of effect sizes and insufficient power to disentangle the effects of closely linked QTL (Beavis 1994; Xu 2003; Slate 2013). The size of the F_2 mapping panel determines the detection power threshold and the extent of the inflation of effect sizes introduced by factors such as the Beavis effect (Slate 2013).

The existence of multiple crossable cichlid species pairs with different divergence times that differ in these same traits offers a unique opportunity to test whether alleles of large additive effect are recruited in the earlier stages of adaptation as predicted

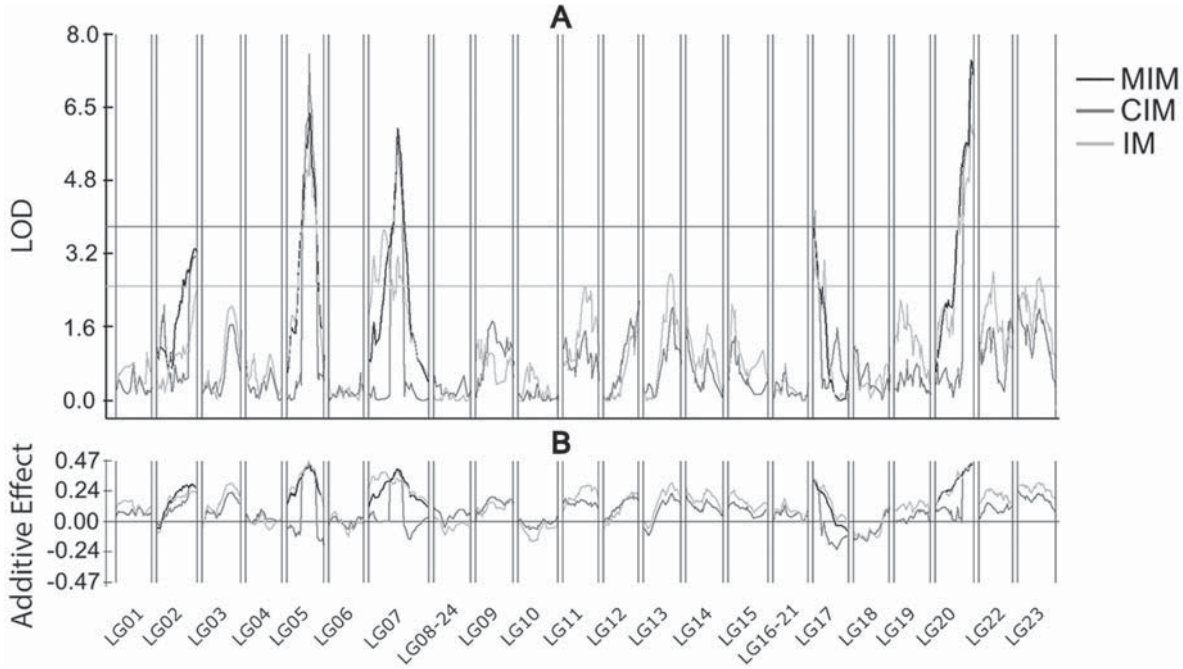

Figure 5. **QTL map of lip area ("LA"). (A) At least five genomic regions underlie phenotypic variation in LA. The LOD profiles for the three different detection methods (IM, CIM, and MIM) are shown and are largely congruent. The dark and light horizontal lines represent the genome-wide (3.7) and chromosome-wide (2.5) significance thresholds for IM and CIM. All MIM QTL that are shown are significant using the BIC criteria. (B) All detected QTL have a positive additive effect (in standard deviation units). The genome-wide profiles of additive effects of all traits is shown in Fig. S7.**

by Fisher's geometric model of adaptive evolution (Orr 2005; Rockman 2012). This is supported, for example by work on sticklebacks (Rogers et al. 2012) and could be tested by further genetic mapping projects in the multiple thick-lipped ecomorphs that occur in other recent radiations such as the ones in Lake Malawi or the Midas cichlid radiation. Multiple ecologically divergent populations—from Lakes Nicaragua and Managua (*Amphilophus citrinellus* and *A. labiatus*), as well as the recently colonized crater lakes—are variable for lip morphology (Machado-Schiaffino et al. 2017). We have recently shown that phenotypic plasticity is an important component of between-morph variation (e.g., Machado-Schiaffino et al. 2014) and that genetic differences also exist between Neotropical morphs (Machado-Schiaffino et al. 2017).

THE CAUSES OF THE REPEATED EVOLUTION OF THICK-LIPPED CICHLIDS

If the evolution of cichlid thick-lipped ecomorphs were facilitated by biases in the origin of selectable variation, one would expect a large contribution from few loci to multiple traits. If covariance dominated the genetic basis of hypertrophic lips, then (a) HM and LM would be expected to be largely positively correlated in F_2s; (b) a significant portion of the covariation between HM and LM would be explained by colocalizing QTL and; (c) the shared QTL would have concordant effects. In contrast, we found that LM and HM segregated largely independently, with the exception of two pairwise comparisons. Additional factors such as environmental variation or measurement error might have contributed to a failure to detect phenotypic associations between HM and the other classes of traits if the impact of these sources of errors be imagined to be largely independent in the different trait groups. However, the high degree of concordance between traits within the same trait groups suggests that measurement error did not have a major role in our analysis.

Seven (out of 13) QTL for HM colocalized with QTL for LM when considering an overlap of 1-LOD intervals but interestingly, three of them had negative effects for HM. The mixture of concordant and discordant effects at shared QTL can result in the masking of genetic correlations at the phenotypic level (Gardner and Latta 2007). Distinguishing between close linkage and pleiotropy depends on the number of observed crossovers and is one of the main limitations of QTL mapping experiments. Nevertheless, the distinction between pleiotropy and linkage relates to how little recombination occurs between loci, with the former representing the extreme case of complete linkage. It is possible that close linkage has a similar effect to pleiotropy in rapid bursts of selection occurring in small populations (Gardner and Latta 2007).

Although the overall level of genetic covariance of LM and HM is unlikely to have a big effect in the response to selection,

the presence of genetic trade-offs and antagonistic pleiotropy might still have an impact on trait evolution (Via and Hawthorne 2002). Overlapping QTL with concordant, positive effects were also found and it would be interesting to test whether these are among the first to be recruited in the initial adaptation or are important in adaptation through introgression. Likewise, lip area was weakly correlated with two measurements of head morphology and it would be interesting to test if this correlation is stronger in earlier instances of adaptation. These hypotheses can be tested for example by selection experiments in recombinant populations to analyze the fitness effects of individual QTL (e.g., Rogers et al. 2012; Arnegard et al. 2014).

The large genetic target size of the phenotypes that we investigated does not support the notion that similar phenotypes will be based on regions that are homologous to those that we have identified, particularly when compared to more divergent taxa (i.e., African vs Neotropical cichlid radiations). However, because sharing of ancient genetic variation and incomplete lineage sorting is rampant in East African cichlids (Brawand et al. 2014) it could be true that the different African radiations have recruited ancient genetic variants. The accumulation of data linking genomic regions to evolutionarily relevant phenotypes in cichlids paves the way for exciting future research testing the importance of introgression and shared ancient genetic variation in cichlid adaptive radiations. It would be interesting to know how often convergent phenotypic evolution between the haplochromine cichlid radiations in Lakes Victoria, Malawi, and Tanganyika involves the recruitment of ancient shared variation, as was shown to be the case in the colonization of freshwater from marine environments in sticklebacks (Colosimo et al. 2005; Jones et al. 2012). Hybridization is a common phenomenon in many groups of organisms, particularly in recently diverged species and its role in adaptation to new environments has been debated for a long time (Lewontin and Birch 1966). However, conclusive evidence of adaptive introgression is restricted to a few systems where the phylogenetic analysis of causal genetic regions in hybridizing species was performed, such as *Heliconius* (Pardo-Diaz et al. 2012). Both contemporary and ancient hybridization seem widespread in cichlid fish (e.g., Koblmuller et al. 2010; Joyce et al. 2011; Genner and Turner 2012; Keller et al. 2013) and it has been proposed to play a crucial role in cichlid adaptive radiations, the "hybrid swarm hypothesis" (Seehausen 2004). Testing for both the role of introgression and incomplete lineage sorting in adaptation can be achieved by *functional phylogenomics*, systematically contrasting the evolutionary histories of several genomic regions identified by forward-genetic screens with random genomic regions using target enrichment (outlined in Henning and Meyer 2014). Despite the decreasing costs for whole-genome sequencing, target enrichment is still more efficient for collecting high-coverage, population-level data from large contiguous genomic regions. It has been used for applications such as phylogenomics, exon sequencing, or population-based fine-mapping (Burbano et al. 2010; Mamanova et al. 2010; Faircloth et al. 2012; Lemmon et al. 2012; Nadeau et al. 2012).

SIGNATURES OF NATURAL SELECTION

Morphological differences in LM, particularly in lip length were strongly associated with foraging success. Genetic variation at the loci underlying morphology could be demonstrated to have an effect on foraging performance. Selection pressures in LM appear to be quite strong in natural conditions. This expectation was also confirmed by analyzing the distribution of effect signs. The null hypothesis of the distribution of QTL additive effect signs could be rejected for LM, thus supporting a role for directional natural selection in the evolution of these species differences. The QTL sign test we employed (QTL-ST) is conservative (Anderson and Slatkin 2003), tests for one particular scenario of natural selection (Orr 1998b) and is sensitive to variance in effect sizes (Rice and Townsend 2012). Therefore, the null hypothesis will only be rejected in extreme cases where the number of detected QTL is high and negative effects are virtually absent (e.g., Muir et al. 2014). Nevertheless, even with these restrictions it was possible to show that the observed abundance of positive effect alleles in LM traits is unlikely to have accumulated by chance. For comparison, although a large number of QTL responded to artificial selection for oil content in Maize in an even shorter timeframe, a great number of QTL (approx. 20%) had negative effects (Laurie et al. 2004). This suggests that the degree of selection for habitat partitioning in cichlid adaptive radiations is incredibly strong. In contrast, the persistence of negative effect alleles for HM, the overall distribution of additive effects and the weaker correlation with foraging performance all suggest a weaker or indirect selection pressure on HM. There are likely to be many additional QTL, given that the distribution of additive effects seems biased toward positive effects also in chromosomes where no QTL was detected (Fig. S7). This suggests that many additional loci have diverged as the result of natural selection in an evolutionary timescale as short as 15,000 years.

Lip traits had the highest overall genome-wide effect with a median genome-wide additive effect = 0.1 (in units of F_2 standard deviation) and a range of 0.07–0.135 for each trait. Despite the low number of detected QTL, foraging performance also had a high overall positive effect (0.087). The net effect of HM was also positive, albeit lower than the previous traits (median = 0.045, range = 0.038–0.05). Although the individual estimates are not independent owing to linkage, the overall median additive effects allows for a straightforward comparison of the influence of natural selection on different traits. When analyzing random traits, it is not possible to polarize trait values in relation to foraging in crevices as we have done for lip and head morphology traits. Nevertheless, species differences that are not the direct products

of natural selection should not be biased toward any particular sign and should yield an overall value close to zero. This was the case with the traits that were taken for comparison (body depth, anal fin base, and caudal peduncle length—phenotypic data not shown) that had a median effect very close to zero (–0.008). Note that this statement concerns only between-species differences and does not imply that these traits evolve randomly.

It was hypothesized based on simulations that selection acting on a large fraction of the genome can lead to a nonlinear and rapid build-up of reproductive isolation during speciation with gene-flow, leading to the process of whole-genome congealing (Flaxman et al. 2013; Feder et al. 2014). This pattern of QTL with biased effect signs throughout the genome has also been described in oral jaw traits that are important in the cichlid adaptive radiations (Albertson et al. 2003a) and could support the model of genome-wide congealing (Flaxman et al. 2013; Brawand et al. 2014; Feder et al. 2014), since the divergence of haplochromines occurred recently and under at least, partial gene flow. The accumulation of anchored genomes and QTL data pave the way for high-resolution studies on natural populations that could provide insights on the degree of genomic divergence that is associated with selection on lip morphology (Seehausen et al. 2014).

CONCLUSIONS

In summary, our results suggest that (i) the loci underlying the morphological adaptations we investigated are numerous and have small additive effects; (ii) foraging performance is functionally and genetically associated with between-species morphological differences, particularly in lip morphology; (iii) the distribution of additive effects suggests that natural selection had a genome-wide effect; and that (iv) variation in lip and head morphology is largely genetically independent. Genetic correlations between lip and head morphology are unlikely to facilitate concerted evolution and in fact might have constrained trait evolution through the tight coupling of discordant alleles or antagonistic pleiotropy. While recent empirical and theoretical work has highlighted the role of large effect variants and pleiotropy in the repeated evolution and the maintenance of adaptations (Ferris et al. 2016 and references therein), the present results show that this is certainly not a requirement for evolutionary convergence in adaptive radiations.

AUTHOR CONTRIBUTIONS

AM, FH and GMS conceived the study. FH, GMS and LB designed the foraging performance trials and analyzed all data. FH drafted the manuscript. All authors read and approved the final version.

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LITERATURE CITED

Albert, A. Y. K., S. Sawaya, T. H. Vines, A. K. Knecht, C. T. Miller, B. R. Summers, S. Balabhadra, D. M. Kingsley, and D. Schluter. 2008. The genetics of adaptive shape shift in stickleback: pleiotropy and effect size. Evolution 62:76–85.

Albertson, R., J. Streelman, and T. Kocher. 2003a. Directional selection has shaped the oral jaws of Lake Malawi cichlid fishes. Proc. Natl. Acad. Sci. USA 100:5252–5257.

———. 2003b. Genetic basis of adaptive shape differences in the cichlid head. J. Hered. 94:291–301.

Albertson, R. C., K. E. Powder, Y. A. Hu, K. P. Coyle, R. B. Roberts, and K. J. Parsons. 2014. Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. Mol. Ecol. 23:5135–5150.

Anderson, E. C., and M. Slatkin. 2003. Orr's quantitative trait loci sign test under conditions of trait ascertainment. Genetics 165:445–446.

Arnegard, M. E., M. D. McGee, B. Matthews, K. B. Marchinko, G. L. Conte, S. Kabir, N. Bedford, S. Bergek, Y. F. Chan, F. C. Jones, et al. 2014. Genetics of ecological divergence during speciation. Nature 511:307–311.

Arnold, S. J. 1983. Morphology, performance and fitness. Am. Zool. 23:347–361.

Baumgarten, L., G. Machado-Schiaffino, F. Henning, and A. Meyer. 2015. What big lips are good for: on the adaptive function of repeatedly evolved hypertrophied lips of cichlid fishes. Biol. J. Linn. Soc. 115:448–455.

Beavis, A. D. 1994. The power and deceit of QTL experiments: lessons from comparative QTL studies. Pp. 250–266. Proceedings of the 49th Annual Corn and Sorghum Research Conference, edited by D. B. Wilkinson. American Seed Trade Association, Washington, DC.

Brakefield, P. M. 2006. Evo-devo and constraints on selection. Trends Ecol. Evol. 21:362–368.

Brawand, D., C. E. Wagner, Y. I. Li, M. Malinsky, I. Keller, S. Fan, O. Simakov, A. Y. Ng, Z. W. Lim, E. Bezault, et al. 2014. The genomic substrate for adaptive radiation in African cichlid fish. Nature 513:375–381.

Broman, K. W., and S. Sen. 2009. A guide to QTL mapping with R/qtl. Springer, Dordrecht.

Burbano, H. A., E. Hodges, R. E. Green, A. W. Briggs, J. Krause, M. Meyer, J. M. Good, T. Maricic, P. L. F. Johnson, Z. Y. Xuan, et al. 2010. Targeted investigation of the Neandertal genome by array-based sequence capture. Science 328:723–725.

Burress, E. 2014. Cichlid fishes as models of ecological diversification: patterns, mechanisms, and consequences. Hydrobiologia 748:1–21.

Catchen, J. M., A. Amores, P. Hohenlohe, W. Cresko, and J. H. Postlethwait. 2011. Stacks: building and genotyping loci de novo from short-read sequences. G3 Genes Genomes Genet. 1:171–182.

Chan, Y. F., M. E. Marks, F. C. Jones, G. Villarreal, M. D. Shapiro, S. D. Brady, A. M. Southwick, D. M. Absher, J. Grimwood, J. Schmutz, et al. 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a Pitx1 enhancer. Science 327:302–305.

Colombo, M., E. T. Diepeveen, M. Muschick, M. E. Santos, A. Indermaur, N. Boileau, M. Barluenga, and W. Salzburger. 2013. The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. Mol. Ecol. 22:670–684.

Colosimo, P. F., K. E. Hosemann, S. Balabahadra, G. Villarreal, M. Dickson, J. Grimwood, J. Schmutz, R. M. Myers, D. Schluter, and D. M. Kingsley. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodyplasin alleles. Science 307:1928–1933.

Cook, L. M., B. S. Grant, I. J. Saccheri, and J. Mallet. 2012. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. Biol. Lett. doi:10.1098/rsbl.2011.1136.

Dittmar, E. L., C. G. Oakley, J. K. Conner, B. A. Gould, and D. W. Schemske. 2016. Factors influencing the effect size distribution of adaptive substitutions. Proc. R. Soc. B 283:20153065. doi:10.1098/rspb.2015.306.

Ellegren, H., L. Smeds, R. Burri, P. I. Olason, N. Backstrom, T. Kawakami, A. Kunstner, H. Makinen, K. Nadachowska-Brzyska, A. Qvarnstrom, et al. 2012. The genomic landscape of species divergence in *Ficedula* flycatchers. Nature 491:756–760.

Endler, J. A. 1986. Natural selection in the wild. Princeton Univ. Press, Princeton, NJ.

Faircloth, B. C., J. E. McCormack, N. G. Crawford, M. G. Harvey, R. T. Brumfield, and T. C. Glenn. 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. Syst. Biol. 61:717–726.

Feder, J. L., P. Nosil, A. C. Wacholder, S. P. Egan, S. H. Berlocher, and S. M. Flaxman. 2014. Genome-wide congealing and rapid transitions across the speciation continuum during speciation with gene flow. J. Hered. 105:810–820.

Ferris, K. G., L. L. Barnett, B. K. Blackman, and J. H. Willis. 2016. The genetic architecture of local adaptation and reproductive isolation in sympatry within the *Mimulus guttatus* species complex. Mol. Ecol. 26:208–224. doi. 10.1111/mec.13763.

Fishman, L., A. J. Kelly, and J. H. Willis. 2002. Minor quantitative trait loci underlie floral traits associated with mating system divergence in *Mimulus*. Evolution 56:2138–2155.

Flaxman, S. M., J. L. Feder, and P. Nosil. 2013. Genetic hitchhiking and the dynamic buildup of genomic divergence during speciation with gene flow. Evolution 67:2577–2591.

Flint, J., and T. F. C. Mackay. 2009. Genetic architecture of quantitative traits in mice, flies, and humans. Genome Res. 19:723–733.

Gardner, K. M., and R. G. Latta. 2007. Shared quantitative trait loci underlying the genetic correlation between continuous traits. Mol. Ecol. 16:4195–4209.

Genner, M. J., and G. F. Turner. 2012. Ancient hybridization and phenotypic novelty within Lake Malawi's cichlid fish radiation. Mol. Biol. Evol. 29:195–206.

Gompel, N., and B. Prud'homme. 2009. The causes of repeated genetic evolution. Dev. Biol. 332:36–47.

Greenwood, A. K., J. N. Cech, and C. L. Peichel. 2012. Molecular and developmental contributions to divergent pigment patterns in marine and freshwater sticklebacks. Evol. Dev. 14:351–362.

Greenwood, A. K., A. R. Wark, K. Yoshida, and C. L. Peichel. 2013. Genetic and neural modularity underlie the evolution of schooling behavior in Threespine Sticklebacks. Curr. Biol. 23:1884–1888.

Hall, M. C., C. J. Basten, and J. H. Willis. 2006. Pleiotropic quantitative trait loci contribute to population divergence in traits associated with life-history variation in *Mimulus guttatus*. Genetics 172:1829–1844.

Hendry, A. P. 2013. Key questions in the genetics and genomics of eco-evolutionary dynamics. Heredity 111:456–466.

Henning, F., H. J. Lee, P. Franchini, and A. Meyer. 2014. Genetic mapping of horizontal stripes in Lake Victoria cichlid fishes: benefits and pitfalls of using RAD markers for dense linkage mapping. Mol. Ecol. 23:5224–5240.

Henning, F., and A. Meyer. 2014. The evolutionary genomics of cichlid fishes: explosive speciation and adaptation in the postgenomic era. Annu. Rev. Genomics Hum. Genet. 15:417–441.

Huang, W., S. Richards, M. A. Carbone, D. H. Zhu, R. R. H. Anholt, J. F. Ayroles, L. Duncan, K. W. Jordan, F. Lawrence, M. M. Magwire, et al. 2012. Epistasis dominates the genetic architecture of Drosophila quantitative traits. Proc. Natl. Acad. Sci. USA 109:15553–15559.

Johnson, T. C., C. A. Scholz, M. R. Talbot, K. Kelts, R. D. Ricketts, G. Ngobi, K. Beuning, I. Ssemmanda, and J. W. McGill. 1996. Late pleistocene desiccation of Lake Victoria and rapid evolution of cichlid fishes. Science 273:1091–1093.

Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, S. White, et al. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature 484:55–61.

Joyce, D. A., D. H. Lunt, M. J. Genner, G. F. Turner, R. Bills, and O. Seehausen. 2011. Repeated colonization and hybridization in Lake Malawi cichlids. Curr. Biol. 21:R108–R109.

Kao, C. H., Z. B. Zeng, and R. D. Teasdale. 1999. Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216.

Keenleyside, M. H. A. 1991. Cichlid fishes: behaviour, ecology and evolution. Chapman & Hall, London.

Keller, I., C. E. Wagner, L. Greuter, S. Mwaiko, O. M. Selz, A. Sivasundar, S. Wittwer, and O. Seehausen. 2013. Population genomic signatures of divergent adaptation, gene flow and hybrid speciation in the rapid radiation of Lake Victoria cichlid fishes. Mol. Ecol. 22:2848–2863.

Koblmuller, S., B. Egger, C. Sturmbauer, and K. M. Sefc. 2010. Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. Mol. Phylogenet. Evol. 55:318–334.

Kocher, T. D., J. A. Conroy, K. R. Mckaye, and J. R. Stauffer. 1993. Similar morphologies of cichlid fish in Lakes Tanganyika and Malawi are due to convergence. Mol. Phylogenet. Evol. 2:158-165.

Kowalko, J. E., N. Rohner, S. B. Rompani, B. K. Peterson, T. A. Linden, M. Yoshizawa, E. H. Kay, J. Weber, H. E. Hoekstra, W. R. Jeffery, et al. 2013. Loss of schooling behavior in cavefish through sight-dependent and sight-independent mechanisms. Curr. Biol. 23:1874–1883.

Laland, K., T. Uller, M. Feldman, K. Sterelny, G. B. Muller, A. Moczek, E. Jablonka, and J. Odling-Smee. 2014. Does evolutionary theory need a rethink?—POINT Yes, urgently. Nature 514:161–164.

Lande, R. 1984. The genetic correlation between characters maintained by selection, linkage and inbreeding. Genet. Res. 44:309–320.

Laurie, C. C., S. D. Chasalow, J. R. LeDeaux, R. McCarroll, D. Bush, B. Hauge, C. Q. Lai, D. Clark, T. R. Rocheford, and J. W. Dudley. 2004. The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. Genetics 168:2141–2155.

Lemmon, A. R., S. A. Emme, and E. M. Lemmon. 2012. Anchored hybrid enrichment for massively high-throughput phylogenomics. Syst. Biol. 61:727–744.

Lewontin, R. C., and L. C. Birch. 1966. Hybridization as a source of variation for adaptation to new environments. Evolution 20:315–336.

Linnen, C. R., Y. P. Poh, B. K. Peterson, R. D. H. Barrett, J. G. Larson, J. D. Jensen, and H. E. Hoekstra. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science 339:1312–1316.

Losos, J. B. 2011. Convergence, adaptation, and constraint. Evolution 65:1827–1840.

Machado-Schiaffino, G., F. Henning, and A. Meyer. 2014. Species-specific differences in adaptive phenotypic plasticity in an ecologically relevant trophic trait: hypertrophic lips in Midas cichlid fishes. Evolution 68:286–291.

Machado-Schiaffino, G., A. Kautt, J. Torres-Dowdall, L. Baumgarten, F. Henning, and A. Meyer. 2017. Incipient speciation driven by hypertrophied lips in Midas cichlid fishes. Mol. Ecol. Accepted Article. doi: 10.1111/mec.14029.

Mamanova, L., A. J. Coffey, C. E. Scott, I. Kozarewa, E. H. Turner, A. Kumar, E. Howard, J. Shendure, and D. J. Turner. 2010. Target-enrichment strategies for next-generation sequencing. Nat. Meth. 7:111–118.

Manousaki, T., P. M. Hull, H. Kusche, G. Machado-Schiaffino, P. Franchini, C. Harrod, K. R. Elmer, and A. Meyer. 2013. Parsing parallel evolution: ecological divergence and differential gene expression in the adaptive radiations of thick-lipped Midas cichlid fishes from Nicaragua. Mol. Ecol. 22:650–669.

Miller, C. T., A. M. Glazer, B. R. Summers, B. K. Blackman, A. R. Norman, M. D. Shapiro, B. L. Cole, C. L. Peichel, D. Schluter, and D. M. Kingsley. 2014. Modular skeletal evolution in sticklebacks is controlled by additive and clustered quantitative trait loci. Genetics 197:405–420.

Muir, C. D., J. B. Pease, and L. C. Moyle. 2014. Quantitative genetic analysis indicates natural selection on leaf phenotypes across wild tomato species (*Solanum sect. lycopersicon*; Solanaceae). Genetics 198:1629–1643.

Nadeau, N. J., A. Whibley, R. T. Jones, J. W. Davey, K. K. Dasmahapatra, S. W. Baxter, M. A. Quail, M. Joron, R. H. Ffrench-Constant, M. L. Blaxter, et al. 2012. Genomic islands of divergence in hybridizing *Heliconius* butterflies identified by large-scale targeted sequencing. Philos. Trans. Royal Soc. B Biol. Sci. 367:343–353.

O'Quin, C. T., A. C. Drilea, M. A. Conte, and T. D. Kocher. 2013. Mapping of pigmentation QTL on an anchored genome assembly of the cichlid fish, *Metriaclima zebra*. BMC Genomics 14:287.

O'Quin, C. T., A. C. Drilea, R. B. Roberts, and T. D. Kocher. 2012. A small number of genes underlie male pigmentation traits in Lake Malawi cichlid fishes. J. Exp. Zool.Part B Mol. Dev. Evol. 318B:199–208.

Oliver, M. K., and M. E. Arnegard. 2010. A new genus for *Melanochromis labrosus*, a problematic Lake Malawi cichlid with hypertrophied lips (Teleostei: Cichlidae). Ichthyol. Explor. Fres. 21:209–232.

Orr, H. A. 1998a. The population genetics of adaptation: the distribution of factors fixed during adaptive evolution. Evolution 52:935–949.

———. 1998b. Testing natural selection vs. genetic drift in phenotypic evolution using quantitative trait locus data. Genetics 149:2099–2104.

———. 2005. The genetic theory of adaptation: a brief history. Nat. Rev. Genet. 6:119–127.

Orr, H. A., and J. A. Coyne. 1992. The genetics of adaptation—a reassessment. Am. Nat. 140:725–742.

Pardo-Diaz, C., C. Salazar, S. W. Baxter, C. Merot, W. Figueiredo-Ready, M. Joron, W. O. McMillan, and C. D. Jiggins. 2012. Adaptive introgression across species boundaries in *Heliconius* butterflies. PLoS Genet. 8: e1002752. doi:10.1371/journal.pgen.1002752.

Parnell, N. F., C. D. Hulsey, and J. T. Streelman. 2012. The genetic basis of a complex functional system. Evolution 66:3352–3366.

Peterson, B. K., J. N. Weber, E. H. Kay, H. S. Fisher, and H. E. Hoekstra. 2012. Double digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. Plos One 7: e37135. doi:10.1371/journal.pone.0037135.

Poelstra, J. W., N. Vijay, C. M. Bossu, H. Lantz, B. Ryll, I. Muller, V. Baglione, P. Unneberg, M. Wikelski, M. G. Grabherr, et al. 2014. The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. Science 344:1410–1414.

Rice, D. P., and J. P. Townsend. 2012. Resampling QTL effects in the QTL sign test leads to incongruous sensitivity to variance in effect size. G3 Genes Genomes Genet. 2:905–911.

Roberts, R. B., J. R. Ser, and T. D. Kocher. 2009. Sexual conflict resolved by invasion of a novel sex determiner in Lake Malawi cichlid fishes. Science 326:998–1001.

Rockman, M. V. 2012. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. Evolution 66:1–17.

Rogers, S. M., P. Tamkee, B. Summers, S. Balabahadra, M. Marks, D. M. Kingsley, and D. Schluter. 2012. Genetic signature of adaptive peak shift in threespine stickleback. Evolution 66:2439–2450.

Rohlf, F. 2010a. tpsDig version 2.16, Department of Ecology and Evolution, State University of New York at Stony Brook. Available at http://life.bio.sunysb.edu/morph/.

———. 2010b. tpsRelw, version 1.49, Department of Ecology and Evolution, State University of New York at Stony Brook. Available at http://life.bio.sunysb.edu/morph/.

Schluter, D. 1996. Adaptive radiation along genetic lines of least resistance. Evolution 50:1766–1774.

Schneeberger, K. 2014. Using next-generation sequencing to isolate mutant genes from forward genetic screens. Nat. Rev. Genet. 15:662–676.

Seehausen, O. 1996. Distribution of and reproductive isolation among color morphs of a rock-dwelling Lake Victoria cichlid (*Haplochromis nyererei*). Ecol. Freshw. Fish. 5:195–202.

———. 2004. Hybridization and adaptive radiation. Trends Ecol. Evol. 19:198–207.

Seehausen, O., and N. Bouton. 1998. The community of rock-dwelling cichlids in Lake Victoria. Bonner Zoologische Beitrage 47:301–312.

Seehausen, O., R. K. Butlin, I. Keller, C. E. Wagner, J. W. Boughman, P. A. Hohenlohe, C. L. Peichel, G.-P. Saetre, C. Bank, A. Brannstrom, et al. 2014. Genomics and the origin of species. Nat. Rev. Genet. 15:176–192.

Servedio, M. R., G. S. Doorn, M. Kopp, A. M. Frame, and P. Nosil. 2011. Magic traits in speciation: 'magic' but not rare? Trends Ecol. Evol. 26:389–397.

Silva, L. D. E., S. Wang, and Z. B. Zeng. 2012. Composite interval mapping and multiple interval mapping: procedures and guidelines for using windows QTL Cartographer. Pp. 75-120 *in* S. A. Rifkin, ed. Quantitative trait loci (QTL): methods and protocols. Humana Press, Springer, New York.

Slate, J. 2013. From Beavis to beak color: a simulation study to examine how much QTL mapping can reveal about the genetic architecture of quantitative traits. Evolution 67:1251–1262.

Stelkens, R. B., K. A. Young, and O. Seehausen. 2010. The accumulation of reproductive incompatibilities in African cichlid fish. Evolution 64:617–632.

Streelman, J. T., R. C. Albertson, and T. D. Kocher. 2003. Genome mapping of the orange blotch colour pattern in cichlid fishes. Mol. Ecol. 12:2465–2471.

Team, R. C. 2014. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

van 't Hof, A. E., N. Edmonds, M. Dalikova, F. Marec, and I. J. Saccheri. 2011. Industrial melanism in British peppered moths has a singular and recent mutational origin. Science 332:958–960.

van Oijen, M. J. P. 1996. The generic classification of the haplochromine cichlids of Lake Victoria, East Africa Zool. Verh. Leiden 302:57–110.

Van Ooijen, J. W. 2006. JoinMap 4. Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen, The Netherlands.

Via, S., and D. J. Hawthorne. 2002. The genetic architecture of ecological specialization: correlated gene effects on host use and habitat choice in pea aphids. Am. Nat. 159:S76–S88.

Weber, J. N., B. K. Peterson, and H. E. Hoekstra. 2013. Discrete genetic modules are responsible for complex burrow evolution in *Peromyscus mice*. Nature 493:402–405.

Witte, F., and M. J. P. Van Oijen. 1990. Taxonomy, ecology and fishery of Lake Victoria haplochromine trophic groups. Zoologische Verhandelingen 262:1–47.

Wray, G. A., H. E. Hoekstra, D. J. Futuyma, R. E. Lenski, T. F. C. Mackay, D. Schluter, and J. E. Strassmann. 2014. Does evolutionary theory need a rethink?—COUNTERPOINT No, all is well. Nature 514:161–164.

Wu, T. D., and C. K. Watanabe. 2005. GMAP: a genomic mapping and alignment program for mRNA and EST sequences. Bioinformatics 21:1859–1875.

Xu, S. Z. 2003. Theoretical basis of the Beavis effect. Genetics 165:2259–2268.

Zeller, M., K. Lucek, M. Haesler, O. Seehausen, and A. Sivasundar. 2012. Little evidence for a selective advantage of armour-reduced threespined stickleback individuals in an invertebrate predation experiment. Evol. Ecol. 26:1293–1309.