

Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish

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Abstract

Genetic adaptation and phenotypic plasticity are two ways in which organisms can adapt to local environmental conditions. We examined genetic and plastic variation in gill and brain size among swamp (low oxygen; hypoxic) and river (normal oxygen; normoxic) populations of an African cichlid fish, *Pseudocrenilabrus multicolor victoriae*. Larger gills and smaller brains should be advantageous when oxygen is low, and we hypothesized that the relative contribution of local genetic adaptation vs. phenotypic plasticity should be related to potential for dispersal between environments (because of gene flow's constraint on local genetic adaptation). We conducted a laboratory-rearing experiment, with broods from multiple populations raised under high-oxygen and low-oxygen conditions. We found that most of the variation in gill size was because of plasticity. However, both plastic and genetic effects on brain mass were detected, as were genetic effects on brain mass *plasticity*. F₁ offspring from populations with the highest potential for dispersal between environments had characteristically smaller and more plastic brains. This phenotypic pattern might be adaptive in the face of gene flow, if smaller brains and increased plasticity confer higher average fitness across environment types.

Introduction

Genetic adaptation and phenotypic plasticity have traditionally been considered two alternate ways in which organisms can adapt to different environments (review: Schlichting & Pigliucci, 1998). On the one hand, natural selection can act on genetic variation in a population over a number of generations to result in a population that is locally adapted to prevailing environmental conditions (e.g. Cain & Sheppard, 1954; Kettlewell, 1958; Antonovics & Bradshaw, 1970; Bürger & Lynch, 1995; Kawecki & Ebert, 2004). On the other hand, adaptive phenotypic change can occur within a generation, producing locally adapted phenotypes without genetic change (e.g. Van Buskirk, 2002; Steinger *et al.*, 2003; Doughty & Reznick, 2004; Ghalambor *et al.*, 2007;

Latta *et al.*, 2007). Yet, in many systems, both plastic and genetic variation contribute to adaptive phenotypic change (co-gradient or counter-gradient variation; Conover & Schultz, 1995; Conover *et al.*, 2009). The relative contribution of plasticity and local adaptation to phenotypic divergence should depend on the costs and benefits of each under specific conditions (reviews: DeWitt *et al.*, 1998; Alpert & Simms, 2002; Doughty & Reznick, 2004; van Kleunen & Fischer, 2005; Richards *et al.*, 2006).

A number of recent studies have focused on the balance between local genetic adaptation and gene flow. Both theoretical studies (e.g. Hendry *et al.*, 2001; Lenormand, 2002; Griswold, 2006) and empirical work (e.g. Riechert, 1993; King & Lawson, 1995; Storfer *et al.*, 1999; Hendry *et al.*, 2002; Hendry & Taylor, 2004; Nosil & Crespi, 2004; Moore *et al.*, 2007) in this area suggest that gene flow between selective environments can reduce local adaptation. We predict that in systems with high gene flow between selective environments, local adaptation might not be apparent because of migration load,

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and instead adaptive phenotypic plasticity would evolve (Sultan & Spencer, 2002; Crispo, 2008). We examine the contribution of local adaptation and phenotypic plasticity to morphological divergence in a system with potential for high gene flow between selective environments.

The African cichlid fish, *Pseudocrenilabrus multicolor victoriae* Seegers, is a widespread haplochromine cichlid found throughout East African rivers, lakes and swamps (Greenwood, 1965; Schierwater & Mrowka, 1987). *Pseudocrenilabrus* is a primarily riverine, relatively basal haplochromine genus (Booton *et al.*, 1999; Salzburger *et al.*, 2002; Joyce *et al.*, 2005; Katongo *et al.*, 2005). Although relatively depauperate, young species flocks have been identified in rivers and lakes (Koblmüller *et al.*, 2008; Stelkens & Seehausen, 2009). In the Mpanga River drainage of western Uganda, *P. multicolor* persists in both high-oxygen (normoxic) rivers and low-oxygen (hypoxic) papyrus swamps and also in areas of the river where dissolved oxygen concentration fluctuates seasonally (Crispo & Chapman, 2008, 2010). Previous studies on this system have shown genetic differentiation at neutral microsatellite markers to be low and temporally variable among oxygen environments (Crispo & Chapman, 2008, 2010). Low levels of genetic differentiation suggest that contemporary gene flow might be high, and we therefore predict that plasticity of ecologically relevant traits is adaptive in this system, particularly for populations located near the junction between the river and swamp, where dispersal between divergent oxygen environments should be highest.

Previous studies have documented high levels of plasticity in *P. multicolor* with respect to traits that should be adaptive in alternate forms under alternate oxygen regimes, including gill size, brain mass and body shape traits (Chapman *et al.*, 2000, 2008). We extend previous analyses on *P. multicolor* by examining gill and brain size plasticity in populations from multiple locations within a single drainage (the Mpanga River drainage). Gills are expected to be larger under hypoxia to facilitate oxygen uptake (Palzenberger & Pohla, 1992; Chapman *et al.*, 2000; Langerhans *et al.*, 2007; Chapman *et al.*, 2008). Brains are expected to be smaller under these same conditions, because of the high metabolic demand of the brain (Chapman & Hulen, 2001; Poulson, 2001; Safi *et al.*, 2005). Smaller brains might occur because large brains cannot develop under hypoxia, or because large brains would decrease energy allocation to other functions. Changes in gills and brains might cause correlated changes in other fitness-related traits. For example, evidence suggests that increased gill size correlates with a decrease in the size of trophic structures, which might limit feeding performance (Schaack & Chapman, 2003; Chapman *et al.*, 2008). Small brains might come at the cost of lowered cognition or sensory ability (Kotrschal *et al.*, 1998; Shumway, 2008; Gonzalez-Voyer *et al.*, 2009). Therefore, we expect that alternate phenotypes (large vs. small gills and brains) reflect local adaptations

to alternate oxygen regimes. Our goal is to determine what proportion of the phenotypic variation is because of genetic differences among populations vs. direct environmental effects (i.e. plasticity) and to relate this information to the potential for dispersal between selective environments.

Methods

Laboratory experiment

We used baited minnow traps to collect live fish from the Mpanga River drainage. Collections were from four sites in June 2006 (Bunoga, Rwebakwata, Kahunge, Kantembwe; Fig. 1) and two sites in May–June 2008 (Kamwenge, Kanyantale; Fig. 1). Kanyantale is a hypoxic swamp site with average oxygen levels of 0.28 mg L^{-1} ($\pm 0.03 \text{ SE}$) and an average per cent oxygen saturation of 3.5% in May 2008. All other sites are described in Crispo & Chapman (2008). Briefly, Kantembwe is a hypoxic swamp site with dissolved oxygen levels similar to those observed in Kanyantale. Rwebakwata and Kahunge are river sites adjacent to the swamp and experience seasonal fluctuations in dissolved oxygen because of flooding of the adjacent swamp and the influx of organic debris during wet seasons. Bunoga

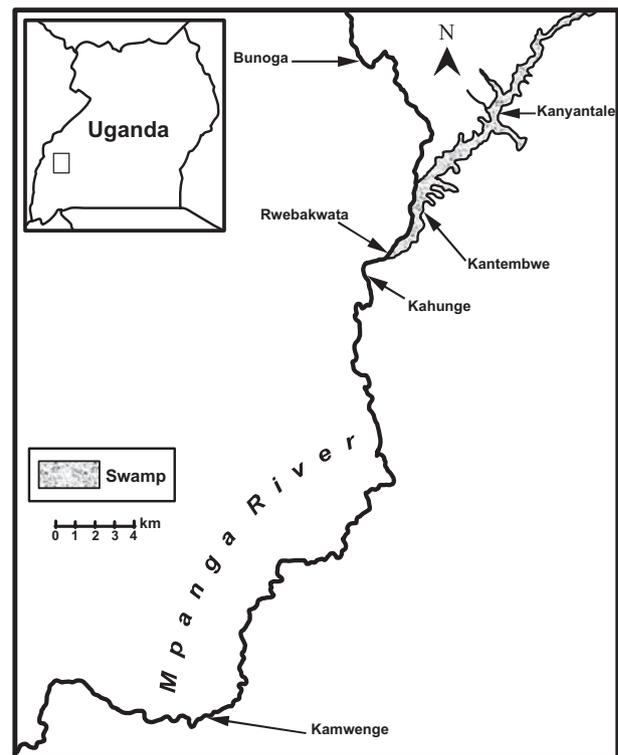


Fig. 1 Map of study sites within the Mpanga River drainage in Uganda. Water flows in the north-south direction. Inset on the map of Uganda depicts the location of the Mpanga River drainage.

is a river site that also experiences some seasonal fluctuation in dissolved oxygen, possibly because of surrounding intensive land use. However, oxygen at these three river sites never reaches levels as low as those in the swamp. Although we have no long-term oxygen data for Kamwenge, we expect that this river site experiences relatively high oxygen levels year-round, given its large geographical distance from the swamp and consistently high oxygen levels during our expeditions to this area. We know of no physical barriers to dispersal that exist among sampled sites, suggesting that contemporary gene flow is possible among all sites, at least during the wet seasons when flooding occurs (Crispo & Chapman, 2008, 2010). Habitat patchiness probably restricts dispersal in the swamps during the dry seasons (L. Chapman, personal observation). A large waterfall separates the Kamwenge site from downstream swamps (L. Chapman, personal observation). We expect dispersal between river and swamp environments to be greatest among the sites located closest to the junction between these two environments.

All experiments took place at McGill University following shipment from Uganda of the collected adults. The experiment was divided into two parts, with Bunoga, Rwebakwata, Kahunge, and Kantembwe broods raised in 2007–2008, and Kamwenge and Kanyantale broods raised in 2008–2009. This split was necessary because of spatial constraints in the laboratory and the logistics of transferring live fish from Uganda. For each collection site, seven full-sib families were raised, where a family consisted of the brood of one male–female pair (i.e. each parent used only once in the experiment). An exception was Kanyantale, for which nine full-sib families were used. *Pseudocrenilabrus multicolor* is a mouth brooder, meaning that fry develop in the mouth of the female parent. Brood sizes reached values of > 60, although minimum brood sizes could not be determined because of cannibalism by the mothers. F₁ broods from each family were split between high-oxygen and low-oxygen treatments 1 week after release from the mouth. A separate 14-gallon tank was used for each combination of family and treatment. Filtration was performed using Hagen Fluval underwater filters. Low-oxygen conditions (tank averages of 0.54–1.29 mg L⁻¹) were maintained using a commercial oxygen controlling system (Point Four Systems Inc., Coquitlam, British Columbia). High-oxygen conditions (tank averages of 7.27–8.07 mg L⁻¹) were maintained via constant bubbling of air through the water column. Fry were fed Hikari First Bites fry food for the first 3 weeks after release from the females' mouths. TetraMin Pro Tropical Crisps were gradually introduced 2 weeks after release and were then fed to the growing offspring for the remaining duration of the experiment. Mortality during the experiment was related primarily to aggression among mature siblings and was not related to oxygen. At approximately 1 month of age, broods were culled to 10 fish per tank. If fewer than 10 fish were

present in a tank, we did not perform culling. Ambient temperature remained constant at 25.5 °C.

Gills

At an age of approximately 1 year, the largest two males in each tank (or one male if there was only one mature male in the tank) were harvested for analysis of morphology. We used only males because mouth brooding in females might have affected the development of the gills (Schwartz, 1995; C. O'Connor, E. Reardon & L. Chapman, unpublished data). Some, but not all, females brooded during the experiment, but whether or not an individual female had brooded was not recorded. Fish were euthanized using buffered tricaine methane-sulfonate (MS222; pH = 7.0) and were preserved in 4% paraformaldehyde (buffered with phosphate buffered saline; pH = 7.0). Gills were dissected out from the branchial basket on one side of the fish (normally the right side, unless the gills were damaged during the dissection process). The four gill arches were separated, and both sides of each arch (hemibranch) were photographed using a Lumenera Scientific Infinity camera attached to a dissecting microscope. Measurements of five gill metrics were made using Motic Images Plus version 2.0, including total gill filament length (TGFL), average gill filament length (AFL), total number of gill filaments (TNF), total hemibranch area (THA) and total perimeter of the hemibranches (TP) (Muir & Hughes, 1969; Hughes, 1984; Langerhans *et al.*, 2007; Chapman *et al.*, 2008). Units were mm or mm². TGFL was quantified by measuring the length of every fifth filament, using the average of two measures to estimate the length of the four filaments between them, summing the lengths of the filaments for one gill, and multiplying by two to obtain the overall length of the filaments for both gills. Similarly, AFL was quantified using this procedure, but the total filament length was divided by the number of filaments on one gill, and the final value was not multiplied by two. TNF was quantified by counting the number of filaments on one gill and multiplying by two. THA was quantified by estimating the area around the filaments for each side of each hemibranch, summing these values and multiplying by two (see Fig. 3 in Langerhans *et al.*, 2007). TP was quantified in a similar way, but using the perimeter of the area measured for THA. All of these metrics were positively correlated with total gill surface area measured in a subset of the fish from Bunoga and Kantembwe (log₁₀-transformed values; Pearson two-tailed correlation: TGFL, $r = 0.908$, $P < 0.001$; THA, $r = 0.923$, $P < 0.001$; TNF, $r = 0.639$, $P = 0.002$; AFL, $r = 0.927$, $P < 0.001$; TP, $r = 0.851$, $P < 0.001$). These metrics were also correlated with total gill surface area in previous studies (Chapman *et al.*, 2000, 2007).

To test for population, treatment and population-by-treatment effects on gill size, we performed a

mixed-model analysis of variance (ANOVA), instead of a multivariate analysis of variance (MANOVA), so that 'family' could be included as a *random* factor. We standardized gill metrics to a common body mass so that we could perform a principle component analysis (PCA) and used the PCA scores as the response variable in the univariate test. Each of the above gill metrics was standardized to a common body mass using the allometric equation: $Y_{std} = Y_{obs}(M_{avg}/M_{obs})^\beta$, where Y represents the gill metric; M represents body mass; subscripts std, obs, and avg refer to the standardized, observed (actual) and average (for all fish) measures, respectively; and β represents the slope of the relationship between the gill metric and body mass across all populations (Reist, 1986; Hendry & Taylor, 2004; Chapman *et al.*, 2008). The β values were obtained from analyses of covariance (ANCOVAs) including population and treatment as fixed factors, family (nested within population) as a random factor, the population-by-treatment interaction, and \log_{10} -transformed body mass as a covariate. All analyses were performed using Type III sums of squares in SPSS version 16.0. All gill metrics were \log_{10} -transformed for the ANCOVAs, but nontransformed trait values were used in the above allometric equation. Size standardization via this approach was appropriate because interactions with body mass were nonsignificant in ANCOVAs (results not shown).

We performed a principal components analysis on the \log_{10} -transformed body mass-standardized gill metrics. We used the correlation matrix and regression method to obtain composite scores. PCA extracted one component (reflecting gill size) with an eigenvalue > 1 (Table 1), and we performed a mixed-model ANOVA using the scores from this single composite variable as the response variable. We included population and treatment as fixed factors, the population-by-treatment interaction, and family (nested within population) as a random factor (Model 1).

Table 1 Loadings (correlations) of each gill metric on the components extracted from the principle component analyses. Only components with eigenvalues > 1 are shown. Gill metrics were body mass-standardized and \log_{10} -transformed as described in the text.

Gill metric	Both treatments		Low oxygen		High oxygen	
	PC1	PC1	PC2	PC1	PC2	
TGFL	0.990	0.969	-0.056	0.954	-0.154	
THA	0.983	0.964	-0.228	0.963	-0.191	
TNF	0.610	0.576	0.780	0.566	0.757	
AFL	0.944	0.851	-0.508	0.663	-0.746	
TP	0.967	0.835	0.307	0.752	0.528	

TGFL, total gill filament length; THA, total hemibranch area; TNF, total number of filaments; AFL, average filament length; TP, total perimeter.

We performed additional analyses to gain insight into family-level variation in plasticity, effects on individual gill metrics and oxygen effects *within* treatments. First, family-by-treatment interactions could not be tested in the above model because family is a random factor nested within population. Thus, we also ran the analysis without the population term, but including family (a random factor), treatment and the family-by-treatment interaction (Model 2). This analysis allowed us to determine whether gill size plasticity varies among families, irrespective of population. We were able to perform this analysis because the population term was nonsignificant (see Results). Second, we performed univariate tests on each gill metric separately to see if treatment and population effects, and their interaction, influence individual gill metrics. These tests included the \log_{10} -transformed nonstandardized gill metrics as dependent variables, \log_{10} -transformed body mass as a covariate, population and treatment as fixed factors, family (nested within population) as a random factor, and the population-by-treatment interaction. Interactions with body mass were nonsignificant (results not shown) and were therefore not included in the model.

Within the low-oxygen treatment, the oxygen controlling system recorded the dissolved oxygen concentration every hour for the duration of the experiment. This was necessary to facilitate precision on the control of oxygen in the low-oxygen tanks. For the high-oxygen treatment, we had weekly oxygen readings that were taken using a hand-held device (OxyGuard Polaris). A commercial controlling system was not used for the high-oxygen treatment because these levels of oxygen were easier to achieve. We performed ANCOVAs *within* each treatment separately to test whether the fine-scale variation in oxygen concentration that occurred within treatments influenced gill size. For these analyses, the standardization and PCA methods described earlier were repeated using only fish from the high-oxygen or low-oxygen treatments. Two PCA components with eigenvalues > 1 were extracted for each treatment (Table 1), and thus two ANCOVAs were performed for each treatment. We performed ANCOVAs with the family mean PCA scores as the response variables, population as a fixed factor, the \log_{10} -transformed mean oxygen concentration (mg L^{-1}) as a covariate and the population-by-oxygen interaction. All effects were nonsignificant for the high-oxygen treatment, and so we do not report the results. These analyses were used to reveal whether observed family effects are because, at least in part, of tank effects related to small differences in dissolved oxygen concentration.

Brain

Brains were extracted from the same fish that were used for the gill metrics, using standard dissection methods (Chapman *et al.*, 2008), and were stored in 4% buffered

paraformaldehyde. We obtained the blotted weight to the nearest 0.1 mg and used the average of five measurements per brain for the analyses. We performed an ANCOVA with \log_{10} -transformed brain mass included as the response variable, population and treatment as fixed factors, family (nested within population) as a random factor, the population-by-treatment interaction and \log_{10} -transformed body mass as a covariate. Interactions with body mass were not significant (results not shown), and so they were not included in the model. We were not able to perform a *post hoc* test for multiple comparisons with the covariate (body mass) included in the model. We therefore also standardized the brain mass to a common body mass, as we did for the gill metrics. We then performed an ANOVA as earlier, using the \log_{10} -transformed body mass-standardized brain mass as the response variable, without the body mass covariate (results not shown). Because population effects were significant (see Results), we followed this with a Tukey honestly significant difference *post hoc* test for multiple comparisons to identify homogenous subsets with respect to population. We did not test for family-by-treatment interactions, as we did for the gills, because population and population-by-treatment effects on brain mass were strong (see Results). Therefore, we could not remove the population effect from the model to test for interactions with family. Using the within-treatment adjusted family means (from the above ANCOVA including the body mass covariate) as the response variables, we also performed ANCOVAs for each treatment separately, including the oxygen covariate, as we did for the gills. Within the high-oxygen treatment, the population effect was significant, but the oxygen covariate was not, and so we do not report the results from the analyses within the high-oxygen treatment.

Results

Gills

PCA analysis on the full dataset extracted one component with an eigenvalue > 1 , and this component explained 82.9% of the variation in the data. All gill metrics were strongly positively correlated with the component scores (Table 1, both treatments combined), and thus, this component reflects variation in overall gill size (after controlling for variation in body mass). Results from ANOVA using the component scores as a response variable indicated that treatment and family effects were strong, but population and population-by-treatment effects were marginally nonsignificant (Model 1; Table 2). Gill metrics were larger for fish raised under low oxygen than for fish raised under high oxygen. Population effects were still not significant when the interaction term was removed from the model, although again they were only marginally nonsignificant (results not shown). When the population term was excluded from the analysis, treatment

Table 2 Results from ANOVAs for F_1 *Pseudocrenilabrus multicolor* from multiple populations raised under high-oxygen or low-oxygen conditions in a laboratory-rearing experiment. The principle component scores for \log_{10} -transformed body mass-standardized gill metrics were used as the response variable. The d.f. are the hypothesis degrees of freedom followed by the error degrees of freedom. η^2 = effect size.

Effect	F	d.f.	P	η^2
Model 1				
Population	2.205	5, 38.945	0.073	0.221
Treatment	921.170	1, 117	< 0.001	0.887
Family (population)	2.177	38, 117	0.001	0.414
Population-by-treatment	1.969	5, 177	0.088	0.078
Model 2				
Treatment	630.729	1, 44.688	< 0.001	0.934
Family	1.744	43, 43	0.036	0.636
Family-by-treatment	1.786	43, 79	0.013	0.493

and family were still significant, but here significant family-by-treatment effects were also identified (Model 2; Table 2). Family-by-treatment effects suggest the potential for the evolution of plasticity, although significant family and family-by-treatment terms might also reflect tank effects related to variation in dissolved oxygen within treatments (see below).

In the univariate tests, treatment and body mass effects were highly significant for each gill metric. Gill metrics were larger under low oxygen, and gill size increased with an increase in body mass (Table 3). The overall variation between treatments relative to gill size under high oxygen, using body mass-standardized values, ranged from a low of 14.9% for the TP to a high of 37.5% and 55.9% for TGFL and THA, respectively. Family effects were also significant for all metrics except TP (Table 3). Population effects were never significant, although they were only marginally nonsignificant for TGFL and THA (Table 3). Population-by-treatment interactions were significant for TGFL, THA and average filament length (Table 3). These interactions were driven primarily by increased plasticity in Kamwenge and Kanyantale, which had the steepest reaction norms (Fig. 2). When these two populations were excluded from the analyses, population-by-treatment interactions were nonsignificant (results not shown). However, larger gills under low oxygen in these two populations might be a reflection of the slightly lower oxygen concentrations under which these populations were raised (Fig. 3).

In the within-treatment PCAs, two components with eigenvalues > 1 were extracted. For the low-oxygen treatment, the first component explained 72.4% of the variation in the data, and the second explained 20.3%. For the high-oxygen treatment, the first component explained 63.3% of the variation in the data and the second explained 29.4%. For both treatments, the first component was similar to that of the full dataset in that all gill metrics were strongly positively correlated with

Table 3 Results from ANCOVAs for F_1 *Pseudocrenilabrus multicolor* from multiple populations raised under high-oxygen or low-oxygen conditions in a laboratory-rearing experiment. The \log_{10} -transformed gill metrics were used as the response variables. The d.f. are the hypothesis degrees of freedom followed by the error degrees of freedom. η^2 = effect size.

Effect	F	d.f.	P	η^2
TGFL				
Population	2.338	5, 39.441	0.060	0.229
Treatment	1070.691	1, 116	< 0.001	0.902
Family (population)	2.790	38, 116	< 0.001	0.478
Population-by-treatment	2.747	5, 116	0.022	0.106
Body mass	214.598	1, 116	< 0.001	0.649
THA				
Population	2.382	5, 39.441	0.056	0.230
Treatment	1510.644	1, 116	< 0.001	0.929
Family (population)	2.211	38, 116	0.001	0.420
Population-by-treatment	3.320	5, 116	0.008	0.125
Body mass	267.806	1, 116	< 0.001	0.698
TNF				
Population	0.976	5, 39.441	0.444	0.108
Treatment	27.715	1, 116	< 0.001	0.193
Family (population)	1.801	38, 116	0.009	0.371
Population-by-treatment	0.954	5, 116	0.449	0.039
Body mass	51.070	1, 116	< 0.001	0.306
AFL				
Population	1.932	5, 39.441	0.111	0.197
Treatment	1173.552	1, 116	< 0.001	0.910
Family (population)	2.390	38, 116	< 0.001	0.474
Population-by-treatment	4.325	5, 116	0.001	0.157
Body mass	140.073	1, 116	< 0.001	0.547
TP				
Population	1.045	5, 39.441	0.405	0.113
Treatment	527.321	1, 116	< 0.001	0.820
Family (population)	1.436	38, 116	0.074	0.320
Population-by-treatment	1.058	5, 116	0.388	0.044
Body mass	229.801	1, 116	< 0.001	0.665

TGFL, total gill filament length; THA, total hemibranch area; TNF, total number of filaments; AFL, average filament length; TP, total perimeter.

component scores (Table 1). Scores from the second component were strongly positively correlated with the total number of filaments, positively correlated with the TP and negatively correlated with the average filament length (Table 1), suggesting that this component represents an elongation of the gills. Results from the ANCOVA using the oxygen covariate within the low-oxygen treatment revealed that gill size (PC1) decreased with increasing oxygen concentration (Table 4; Fig. 3), which is striking given the small variation in oxygen concentration among tanks (see Methods). After controlling for oxygen concentration in this analysis, population variation was no longer present under low-oxygen conditions. Variation among populations and families under low oxygen therefore probably reflects small variation in oxygen concentration among tanks. Interactions between population and oxygen concentration did not

have an effect on gill size, suggesting no variation in plasticity among populations (Table 4). Results from the ANCOVA using PC2 scores as the response variable, and from the ANCOVAs using data from the high-oxygen treatment, were always nonsignificant and are thus not reported.

Brain

Population and treatment effects on brain mass were strong, as was the population-by-treatment interaction (Table 5). The overall variation between treatments (relative to brain mass under high oxygen), using body mass-standardized values, was 10.3%. Family effects were nonsignificant, meaning that genetic variation for brain mass within populations, and tank effects within treatments, were not detected (Table 5). These results were similar regardless of whether the body mass covariate was included in the model (Table 5) or whether brain mass was standardized to a common body mass prior to the analysis (not shown). Brain mass increased with an increase in body mass (Table 5).

The test using the body mass-standardized values uncovered four homogenous subsets for the populations. Kamwenge and Kanyantale had the heaviest brains overall (i.e. averaged between treatments; $P = 0.831$); and Kahunge, Kantembwe and Bunoga had the lightest ($P = 0.311$). Other subsets included Kanyantale and Rwebakwata ($P = 0.242$) and Rwebakwata, Bunoga and Kantembwe ($P = 0.161$; Fig. 2). The populations with the most divergent slopes (i.e. reaction norms) for the relationship between brain mass and oxygen treatment included Kantembwe (steeper slope) and Kamwenge and Kanyantale (shallower slopes) (Fig. 2).

Results from the ANCOVA using the oxygen covariate within the low-oxygen treatment revealed that brain mass increased with increasing oxygen concentration (Table 6; Fig. 3). Population variation was present under low-oxygen conditions, but the interaction between population and oxygen concentration was not significant (Table 6). Under high-oxygen conditions, population variation was present when the interaction term (nonsignificant) was not present in the model, but the effects of oxygen concentration were never significant (results not shown). These results suggest that genetic variation in brain mass exists among populations after controlling for tank effects related to oxygen concentration.

Discussion

Our results revealed high levels of phenotypic plasticity for both gill size and brain mass in *P. multicolor*. However, differences among populations both in the size of these organs and in their plasticity differed between the gills and brain. Most notably, gill size and gill size plasticity varied little among populations, whereas brains were heavier and less plastic in two populations that were

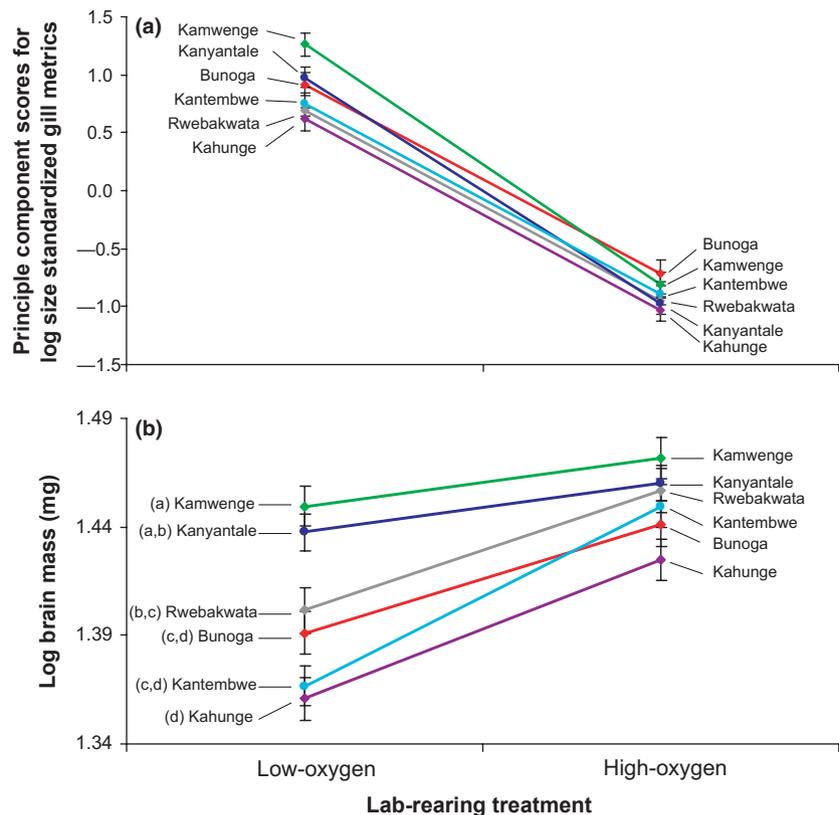


Fig. 2 Adjusted mean values (\pm standard error) from the mixed-model AN(C)OVAS including the population-by-treatment interaction, as described in the Methods, for F_1 *Pseudocrenilabrus multicolor* raised under high-oxygen or low-oxygen conditions in a laboratory-rearing experiment. The response variables were the principle component scores for the \log_{10} -transformed body mass-standardized gill metrics (a) and the \log_{10} -transformed brain mass (b). (Values for \log_{10} -transformed brain mass in panel b were standardized to a common body mass via the inclusion of \log_{10} -transformed body mass as a covariate in the ANCOVA.) Letters in panel b denote homogenous subsets in the Tukey *post hoc* test.

distant from the junction between the swamp and river. These differences might be because of variation in associated trade-offs, which occur when a change in size results in both costs and benefits to the organism. Evolution of increased or decreased size of these organs, or evolution of their plasticity, would therefore depend on the balance between costs and benefits. The brain has a variety of diverse functions including cognition, olfaction, vision, taste, mechanosensation and motor control (e.g. van Staaden *et al.*, 1994; Shumway, 2008; Gonzalez-Voyer *et al.*, 2009). Therefore, it might be beneficial to have a large brain, if energetically possible, regardless of the oxygen environment. Gills, on the other hand, have more limited functions, which include respiration and ion exchange (Palzenberger & Pohla, 1992; Nilsson, 2007). Costs of size differences (plastic or genetic) in some contexts might therefore be higher for the brain than for the gills. In addition, different traits might differ in their heritability (e.g. Mousseau & Roff, 1987; Houle, 1992; Roff & Gélinas, 2003), and in the heritability of their plasticity (e.g. Brommer *et al.*, 2005; Nussey *et al.*, 2005; Pelletier *et al.*, 2007; Winterhalter & Mousseau, 2007), so that local adaptation, or adaptive plasticity, might be more achievable for some traits than others. We therefore expected different patterns of evolution of gill and brain size, and gill and brain size plasticity, among populations from different localities.

Gills

Our results unequivocally show that gill size variation between high-oxygen and low-oxygen treatments is driven by direct environmental effects (i.e. phenotypic plasticity). Gill size varied with oxygen concentration even *within* the low-oxygen treatment. Any genetic differences in gill size are minimal compared to the overwhelming effects of plasticity. Of course, we did not directly test whether differences in gill size are adaptive, but a larger gill size increases the surface area for oxygen uptake from the environment (Hughes & Morgan, 1973), and thus it is intuitive that larger gills should be advantageous under hypoxic conditions. Smaller gills should be beneficial when large gills are not needed for enhanced oxygen uptake, because of spatial constraints in the head of the fish that could correlate with the reduced size of trophic structures (Chapman *et al.*, 2000, 2008), or because of osmoregulatory constraints that are associated with large gill surface area (Nilsson, 2007). We therefore conclude that the phenotypic variation in gill size in our experiment is most likely because of *adaptive* plastic responses.

High levels of gill size plasticity were observed in *P. multicolor* in other studies (Chapman *et al.*, 2000, 2008). Evidence for genetic variation in gill size was also detected in a study that examined *P. multicolor* populations

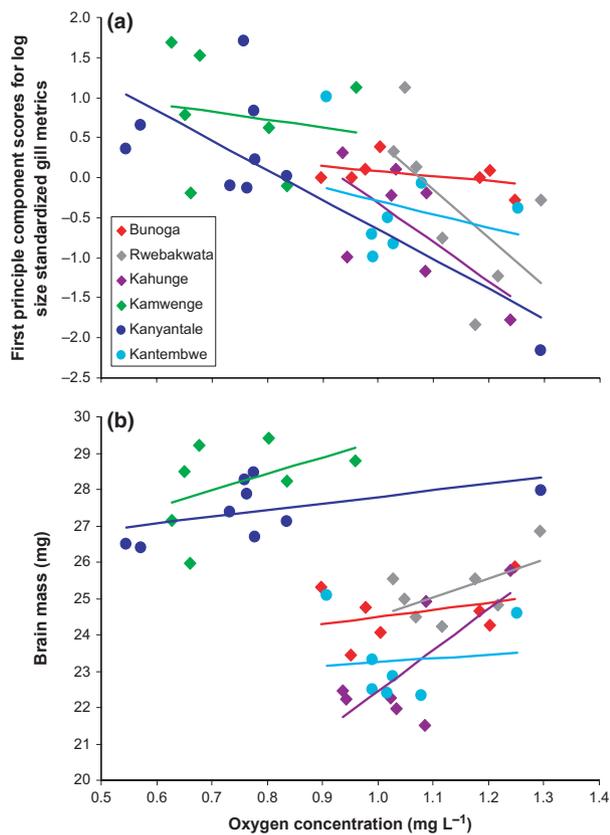


Fig. 3 Gill size and brain mass for *F₁ Pseudocrenilabrus multicolor* raised under the low-oxygen treatment, as a function of the average dissolved oxygen concentration (mg L^{-1}) under which fish were raised. Shown are the family means of the first principal component scores for \log_{10} -transformed body mass–standardized gill metrics (a), and the anti-logged values of the adjusted family means of the \log_{10} -transformed brain mass (b). (Values for \log_{10} -transformed brain mass in panel b were standardized to a common body mass via the inclusion of \log_{10} -transformed body mass as a covariate in the ANCOVA.) River populations are denoted by diamonds, and swamp populations are denoted by circles.

from a lake, a river and a swamp site that are disconnected from each other. However, population effects on gill size were weak relative to the environmentally induced changes, which is consistent with the present study (Chapman *et al.*, 2008). In the present study, local adaptation might have been constrained by gene flow (Crispo & Chapman, 2008, 2010). Although variation was observed among families, this effect was probably because of the slight variation in oxygen concentration among tanks within treatments. Thus, we conclude that genetic effects on gill size are small in this system.

We also observed little variation in plasticity among populations. One might expect higher levels of plasticity in temporally fluctuating environments, such as the river sites located adjacent to, or immediately downstream from, the swamp (Crispo & Chapman, 2008, 2010). Yet

Table 4 Results from ANCOVAs for *F₁ Pseudocrenilabrus multicolor* from multiple populations raised under low oxygen in a laboratory-rearing experiment. Family mean principle component scores for the first principle component for \log_{10} -transformed body mass–standardized gill metrics were used as the response variable, and the \log_{10} -transformed average oxygen concentration was used as a covariate. η^2 = effect size.

Effect	<i>F</i>	d.f.	<i>P</i>	η^2
Population	1.405	5	0.249	0.180
Oxygen	10.844	1	0.002	0.253
Population-by-oxygen	0.675	5	0.645	0.095
Error		32		

Table 5 Results from ANCOVAs for *F₁ Pseudocrenilabrus multicolor* from multiple populations raised under high-oxygen or low-oxygen conditions in a laboratory-rearing experiment. The \log_{10} -transformed brain mass was included as the response variable. The d.f. are the hypothesis degrees of freedom followed by the error degrees of freedom. η^2 = effect size.

Effect	<i>F</i>	d.f.	<i>P</i>	η^2
Population	12.515	5, 41,587	< 0.001	0.601
Treatment	81.255	1, 116	< 0.001	0.412
Family (population)	1.132	38, 116	0.303	0.270
Population-by-treatment	3.410	5, 116	0.007	0.128
Body mass	86.347	1, 116	< 0.001	0.427

Table 6 Results from ANCOVAs for *F₁ Pseudocrenilabrus multicolor* from multiple populations raised under low oxygen in a laboratory-rearing experiment. Adjusted family mean \log_{10} -transformed brain mass was used as the response variable, and the \log_{10} -transformed average oxygen concentration was used as a covariate. η^2 = effect size.

Effect	<i>F</i>	d.f.	<i>P</i>	η^2
Population	19.958	5	< 0.001	0.757
Oxygen	8.073	1	0.008	0.201
Population-by-oxygen	1.237	5	0.315	0.162
Error		32		

we have no evidence for adaptive divergence in plasticity of gill size among populations of *P. multicolor* from the Mpanga drainage. The earlier study on three populations of *P. multicolor* also found no evidence for higher levels of plasticity in the populations that experienced the most temporally fluctuating environment (Chapman *et al.*, 2008).

Low genetic divergence at microsatellite markers in the Mpanga River drainage suggests that either contemporary gene flow is high, or colonization was recent, followed by insufficient time for significant neutral divergence to occur (Crispo & Chapman, 2008, 2010). Gill size plasticity in *P. multicolor* might have facilitated colonization of the swamp environment, and/or contemporary gene flow between environments might result

in selection for plasticity in the meta-population. Positive effects of plasticity on colonization ability have been suggested in several natural systems (e.g. Yeh & Price, 2004; Wund *et al.*, 2008; Whiteley *et al.*, 2009). High levels of plasticity in gill size in *P. multicolor* might be because of low costs of plasticity (Edelaar *et al.*, 2005), in the absence of genetic drift (Masel *et al.*, 2007), following colonization. If plasticity facilitates colonization of new environments, it seems likely that plasticity could be adaptive in response to contemporary gene flow between environments (Crispo, 2008). Therefore, historical contingency and contemporary gene flow are both plausible reasons for the high levels of plasticity and the limited local adaptation in gill size observed in this system.

Brain

As with the gills, above, our results revealed plasticity in brain mass between high-oxygen and low-oxygen treatments and also *within* the low-oxygen treatment. Brains were heavier under high-oxygen conditions, presumably because an abundance of oxygen permitted the production of large brains, whereas limited oxygen restricted it. Significant plasticity was observed even within the two least plastic populations (Kamwenge and Kanyantale; results not shown). Brain size has been associated with oxygen availability in other laboratory-raised populations of *P. multicolor* (Chapman *et al.*, 2008) and in other fish species in the wild (Albert *et al.*, 1997; Chapman & Hulén, 2001). In vertebrates, the brain consumes approximately 2–8% of the total energy at rest, although the brain typically consists of < 2% of the total body mass (Mink *et al.*, 1981). In cave fishes (Amblyopsidae), a reduction in overall metabolic rate was more strongly related to a reduction in brain oxygen consumption than to a reduction in gill surface area or in muscle oxygen consumption (Poulson, 2001). Taken together, these observations suggest that the brain is a metabolically costly organ. We might therefore conclude that a reduction in brain size under low oxygen is adaptive, so that increased metabolic energy can be allocated to other functions. However, this reduction might also reflect an inability to develop adequately when resources are restricted (i.e. 'passive' plasticity; van Kleunen & Fischer, 2005).

As outlined in our predictions, large brains might have evolved in the Kamwenge population because it likely experiences high-oxygen conditions year-round and therefore is not subject to restrictions in oxygen that might preclude the development of a large brain. Also, this population probably experiences increased competition and predation relative to the other populations (L. Chapman, personal observation); and therefore, larger brains might be needed for increased cognition to acquire resources and escape predation (reviews: Kotrschal *et al.*, 1998; Shumway, 2008). The Kanyantale site, on the other hand, is probably chronically hypoxic year-round,

as are other sites that we sampled throughout the year within this swamp. We might therefore have predicted the evolution of *smaller* brains for increased energy allocation for other functions; yet our results did not match this prediction. The presence of large brains in this population might be made possible via the evolution of other traits that allow for enhanced oxygen acquisition and utilization, so that metabolic oxygen for the brain is not limited. These might include physiological mechanisms that were not considered in the present study. For example, *P. multicolor* sampled from a hypoxic *Miscanthidium* swamp had increased haematocrit and lactate dehydrogenase activity relative to those sampled in a nearby normoxic lake (Martínez *et al.*, 2009). Local adaptation of these physiological mechanisms might be restricted in the populations near the junction between the river and swamp because of gene flow between the two environments. Because the brain is used for a wide variety of functions, such as cognition and social interactions (van Staaden *et al.*, 1994; Kotrschal *et al.*, 1998; Shumway, 2008; Gonzalez-Voyer *et al.*, 2009), it might be beneficial for fish to produce large brains when possible.

In addition to variation in brain mass among populations, we also uncovered variation in brain mass *plasticity*. Higher levels of brain mass plasticity observed in populations near the junction between the river and swamp could be because of gene flow between high-oxygen and low-oxygen environments. Gene flow might result in selection for increased phenotypic plasticity if the development of small brains is generally adaptive under hypoxia and the development of large brains is generally adaptive under normoxia, all else being equal. A positive role for gene flow on plasticity has also been invoked in lake-stream stickleback (*Gasterosteus aculeatus*). Gene flow in a lake-stream stickleback system is unidirectional, with gene flow occurring from a lake into an outlet stream (Hendry *et al.*, 2002). Differences in trophic morphology and body shape are observed between the relatively isolated inlet stream and the lake populations, but in the outlet stream population, local adaptation is constrained by gene flow from the lake (Hendry *et al.*, 2002; Moore & Hendry, 2005). Differences in body shape between the inlet and lake have a strong genetic basis, but the differences in the outlet are largely driven by plasticity (Sharpe *et al.*, 2008). Thus, plasticity might evolve when gene flow is high, if the most plastic individuals dispersing into a new environment have increased fitness relative to nonplastic individuals. Even if plasticity was developmental (i.e. the phenotypic trajectory is determined during development and is irreversible), it could be advantageous if juveniles disperse, or if the plastic response increased fitness in the offspring of dispersing individuals (Crispo, 2008).

It remains possible that different patterns for the brain in two populations (Kamwenge and Kanyantale) are because of year effects, perhaps associated with flooding that occurred between sampling years (Crispo & Chapman,

2010). That is, environmental variation between years might have selected individuals with larger and less plastic brains. Temporal variation in both the direction and form of selection is widespread in nature (Siepielski *et al.*, 2009). If a selective sweep had occurred, we would predict (1) lower within-population variation in our second sampling year than in our first, and (2) variation in the second year that falls within the range of values for the first year. In our laboratory-rearing experiment, the observed variation in brain mass within populations was not substantially lower for the two populations raised in the second year (Kanyantale and Kamwenge; Fig. 3b). Also, brain mass for Kanyantale and Kamwenge fell outside of the range observed for the other populations at a given oxygen concentration. This was true for values observed under the low-oxygen treatment, where most of the variation in brain mass occurred (Figs 2b and 3b). Therefore, the spatial variation in brain mass observed in the present study reflects actual geographical variation. In terms of brain mass *plasticity*, however, the above patterns are less clear. Specifically, the variation in plasticity for Kanyantale and Kamwenge families fell within the range of variation for the other four populations. Also, the within-population variation in plasticity was lower for Kamwenge than for the other populations (data not shown; available upon request from EC). Therefore, it is difficult to discern spatial patterns from temporal patterns of selection on brain mass plasticity.

Although we documented plastic and genetic differences in overall brain mass, we have not measured changes in the relative size of different components of the brain. Differences among environments in not only oxygen concentration, but also other selective pressures, such as competition, predation, prey availability and habitat structural complexity, might differentially affect brain components (Kotrschal *et al.*, 1998; Shumway, 2008; Gonda *et al.*, 2009a). As an example, differences in both the size and the *plasticity* of size of different brain components were observed between marine and pond populations of nine-spined sticklebacks (*Pungitius pungitius*), which might reflect differences in habitat complexity in the home environments (Gonda *et al.*, 2009a,b). As another example, differences in the size of the telencephalon were observed between two lake populations of an East African cichlid (*Astatoreochromis alluaudi*), and this brain component was smaller under low oxygen than under high-oxygen conditions in a laboratory-rearing experiment (C. Robineau & L. Chapman, unpublished data). Future study should focus on intraspecific differences in brain components and relate these differences to multiple selective pressures imposed by the environment.

Conclusions

Although most studies of adaptation in natural systems find that phenotypic variation is explained by a combi-

nation of genetic and plastic responses (reviewed by: Price *et al.*, 2003; Pigliucci *et al.*, 2006; Crispo, 2007; Ghalambor *et al.*, 2007; Crispo, 2008), some studies have found that all of the variation in some traits is because of plasticity (e.g. Charmantier *et al.*, 2008; Neufeld & Palmer, 2008). Plasticity can be used in some cases as a strategy for adaptation to alternate selective regimes. This strategy might be particularly likely in cases where meta-population structure (i.e. high gene flow) is present across environmental gradients. Yet, in other systems, local adaptation can be prevalent even when organisms are phenotypically plastic and gene flow between selective regimes is high (e.g. Jiménez-Ambriz *et al.*, 2007; Magalhaes *et al.*, 2009), or gene flow can be restricted between selective regimes even when plasticity is prevalent (e.g. Tobler *et al.*, 2008). The evolution of plasticity should depend on its relative costs and benefits (DeWitt *et al.*, 1998; Van Buskirk & Steiner, 2009), which should differ among traits. We did find stronger evidence for genetic divergence in brain mass and its plasticity than in gill size and its plasticity, which might reflect differences in the costs and benefits of alternate forms (large vs. small, plastic vs. nonplastic) of these two organs. Additional studies will provide insight into how common the evolution of plasticity is as an adaptive strategy, and the conditions under which it is expected to evolve.

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