Repeated Divergence in Opsin Gene Expression Mirrors Photic Habitat Changes in Rapidly Evolving Crater Lake Cichlid Fishes

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ABSTRACT: Selection pressures differ along environmental gradients, and traits tightly linked to fitness (e.g., the visual system) are expected to track such variation. Along gradients, adaptation to local conditions might be due to heritable and nonheritable environmentally induced variation. Disentangling these sources of phenotypic variation requires studying closely related populations in nature and in the laboratory. The Nicaraguan lakes represent an environmental gradient in photic conditions from clear crater lakes to very turbid great lakes. From two old, turbid great lakes, Midas cichlid fish (*Amphilophus* cf. *citrinellus*) independently colonized seven isolated crater lakes of varying light conditions, resulting in a small adaptive radiation. We estimated variation in visual sensitivities along this photic gradient by measuring cone opsin gene expression among lake populations. Visual sensitivities observed in all seven derived crater lake populations shifted predictably in direction and magnitude, repeatedly mirroring changes in photic conditions. Comparing wild-caught and laboratory-reared fish revealed that 48% of this phenotypic variation is genetically determined and evolved rapidly. Decreasing intrapopulation variation as environments become spectrally narrower suggests that different selective landscapes operate along the gradient. We conclude that the power to predict phenotypic evolution along gradients depends on both the magnitude of environmental change and the selective landscape shape.

Keywords: local adaptation, phenotypic evolution, environmental gradient, sensory ecology, visual sensitivity.

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Introduction

Abiotic factors are widely recognized selective agents influencing biological diversity by affecting fitness and thus driving evolution by natural selection (Haldane 1948; Endler 1986; Schluter 2000; Maccoll 2011). Evolutionary theory predicts that as long as enough genetic variation for a trait exists, phenotypes under selection would be expected to match local optima, particularly in the absence of gene flow (Fisher 1930; Williams 1966; Kawecki and Ebert 2004). Most commonly, local adaptation has been studied focusing on the ends of the environmental continuum in a dichotomous way (e.g., Reznick and Endler 1982; Girvan and Braithwaite 1998; Filchak et al. 2000; Hoekstra et al. 2004; Barrett et al. 2008; Tobler et al. 2018). Although analyzing extreme habitats increases the power to detect adaptation, it might not suffice to explain the range of phenotypic variation observed across natural populations (Becker et al. 2006; Hereford and Winn 2008; Riesch et al. 2018). For instance, habitat conditions that vary in a continuous manner might result in gradual variation for selected traits (Huxley 1938; Endler 1977; Sotka 2008). Several classic studies have identified local adaptation along environmental gradients in different taxonomic groups (Clausen et al. 1948; McNeilly 1968; Bishop 1972; Sand et al. 1995; Huey et al. 2000; Mullen and Hoekstra 2008). Leveraging such systems allows us to test how closely organisms track environmental changes and contributes to broadening our understanding about the predictability of evolutionary outcomes (Blount et al. 2018; Losos 2018; Nosil et al. 2018). In theory, adaptive phenotypic evolution should mirror environmental pressures and facilitate contemporary evolution, especially in the presence of geographic barriers, where gene flow cannot counteract the effect of selection (García-Ramos and Kirkpatrick 1997).

The visual system is a particularly fitting trait to study evolution by natural selection given its crucial role in fitness-related tasks, such as mate choice, foraging behavior, predator avoidance, and conspecific recognition (Cronin et al. 2014). In particular, aquatic organisms are often exposed to an especially wide range of photic conditions (Kirk 2010). When traveling through water, light is strongly attenuated over short depths and depleted of certain wavelengths. This creates a diversity of photic environments found across aquatic ecosystems (Loew and McFarland 1990; Partridge and Cummings 1999). Therefore, aquatic organisms are a good candidate to study how visual systems respond to spectral variability, a key abiotic factor that can be quantified and compared across environments (Carleton et al. 2020). By addressing opposite ends of the photic continuum, several studies in fish have reported divergence in the visual system between different light conditions (Fuller et al. 2004; Spady et al. 2005; Rennison et al. 2016; Hahn et al. 2017; Marques et al. 2017; Torres-Dowdall et al. 2017). In those studied cases, vision is tuned in a discrete manner by structural changes in key proteins or by regulating gene expression in response to different photic conditions. In contrast, studying a broader range of photic conditions allows testing how predictably phenotypes change in response to gradual environmental variation.

Color vision is initiated in response to light in cone photoreceptor cells found in the retina. Cone photoreceptors contain visual pigments, light-sensitive molecules consisting of an opsin protein bound to a chromophore (Bowmaker 1990). The amino acid residues of the opsin protein and the type of chromophore determine the probability of a visual pigment absorbing a photon of a given wavelength, in other words, the spectral sensitivity of the photoreceptor (Wald 1939; Davies et al. 2012). Therefore, the ability of organisms to detect color relies in the first place on the expression of cone opsin genes with different spectral sensitivities across photoreceptor cells (Lythgoe 1979; Johnsen 2012; Cronin et al. 2014; Schweikert et al. 2018; Carleton et al. 2020; Musilova et al. 2021). The wavelength-dependent photoreceptor signals are then processed downstream by bipolar and ganglion cells and finally in the brain to generate color perceptions (Baden and Osorio 2019; Baden 2021). Despite the complexity of the neural processes associated with color vision, the association between retinal expression of certain cone opsin genes and the overall visual sensitivity of an organism have been extensively studied (Cronin et al. 2014; Carleton and Yourick 2020; Baden 2021). For instance, sensitivity to ultraviolet light (UV) was described in several species on the basis of behavioral experiments before the expression of UV-sensitive opsin genes (*sws1*) could be reliably quantified, and the physiological relevance of *sws1* has been experimentally linked to foraging performance (Cronin and Bok 2016; Novales Flamarique 2016).

Similar associations have been made for sensitivity to long-wavelength regions of the spectrum and the expression of red-sensitive cone opsin genes (Smith et al. 2012; Sakai et al. 2018). The relationship between spectral sensitivity and the relative expression of cone opsin genes in the retina has also been demonstrated via electrophysiological measurements (Sabbah et al. 2010). Furthermore, measurements of retinal opsin gene expression using quantitative real-time polymerase chain reaction (qPCR) have been shown to reflect the number of photoreceptors in each spectral class using RNA fluorescence in situ hybridization and to be correlated with RNA sequencing data (Härer et al. 2018; Karagic et al. 2018). Hence, while the detailed mechanisms involved in visual sensitivity are still a matter of ongoing research, there is a logical link among retinal opsin gene expression, the spectral sensitivity of the cone photoreceptors, and the overall visual sensitivity of the retina.

Due to both gene gains and losses and sequence divergence, the so-called visual opsins within the opsin gene family vary substantially across lineages, resulting in spectral sensitivities that extend over much of the visible spectrum (Yokoyama 2000). Among vertebrates, teleost fishes have a highly diverse number of visual opsins, with a median of six cone opsin genes found in their genomes (Yokoyama 2008; Musilova et al. 2019). Cichlid fishes (family Cichlidae) typically possess seven cone opsin genes—three short-wavelength sensitive ones expressed in single cones (*sws1*, *sws2a*, *sws2b*) and four mid- to long-wavelength sensitive ones expressed in double cones (*rh2b*, *rh2aα*, *rh2aβ*, *lws*)—although not all of these genes are usually expressed simultaneously. In lineages of African cichlids, opsin genes seem to be expressed in fixed combinations (i.e., visual palettes; Carleton et al. 2010), with visual sensitivities being discretely distributed in short-wavelength (*sws1*, *rh2b*, *rh2a*), middle-wavelength (*sws2b*, *sws2a*, *rh2a*), or long-wavelength (*sws2a*, *rh2a*, *lws*) clusters (Hofmann et al. 2009). The modularity of the palettes contrasts with visual sensitivities in some Neotropical cichlids, where opsin expression is not compartmentalized into palettes but seems to be more tunable (Torres-Dowdall et al. 2021). Given that visual sensitivities represent an integrated phenotype composed of a limited number of opsins, the distinct modularities described so far should mirror environmental gradients in different ways. If visual sensitivities are constrained, modularity should result in a limited number of phenotypes along gradients (Hofmann et al. 2009; O'Quin et al. 2010). Instead, gradual phenotypic variation might be expected if visual sensitivities are not restricted to the modular expression of opsin genes (e.g., Torres-Dowdall et al. 2021). The degree to which visual sensitivities can be tuned will inevitably shape the variation available for natural selection to act on and thus the phenotypic range in the wild.

A useful framework to study visual sensitivities in the wild is referred to as the "sensitivity hypothesis," which states that organisms' retinal sensitivities should be optimized to maximize sensitivity to the predominant background spectral regions (Bayliss et al. 1936; Clarke 1936; Crescitelli et al. 1985). Under this hypothesis, natural selection might favor visual sensitivities that match the local photic environment consequently shaping phenotypic variation (Lythgoe 1979; Bowmaker 1990;Cronin et al. 2014). Furthermore, while spectrally narrow environments have only a small subset of wavelengths available for visual systems to exploit, broadband environments offer more spectral regions for visual sensitivities to diversify (Loew 1995; O'Quin et al. 2010; Carleton et al. 2016). As an analogy, one could regard photons as a resource available for pigments to be exploited (Stomp et al. 2007), where broadband photic environments represent multiple photon-rich spectral regions that contrast with the resource-poor tightly allocated photons in narrow conditions. In this case, increased diversity of visual sensitivities would be expected in broadband environments, given that multiple wavelengths can be matched by phenotypes (McFarland and Munz 1975b). In contrast, spectrally narrow photic conditions should limit the evolution of phenotypes around one single "sensitivity optimum." While phenotypic evolution might be easier to predict when a reduced number of phenotypes are clearly favored, multiple simultaneous selective pressures might obscure determinism in variable heterogeneous environments (Bell 2010; Nosil et al. 2018; Reimchen and Bergstrom 2023).

Here, we investigate the role of photic environments as drivers of phenotypic evolution in visual sensitivities along a photic gradient placed within a natural experiment. For this, we measured the difference in photic conditions between great and crater lakes and determined the divergence in visual sensitivity between pairs of source and derived populations of wild-caught and laboratory-reared Midas cichlid fish (*Amphilophus* cf. *citrinellus*) to test the following hypotheses: (1) phenotypic changes in the visual system of Midas cichlids are in the same direction as changes in photic conditions and thus are potentially adaptive; (2) the degree of phenotypic change is correlated with the degree of environmental change; and (3) most of the variation in visual sensitivity can be explained by the photic conditions at the lake of origin rather than by rearing condition (i.e., there is a strong genetic component). Overall, we provide evidence that visual sensitivities vary continuously by fine-tuning the expression of opsin genes and that their evolution is consistent with the sensitivity hypothesis. We show that while visual sensitivities match photic conditions in a predictable manner, their phenotypic range within a given habitat is influenced by environment-specific selection regimes, with spectrally narrow habitats exhibiting reduced intrapopulation phenotypic variation.

Material and Methods

Study Design

In Nicaragua, a natural experiment occurred, where from a common source population in the great lakes Managua and Nicaragua seven isolated crater lakes were independently colonized by Midas cichlid fish (*Amphilophus* cf. *citrinellus*) between 4,700 to 800 years ago (Barluenga et al. 2006; Kautt et al. 2016, 2020). The young radiation of Midas cichlids currently encompasses 13 nominal species characterized by genomic and morphological differentiation among lakes, with sympatric and allopatric species showing divergence in traits related to lip size, body shape, pharyngeal morphology, or body coloration (fig. 1*A*; Torres-Dowdall and Meyer 2021). Given the isolated nature of the crater lakes, their colonization from common source populations and their geomorphological similarities, crater lakes have been considered as natural replicates regarding many of their environmental factors (Kautt et al. 2018). However, photic conditions differ widely among Nicaraguan lakes. The great lakes are big and shallow, and the winds create waves that constantly stir up the sediments, making them very turbid (Elmer et al. 2010). Similar light conditions can be found in River San Juan, a major river connected to Lake Nicaragua that is also inhabited by Midas cichlids. In contrast, the crater lakes are very deep, and thus sediments are deposited at depths far from the influence of waves; this might contribute to the observation that most crater lakes are clearer than the great lakes, but there is still substantial variation among crater lakes (Torres-Dowdall and Meyer 2021). Studies of the visual ecology of the two oldest crater lakes, Apoyo and Xiloá, found convergent changes in the visual system of these populations regardless of their color morphs, suggesting a strong effect of the ambient light environment (Torres-Dowdall et al. 2017; Härer et al. 2018). However, given the diversity of photic environments found among crater lakes, their population isolation (i.e., absence of gene flow), and their recent and independent colonization from a common source, the system provides an excellent opportunity to investigate the predictability of phenotypic evolution in visual systems along a wide range of photic conditions by asking the following question: how closely do visual systems track local conditions along a natural gradient?

Characterization of the Photic Environments

To characterize the photic conditions found among the Nicaraguan lakes, underwater irradiance was measured from both great lakes, Managua and Nicaragua; seven neighboring crater lakes; and one riverine population (fig. 1*B*). Absolute irradiance was measured using a spectrometer (FLAME-S-XR1-ES; Ocean Insight, Orlando, FL) connected to a 25-m
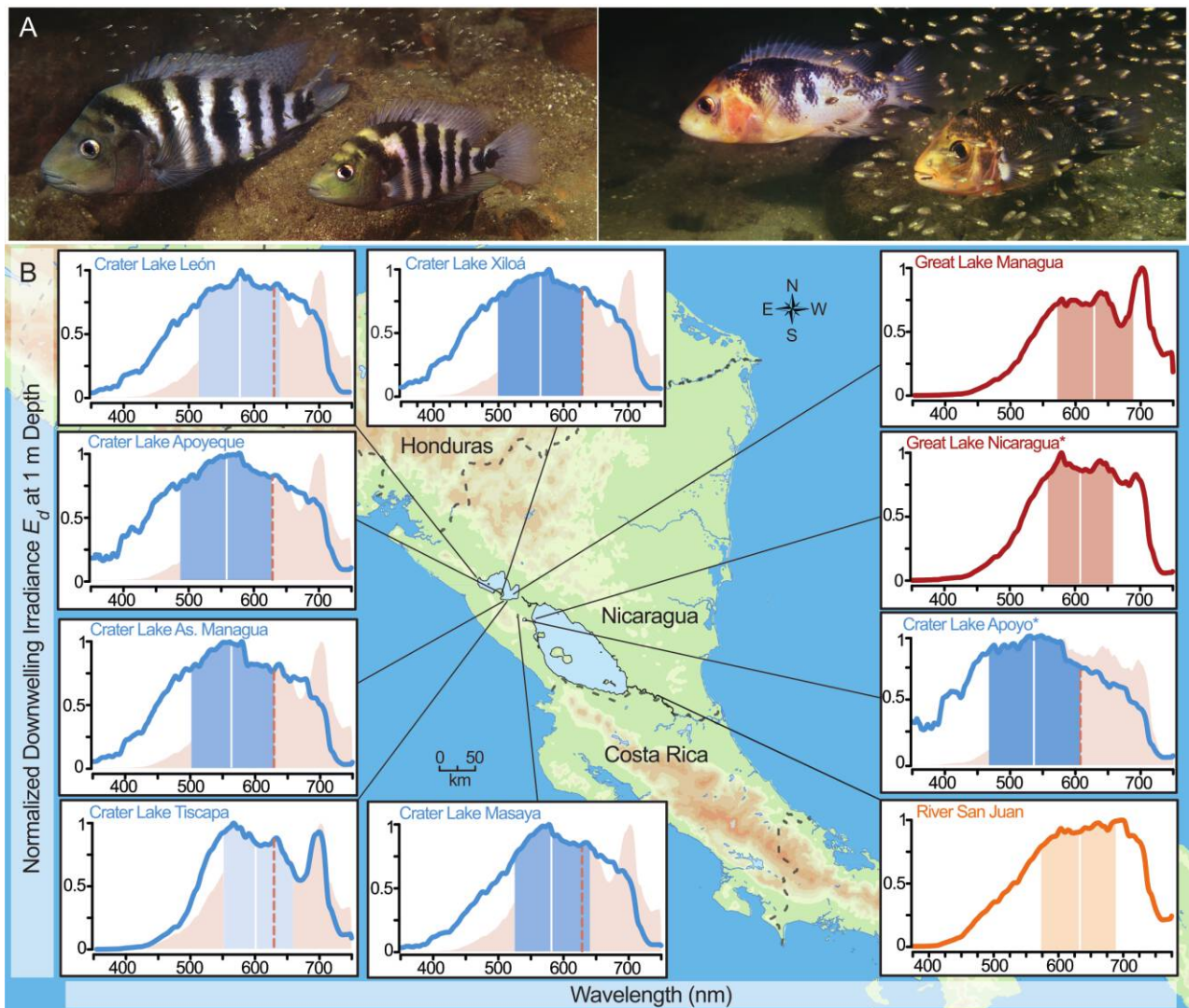
Figure 1: *A*, Breeding pairs of dark (*left*) and gold (*right*) color morphs of *Amphilophus sagittae* (photos by Ad Konings). *B*, Map showing the Nicaraguan great lake populations, crater lake populations, and one riverine population. Inserts depict the photic environment at a depth of 1 m, with spectral curves showing the normalized downwelling irradiance (E_d). Vertical solid lines below each spectral curve represent the spectrum-halving wavelength (λP_{50}) within the colored area depicting the spectral bandwidth and its color intensity representing the relative luminosity (%E_d). The shaded area in the background of the crater lake inserts shows the photic environment plus λP_{50} (dashed line) of the respective source great lake.

UV-VIS optical fiber (OCF-104472; Ocean Insight) with a cosine corrector (CC-3-UV-S; Ocean Insight). Multiple consecutive measurements during daytime between 10 a.m. and 2 p.m. were performed at 0.15-, 1-, 3-, 5-, 10-, 15-, 20-, and 25-m depths. Sites with a depth less than 25 m were measured until their deepest point. The measurements were performed by orienting the sensor upward (downwelling irradiance, E_d), sideways (sidewelling irradiance, E_s), and downward (upwelling irradiance, E_u). Absolute irradiance measurements were corrected for integration time and

converted to E (photons/cm²/s/nm) on the basis of Johnsen (2012), as follows:

$$E = W(\lambda)\left(\frac{1}{h \cdot c}\right), \tag{1}$$

where W represents the irradiance in energy units (W/m²/nm) at each wavelength λ, h represents Planck's constant (m²·kg/s), and c is the speed of light (m/s). To minimize the effect of outliers due to handling of the spectrometer,

the median absolute irradiance of 3–10 measurements for each depth was used and smoothed using a rolling mean over 5 nm following the manufacturer's instructions (http://oceaninsight.com; fig. S1; figs. S1–S11 are available online). Only wavelengths within the visible spectrum (350–700 nm) were used, based on the peak sensitivity of visual pigments in fish (Rennison et al. 2016; Carleton et al. 2020). To allow the comparison of spectral shape across sites, absolute spectra were divided by their respective maximal value, resulting in normalized irradiance (fig. 1*B*).

Opsin Gene Expression

To determine the degree of variation in cone opsin expression in populations of Midas cichlids, six to eight wild-caught adult fish per site were collected in January and February 2018 from 10 locations across Nicaragua for a total of 78 individuals (table S1; tables S1–S6 are available online). In the rest of the text, the term "population" is used to refer to this level of sampling (i.e., locations), although some of the sampled populations correspond to formally described species within the Midas cichlid radiation. Additionally, 62 laboratory-reared adults from nine populations raised for at least two generations in the animal research facility at the University of Konstanz were included in our study. These laboratory experiments were done to measure phenotypic variability in the absence of developmental noise due to the different light conditions fish experience in the wild. Only sexually mature fish (at least 2 years old) were used given that opsin gene expression varies during ontogeny, eventually reaching a developmental plateau at adulthood (Härer et al. 2017, 2019). Fish were sampled during the same daytime period (11 a.m. to 3 p.m.) to control for diurnal variation in gene expression (Yourick et al. 2019), and they were euthanized by applying an overdose of MS-222 and subsequent cervical dislocation. The retinas were removed and stored in RNAlater (Sigma-Aldrich, Burlington, MA) at $-20°C$ until extraction. RNA was extracted using a standard TRIzol-chloroform protocol based on Rio et al. (2010). For each sample, 200 ng of total RNA was used to synthesize first-strand complementary DNA using the manufacturer's protocol (GoScript Reverse Transcription System; Promega, Madison, WI). Gene expression of six cone opsin genes (*sws1*, *sws2b*, *sws2a*, *rh2aβ*, *rh2b*, and *lws*) and two reference genes (*gapdh* and *imp2*) was measured using qPCR for 40 cycles (CFX96; Bio-Rad Laboratories, Hercules, CA) following Härer et al. (2017). Expression of the paralog *rh2aα* was not measured since it is not expressed in Midas cichlids (Torres-Dowdall et al. 2017). Mean threshold cycle (Ct) values from three technical replicates were used for analysis. Primer sequences, amplification efficiencies, and mean expression of reference genes are reported in the supplemental PDF (tables S2, S3; fig. S3).

Proportional opsin expression for each individual was calculated as the amount of each cone opsin (T_i) relative to the total cone opsin expression (T_{all}), as in Fuller et al. (2004):

$$\frac{T_i}{T_{all}} = \frac{1/\left((1 + E_i)^{Ct_i}\right)}{\sum \left(1/\left((1 + E_i)^{Ct_i}\right)\right)}, \qquad (2)$$

where E_i is the efficiency of primer i, Ct_i is the critical cycle number for gene i, and the overall sum of the proportional expressions of the six opsin genes equals 1. To test for divergence among lakes due to photic conditions, opsin expression was analyzed using ANOVA (type II) with lake of origin as the predictor variable and proportional opsin expression as the response variable. When significant, pairwise comparisons were analyzed using Tukey's honestly significant difference post hoc test.

Testing the Association between Photic Changes and Sensitivity Shifts

To determine whether changes in the visual system between ancestral and derived populations are correlated with differences in the photic conditions between the source and the derived habitats, we took advantage of our knowledge about the demographic history of Midas cichlids (Kautt et al. 2020). Extensive population genomic analyses showed that crater lake Apoyo was colonized from great lake Nicaragua and that all other crater lakes were colonized from great lake Managua. While the ancestry of crater lake Masaya is admixed, great lake Managua is considered its main population source for analysis in this study because of the small contribution of great lake Nicaragua (~22%; Kautt et al. 2018, 2020). We asked whether photic habitat differences drive the phenotypic divergence seen between source and derived populations of Midas cichlids. For this, we calculated the degree of correlation between spectral attenuation coefficients (K_d) and estimated spectral sensitivity curves (ΔSSC_j). Spectral attenuation coefficients represent the extinction of ambient downwelling light with depth and are less prone to background noise (e.g., atmospheric events, waves) than irradiance, making it a more robust estimate of the spectral characteristics of water bodies (Mobley 1994; Rennison et al. 2016). The localized spectral attenuation coefficient, K_d, was calculated on the basis of Sabbah et al. (2011), as follows:

$$K_d(\lambda) = \frac{1}{z}\ln[E_{d,z}(\lambda) - E_{d,0}(\lambda)], \qquad (3)$$

where $E_{d,z}$ is the downwelling irradiance at depth z (1 m in this study) and $E_{d,0}$ is the downwelling irradiance 15 cm below the water surface for each wavelength λ ranging from 350 to 750 nm (fig. S4). Next, we determined the change in predicted visual sensitivity experienced by fish

from derived populations compared with the source populations. For this, the individual continuous estimates of visual sensitivity, the spectral sensitivity curves (SSC$_j$) were estimated for each specimen following Rennison et al. (2016). In short, absorbance templates from Govardovskii et al. (2000) and absorption peaks from Torres-Dowdall et al. (2017) for each opsin were used. We simulated scenarios assuming either only A$_1$ or only A$_2$ chromophore usage. The sensitivity curve was then weighted by the proportional expression of each opsin, and the sensitivity curves of the six expressed opsins were added (fig. S5). Subsequently, shifts in spectral sensitivity curves (ΔSSC$_j$) were estimated as the difference between the median spectral sensitivity of the source population and the individual spectral sensitivities of each fish in the derived populations, as in Rennison et al. (2016):

$$\Delta SSC_j(\lambda) = \left[\tilde{x}_S(\lambda)\right]_{\text{Source}} - \left[SSC_j(\lambda)\right]_{\text{Derived}}, \quad (4)$$

where SSC$_j$ is the individual spectral sensitivity curve. Finally, we ran Pearson's correlation tests between shifts in spectral sensitivity (ΔSSC$_j$) and changes in attenuation coefficients (ΔK_d) for each individual. Similar analyses but using changes in spectral irradiance (ΔE_d) instead of ΔK_d are reported in the supplemental PDF (fig. S6). All P values were corrected for multiple testing using the Benjamini-Hochberg false discovery rate method (Benjamini and Hochberg 1995). All statistical analyses were performed in R (R Core Team 2020).

Predictability of Visual Sensitivity Based on Photic Conditions

To test whether the spectral sensitivity of fish can be predicted on the basis of their local photic conditions, we used point estimates for visual sensitivity and regressed on a composite axis of the photic conditions at their lake of origin at a depth of 1 m. This depth was chosen as a compromise to include all sites (shallow habitats: depth of ~2 m) and in agreement with the habitat ecology of Midas cichlids (Oldfield et al. 2006; Dittmann et al. 2012). The composite axis was generated using correlation-based principal component analysis (PCA) of seven z-standardized variables. We used down- and sidewelling λP_{50} as the spectrum-halving wavelength that summarizes the photon distribution into a single value indicating short- or long-wavelength predominant spectra (McFarland and Munz 1975a). We also included λP_{25} and λP_{75}, the wavelengths within which 50% of the photons are found (i.e., spectral broadness). Finally, the percentage of downwelling photons available at 1 m compared with 15 cm below the water surface, an estimate of luminosity %E_d, was also included in the PCA (table S4). The response variable predicted sensitivity

index (PSI) was defined as the sum of peaks in absorption of each opsin weighted by its proportional expression in the retina (Hofmann et al. 2009) and calculated using the following equation:

$$\begin{aligned} PSI_j = {} & PE_{sws1} \times 360 \text{ nm} + PE_{sws2b} \times 440 \text{ nm} \\ & + PE_{sws2a} \times 466 \text{ nm} + PE_{rh2b} \times 500 \text{ nm} \quad (5) \\ & + PE_{rh2a\beta} \times 555 \text{ nm} + PE_{lws} \times 610 \text{ nm}, \end{aligned}$$

where PE is the proportional cone opsin expression for each individual using opsin absorbance peaks assuming A$_2$ chromophore usage from Torres-Dowdall et al. (2017).

To determine the genetic component of the phenotypic variation found in the visual system of Midas cichlids, we also estimated the PSI of individuals reared in the laboratory. Given that phenotypic divergence in the wild could also be mediated by environmentally induced changes, phenotypic variation measured under common garden conditions informs about its genetic component. We used a linear mixed effects model to determine the percentage of phenotypic variation that is explained by native photic conditions independently of rearing conditions. The model considered the PSI as the response variable and photic environment (PC1), rearing environment (i.e., wild or lab), and their interaction as predictor variables (fixed effects); lake of origin was used as a random intercept. The relative importance of each regressor to the amount of explained variance was estimated on the basis of Stoffel et al. (2021). Confidence intervals (CIs) for mean regression lines accounting for the standard error of the regression line and intercept on each population were calculated following Breheny and Burchett (2017). To account for potential bias in predicting spectral sensitivity from gene expression data, a sensitivity analysis to assess robustness of predictors and outcome to unobserved confounding factors was performed as in Cinelli and Hazlett (2020). Additionally, we regressed the coefficients of variation in our estimates of visual sensitivity (PSI) within each population against the photic axis (PC1) to test whether phenotypic variation is reduced in spectrally narrow environments. Diagnostic plots are provided in the supplemental PDF (figs. S10, S11).

Results

The Photic Gradient along the Nicaraguan Great and Crater Lakes

The photic gradient of the Nicaraguan lakes consists of a composite axis that ranges from broad, short-wavelength-shifted, and bright crater lakes to narrow, long-wavelength-shifted, and dim great lakes (fig. 1; table S4). The PCA including all photic parameters at a depth of 1 m identified one main axis explaining most of the variation (PC1 = 93%) and was mainly driven by photon distribution of both
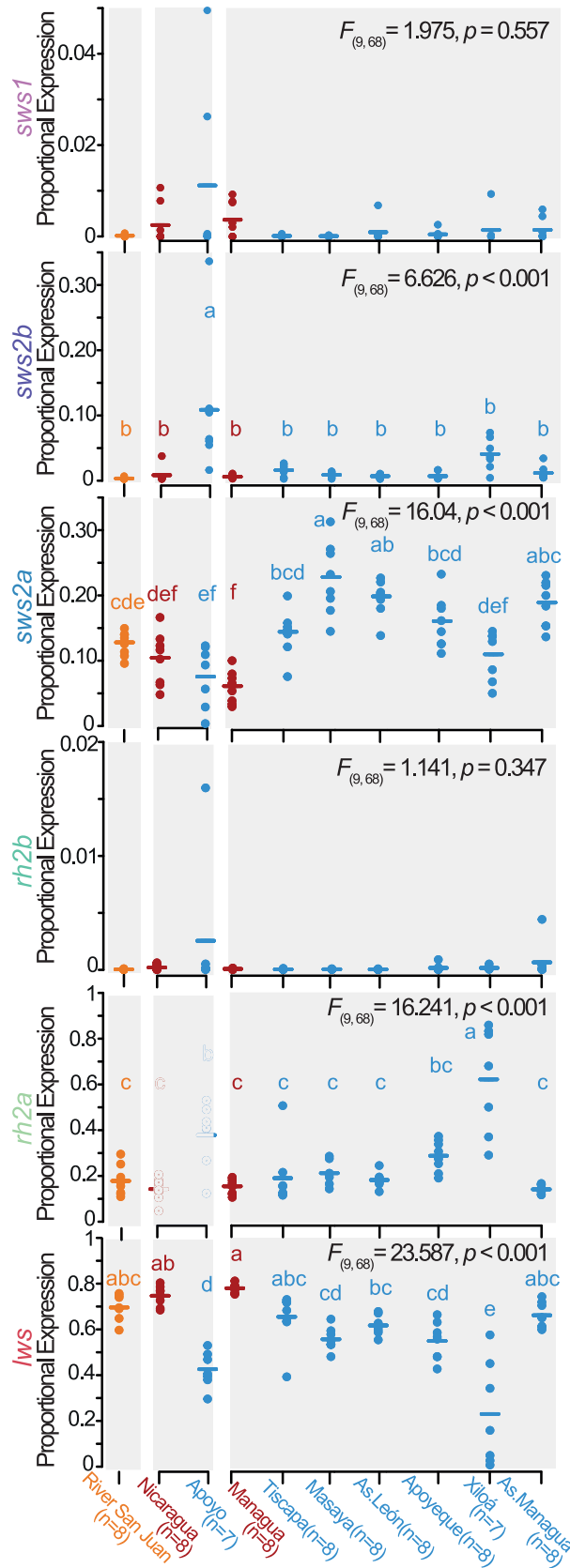
down- and sidewelling irradiance (fig. S7). In contrast, PC2 explained only 4.86% of the variance in photic conditions and was mainly driven by relative downwelling luminosity (table S4). Within the photic gradient (e.g., PC1), crater lake Apoyo represents the most blue-shifted environment, followed by crater lakes As. Managua, Apoyeque, and Xiloá. Crater lakes As. León and Masaya represent intermediate lakes within the photic gradient (PC1). Crater lake Tiscapa has extremely low luminosity values but shows similar spectral properties as great lake Nicaragua and River San Juan. Great lake Managua represents the most red-shifted environment (table S4; fig. S7). The presence of phototropic microorganisms, specifically cyanobacteria and green algae (Stomp et al. 2007), contributes to the spectral peaks around 640 and 700 nm seen in some lakes (i.e., Tiscapa and Managua; figs. 1*B*, S2).

Opsin Gene Expression Varies Significantly across Nicaraguan Lakes

We found significant variation in opsin gene expression in wild-caught individuals, where for at least one opsin gene all derived crater lake populations show divergence from the source great lakes (fig. 2). Fish from short-wavelength-shifted lakes tend to express more *sws2b* and *rh2a* (and less *sws2a* and *lws*) in single and double cones, respectively. These patterns of opsin gene expression were also observed in individuals from the different lakes reared under common garden conditions, which suggests a strong genetic component. Although the laboratory-reared fish experienced the same photic conditions during development, the distinct populations still showed significant variation in opsin gene expression for most opsins (fig. S8).

Shifts in Spectral Sensitivity Are Correlated with Changes in Photic Conditions following Colonization Events

Our results show that spectral sensitivities from the source populations in the great lakes have shifted in the same direction as the changes in photic conditions, suggesting the repeated and independent evolution of visual sensitivity in all seven crater lake populations. Based on the known colonization history of Midas cichlids

Figure 2: Proportional opsin gene expression from wild-caught fish showing groups of a derived crater lake (blue) and its respective source great lake (red) within each panel. River San Juan is shown in orange. Bars represent the mean value for each population. The upper right corner shows *F* values and significance from an ANOVA (type II) using location as the predictor variable. Letters display groups based on Tukey's honestly significant difference test. Great lake Managua is considered the predominant source population for the admixed crater lake Masaya on the basis of Kautt et al. (2018, 2020).
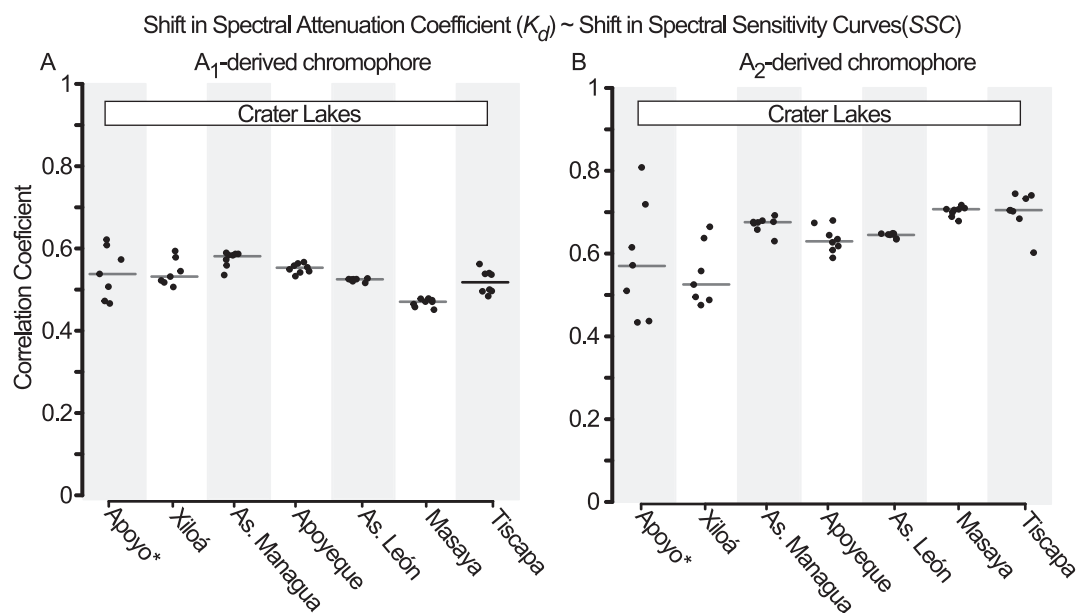
Shift in Spectral Attenuation Coefficient (K_d) ~ Shift in Spectral Sensitivity Curves(SSC)

Figure 3: Correlation of shift in spectral sensitivity curves (ΔSSC; fig. S5) and localized spectral attenuation coefficients (ΔK_d; fig. S4) between derived populations and their source population, either great lake Nicaragua (crater lake Apoyo, depicted with an asterisk) or great lake Managua. Great lake Managua is considered the predominant source population for the admixed crater lake Masaya on the basis of Kautt et al. (2018, 2020).

across the Nicaraguan lakes (Kautt et al. 2020), we asked whether the modulation in opsin expression across lakes resulted in changes in visual sensitivity that correlate with the changes in the photic conditions (spectral attenuation coefficients, K_d) fish experienced after colonizing the crater lakes (figs. S4, S5). Coefficients were significantly positively correlated, averaging 0.53 ± 0.01 and 0.65 ± 0.01 (mean $\pm$ SE) assuming A₁ and A₂ chromophore usage, respectively (fig. 3). These results were sustained, although weakened, when irradiance was used as a measure of the photic environment rather than K_d (fig. S6).

Spectral Sensitivity of Midas Cichlids Is Predicted by Photic Conditions at Their Lake of Origin

If variation in the visual system is driven by the light environment, differences in visual sensitivity among populations should be most strongly predicted by their native photic conditions. We tested this by using a linear mixed effects model with PC1, the main environmental composite axis of photic conditions (table S4; fig. S8), as an explanatory variable for the variation in the PSI (fig. 4A). The fixed effects in our model explained on average about 64% of the overall variation in PSI, with a 51%–74% CI based on 1,000 parametric bootstrapping iterations (fig. S9). About 48% of the variation in visual sensitivity of Midas cichlids across populations was explained by the photic environment in their native habitat

(34%–62% CI; fig. S9). We also found that the visual sensitivity of fish varies depending on rearing conditions (e.g., wild caught or laboratory reared), demonstrating that there is an environmental component that explains on average 13% of the overall variance (0%–33% CI; fig. S9). There is a nonsignificant interaction ($F_{1, 130.07} = 3.407$, $P = .067$) between the native photic environment and rearing conditions, which explained less than 1% of the variation in PSI (0%–22% CI; fig. S9). Furthermore, sensitivity analysis revealed that confounding factors accounting for less than 54% of the residual variance of both photic conditions and predicted sensitivity would not suffice to deem the estimates as statistically not significant ($RV_{q=1,\alpha=0.05} = 54.9\%$; table S6). This implies that even confounders that explain all the residual variation of the outcome and are as strong as the rearing effect are not sufficient to override the effect of the photic environment in our model and thus our conclusions (i.e., $R^2_{Y \sim Z|X,D} < R^2_{Y \sim D|X}$; table S5). Additionally, we tested whether variation in PSI within each population changed in response to the photic conditions, specifically whether spectrally broad environments show higher variation in PSIs than narrow ones. Our results show that coefficients of variation in PSI are significantly affected by photic conditions (PC1) in wild-caught fish (fig. 4A; $F_{1, 8} = 13.25$, $P = .0066$) but not in laboratory-reared fish ($F_{1, 7} = 2.47$, $P = .16$). According to our results, most of the variation in visual sensitivity seen across populations of Midas cichlids along the photic gradient has a strong genetic component and a
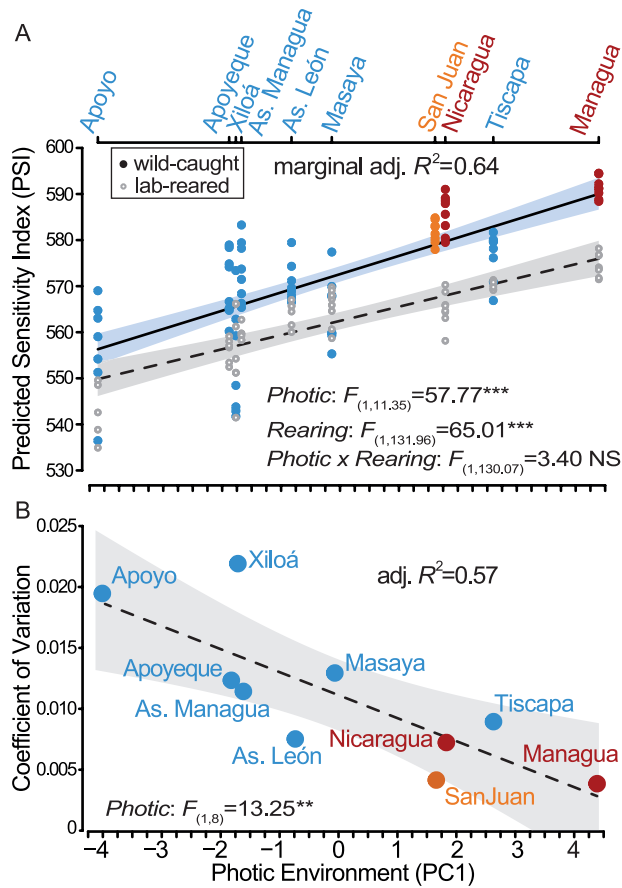
Figure 4: *A,* Individual sensitivity index (predicted sensitivity index [PSI]) is predicted by the photic environment at the lake of origin (*z*-scores of PC1 from photic parameters; table S4). Shown are mean regression lines and 95% confidence intervals (CIs) for wild-caught (solid line) and laboratory-reared (dashed line) individuals. The lower right corner shows *F* values (ANOVA type III) for predictor variables: photic environment, rearing environment, and their interaction. *B,* Mean regression line and 95% CI of coefficients of variation in PSI within each population for wild-caught fish in response to ambient photic environment (PC1). The lower left corner shows *F* values (ANOVA type II). ***$P < .001$; **$P < .01$; NS = not significant ($P > .05$).

relatively smaller environmental effect (fig. 4*A*). Furthermore, the phenotypic range of visual sensitivities within each population is clearly shaped by ambient photic conditions, with decreasing diversity as environments become more spectrally restricted (fig. 4*B*).

Discussion

The visual sensitivity of fishes often varies across light environments, and this variation is commonly interpreted as adaptive (reviewed in Carleton et al. 2010; Carleton and Yourick 2020; Musilova et al. 2021). Most studies in visual ecology have focused on comparisons between strongly

contrasting habitats, such as marine versus freshwater (Rennison et al. 2016), shallow versus deep water (Sugawara et al. 2005), and stained/turbid versus clear waters (Fuller et al. 2004; Torres-Dowdall et al. 2017). By analyzing the photic environments in a dichotomous manner, these studies maximize the power to identify adaptive patterns in the visual system. However, fishes inhabit a wide range of conditions that need to be considered to fully understand the diversity of visual systems seen across natural populations (Kirk 2010; Sabbah et al. 2011; Flamarique et al. 2013). By focusing on how visual sensitivities vary in response to a gradient in photic conditions, this study is able to determine aspects about the evolution of visual systems that were not evident by simply comparing the ends of these gradients (Torres-Dowdall et al. 2017). Below, we discuss how studying variation in the visual system of closely related species living under a wide range of photic conditions allows the identification of links among genotype, phenotype, and fitness, providing novel insights into the mechanisms and pace of adaptive evolution.

The predictability of evolutionary outcomes depends on multiple factors, such as the strength of natural selection or the timescale over which evolutionary changes are observed (Grant and Grant 2002; Reimchen and Bergstrom 2023). Strong selective pressures due to colonization of novel habitats might determine the direction of evolution over short periods of time. However, many other factors influence evolutionary outcomes and often hinder the predictability of long-term evolutionary trajectories (Bell 2010; Nosil et al. 2018). Hence, when repeated or convergent evolution of phenotypes is observed, it is often considered a signature of determinism in the evolutionary responses of organisms to common environmental pressures (Blount et al. 2018; Losos 2018). Here, we show that the visual systems of all seven derived crater lake populations have repeatedly shifted their visual sensitivity in a predictable manner, reflecting changes in photic conditions. Our study highlights the role of the photic environment in imposing selection on the visual system, driving predictable phenotypic evolution of visual sensitivities along an environmental gradient in the absence of gene flow.

While there is a predictable change in phenotypes along the photic gradient, the phenotypic range observed within populations suggests a more complex scenario. Understanding how phenotypic variation is maintained in the wild requires information about the fitness consequences of environmental pressures, which can be visualized as fitness landscapes (Wright 1932). While the relative fitness of certain phenotypes is often unknown, the distribution of phenotypes within a population can inform about the shape of the selective landscape (Svensson 2023). For instance, habitats with single steep adaptive peaks might select for a modal phenotype, whereas broad, "flat" selective landscapes

should allow for multiple phenotypes of similar fitness (Reimchen and Bergstrom 2023). Our data suggest that selective landscapes vary along the photic gradient, becoming less defined around a single modal phenotype as the environment becomes more spectrally heterogeneous. Spectrally broader environments may exert weaker selective pressures, allowing the emergence of distinct visual sensitivities that may be selected for by other factors (e.g., diet, mate choice, microhabitat). This is expected to reduce predictability based solely on photic conditions. In contrast, as turbidity increases, the spectral range is narrowed and luminosity reduced, with fewer remaining photons being distributed among fewer wavelengths. Those spectrally narrow habitats seem to select for reduced variation in visual sensitivity around a modal phenotype tuned to the specific spectral range available (e.g., fig. 4A).

The photic gradient along the Nicaraguan lakes is best represented as a composite axis that ranges from the clearest, short-wavelength-shifted, spectrally broad, and relatively bright crater lake Apoyo to the most turbid, long-wavelength-shifted, spectrally narrow, and dim great lake Managua. In this regard, the strong associations between changes in visual sensitivity and photic habitat seen when comparing ancestral and derived populations of Midas cichlids suggest that their visual systems are locally adapted (fig. 3). Using this same approach, Rennison et al. (2016) identified patterns of rapid adaptive evolution in the visual system of freshwater versus marine threespine stickleback (*Gasterosteus aculeatus*). However, the habitats compared in that study represented rather clear aquatic habitats. This contrasts with some of the drastic differences in photic conditions between crater lakes and great lakes seen in our study, which could enhance our power to detect significant correlations between opsin gene expression and photic changes. Alternatively, if our findings that broad-spectrum environments facilitate the emergence of multiple visual sensitivities of similar fitness are generalizable to other systems (Loew 1995; Carleton et al. 2016; Schneider et al. 2020; Reimchen and Bergstrom 2023), this could also help explain the greater intrapopulation variation found in Rennison et al. (2016) compared with this study. The narrower spectra of some of the Nicaraguan lakes might select for sensitivities tuned to the specific spectral band available, resulting in stronger correlations between habitat conditions and visual sensitivity (Bowmaker 1990). Hence, our ability to predict phenotypic change—a major goal in biology, especially in the context of environmental perturbations to aquatic ecosystems (Seehausen et al. 1997; Solomon et al. 2015; Bunnell et al. 2021)—might depend on both the magnitude of the environmental change and the narrowness of the adaptive peak.

Even in the presence of strong deterministic selective pressures, phenotypic evolution might not occur as pre-

dicted. Adaptive evolution requires heritable traits to be selected over multiple generations to "optimize" phenotypes, while phenotypic plasticity could also tune phenotypic variation within an organism's lifetime (Fisher 1930). Thus, understanding both genetic and plastic contributions to phenotypic variation is critical for assessing the likelihood and speed of adaptive evolution (Hendry and Kinnison 1999; Ghalambor et al. 2007). Given the correlated changes in visual sensitivity of Midas cichlids in response to the colonization of crater lakes, we asked how closely these independent populations tracked the observed gradient in photic conditions. Our model including rearing conditions (wild vs. common garden) and photic conditions at the lake of origin explained 64% of the variation found in visual sensitivity (fig. 4A). Overall, 48% of the variation was explained by the photic conditions in the lake of origin, suggesting a strong genetic component. Although photic conditions have a strong power to explain phenotypic variation in the visual sensitivity of Midas cichlids, there is still some unexplained variation that could arise from other factors proposed in the literature (e.g., diet, mate choice; Carleton and Yourick 2020), which may be particularly important in clear lakes. Among others, spectral tuning in cichlid vision has been associated with foraging habits (Hofmann et al. 2009; Irisarri et al. 2018), mate choice (Seehausen et al. 2008; Schneider et al. 2020), and ecological and lineage-specific factors (Schott et al. 2014; Torres-Dowdall et al. 2015). However, the 13 described species in the small Midas cichlid radiation overlap in their dietary requirements and show almost no sexual dimorphism in coloration (fig. 1A; Torres-Dowdall and Meyer 2021), supporting the conclusion that these factors may be only minor drivers of visual divergence in our system. Furthermore, while adaptive evolution appears to be the main driver of phenotypic variation, the role of plasticity in the visual system must also be considered. Even when plasticity is incomplete and thus not sufficient for organisms to reach certain adaptive peaks (Fisher 1930; Ghalambor et al. 2007), it might facilitate the emergence and maintenance of variation within populations, particularly in spatially and temporally heterogeneous environments (Pigliucci 2001). For instance, reductions in water clarity have been reported in our system due to algal blooms (e.g., As. Managua; Torres-Dowdall et al. 2014) and modern anthropogenic pollution (e.g., Tiscapa; García Espinoza 2020). In theory, changes in water clarity should have the strongest visual repercussions in clear environments (Lythgoe 1979; Mobley 1994; Kirk 2010). This may explain the increased variation in visual sensitivity observed in broadband environments (fig. 4B), as spatial or temporal heterogeneity could allow multiple sensitivity phenotypes to be adaptive and/or favor plasticity (Fuller and Claricoates 2011; Härer et al. 2017).

Given the known demographic history of Midas cichlids (Kautt et al. 2016, 2020), which rules out the possibility of crater lake to crater lake dispersal and thus any gene flow between crater lakes, we were able to estimate evolutionary rates for all derived populations (0.0009–0.0121 haldanes; table S5), highlighting that phenotypic evolution has occurred rapidly and independently in all seven newly colonized crater lakes (for comparison, see Hendry and Kinnison 1999). The most common shifts in visual sensitivity among Midas cichlid species are achieved by modulating the expression of the three most abundant opsins, namely, *sws2a* in single cones and *rh2a* and *lws* in double cones (fig. 2). Overall, this suggests that by modulating the relative expression of opsin genes, phenotypic variation in visual sensitivity can be gradually tuned across populations (Veen et al. 2017). However, populations such as that in crater lake Apoyo show conditional expression of additional opsins (fig. S8). Interestingly, the gradual phenotypic variation observed across populations seems to be achieved by two nonexclusive mechanisms, namely, the conditional expression of different genes (e.g., *sws2b* paralog in Apoyo) and the quantitative regulation of the predominantly expressed cone opsin genes (i.e., *sws2a*, *rh2a*, *lws*). The phenotypic variation in Midas cichlids along the photic gradient suggests that visual sensitivity in these fish is a continuous trait rather than discrete phenotypes compartmentalized into palettes (fig. 4).

Overall, we documented compelling evidence that selection pressures imposed by light environments have resulted in rapid phenotypic divergence in the visual system of Midas cichlids. In the source populations inhabiting turbid great lakes with low light availability and narrow spectral conditions, fine-tuning visual sensitivity to maximize photon catch resulted in little phenotypic variation around a modal phenotype. As fish began to colonize the newly formed crater lakes, they encountered a variety of photic conditions. Here, Midas cichlids repeatedly and predictably adjusted their visual sensitivity as they encountered these novel habitats, as evidenced by the changes in spectral sensitivity positively correlating with photic changes (fig. 3). Our results further suggest that predictability might depend not only on the organisms but also on the environment itself, as less selective environments might result in a wider array of potentially equally well adapted phenotypes (Nosil et al. 2018; Reimchen and Bergstrom 2023). Accordingly, in spectrally broader environments, weaker selection toward a modal phenotype also allowed for more variable phenotypes to emerge. The evolutionary history of the Midas cichlid visual system relied on the modulatory expression of visual opsin genes to generate tuned sensitivities. The change in visual sensitivity was mainly driven by rapid phenotypic evolution rather than plasticity, as the phenotypic variation in the Midas cichlid visual system shows a strong heritable component inferred from individuals reared under common garden conditions (fig. 4*A*). In conclusion, the repeated divergence of visual sensitivities among crater lake populations of Midas cichlids by modulating their expression of cone opsin genes provides an evolutionary lesson on the mechanisms and reasons that facilitate phenotypic variation closely tracking environmental conditions.

Statement of Authorship

J.T.-D., A.H., and A.M. developed the original project and performed the fieldwork; J.T.-D., N.K., and C.B. reared fish and dissected the lab specimens; J.T.-D. and A.M. coordinated the experiments and supervised the project; C.B., A.H., J.T.-D., and N.K. collected the data; and C.B. analyzed the data and led the writing of the manuscript. All authors contributed critically to the draft and gave final approval for publication.

Data and Code Availability

Data and code associated with this study are publicly available in the Dryad Data Repository (https://doi.org/10.5061 /dryad.j3tx95xgk; Bertinetti et al. 2023).

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