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Rapid adaptation to a novel light environment: The importance of ontogeny and phenotypic plasticity in shaping the visual system of Nicaraguan Midas cichlid fish (*Amphilophus citrinellus* spp.)

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Abstract

Colonization of novel habitats is typically challenging to organisms. In the initial stage after colonization, approximation to fitness optima in the new environment can occur by selection acting on standing genetic variation, modification of developmental patterns or phenotypic plasticity. Midas cichlids have recently colonized crater Lake Apoyo from great Lake Nicaragua. The photic environment of crater Lake Apoyo is shifted towards shorter wavelengths compared to great Lake Nicaragua and Midas cichlids from both lakes differ in visual sensitivity. We investigated the contribution of ontogeny and phenotypic plasticity in shaping the visual system of Midas cichlids after colonizing this novel photic environment. To this end, we measured cone opsin expression both during development and after experimental exposure to different light treatments. Midas cichlids from both lakes undergo ontogenetic changes in cone opsin expression, but visual sensitivity is consistently shifted towards shorter wavelengths in crater lake fish, which leads to a pedomorphic retention of their visual phenotype. This shift might be mediated by lower levels of thyroid hormone in crater lake Midas cichlids (measured indirectly as *dio2* and *dio3* gene expression). Exposing fish to different light treatments revealed that cone opsin expression is phenotypically plastic in both species during early development, with short and long wavelength light slowing or accelerating ontogenetic changes, respectively. Notably, this plastic response was maintained into adulthood only in the derived crater lake Midas cichlids. We conclude that the rapid evolution of Midas cichlids' visual system after colonizing crater Lake Apoyo was mediated by a shift in visual sensitivity during ontogeny and was further aided by phenotypic plasticity during development.

KEYWORDS

development and evolution, neotropical cichlids, pedomorphosis, phenotypic plasticity, visual system

1 | INTRODUCTION

Adaptation to changing environmental conditions, for example, after colonization of a novel habitat, is crucial for population persistence and diversification and can result in population differentiation in the

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absence of gene flow (reviewed in Kawecki & Ebert, 2004). Hence, it is crucial to understand the sources of phenotypic variation to further our understanding of how organisms adapt to such new environmental factors. These sources of variation include, among others, developmental shifts (e.g., heterochrony) and environmentally induced changes (i.e., phenotypic plasticity) which can play an important role in local adaptation (Ghalambor et al., 2015; Price, Qvarnstrom, & Irwin, 2003; Schaum & Collins, 2014; Torres-Dowdall, Handelsman, Reznick, & Ghalambor, 2012). However, the contribution of development and phenotypic plasticity to adaptive evolution has commonly received little attention, but incorporating these could help us to better comprehend how organisms adapt to novel and potentially challenging environments.

Developmental changes during ontogeny obviously represent an important source for phenotypic diversity and possibly evolutionary diversification (Shapiro et al., 2004; Tanaka, Barmine, & Kopp, 2009; West-Eberhard, 2003). Adult phenotypes can develop by either of two processes: they are already present in the adult form early in larval or juvenile developmental stages (direct development) or are the result of changes during development (ontogenetic change). By comparing developmental trajectories among closely related species, one can make inferences about shifts in the relative timing and rate of developmental processes, termed heterochrony (e.g., Gould, 1977; West-Eberhard, 2003). Heterochrony has the potential to bring about novel adult phenotypes by maintaining juvenile characteristics into adulthood (paedomorphosis) or by undergoing extended development beyond the previous state (peramorphosis; Gould, 1977; Kollman, 1885). Gould argues that variation in rate and timing of developmental processes provides raw material upon which natural selection can act to produce new phenotypic variants. Hence, heterochrony might facilitate evolutionary change (Gould, 1977; Klingenberg, 1998; Raff & Wray, 1989).

Another mechanism by which different phenotypes can arise is phenotypic plasticity, the ability of one genotype to produce more than one phenotype when exposed to different environmental conditions (Pigliucci, Murren, & Schlichting, 2006). The importance of phenotypic plasticity in promoting adaptive evolutionary change is still highly contentious (e.g., Ancel, 2000; Ghalambor et al., 2015; Paenke, Sendhoff, & Kawecki, 2007; Price et al., 2003), particularly, after colonization of novel habitats with fitness optima differing from the original habitat. In such scenarios, population sizes are commonly small, restricting a population's potential to genetically adapt to the new environment due to low levels of standing genetic variation (Nei, Maruyama, & Chakraborty, 1975) and, consequently, low efficiency of natural selection (Charlesworth, 2009). Some evolutionary biologists argue that adaptive phenotypic plasticity might allow populations to persist in novel environments and, hence, represents a crucial step during adaptive evolution (Ghalambor, McKay, Carroll, & Reznick, 2007; Losos et al., 2000; Pigliucci & Murren, 2003). Already in the late 19th century, J. M. Baldwin proposed a theory concerned with the effects of phenotypic plasticity on evolutionary change (Baldwin, 1896), later termed the "Baldwin effect" (Simpson, 1953). According to Baldwin, adaptive phenotypic plasticity allows

populations to persist and increases survival and reproduction of the most plastic individuals (Crispo, 2007). Over time, selection on standing genetic variation within populations could subsequently lead to evolutionary change in the direction of the initial plastic response (Crispo, 2007; Schneider & Meyer, 2017). During this process, the reaction norm, that is the extent of phenotypic change among environments, would either stay the same or even increase due to a selective advantage of the most plastic individuals (Nussey, Postma, Gienapp, & Visser, 2005; Schneider & Meyer, 2017). In contrast, others have claimed that nonadaptive plasticity, which moves environmentally induced phenotypes away from a local optimum, promotes rapid evolutionary change by increasing strength of selection (Ghalambor et al., 2015).

Cichlid fishes are famous for their phenotypic diversity (e.g., Fryer & Iles, 1972; Meyer, 1993) and have been investigated also in terms of phenotypic plasticity. Some lineages more than others are phenotypically plastic in their ecologically crucial tooth morphology (Meyer, 1987; Schneider, Li, Meyer, & Gunter, 2014), but also hypertrophied lips, that play an essential role both in terms of natural and sexual selection (Machado-Schiaffino, Henning, & Meyer, 2014; Machado-Schiaffino et al., 2017). Besides, several other traits were shown to be particularly plastic in cichlids (reviewed in Schneider & Meyer, 2017). In fact, it has recently been suggested that this degree of plasticity coupled with genetic assimilation might have contributed to the evolutionary success of cichlids (Schneider & Meyer, 2017).

Heterochrony and phenotypic plasticity have also been shown to contribute to shaping the visual system of cichlid fishes (Carleton et al., 2008; Dalton, Lu, Leips, Cronin, & Carleton, 2015; Hofmann, O'Quin, Smith, & Carleton, 2010; O'Quin, Smith, Sharma, & Carleton, 2011). Cichlids are an interesting group to study visual ecology due to a high variability of the visual system among species (Carleton, Dalton, Escobar-Camacho, & Nandamuri, 2016), which enables studying molecular mechanisms promoting adaptive evolutionary change (Hofmann & Carleton, 2009; Hofmann et al., 2009; Terai et al., 2006). In cichlids, the retina is highly organized and is composed of single and double cones responsible for colour vision, as well as dim-light-sensitive rods (Fernald, 1981). Vision is initiated when a photon reaches a visual pigment, which is composed by a light-sensitive chromophore covalently bound to an opsin protein, in the photoreceptors (Ebrey & Koutalos, 2001; Wald, 1968; Yokoyama, 2000). Structural changes and expression differences of genes coding for opsin proteins were shown to be the determinants of most of cichlids' variation in visual sensitivity. Cichlids possess seven cone opsins absorbing light at different wavelengths of the light spectrum, although they commonly express three of these at the same time. One short wavelength-sensitive opsin (*sws1*, *sws2a* or *sws2b*) is expressed in single cones, and two types of longer wavelength-sensitive opsins (*rh2b*, *rh2aβ*, *rh2aα* and *lws*) are expressed in double cones (Carleton & Kocher, 2001; Parry et al., 2005). It has been proposed that heterochronic shifts in opsin expression are involved in shaping some of the impressive phenotypic variation observed in adult visual sensitivity among cichlid species (Carleton et al., 2008). Moreover, opsin expression is phenotypically plastic in some Lake Malawi cichlids, with varying degrees of plasticity among species (Hofmann et al., 2010). In most

cases, the underlying molecular mechanisms shaping the visual system remain elusive, but a few studies have shown that thyroid hormone (TH) signalling regulates cone opsin identity during development (Ng et al., 2001), controls cone opsin expression (Glaschke et al., 2011) and shifts visual sensitivity towards longer wavelengths (Roberts, Srinivas, Forrest, Morreale de Escobar, & Rhe, 2006). To summarize, previous work on African cichlids suggests that both heterochrony and phenotypic plasticity of the visual system might have facilitated ecological diversification. However, the evolutionary significance of these two mechanisms in initial periods after colonizing novel habitats remains to be investigated.

The Neotropical Midas cichlids of the *Amphilophus* cf. *citrinellus* species complex represent an ideal model system to study the divergence of the visual system in different light environments. Midas cichlids inhabit multiple lakes on the western coast of Nicaragua (e.g., Barlow, 1976; Barluenga & Meyer, 2004, 2010). These include ancient great lakes, Lake Managua and Lake Nicaragua, with an age of approximately 500,000 years and numerous, relatively young crater lakes. The oldest of these crater lakes is Lake Apoyo with an estimated age of 23,000 years (Bice, 1985). Midas cichlids colonized Lake Apoyo from Lake Nicaragua approximately 1,700 generations ago (Kautt, Machado-Schiaffino, & Meyer, 2016). The light spectrum in the turbid great lakes is red-shifted compared to the clear crater lakes (Figure 1, white inset). Recent work has shown that Midas cichlids possess, like African cichlids, seven cone opsins (Torres-Dowdall, Pierotti, et al., 2017). This study further revealed that species from different lakes (Lake Nicaragua and Lake Apoyo) differ in photopigment sensitivity (Torres-Dowdall, Pierotti, et al., 2017). These differences in visual sensitivity can mostly be explained by differential cone opsin expression among species from the two lakes (Figure 1). As these differences are maintained when fish are reared in a common light environment, species-specific cone opsin expression patterns are assumed to be genetically determined (Torres-Dowdall, Pierotti, et al., 2017). However, it is still unknown whether cone opsin expression changes during development and what impact the environment has on shaping the visual system.

Here, we experimentally investigated the contributions of development and phenotypic plasticity to the evolution of visual system

divergence among closely related Midas cichlid species. Particularly, we determined whether cone opsin expression changes during ontogeny or develops directly and whether colour vision is phenotypically plastic in these species. To this end, we investigated (i) cone opsin expression and TH levels during ontogeny of great lake Midas cichlids (*Amphilophus citrinellus* from Lake Nicaragua) and crater lake Midas cichlids (*Amphilophus astorquii* from Lake Apoyo) and (ii) determined whether different light treatments affect cone opsin expression in three developmental stages.

2 | METHODS

2.1 | Rearing conditions

Previous results showed differences in cone opsin expression between adult fish (older than 1 year) of *A. citrinellus* from Lake Nicaragua (further referred to as great lake Midas cichlids) and *A. astorquii* from Lake Apoyo (further referred to as crater lake Midas cichlids; Figure 1; Torres-Dowdall, Pierotti, et al., 2017). Here, we investigated gene expression in second-generation laboratory born descendants of wild caught fish during ontogeny and as a response to different light treatments. All fish used in this study (and their parental generation) were reared under standard fluorescence lamp illumination (Fig. S1) at the animal facility of the University of Konstanz, Germany, before they were used for this study. Our first goal was to determine cone opsin expression during ontogeny of the two studied species; therefore, samples were collected at the ages of 7 days, 14 days, 6 months and older than 1 year ($n = 6$ for the first three time points and $n = 8$ for fish older than 1 year). With the first two time points, we aimed at obtaining information about early stages of development. We chose the 6 months time point because opsin expression in African cichlids reaches the adult phenotype (Carleton et al., 2008; O'Quin et al., 2011) and many cichlid species are sexually mature at this age. To verify whether Midas cichlids, like African cichlids, already have an adult phenotype at the age of 6 months, we included data recently published on opsin expression profiles of

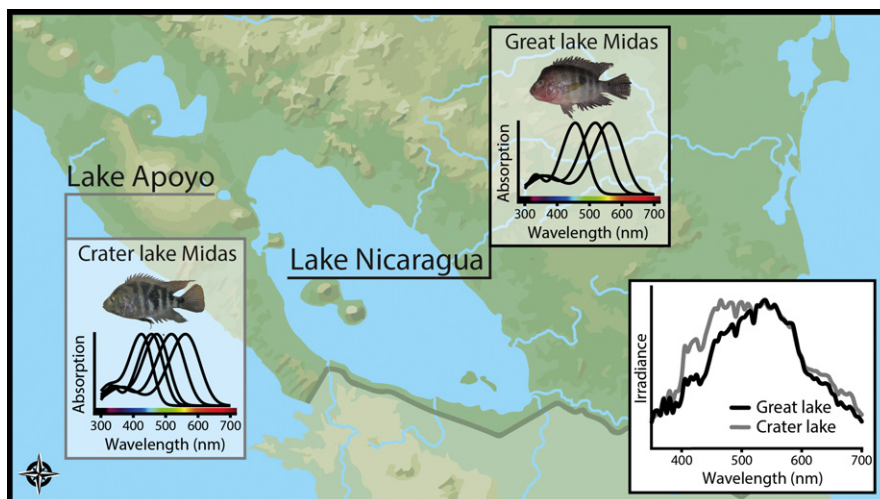


FIGURE 1 Midas cichlids from Lake Nicaragua (*Amphilophus citrinellus*) and Lake Apoyo (*Amphilophus astorquii*) differ in cone opsin expression. The photic environment in Lake Nicaragua (Great lake, white inset) is red-shifted compared to Lake Apoyo (Crater lake, white inset). Cone opsin expression and relative irradiance data were obtained from Torres-Dowdall, Pierotti, et al. (2017)

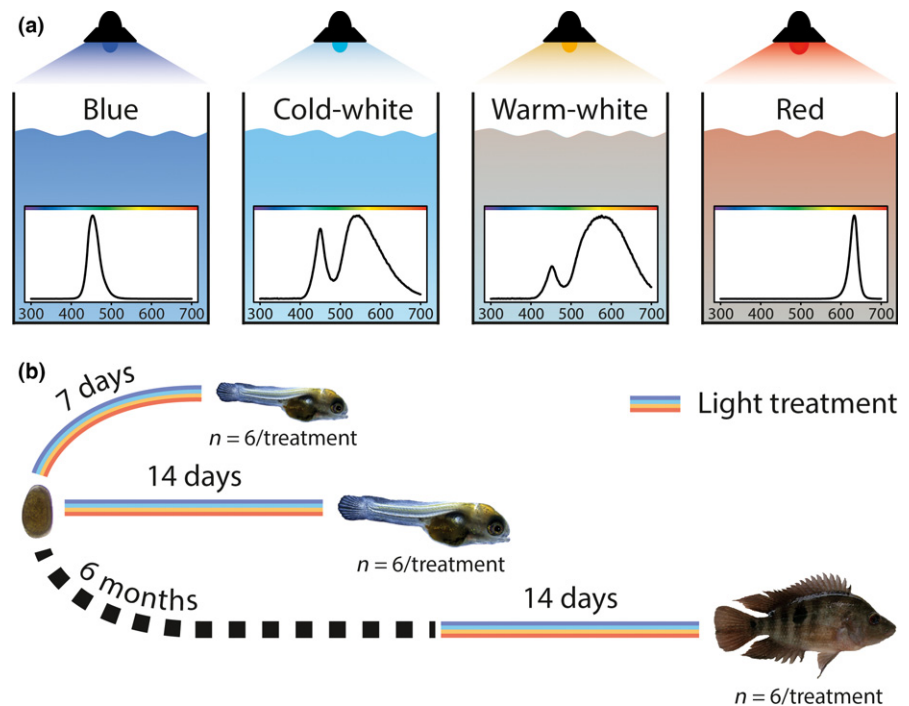


FIGURE 2 To investigate phenotypic plasticity, specimens were randomly assigned to one of four light treatments, each covering a particular part of the light spectrum (a). Fish were either introduced to the light treatment shortly before hatching (7 days and 14 days groups) or at the age of 6 months (b). Light treatments lasted either for 7 days (7 days group) or 14 days (14 days and 6 months group)

adult Midas cichlids (older than 1 year; Torres-Dowdall, Pierotti, et al., 2017).

Our second goal was to determine whether cone opsin expression is affected by the light environment. For this experiment, cone opsin expression was analysed at the same three time points as described above: 7 days, 14 days and 6 months. Specimens were randomly divided into four groups and exposed to different light conditions ($n = 6/\text{treatment}$) covering a particular range of the light spectrum (Figure 2a). One 6-month-old *A. astorquii* specimen from the blue light treatment represented an extreme outlier and, thus, was excluded from all statistical analyses. Two treatments had broad light spectra simulating natural divergence across habitats (cold-white, warm-white), and two had narrow spectra at the extremes of the visible light spectrum (blue, red; Figure 2a). Tanks were illuminated with wavelength-specific LEDs (Cree Inc., Durham, NC; Table S1). Fish were either introduced to the respective light conditions immediately after hatching (for the 7 days and 14 days groups) or at the age of 6 months (for the 6 months group) and treatments lasted either 1 week (for the 7 days group) or 2 weeks (for the 14 days and 6 months group) using a 12-hr:12-hr light: dark cycle (Figure 2b). Relative quantum catch was calculated for each cone opsin in the four light treatments (Fig. S2).

All specimens were sacrificed by applying an overdose of MS-222 (400 mg/L) and subsequent cutting of the vertebral column (in 6-month-old fish). For the 7 days and 14 days groups, whole fish were used; for 6-month-old fish, only retinas were dissected and further used for expression analyses. Opsins are predominantly expressed in the retina; hence, extracting RNA from the whole body of larvae will not affect the analysis of cone opsin expression. All tissues were stored in RNAlater (Sigma-Aldrich, St. Louis, MO, USA) until RNA extraction.

2.2 | Opsin expression analysis

RNA was extracted using the commercial RNeasy Mini Kit (Qiagen, Hilden, Germany), RNA quality was checked by visually examining 28S and 18S rRNA bands after agarose gel electrophoresis (1% agarose gel), and the respective concentrations were measured using the Colibri Microvolume Spectrometer (Titertek Berthold, Pforzheim, Germany). 500 ng–1 µg of total RNA was reverse-transcribed with a first-strand cDNA synthesis kit according to the manufacturer's protocol (GoScript™ Reverse Transcription System; Promega, Madison, Wisconsin) and diluted to a final concentration of 5 ng/µl. Quantitative real-time PCR (qPCR) was performed to quantify proportional expression (PE) levels for six cone opsin genes (*sws1*, *sws2b*, *sws2a*, *rh2b*, *rh2aβ* and *lws*). *rh2aα* is not expressed in adult Midas cichlids (Torres-Dowdall, Pierotti, et al., 2017), and we confirmed this in all fish used in this study. We found that *rh2aα* expression did not exceed 1% of total *rh2a* expression in any sample; hence, it was not included in our analyses. After an initial denaturation step (95°C for 2 min), qPCRs were run for 40 cycles (95°C for 15 s, 60°C for 1 min; CFX96™ Real-Time System; Bio-Rad Laboratories, Hercules, CA, USA) using specifically designed primers (Table S2) for which amplification efficiencies were previously determined (Table S3). qPCR was followed by a melt curve analysis to test for amplification specificity. The two primers of each pair were designed to be located on adjacent exons to detect possible genomic DNA contamination. Specificity of amplification was further checked by Sanger sequencing of purified PCR products on an ABI 3130xl Genetic Analyzer (Life Technologies, Carlsbad, USA). Expression levels of opsin genes were quantified with three technical replicates, and mean threshold cycle (Ct) values were used for further analysis. If reactions did not amplify and, hence, no Ct values could be

determined, Ct values for the respective genes were assigned a value of 40 for calculations of proportional cone opsin expression. Although this represents an overestimation of gene expression, opsins with a Ct value of 40 never exceeded 1–10% of total cone opsin expression. The total volume for each reaction was 20 µl consisting of 2 µl cDNA (5 ng/µl), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM), 10 µl GoTaq qPCR Master Mix, 2× (GoTaq qPCR Master Mix; Promega, Madison, WI, USA) and 7 µl Nuclease-free H₂O. Proportional opsin expression was determined for each specimen by calculating the proportion of each cone opsin (T_i) relative to the total cone opsin expression (T_{all}), but note that RH2Aα expression is not included in this calculation as described above, after Fuller, Carleton, Fadool, Spady, and Travis (2004) using the following equation:

$$\frac{T_i}{T_{all}} = \frac{(1/((1 + E_i)^{Ct_i}))}{\sum (1/((1 + E_i)^{Ct_i}))}$$

E_i represents the primer efficiency for primer i , and Ct_i is the critical cycle number for gene i (the PE values of the six cone opsins add up to 1 for each specimen).

2.3 | Predicted sensitivities of single and double cones

Predicted single- and double-cone sensitivities for each specimen were calculated according to Hofmann et al. (2009) using λ_{max} values previously reported for Midas cichlids (Torres-Dowdall, Pierotti, et al., 2017): SWS2B = 425 nm, SWS2A = 456 nm, RH2B = 472 nm, RH2Aβ = 517 nm and LWS = 560 nm. Because λ_{max} of SWS1 is not known for Midas cichlids, the Nile Tilapia value was used instead (360 nm; Spady et al., 2006). Predicted visual sensitivities were calculated for each fish by taking into account peaks of maximum light absorption and PE of each cone opsin using the following equations for single cones

$$\lambda_{max}(SC) = \frac{PE(SWS1) * 360 + PE(SWS2B) * 425 + PE(SWS2A) * 456}{PE(SWS1) + PE(SWS2B) + PE(SWS2A)}$$

and double cones

$$\lambda_{max}(DC) = \frac{PE(RH2B) * 472 + PE(RH2A\beta) * 517 + PE(LWS) * 560}{PE(RH2B) + PE(RH2A\beta) + PE(LWS)}$$

By conducting Shapiro–Wilk tests, we found that our data were not normally distributed (Shapiro & Wilk, 1965). Hence, to test whether age and species identity (ontogenetic change experiment) or age and light conditions (phenotypic plasticity experiment) affect predicted single- and double-cone sensitivities, we used a nonparametric equivalent to a two-way ANOVA, the Scheirer–Ray–Hare test (Scheirer, Ray, & Hare, 1976). We tested all linear regression models for heteroscedasticity with Breusch–Pagan tests (Breusch & Pagan, 1979). Only the model for predicted *A. citrinellus* single cone sensitivity during ontogeny showed heteroscedasticity ($p = .02$); however, diagnostic plots did not reveal any

aberrant patterns (Fig. S3). All p values were corrected for multiple comparisons using FDR. Statistical analyses were performed in R (R Core Team 2015).

2.4 | Thyroid hormone signalling

Current methods to determine TH levels in fish require small but significant amounts of blood serum (Noyes, Lema, Roberts, Cooper, & Stapleton, 2014), which cannot be obtained from cichlid larvae (14-day-old larvae are approximately 7 mm long and weigh 2 mg). Thus, we had to rely on proxies for TH levels. Two genes have been commonly used for this purpose, the iodothyronine deiodinase type 2 (*dio2*) and type 3 (*dio3*). *Dio2* and *Dio3* enzymes are involved in regulation of TH signalling by transforming the inactive prohormone thyroxine (T4) into the active form triiodothyronine (T3) and by catabolizing T3, respectively (Bianco & Larsen, 2005; Jarque & Pina, 2014). More importantly, TH is known to downregulate *dio2* and upregulate *dio3* gene expression; this has been shown in multiple teleost species (Garcia, Jeziorski, Valverde, & Orozco, 2004; Johnson & Lema, 2011; Marlatt et al., 2012) as well as other vertebrates (reviewed in Gereben, Zeold, Dentice, Salvatore, & Bianco, 2008; Glaschke et al., 2011). To validate this in our study system, we experimentally exposed 1-week-old Midas cichlid larvae to thyroxine (T4; Sigma-Aldrich) for a time period of 2 weeks. T4 was added directly to the water to a final concentration of 300 µg/L. T4 treatment did significantly downregulate expression of *dio2* (Fig. S4; Wilcoxon rank-sum test, $p = .008$) and upregulate *dio3* ($p = .002$). Thus, we conclude that *dio2* and *dio3* are valid proxies for TH. Hence, we measured gene expression of *dio2* and *dio3* for specimens in the ontogenetic change experiment (i.e., a broad light spectrum environment) to determine whether TH levels can explain some of the variation observed between the studied Midas cichlid species. Expression levels were normalized with the geometric mean of two housekeeping genes (HKGs), *gapdh2* and *imp2*. Stability of HKGs was confirmed by calculating the Pearson correlation coefficient in R (R Core Team 2015). Across all samples, we find a strong and significant correlation ($r = .827$, $p < .001$) between gene expression of *gapdh2* and *imp2*. Normalization was carried out using the following equation:

$$RQ_i = \frac{2^{Ct_{HKG}}}{2^{Ct_i}}$$

where Ct_{HKG} is the geometric mean of two housekeeping genes, Ct_i is the critical cycle number for gene i . As we were interested in comparing relative expression of the two deiodinase genes in the two Midas cichlid species at the three different ages, we report (for each age group separately) *dio2* and *dio3* expression levels relative to that seen in great lake Midas cichlids, the ancestral population. Hence, crater lake Midas cichlid *dio2* and *dio3* expression values can be regarded as fold change compared to great lake Midas cichlids. Relative expression was compared between species for each age group using Wilcoxon rank-sum tests. All p values were corrected for multiple comparisons using FDR.

3 | RESULTS

3.1 | Ontogenetic changes in cone opsin expression

To determine the contribution of ontogenetic changes to the divergence in visual sensitivity observed in Nicaraguan Midas cichlids, we analysed predicted visual sensitivities and cone opsin expression during ontogeny of great lake Midas cichlids (*A. citrinellus*) and crater lake Midas cichlids (*A. astorquii*). Both species underwent ontogenetic changes in predicted visual sensitivities, shifting from short wavelengths to longer wavelengths, both in single cones and in double cones (Figure 3a,b). Nonetheless, throughout ontogeny, great lake Midas cichlids (Figure 3a,b, black bars) were sensitive towards longer wavelengths compared to crater lake Midas cichlids. No differences in the rate of progression were found among species (age \times species interaction was nonsignificant in all cases).

The ontogenetic changes in predicted sensitivities were due to differences in the set of opsins expressed at different ages (Figure 3c,d). In single cones, expression of the UV-sensitive *sws1* and the blue-sensitive *sws2a* decreased and increased with age,

respectively (Figure 3c). However, *sws1* expression seemed to be higher and *sws2a* expression lower in crater lake Midas cichlids compared to great lake Midas cichlids. The violet-sensitive *sws2b* was only expressed at early stages (7 and 14 days) in great lake Midas cichlids, but expression was highest at 6 months in crater lake Midas cichlids and decreased thereafter. In contrast to great lake Midas cichlids, both *sws2a* and *sws2b* were expressed in crater lake Midas cichlids older than 1 year.

In double cones, expression of the blue-green-sensitive *rh2b* was restricted to younger ages and decreased with time in both species. Expression levels appeared to be overall higher in crater lake Midas cichlids, and at the age of 6 months, it was only expressed in this species (Figure 3d). Expression of the red-sensitive *lws* increased with age in both species, and expression was seemingly higher in great lake Midas cichlids. The green-sensitive *rh2a β* had highest expression levels in early ages and decreased afterwards. Expression of *rh2a β* was apparently higher in crater lake Midas cichlids, and these differences were most pronounced in fish older than 1 year (mean PE of 10% in great lake Midas cichlids and 47% in crater lake Midas cichlids).

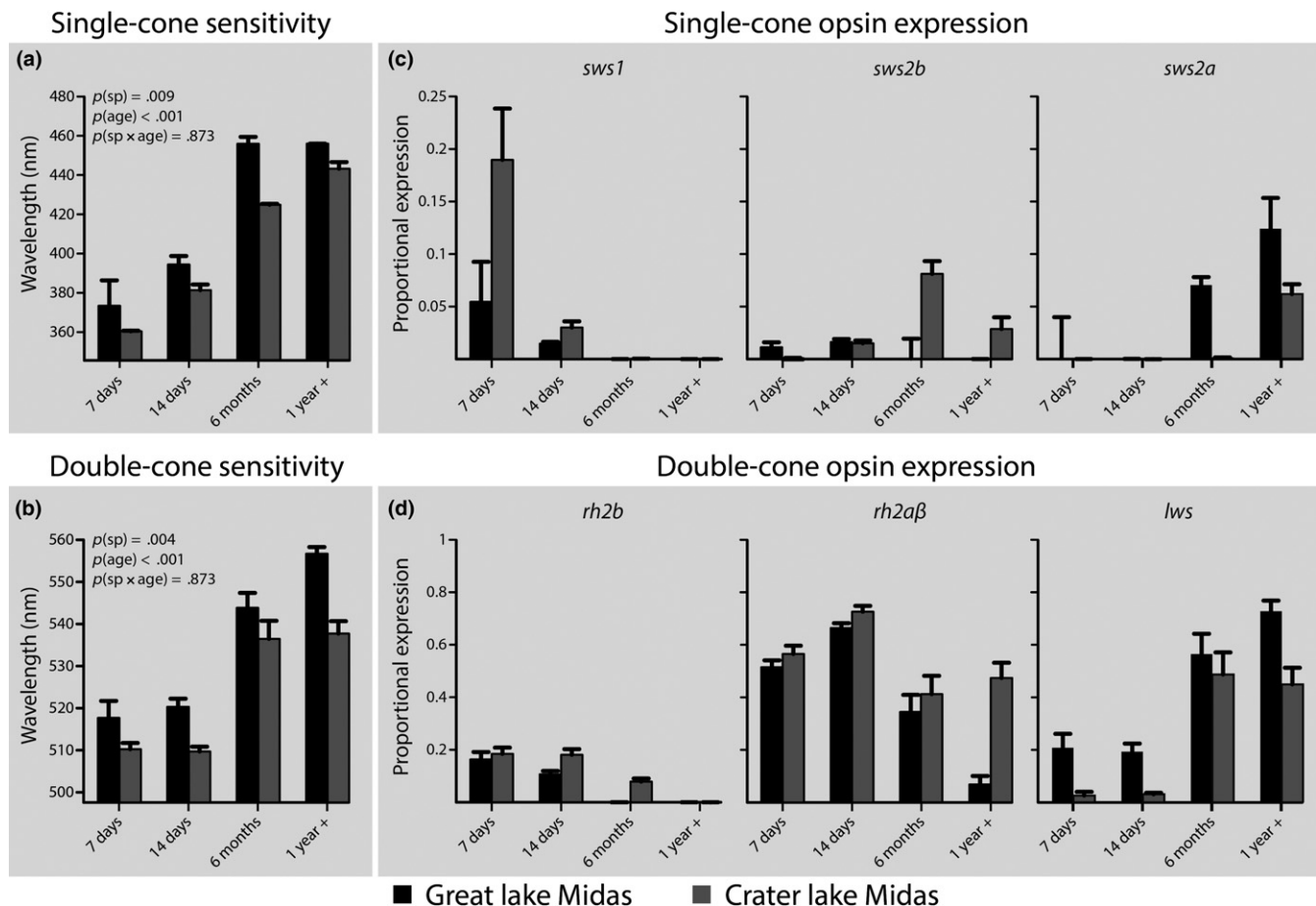


FIGURE 3 Predicted sensitivity (a and b) and proportional expression (PE) of single- and double-cone (c and d) opsins during ontogeny in great lake Midas cichlids and crater lake Midas cichlids. The effects of species identity and age were tested with Scheirer–Ray–Hare tests and significance values (FDR corrected) are indicated. PE values for each cone opsin are relative to overall cone opsin expression, that is, expression values sum up to 1 for each individual (across c and d)

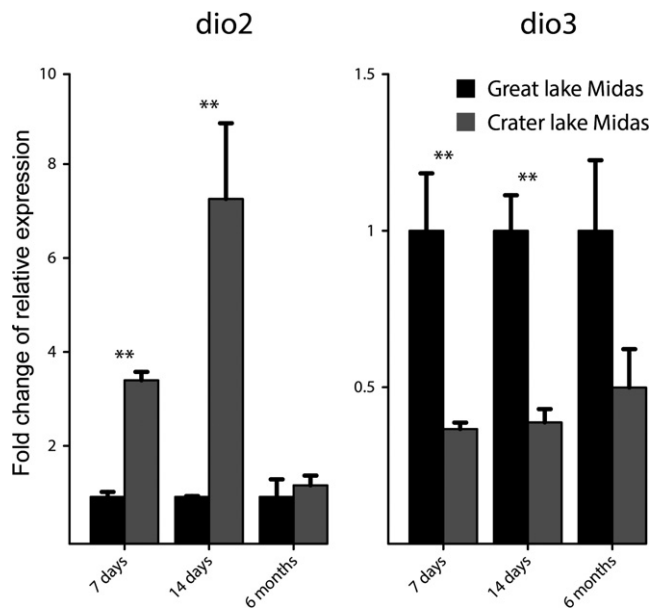


FIGURE 4 Relative expression of *dio2* and *dio3*, two genes involved in the thyroid hormone signalling pathway. Expression data were normalized with the geometric mean of two housekeeping genes. Expression levels are reported relative to the mean of great lake Midas cichlids, the ancestral population, for each time point, that is, crater lake Midas cichlid expression values are fold change compared to great lake Midas cichlids. Normalized expression values were compared between species at each time point (Wilcoxon rank-sum test, ** $p < .01$, FDR corrected)

3.2 | *dio2* and *dio3* expressions as a proxy for TH signalling

We measured *dio2* and *dio3* gene expressions during ontogeny as proxies for TH levels as transcription of these genes is down- and upregulated by TH, respectively (Fig. S4). In great lake Midas cichlids, expression of *dio2* (negatively regulated by TH) was significantly lower compared to crater lake Midas cichlids in 7 days (Wilcoxon rank-sum test, $p = .007$) and 14 days old fish ($p = .007$; Figure 4). In contrast, *dio3* (positively regulated by TH) showed higher expression in great lake Midas cichlids at 7 days ($p = .006$) and 14 days ($p = .006$) compared to crater lake Midas cichlids. In retinas of 6-month-old fish, expression of *dio2* and *dio3* showed the same pattern as at earlier ages; however, interspecific expression differences were not statistically significant (Figure 4). Taken together, these results suggest that TH levels are higher in great lake Midas cichlids than in crater lake Midas cichlids during early stages of development.

3.3 | Effects of light environment on cone opsin expression

We determined the effects of an environmental cue (spectrum of ambient light) on the visual system of great lake Midas cichlids (from turbid Lake Nicaragua) and crater lake Midas cichlids (from clear Lake Apoyo) by analysing predicted visual sensitivities under

different light conditions (Figure 5). For both species, light treatment and age had highly significant effects on predicted sensitivities (Figure 5). Light treatments did affect the ontogenetic progression from short to long wavelength sensitivity. In the blue treatment, this progression was slowed down, whereas in the red treatment, it was accelerated. In great lake Midas cichlids, we also found a significant interaction between light treatment and age. This can be explained by the fact that light treatments did induce strong changes in predicted sensitivities during early stages of development but not at 6 months (Figure 5, left column). At this age, predicted sensitivities were much less variable in great lake Midas cichlids compared to earlier stages. In contrast, predicted sensitivities were still affected in crater lake Midas cichlids at the age of 6 months; single cone sensitivities were altered by the red treatment (Figure 5, right column).

Additionally, we determined proportional cone opsin expression in different light treatments (Fig. S6). Both in single and double cones, the opsins most sensitive to the shortest wavelengths (*sws1* and *rh2b*, respectively) appeared to show elevated expression in short wavelength treatments, but lower levels in the long wavelength treatments. The opposite was true for cone opsins most sensitive to the longest wavelengths (*sws2a* and *lws* in singles and double cones, respectively). Besides, expression of *sws1*, *sws2a*, *rh2b* and *lws* seemingly changed with age in both species and additionally, and *sws2b* changed in crater lake Midas cichlids (Fig. S6).

4 | DISCUSSION

Midas cichlids inhabit multiple lakes in Nicaragua and have been established as a model system to study early stages of divergence and sympatric speciation (Barluenga, Stolting, Salzburger, Muschick, & Meyer, 2006; Elmer, Kusche, Lehtonen, & Meyer, 2010; Elmer et al., 2014; Klingenberg, Barluenga, & Meyer, 2003). Midas cichlids colonized crater Lake Apoyo from great Lake Nicaragua around 1,700 generations ago (Kautt et al., 2016). These two lakes differ strongly in their photic environment with the irradiance spectrum in Lake Apoyo shifted towards shorter wavelengths compared to Lake Nicaragua (inset in Figure 1; Torres-Dowdall, Pierotti, et al., 2017). Recent work suggests that adult Midas cichlids from the two lakes differ in cone opsin expression patterns, consistent with differences in the photic environment (Figure 1). These interspecific differences are maintained when fish are reared in a common light environment, arguing for a genetic basis for the observed phenotypic divergence (Torres-Dowdall, Pierotti, et al., 2017). Due to their known phylogenetic history and very recent divergence among species, Midas cichlids represent an ideal system to study the role of ambient light in shaping the visual system at a very early stage of population divergence and the effects of development in producing interspecific differences. As Midas cichlids colonized Lake Apoyo from Lake Nicaragua, visual sensitivity in great lake Midas cichlids represents the ancestral state and crater lake Midas cichlids have a derived phenotype.

In this study, we show that great lake Midas cichlids and crater lake Midas cichlids undergo an ontogenetic change in cone opsin

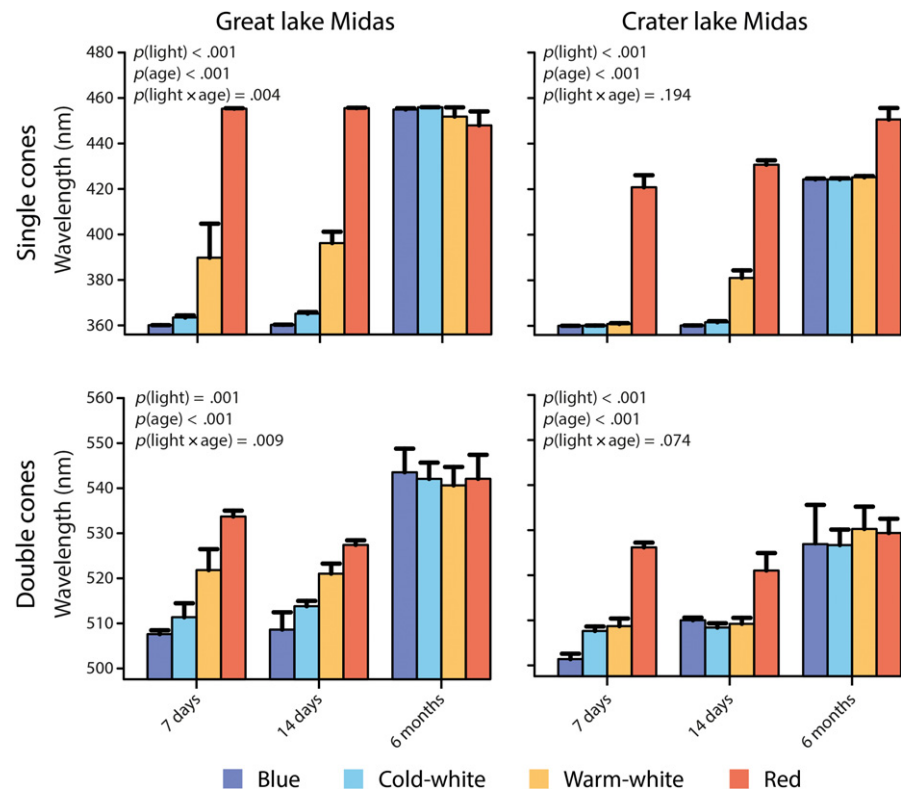


FIGURE 5 Plasticity in the predicted visual sensitivity for single (top row)- and double-cone opsins (bottom row) of great lake Midas cichlids (left column) and crater lake Midas cichlids (right column). The effects of light treatment and age were tested with Scheirer–Ray–Hare tests, and significance values (FDR corrected) are indicated

expression from short to long wavelength sensitivity. Many species of African cichlids have a direct development of cone opsin expression, especially those inhabiting the African great lakes (Lake Malawi and Lake Victoria; Carleton et al., 2016). However, riverine lineages of cichlids commonly show ontogenetic changes in cone opsin expression similar to that observed in Midas cichlids (Carleton et al., 2008, 2016). The developmental progression in visual sensitivity might be an adaptation to shifts in food sources. These fish commonly have a UV-sensitive phenotype in larvae, but UV sensitivity is lost later in life. Larvae typically feed on zooplankton, and the presence of UV-sensitive cones improves foraging performance in zebrafish and rainbow trout larvae (Novales Flamarique, 2013, 2016). Midas cichlid skin reflects UV light (Torres-Dowdall, Golcher-Benavides, & Meyer, 2017) and as larvae feed on the mucus of their parents' skin, UV sensitivity might enhance the ability to find their parents and thereby improve feeding success. The ontogenetic change observed in lacustrine Neotropical Midas cichlids has also, as mentioned above, been described in few species of riverine African cichlids, whereas the majority of cichlid species from the African great lakes show direct development. Hence, our results illustrate that the ontogenetic change in cone opsin expression might be a more common phenotype and is not restricted to riverine species.

Developmental shifts might represent a source of phenotypic variation in adult cone opsin expression profiles (Carleton et al., 2008; Matsumoto & Ishibashi, 2016; O'Quin et al., 2011; Shand et al., 2008; Spady et al., 2006). Particularly, by comparing closely related species insights into developmental changes of the visual system can be obtained. Predicted visual sensitivities in recently diverged Midas cichlids are consistently shifted towards shorter

wavelengths during development of the derived crater lake species compared to the ancestral species. However, the rate of ontogenetic change is similar in both species, hence, providing no evidence for a heterochronic shift, as defined by a change in relative rate of developmental progression (Rice, 1997). Nonetheless, the progression of cone opsin expression towards long wavelength sensitivity is terminated early in crater lake Midas cichlids and, hence, adults of this species pedomorphically resemble juveniles of great lake Midas cichlids regarding their visual phenotype (compare opsin expression profiles of 6-month-old great lake Midas cichlids and crater lake Midas cichlids older than 1 year in Figure 3). Apparently, after colonizing crater Lake Apoyo, Midas cichlids shifted their visual sensitivity throughout ontogeny leading to a pedomorphic adult phenotype that is more sensitive to shorter wavelengths. This shift is most likely adaptive because the light environment in crater Lake Apoyo is blue-shifted compared to great Lake Nicaragua (Torres-Dowdall, Pierotti, et al., 2017). Interestingly, the observed differences in visual sensitivities of Midas cichlids evolved within less than 1,700 generations (Kautt et al., 2016), suggesting that the visual system of Midas cichlids can rapidly adapt to novel light environments by altering the developmental progression from short to longer wavelength sensitivity. A similar pattern, where adults show a pedomorphic phenotype, can be found in some cichlids from the African great lakes. In these lakes, some species do not undergo the ancestral developmental progression from short to long wavelength sensitivity, but retain either a short or medium wavelength-sensitive visual system, emphasizing that ontogenetic changes might be a common mechanism underlying phenotypic divergence in both African and Neotropical cichlid fishes (Carleton et al., 2016).

Yet, the molecular mechanisms underlying this shift in cone opsin expression remain to be investigated, but TH has been shown to shift visual sensitivity from short to long wavelengths in multiple species. TH signalling affects cone opsin identity during development of the model systems mice and zebrafish, but also other fish species such as rainbow trout and coho salmon (Cheng, Gan, & Flammarique, 2009; Ng et al., 2001; Suliman & Flammarique, 2014) and adult cone opsin expression in mice (Glaschke et al., 2011). The active form of the hormone, triiodothyronine (T3), is synthesized from the prohormone thyroxine (T4) by the enzyme iodothyronine deiodinase type 2 (Dio2), whereas the enzyme iodothyronine deiodinase type 3 (Dio3) catabolizes T3 (Bianco & Larsen, 2005; Jarque & Pina, 2014). Knockdown of deiodinases in zebrafish causes morphological defects of the eye and disrupted visual function (Houbrechts et al. 2016). Dio3-deficient mice have degenerated cone cells, but deletion of Dio2 in this strain recovered a normal phenotype (Ng, Liu, St. Germain, Hernandez, & Forrest, 2017). These results emphasize the crucial role of TH and its regulation via deiodinases in eye development. Moreover, expression of the two deiodinases is controlled by TH via feedback loops, and increased levels of TH downregulate *dio2* and upregulate *dio3* gene transcription in Midas cichlids (Fig. S4), other fishes (Garcia et al., 2004; Johnson & Lema, 2011; Marlatt et al., 2012) and terrestrial vertebrates (Gereben et al., 2008; Glaschke et al., 2011). In developing mouse retinas, TH inhibits expression of short wavelength-sensitive cone opsins and activates expression of medium wavelength-sensitive cone opsins, thereby causing a shift in visual sensitivity towards longer wavelengths (Roberts et al. 2006). We validated this in Midas cichlids, where treatment with TH increased relative expression of the cone opsins most sensitive to the longest wavelengths in single cones (*sws2a*) and double cones (*lws*; Fig. S4). We hypothesized that the observed differences between great lake Midas cichlids and crater lake Midas cichlids might be caused by changes in TH levels during development. To this end, we used two deiodinase genes (*dio2* and *dio3*) as proxies for TH levels (see Methods and Fig. S4) and found that *dio2* expression is significantly higher in developing crater lake Midas cichlids, while *dio3* is significantly lower compared to great lake Midas cichlids (Figure 4). Moreover, there is some evidence that expression of *dio2* (indicative of high TH levels) is positively correlated with a short wavelength-sensitive cone opsin in single cones (*sws2b*), and expression of *dio3* is positively correlated with more long wavelength-sensitive cone opsins in single (*sws2a*) and double cones (*rh2aβ* and *lws*; Fig. S5), which are commonly expressed in adult fish. These results suggest that circulating TH levels are lower in developing crater lake Midas cichlids compared to great lake Midas cichlids and that TH levels might affect cone opsin expression. The lower TH levels we inferred during development of crater lake Midas cichlids might have promoted the retention of short wavelength shifted sensitivity. This, in turn, could have contributed to the paedomorphic phenotype in adult crater lake Midas cichlids (Figure 3). However, further experiments are needed to validate these hypotheses.

We observed that the light environment affects the visual system of developing Midas cichlids. These differences were most pronounced when fish were exposed to light conditions at the extremes

of the visible spectrum (Figure 5), but also broad light conditions resembling variation in the natural environment induced changes in cone opsin expression. Short wavelength shifted light caused a retention of the larval phenotype and a delay of the progression from short to long wavelength sensitivity (Figure 5), resulting in a paedomorphic visual phenotype (Gould, 1977; Alberch, Gould, Oster, & Wake, 1979; Rice, 1997). In contrast, exposure to long wavelength shifted light caused a faster progression of cone opsin expression due to acceleration. As in all cases, rate and timing of cone opsin expression are changed during development, the ambient light environment induced plastic heterochronic shifts in cone opsin expression (Gould, 1977).

These plastic shifts in visual sensitivity as a response to different light treatments occurred in the direction we expected; that is, fish in the blue and red light treatments expressed more short and long wavelength-sensitive opsins, respectively. The shift in the blue light treatment resembles what has been observed in adult crater lake Midas cichlids with their short wavelength shifted phenotype (Torres-Dowdall, Pierotti, et al., 2017), supporting the idea that Midas cichlids are able to adaptively adjust their visual system to the prevalent light environment. The ability to change cone opsin expression patterns in a novel light environment might have facilitated colonization of crater Lake Apoyo, emphasizing the importance of adaptive phenotypic plasticity.

The effects of phenotypic plasticity in evolution are a matter of debate (e.g., Ancel, 2000; Ghalambor et al., 2015; Paenke et al., 2007; Price et al., 2003). Some claim that adaptive phenotypic plasticity might facilitate population persistence by moving organisms closer to a new adaptive peak (Amarillo-Suarez & Fox, 2006; Robinson & Dukas, 1999), thereby acting as a driving force for further evolutionary change (Baldwin, 1896; Crispo, 2007). Others have stated that nonadaptive plasticity promotes evolutionary change by moving environmentally induced phenotypes further away from adaptive peaks, thereby increasing strength of selection (Ghalambor et al., 2015). In this context, we argue that in Midas cichlids, adaptive phenotypic plasticity of the visual system has facilitated evolutionary change that led to species divergence after colonization of a novel light environment. Accordingly, high levels of phenotypic plasticity might have been advantageous in the early stages after colonizing crater Lake Apoyo and enabled Midas cichlids to slow down the ontogenetic change of cone opsin expression leading to a shorter wavelength-sensitive adult phenotype. Subsequently, selection on standing genetic variation could have led to genetic assimilation of the initially environmentally induced plastic response (Crispo, 2007; Ghalambor et al., 2007), causing the interspecific differences in adult Midas cichlids (Torres-Dowdall, Pierotti, et al., 2017). Notably, after colonization of crater Lake Apoyo, Midas cichlids formed a small adaptive radiation and five distinct genetic clusters have been reported, most probably referring to different species (Kautt et al., 2016). It has recently been proposed that high levels of phenotypic plasticity are correlated with evolutionary diversification of cichlid fishes, according to the flexible stem model (Schneider & Meyer, 2017). The contribution of Midas cichlids' plastic visual system to the adaptive radiation in crater Lake Apoyo remains to be assessed.

Interestingly, we found that the ancestral great lake Midas cichlids have a reduced plastic response at the juvenile/adult stage (>6 months) compared to earlier stages of development. In fact, the light treatments did not elicit any effect in the expression of opsin genes at this stage. However, when analysing 6-month-old derived Midas cichlids from crater Lake Apoyo, we found that at this age, specimens retained the ability to plastically respond to the light environment (Figures 5 and S6). Thus, plasticity itself appears to have evolved after the colonization of crater Lake Apoyo with its blue-shifted light environment. Although it will be necessary to test plasticity under different light conditions in adult fish, there is some evidence that plasticity might be retained also in adults as there are differences in the expression levels of cone opsin genes between laboratory reared and wild caught fish. These differences tend to be higher in the derived crater lake populations than in the ancestral great lake Midas cichlids (Torres-Dowdall, Pierotti, et al., 2017). This pattern of increased plasticity in derived populations after the colonization of a new environment, as we see in crater lake Midas cichlids, is consistent with the “Baldwin effect” (Baldwin, 1896; Crispo, 2007; Torres-Dowdall et al., 2012), but inconsistent with models predicting genetic assimilation (e.g., Lande, 2009). The retention of plasticity into juvenile/adult stages in the crater lake Midas cichlids is in agreement with our interpretation of these fish having a paedomorphic visual system compared to the source population from the turbid great lake. Thus, increased selection for a short wavelength-sensitive phenotype in the clear water crater lake might have resulted in a correlated increase of plasticity. Alternatively, plasticity itself could have been selected for, if the crater lakes have a more variable light environment. This is a possible explanation given that light environment is expected to greatly vary with depth in the deep crater lakes. Nonetheless, this hypothesis remains to be tested.

To summarize, Midas cichlids tuned their visual system after colonizing crater Lake Apoyo, which represents a novel light environment, by altering cone opsin expression compared to the ancestral great lake Midas cichlids. Our results show that the interspecific differences in adult cone opsin expression patterns are brought about by shifting visual sensitivity towards shorter wavelengths throughout development, possibly mediated by TH signalling. Moreover, cone opsin expression is plastic in early development of Midas cichlids and changes according to the ambient light. Hence, phenotypic plasticity can be considered adaptive and might have, via interaction with development, facilitated colonization, population persistence and subsequently speciation in the novel environment of crater Lake Apoyo. Remarkably, these changes evolved in a very short time frame of less than 1,700 generations, representing a case of exceptionally rapid adaptive evolution that was possibly aided by developmental phenotypic plasticity.

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DATA ACCESSIBILITY

Raw qPCR data are provided in Table S3.

AUTHOR CONTRIBUTIONS

A.H. and J.T.D. designed the experiments, collected the data, and performed the analyses. A.H. wrote the first draft of the manuscript with contributions from J.T.D. All the authors read and edited the final version.

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