Developmental and environmental plasticity in opsin gene expression in Lake Victoria cichlid fish

Irazábal-González, Lucia; Wright, Daniel S; Maan, Martine E

Published in:
Evolution & development

DOI:
10.1111/ede.12465

Publication date:
2024

Received: 18 October 2021 | Accepted: 15 November 2023

DOI: 10.1111/ede.12465

RESEARCH ARTICLE

WILEY

Developmental and environmental plasticity in opsin gene expression in Lake Victoria cichlid fish

Lucia Irazábal-González | **Daniel S. Wright** | **Martine E. Maan**

Groningen Institute for Evolutionary Life Sciences (GELIFES), University of Groningen, Groningen, The Netherlands

Correspondence
Lucia Irazábal-González, Groningen Institute for Evolutionary, Life Sciences, University of Groningen, Groningen, The Netherlands.
Email: l.irazabal.gonzalez@rug.nl

Present address
Daniel S. Wright, Division of Evolutionary Biology, Ludwig Maximilian University of Munich, Munich, Germany.

Funding information
Financial support came from the Swiss National Science Foundation, Grant/Award Number: (SNSF PZ00P3-126340; to MM); Netherlands Foundation for Scientific Research, Grant/Award Number: (NWO VENI 863.09.005; to MM); Rijksuniversiteit Groningen

Abstract

In many organisms, sensory abilities develop and evolve according to the changing demands of navigating, foraging, and communication across different environments and life stages. Teleost fish inhabit heterogeneous light environments and exhibit a large diversity in visual system properties among species. Cichlids are a classic example of this diversity; visual system variation is generated by different tuning mechanisms that involve both genetic factors and phenotypic plasticity. Here, we document the developmental progression of visual pigment gene expression in Lake Victoria cichlids and test if these patterns are influenced by variation in light conditions. We reared two sister species of *Pundamilia* to adulthood in two distinct visual conditions that resemble the light environments that they naturally inhabit in Lake Victoria. We also included interspecific first-generation hybrids. We focused on the four opsins that are expressed in *Pundamilia* adults (using real-time quantitative polymerase chain reaction (RT-qPCR)) (*SWS2B*, *SWS2A*, *RH2A*, and *LWS*) at 17 time points. We find that opsin expression profiles progress from shorter-wavelength sensitive opsins to longer-wavelength sensitive opsins with increasing age, in both species and their hybrids. The developmental trajectories of opsin expression also responded plastically to the visual conditions. Developmental and environmental plasticity in opsin expression may provide an important stepping stone in the evolution of cichlid visual system diversity.

KEYWORDS
adaptation, heterochrony, light, *Pundamilia*, vision

1 | INTRODUCTION

Animal sensory systems mediate interactions with the environment, contributing to foraging, navigation, predator avoidance, and mate selection. Sensory systems are highly diverse within and between species, associated with differences in ecological niche and life history (Stevens, 2013). For example, the visual system rapidly adapts to variation in local light conditions, resulting in inter- and intraspecific variation in visual system properties (Bowmaker, 2008; Carleton et al., 2016; Chang et al., 2021; Veilleux & Kirk, 2014). In water, where light

transmission is poorer than in air, there is substantial heterogeneity in light conditions, mediated by water depth and transparency. As a result, variation in visual properties is high across aquatic vertebrates, compared with other groups (Bowmaker et al., 1994; Loew & McFarland, 1990).

Cichlids are a family of teleost fish that has rapidly diverged into numerous species across Africa, Asia, South America, and Central America (Kocher, 2004; Salzburger, 2018; Seehausen, 2006). The species inhabit different photic environments, and their visual system properties are highly diverse (Carleton & Yourick, 2020). Some of this diversity is genetically encoded, such as variation in the opsin gene sequences that affect the wavelength absorption properties of the visual pigments, and the subset of opsin genes actually expressed (Carleton et al., 2005, 2008, 2016; Hofmann et al., 2009). Other aspects of the visual system can be influenced by developmental or environmental plasticity, including the expression levels of the opsin genes and the use of alternative chromophores (Carleton & Kocher, 2001; Carleton et al., 2016; Halstenberg et al., 2005; Härer et al., 2017; Hofmann et al., 2009). In this study, we examine the developmental trajectory of relative opsin expression in two Lake Victoria cichlid species (genus *Pundamilia*) and their hybrids, and investigate the influence of light-induced plastic variation in opsin expression.

Cichlids have seven distinct cone opsin genes: the short-wavelength-sensitive opsins *SWS1* (UV), *SWS2B* (violet), *SWS2A* (blue); the medium-wavelength-sensitive opsins *RH2Aα*, *RH2Aβ*, *RH2B* (green); and the long-wavelength-sensitive opsin *LWS* (red) (Carleton et al., 2005, 2016). Different species express different subsets of these, typically consisting of three or four opsins (Carleton et al., 2016; Hofmann et al., 2009). The subset of four opsins that *Pundamilia* express include: two short-wavelength-sensitive opsins (*SWS2B* and *SWS2A*), one medium-wavelength-sensitive opsin (*RH2A*), and one *LWS* (Carleton et al., 2005; Hofmann et al., 2009). During development, opsin expression profiles may change and species differ in their developmental patterns. This variation (heterochrony) may provide a target for divergent selection and contribute to the evolution of differences in opsin gene expression observed across species (Carleton et al., 2008; Härer et al., 2017; O'Quin et al., 2011; Sandkam et al., 2020).

Carleton et al. (2008) characterized the developmental patterns of opsin expression in two Lake Malawi cichlid clades (sand-dwelling *Dimidiochromis compressiceps* and *Tramitichromis intermedius*, and rock-dwelling *Metriaclima zebra* "gold," *Metriaclima zebra*, *Metriaclima benetos*, and *Labeotropheus fuelleborni*) and an ancestral riverine cichlid lineage (Tilapia, *Oreochromis niloticus*). *O. niloticus* expressed predominantly short-wavelength-sensitive opsins as larvae (*SWS1* and *RH2B*), increased the expression of medium-wavelength-sensitive opsins as juveniles (*SWS2B* and *RH2A*), and finally expressed high amounts of long-wavelength-sensitive opsins as adults (*SWS2A*, *RH2A*, and *LWS*) (Carleton et al., 2008). Rock-dwelling Lake Malawi larvae and juveniles expressed short- and medium-wavelength-sensitive opsins (*SWS1*, *SWS2B*, and *RH2A*), and maintained this expression pattern until adulthood. Larvae and juveniles of the sand-dwelling species expressed long-wavelength-sensitive opsins (*SWS2A*, *RH2A*, and *LWS*) and maintained this expression profile throughout development. Carleton et al. (2008) hypothesized that the developmental pattern of opsin expression in Lake Victoria cichlids may resemble the one observed in the Lake Malawi sand-dwellers, but the available data came from only a small number of Lake Victoria juveniles sampled at a single time point (Carleton et al., 2005, 2008). Therefore, the developmental pattern of opsin expression in Lake Victoria cichlids remains to be established.

Research highlights

In Lake Victoria cichlid fish, expression levels of opsin genes (encoding visual pigments) differ between developmental stages and between experimental light treatments. This plasticity may contribute to the evolution of cichlid visual system diversity.

Pundamilia pundamilia and *Pundamilia nyererei* are two closely related cichlid species that co-occur at several rocky islands in southeast Lake Victoria, in the Mwanza and Speke Gulfs (Seehausen et al., 2008). Water transparency differs between sites, with turbid waters in the south and clearer waters in the north (Castillo Cajas et al., 2012). Both species are consistently depth segregated at all locations but with more overlap in more turbid locations. *P. pundamilia* is restricted to shallow waters (0–2 m), whereas *P. nyererei* is most abundant at 3–10 m depth, where the light spectrum is shifted towards longer wavelengths and is largely lacking short-wavelength light (Seehausen et al., 2008). Anatomically, both species are similar, but they differ in male coloration. *P. pundamilia* males are metallic blue with a blue dorsal fin and *P. nyererei* males are red with yellowish flanks. Females of both species are cryptic yellow/gray.

In this study, we use fish from Python Island, where until recently, the blue and red species were thought to be

P. pundamilia and *P. nyererei*, respectively. However, Meier et al., (2017, 2018) showed that the population at Python Island represents a separate speciation event and is, therefore, referred to as *P.* sp. "*pundamilia-like*" and *P.* sp. "*nyererei-like*." Across *Pundamilia* populations, and consistently linked to male color, depth, and water clarity, the *LWS* opsin gene has sequence variations that alter pigment sensitivity (Hofmann et al., 2009; Seehausen et al., 2008; Wright et al., 2019). Wild populations also differ in opsin expression within and between locations (Hofmann et al., 2009; Wright et al., 2019). In the laboratory, we mimicked the natural shallow (broad-spectrum) and deep (red-shifted) light environments of Python Island and observed evidence of opsin expression plasticity: adult fish expressed more *LWS* and less *SWS2A* when reared in red-shifted light conditions, compared with their counterparts from broad- spectrum light conditions (Wright et al., 2020).

Here we characterize the developmental patterns of opsin expression in *P.* sp. "*pundamilia-like*," *P.* sp. "*nyererei-like*," and their hybrids. We hypothesize that the developmental patterns of both species and their hybrids will resemble those of the Lake Malawi sand-dwellers. We also explore the extent of environmental plasticity during development by rearing the fish in broad-spectrum and red-shifted light conditions (Maan et al., 2017; Wright et al., 2017). We expect longer wavelength sensitivities in the red-shifted light condition compared to the broad-spectrum light condition. Moreover, a study in neotropical Midas cichlids suggests that the transition to the adult expression profile may be delayed in short-wavelength-rich light environments and accelerated in long-wavelength-rich environments (Härer et al., 2017), Therefore, we predict that *LWS* expression increases faster in the red-shifted light condition. For both overall opsin expression and its developmental pattern, we expect hybrids to respond similarly to the parental species.

2 | MATERIAL AND METHODS

2.1 | Fish species and breeding

We used first- (F1) and second- (F2) generation aquarium-reared offspring of wild caught *P.* sp. "*pundamilia-like*" and *P.* sp. "*nyererei-like*" collected from Python Island in 2014 (−2.6238, 32.8567).

For breeding, we housed several females with a single male. All fish were tagged (PIT tags, Passive Integrated Transponder, Biomark, and Dorset Identification, Aalten, Netherlands). Haplochromine cichlids are maternal mouthbrooders; about 5 days after fertilization, the eggs were removed from the mother's buccal cavity and divided equally between light treatments (described below).

We reared both species, as well as their reciprocal hybrids, in both light environments. The analyzed samples came from 30 aquarium-reared F1 and F2 families, with 22 dams and 17 sires (Supporting Information S1: Table S1). The animal experiments that we performed for this study were approved by the animal ethics committee of the University of Groningen (DEC 6205B; AVD105002016464).

2.2 | Housing and light conditions

All fish were maintained at 25 ± 1°C, on a 12:12 h day:night light cycle. In both light treatments (mimicking the shallow and deep waters at Python Island), the tanks were lit with halogen bulbs (Philips Halogen Masterline ES, 35W) that were differently filtered depending on the treatment. In the deep treatment, the light was filtered with yellow (no. 15 by LEE, Andover, UK) and green filters (LEE no. 243), generating a red-shifted light spectrum (Supporting Information S1: Figure S1). In the shallow treatment, the light was filtered with the green filter only. Halogen bulbs have a limited short-wavelength radiance, so the short wavelengths were supplemented with blue lights (Paulmann 88090 ESL Blue spiral 15W) in the broad-spectrum light environment (Supporting Information S1: Figure S1). Further details on the light treatments and a comparison with the spectral conditions at Python Island are provided in the Supporting Information.

2.3 | Samples

All samples were collected between February 3, 2017 and May 28, 2018. Fish were sampled at 14 different time points, ranging from 10 to 192 days post fertilization (dpf) (Supporting Information S1: Table S2; *Pundamilia* reach sexual maturity at ~8 months of age; i.e., ~240 dpf). To control for variation between families, we aimed to include samples from at least two different families at each time point. Based on previous studies (Carleton et al., 2008; O'Quin et al., 2011) and on pilot trials of total RNA isolation from fish of various ages, the number of eyes sampled for a single data point differed between time points: we pooled both eyes from two individuals at 10–20 dpf (i.e., four eyes represent one data point), both eyes from one individual at 30–60 dpf, and used one eye per individual from 70 dpf onwards. Whole eyes were

used up to 90 dpf and retinal tissue was used from 120 dpf onwards.

Fish were euthanized in the environments that they were reared in. We used an overdose of MS-222 buffered with sodium bicarbonate, and the eyes were removed, preserved in RNALater (Ambion®), and frozen until quantitative polymerase chain reaction (qPCR) analysis (detailed below). Previous studies have shown a circadian rhythm in opsin expression (Halstenberg et al., 2005). To minimize variation and maximize yield, all samples were collected between 4:00 p.m. and 6:00 p.m.

2.4 | Quantification of opsin expression by real-time qPCR (RT-qPCR)

Following previous studies (Wright et al., 2019, 2020), we isolated total RNA with Trizol (Ambion) and reverse-transcribed 1 μg of RNA from each sample with Oligo $(dT)_{18}$ (100 μM) (Thermo Scientific, Life Technologies) and RevertAid H minus RT reverse transcriptase (Thermo Scientific, Life Technologies) at 45°C.

We performed RT-qPCR to quantify the relative expression of the four opsins present in adult Lake Victoria cichlids (*SWS2B*, *SWS2A*, *RH2A*, and *LWS* (Hofmann et al., 2009)), using gene-specific TaqMan® primers and probes (Supporting Information S1: Table S3). *RH2Aα* and *RH2Aβ* were analyzed together as *RH2A*, because they are highly similar in sequence and function (Parry et al., 2005). Previous studies have shown that *RH2B* and *SWS1* were not expressed in adult *Pundamilia* cichlids (Carleton et al., 2005; Hofmann et al., 2009). To verify that *RH2B* and *SWS1* are indeed not expressed, even in fry and juveniles, we conducted an additional series of PCR (RT-qPCR) analyses for these genes on a subset of the samples (42 samples). These analyses showed that the expression of both genes is negligible (*RH2B*: 0.083 ± 0.015%; *SWS1*: 0.39 ± 0.068%). For details, see Supporting Information. Furthermore, the joined relative expression levels of *RH2A* and *RH2B* observed in *Pundamilia* juveniles in another study (Carleton et al., 2005) correspond very well to the proportional expression levels of *RH2A* we observed in the present study. Fluorescence was monitored during 50 cycles with a BIO-RAD C1000 Thermal Cycler (CFX96 Real Time System) (95°C for 2 min, 95°C for 50 s, and 60°C for 1 min).

We used LinRegPCR® to determine the critical threshold for each sample. This program examines the log-linear part of the PCR curve for each sample to determine the upper and lower limit of the "window of linearity." Linear regression analysis is then used to calculate the intercept (i.e., the estimated initial concentration) (Ramakers et al., 2003). We used the same approach to calculate the slope and the intercept of a serially diluted construct of the four opsin genes ligated together. We used the following equation to calculate the relative opsin gene expression:

$$\frac{N_0}{N_{\text{all}}} = \frac{e^{\left(\frac{Cti-b}{m}\right)}}{\sum e^{\left(\frac{Cti-b}{m}\right)}}$$

where N_0/N_{all} represents the expression of each opsin gene, relative to total opsin expression. *Cti* is the threshold cycle number for the focal opsin, *b* is the intercept of the mean *Cti* of each diluted point of the ligated standard, and *m* the slope of this ligated standard. All samples were run twice.

2.5 | Data analysis

We discarded samples with PCR efficiencies below 1.75 and above 2.25, and when the *Cq* SD between replicates was >0.5. To avoid unwarranted exclusion of low expression levels, we did not apply these rules for opsins with <1% of the total expression (efficiencies decrease and error rates increase at such low concentrations). The relative expression of each opsin is defined in relation to the other three opsins, so discarding a sample for one opsin means discarding the entire sample. The RT-qPCR from the discarded samples was repeated once for any opsin that fell outside of the parameters mentioned above. After applying these quality thresholds, 27 of 239 samples were discarded.

Before statistical analysis, we performed an outlier check with Tukey's method identifying the outliers that fell above and below the 1.5 interquartile range. For this procedure, we divided the data set by species and treatment. Moreover, due to the change in opsin expression with developmental time (see Section 3), the data were divided by age of sampling (10–30 and 40 dpf onwards). This analysis was performed to ensure that the data set did not contain artefacts from the sampling, RT-qPCR or the methodology (as discussed in Carleton et al., 2005; Hofmann et al., 2009; Wright et al., 2019, 2020). Thirty-four outliers were removed, leaving 178 samples for the analyses (Supporting Information S1: Table S1). We used linear mixed modeling (in R, R Development Core Team 2017, lmer, package lme4), separately for each opsin, with the time (dpf), species (*P.* sp. "*pundamilia-like*," *P.* sp. "*nyererei-like*," or hybrid) and light treatment (broad-spectrum or red-shifted) as fixed effects, and mother and father ID as random effects ($expression \sim treatment \times species \times time + (1|momID) + (1|dadID)$). Visual checks (*Q*–*Q* and

residual plots) and akaike information criterion model comparisonsshowed that for the fixed effect *time*, a log10 transformation was the best fit for all opsins. The response variable *relative SWS2B expression* was also log10 transformed to satisfy the assumptions of linear analysis; the remaining three opsins were analyzed without transformation. We used stepwise backward selection based on statistical significance (in R, "MASS" package version 7.3-45; [Venables & Ripley, 2002]) to determine the minimum adequate model (retaining parental identities in all models to account for pseudo replication). Finally, we performed "KRmodcomp" to estimate the parameter effects, *p-values*, and degrees of freedom based on the Kenward–Roger approximation (in R, package "pbkrtest" version 0.4-6; [Halekoh and Højsgaard, 2014]).

3 | RESULTS

3.1 | *Pundamilia* opsin expression throughout development

Relative expression levels of all four opsins changed significantly over time, as evidenced by the significant effect of time in all models (Figure 1 and Table 1). Throughout development, *Pundamilia* expressed high proportions of *LWS*, followed by *RH2A*, *SWS2A*, and *SWS2B*. With time, relative *RH2A* expression decreased from ~25% to below 20%, and *LWS* increased from ~60% to ~75%. The relative expression of both *SWS2* opsins was low in early development (~5%). *SWS2A* increased to about 20%, whereas *SWS2B* decreased to nearly 0 at 50 dpf. The expression of all four opsins stabilized after 200 dpf, reaching the levels previously established for adult fish (Figure 1).

3.2 | Species differences

Relative expression levels of *LWS* and *RH2A* differed significantly between the species groups (Table 1 and Figure 2), and posthoc tests showed that all pairwise comparisons were significant (all $p < 0.001$). *LWS* expression was highest in *P.* sp. "*pundamilia-like*," lowest in *P.* sp. "*nyererei-like*," and the hybrids were intermediate. *RH2A* showed the opposite pattern: it was highest in *P.* sp. "*nyererei-like*," lowest in *P.* sp. "*pundamilia-like*," and the hybrids were in-between. Expression levels of the short wavelength sensitive opsins were similar for both species and their hybrids (Table 1).

Despite the differences in relative opsin expression levels, there were no differences in the developmental patterns between species: the interaction between species and time did not significantly affect the expression level of any of the opsins (Table 1).

3.3 | Effects of the light environment

We found significant effects of the light treatments on the relative expression levels of *SWS2B*, *SWS2A*, and *RH2A* (see Figure 3 and Table 1). In broad-spectrum light, fish expressed higher proportions *SWS2B* and lower proportions of *SWS2A* and *RH2A*, compared with their counterparts from the red-shifted light environment.

The developmental patterns of opsin expression were similar between light conditions (Figure 3), but the rate of change in opsin expression differed significantly. Overall, the proportion of *SWS2A* increased in both light conditions, but the rate of change differed (Figure 3 and Table 1). In broad-spectrum light, *SWS2A* expression was initially lower, but it rapidly increased (during 10–50 dpf) until the expression levels were similar in both light conditions. The increase in *SWS2A* expression was less steep in the red-shifted light condition, where expression remained relatively stable throughout development. Proportional *LWS* expression was lower in early development in the red-shifted light condition and increased more steeply than in the broad-spectrum light environment. The developmental

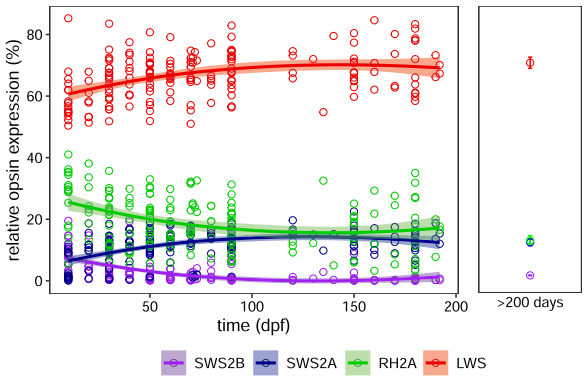

FIGURE 1 *Pundamilia* opsin expression throughout development. *P.* sp. "*pundamilia-like*," *P.* sp. "*nyererei-like*" and hybrids are pooled. The relative expression of all four opsins (*SWS2B*, *SWS2A*, *RH2A*, and *LWS*) changed during development, as highlighted by the significant effect of time (dpf, days post fertilization) in all models. Each symbol represents an individual (two individuals [four eyes] pooled for time points 10 and 20 dpf, one individual [two eyes] for time points 30–60 dpf, one individual [one whole eye until 90 dpf], and one individual [retinal tissue] from 120 dpf onwards), with shaded areas indicating 95% confidence interval (95% CI). Opsin expression profiles of lab-reared adults (>200 dpf) are provided for reference (from Wright et al., 2020). Error bars represent 95% CI. dpf, days post fertilization.

TABLE 1 Parameter estimates from the linear mixed models explaining variation in opsin expression.

SWS2B	***F***	**Df**	**Df resid**	***p***
Time	172.75	1	170.18	<2.2e−16***
Treatment	110.62	1	156.06	<2.2e−16***
Removed factors	***F***	**ndf**	**ddf**	***p***
Time:treatment	1.34	1	154.71	0.25
Time:species	0.97	2	19.76	0.40
Treatment:species	1.01	4	29.49	0.42
Species	1.41	2	11.04	0.28
SWS2A	***F***	**Df**	**Df resid**	***p***
Time	40.32	1	170.59	1.880e−09***
Treatment	6.62	1	156.53	0.01101*
Time:treatment	23.61	1	156.21	2.852e−06***
Removed factors	***F***	**ndf**	**ddf**	***p***
Species	1.12	2	10.29	0.36
Time:species	1.33	2	13.15	0.30
Treatment:species	1.42	4	27.55	0.25
RH2A	***F***	**Df**	**Df resid**	***p***
Time	43.55	1	169.06	5.108e−10***
Treatment	9.86	1	156.68	0.002019**
Species	49.11	2	9.93	7.090e−06***
Treatment:species	5.58	2	157.20	0.00456**
Removed factors	***F***	**ndf**	**ddf**	***p***
Time:species	1.93	2	137.72	0.15
Time:treatment	1.472	1	155.23	0.23
LWS	***F***	**Df**	**Df resid**	***p***
Time	48.4058	1	159.975	8.41e−11***
Treatment	0.1718	1	155.660	0.68
Species	31.4869	2	9.296	7.25e−05***
Time:treatment	4.7352	1	155.539	0.031*
Treatment:species	3.8317	2	156.089	0.024*
Removed factors	***F***	**ndf**	**ddf**	***p***
Time:species	0.3093	2.0000	127.8492	0.735

Note: Time represents the days post fertilization, treatment represents the light conditions (broad-spectrum or red-shifted), and species represents the different crosses (*P.* sp. "*pundamilia-like*" or *P.* sp. "*nyererei-like*" or their hybrids). We used the ANOVA () function ("car" package) to estimate the parameter effects, degrees of freedom, and *p*-values of the significant factors (significance codes are represented by asterisk symbols: "*" .01; "**" .001; "***" .0001), and we used "KRmodcomp" to compare the minimum adequate model with a model containing the removed factor(s).

Abbreviations: ANOVA, analysis of variance; ddf, denominator degrees of freedom; ndf, numerator degrees of freedom.

patterns of proportional *SWS2B* and *RH2A* expressions were not affected by the light treatments.

We also examined whether the two *Pundamilia* species and their hybrids responded differently to the light treatments. We found significant interaction effects (species by treatment) for proportions of *RH2A* and *LWS* expression (Table 1 and Figure 3). However, Tukey's posthoc tests showed that only hybrids differed in

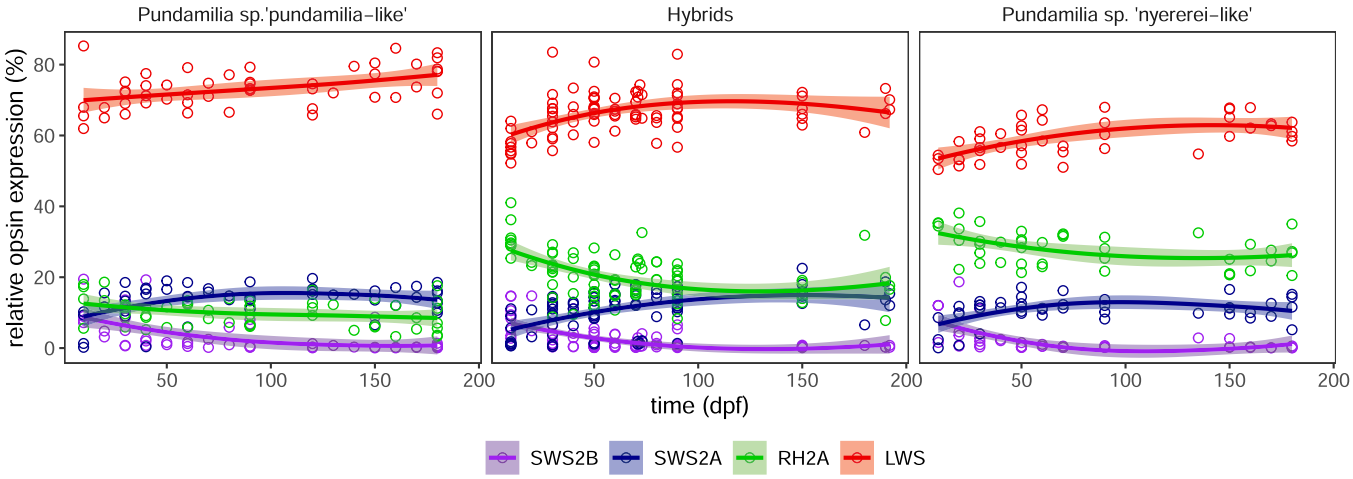

FIGURE 2 Opsin expression throughout development for *P.* sp. "*pundamilia-like*," *P.* sp. "*nyererei-like*," and their hybrids in both light treatments. Relative *LWS* and *RH2A* expression differed significantly between the species groups, but the developmental patterns were similar. Note that these are the same data represented in Figure 1, but separated by species. Each symbol represents an individual (two individuals pooled for time points 10 and 20 dpf), with shaded areas indicating 95% CI.

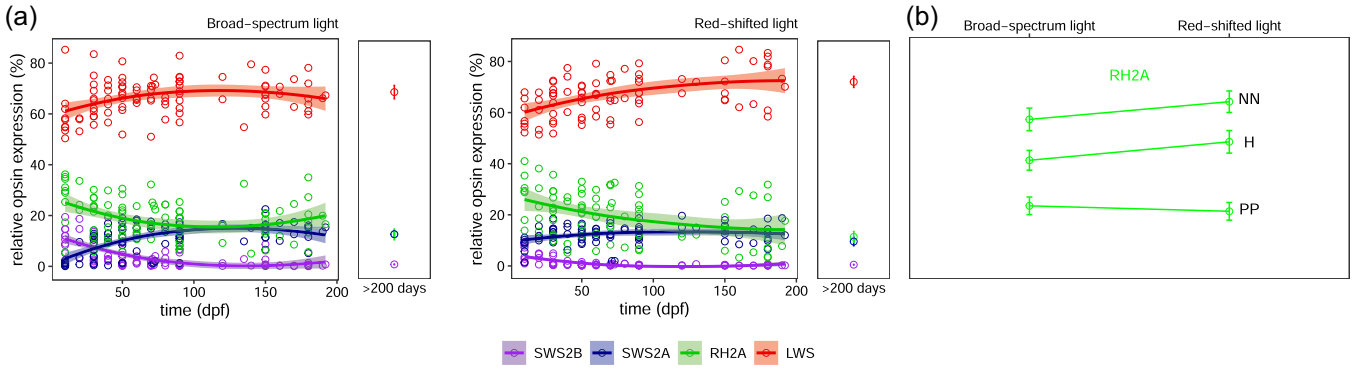

FIGURE 3 Effects of the light treatments on the developmental patterns of *Pundamilia* opsin expression. (a) Opsin expression patterns of fish housed in broad-spectrum (left panel) versus red-shifted light (right panel). *P.* sp. "*pundamilia-like*," *P.* sp."*nyererei-like*," and hybrids are combined. The proportional expression levels of *SWS2B*, *SWS2A*, and *RH2A* were significantly affected by the light treatments. Shaded areas indicate 95% confidence interval (95% CI). Note that these are the same data as in Figures 1 and 2, but separated by light treatment. The opsin expression profiles of lab-reared adults (>200 dpf) are provided for reference (from Wright et al., 2020). (b) The effect of rearing light treatments differed in hybrids for *RH2A* and it did not affect the relative opsin expression of *P.* sp "*pundamilia-like*," or *P.* sp. "*nyererei-like*." In the red-shifted light condition, hybrids expressed relatively higher levels of *RH2A* than their counterparts in broad-spectrum light condition. Error bars represent 95% CI.

relative *RH2* expression between light treatments ($p = 0.006$). The hybrids had higher *RH2A* expression in the red-shifted light environment, whereas the *RH2A* expression levels of *P.* sp. "*nyererei-like*" and *P.* sp. "*pundamilia-like*" did not differ between conditions.

4 | DISCUSSION

In this study, we characterized the developmental pattern of opsin expression in two Lake Victoria haplochromines, *P.* sp. "*pundamilia-like*" and P. sp. "*nyererei-like*," and their hybrids in two distinct light conditions. Our results show that opsin expression changes over developmental time and is also influenced by environmental light.

4.1 | *Pundamilia* opsin expression throughout development

We hypothesized that the relative opsin expression in *Pundamilia* cichlids (*SWS2B*, *SWS2A*, *RH2A*, and *LWS*) would remain fairly constant across development, with

relatively high long-wavelength sensitivity throughout, as previously documented in sand-dwelling cichlids from Lake Malawi (Carleton et al., 2008). Our results are partly consistent with this hypothesis: throughout development, relative expression of the long-wavelength sensitive opsin (*LWS*) was high, compared with the medium- and short-wavelength sensitive opsins (*RH2A*, *SWS2B*, and *SWS2A*). The *Pundamilia* expression pattern is similar to that of the Lake Malawi sand-dwellers. However, the pattern in *Pundamilia* has a more noticeable progression from short-wavelength sensitive opsins in early stages (10 dpf) towards long-wavelength sensitive opsins in the adult stage. In that sense, they also resemble the developmental pattern of *O. niloticus* (Carleton et al., 2008; O'Quin et al., 2011). Therefore, the developmental pattern of *Pundamilia* cichlids falls in-between *O. niloticus* and Lake Malawi sand-dwellers. When comparing our present findings to the opsin expression levels we documented in adults (Wright et al., 2020), we observe that the opsin expression profile stabilizes at around 200 dpf.

At 10 dpf, relative *SWS2B* expression was relatively high (~8%) but quickly decreased to zero at ~100 dpf. A possible adaptive explanation for high *SWS2B* expression in young fish might be an ontogenetic shift in the vertical distribution in the water column. In several haplochromine species from Lake Victoria, larvae and juveniles have shallower depth distributions than adults (Goldschmidt et al., 1990), and shallow waters are relatively rich in short-wavelength light (captured by *SWS1*, *SWS2B*, and *SWS2A*). The depth distribution of *Pundamilia* juveniles is unknown but high *SWS2B* expression may suggest that they inhabit relatively shallow waters. *SWS2B* expression may also contribute to foraging efficiency; cichlids and other teleost fish change their foraging strategies as they develop and zooplankton is an important component of larval and juvenile diets (Fryer & Iles, 1972). Short-wavelength vision particularly aids the detection of small and translucent objects (Britt et al., 2001; Browman et al., 1994; Carleton et al., 2008; Loew & Wahl, 1991; Flamarique, 2013).

4.2 | Species differences

The parental species differed in the proportions of *LWS* and *RH2A* expression: *P.* sp. "*pundamilia-like*" had higher relative *LWS* expression than *P.* sp. "*nyererei-like*," whereas *RH2A* exhibited the opposite pattern. This is in line with earlier studies of wild caught (Wright et al., 2019) and laboratory-reared fish (Hofmann et al., 2009; Wright et al., 2020). As hypothesized, the developmental patterns of opsin expression were similar in both species.

4.3 | Effects of the light environment

The fish were reared in two light environments mimicking the natural shallow (broad-spectrum) and deep (red-shifted) light environments of Lake Victoria. These light treatments influenced opsin expression. Relative *SWS2B* expression was higher in broad-spectrum light than in red-shifted light, whereas *SWS2A* and *RH2A* followed the opposite pattern. Higher *SWS2A* expression in the red-shifted condition contrasts with what was previously observed in *Pundamilia* adults (Wright et al., 2020); we discuss this further below.

In addition to the overall expression levels, the developmental patterns of *LWS* and *SWS2A*, were affected by our light treatments. Relative *LWS* expression increased faster in the red-shifted light condition, similar to the pattern observed in Midas cichlids (Härer et al., 2017). However, *SWS2A* showed a different effect, changing faster in the broad-spectrum light conditions: relative *SWS2A* increased faster. It seems that the proportions of *LWS* and *SWS2A* expression increase faster in the light conditions where these opsins would confer greater photon capture, i.e., *LWS* expression changed faster in the red-shifted environment, which is dominated by long wavelengths (and lacks short wavelengths), and *SWS2A* expression increased faster in the broad-spectrum environment, which includes short wavelengths. Thus, our findings do not correspond to the general pattern suggested for neotropical cichlids, where progress towards the adult phenotype was accelerated in light conditions rich in long wavelengths (Härer et al., 2017). Another interpretation of our results would be in line with the results from Härer et al. (2017). The relative expression levels of *SWS2A* at the start of the development in the red-shifted light condition resembled those of adult *Pundamilia*, whereas those levels were reached later in the broad-spectrum light. This might suggest that the development towards adult phenotypes was arrested in the broad-spectrum light condition. However, we cannot be certain about this because we lacked time points before 10 dpf. Thus, for *Pundamilia* in the red-shifted light condition, we do not know whether the relative expression of *SWS2A* stays stable before 10 days and increases slowly, agreeing with our first interpretation, or if it increases very fast in the days before 10 dpf, which would suggest that development is accelerated. To test this interpretation, experiments are needed to quantify fishes' visual performance in different visual

conditions and at different developmental stages, such as before 10 dpf.

When analyzing the interaction between environmental light and species (*P.* sp. "*pundamilia-like*," *P.* sp. "*nyererei-like*," or hybrids), we found that *LWS* and *RH2A* expression were both species- and environment-specific (see Figure 3 and Table 1, treatment:species). However, only hybrids differed in *RH2A* expression between treatments. Hybrids had higher proportional *RH2A* expression in red-shifted light than their counterparts in broad-spectrum light, while *P.* sp. "*nyererei-like*" and *P.* sp. "*pundamilia-like*" expression did not differ between the treatments. These results suggest that *Pundamilia* hybrids respond plastically to the light treatments, showing longer-wavelength sensitivity in the red-shifted light environment. In the adults, *P.* sp. "*nyererei-like*" showed stronger effects of the rearing light at the spectrum extremes: *LWS* was expressed in higher proportions in the red-shifted condition than in the broad-spectrum condition and *SWS2A* was expressed in lower proportions in the red shifted light condition than in the broad-spectrum light condition (Wright et al., 2020). Hybrids and *P.* sp. "*pundamilia-like*" did not show any significant differences between light environments. Our results coincide with the lack of plasticity in the opsin expression of *P.* sp. "*pundamilia-like*." In the case of *P.* sp. "*nyererei-like*," the mismatch between the lack of plasticity in our results and the data from the adults might be explained by the lack of data points between 192 dpf and the adult stage (240 dpf and older). Possibly, *P.* sp. "*nyererei-like*" increase their *LWS* expression during this time. Additional sampling during this developmental period, as well as transfer experiments (e.g., Härer et al., 2019), are required to establish the sensitive window for the effects of light conditions on opsin expression and potential species differences in this regard.

Heterochronic changes are modifications in developmental pattern compared with the ancestral state (McKinney & McNamara, 1991). For haplochromine cichlids, *O. niloticus* is considered to represent the ancestral pattern of opsin expression development (Carleton et al., 2008; O'Quin et al., 2011). Rock- and sand-dwelling species from Lake Malawi differ greatly from *O. niloticus*, and differences in their developmental patterns can be interpreted as adaptations to different light environments. *P.* sp. "*pundamilia-like*" and *P.* sp. "*nyererei-like*" also differ from *O. niloticus* in the combination and proportion of opsins expressed during development. Yet, even though the two *Pundamilia* species inhabit very different natural light environments, there is little difference in their developmental patterns. We do find that they respond somewhat differently to the light treatments. These plastic responses may provide a target for selection and allow the evolution of species-specific developmental trajectories; representing a possible starting point for heterochronic shifts in *Pundamilia.*

5 | CONCLUSION

Heterochrony is evolutionary important because it produces developmental patterns that differ from the ancestral lineage pattern. During these different developmental patterns new phenotypes might appear, and if they are beneficial in a given environment, they can then become targets of selection, potentially leading to arrested or accelerated development. Likewise, environmentally-induced phenotypes, throughout development, are "seen" by natural selection and can provide stepping stones for evolutionary adaptation (West-Eberhard, 2003). In this study, we documented the developmental pattern of relative opsin expression in two species of Lake Victoria cichlids and their reciprocal hybrids. We found that *Pundamilia* cichlids progress from high levels of short-wavelength-sensitive opsin expression as larvae and juveniles to high levels of long-wavelength-sensitive opsin expression as (sub)adults. This pattern may reflect differences between life stages in water depth distribution, where larvae and juveniles reside in more shallow waters, with broad-spectrum light, compared with adults. Developmental patterns were similar between the species, but the overall opsin expression levels differed between them, consistent with prior work. The developmental trajectory of opsin expression of hybrids responded plastically to the visual conditions.

ACKNOWLEDGMENTS

We acknowledge Sjoerd Veenstra and Brendan Verbeek for taking care of the fish. Financial support came from the Swiss National Science Foundation (SNSF PZ00P3-126340; to Martine E. Maan), the Netherlands Foundation for Scientific Research (NWO VENI 863.09.005; to Martine E. Maan), and the University of Groningen.

DATA AVAILABILITY STATEMENT

All the data will be submitted to the archive www.dataverse.nl. The link for the data deposition will be available in https://hdl.handle.net

ORCID

Lucia Irazábal-González http://orcid.org/0000-0001-8909-8195

REFERENCES

Bowmaker, J. K. (2008). Evolution of vertebrate visual pigments. *Vision Research*, *48*(20), 2022–2041.

Bowmaker, J. K., Govardovskii, V. I., Shukolyukov, S. A., L.V. Zueva, J., Hunt, D. M., Sideleva, V. G., & Smirnova, O. G. (1994). Visual pigments and the photic environment: The cottoid fish of Lake Baikal. *Vision Research*, *34*(5), 591–605.

Britt, L. L., Loew, E. R., & McFarland, W. N. (2001). Visual pigments in the early life stages of Pacific northwest marine fishes. *Journal of Experimental Biology*, *204*(14), 2581–2587.

Browman, H. I., Novales-Flamarique, I., & Hawryshyn, C. W. (1994). Ultraviolet photoreception contributes to prey search behaviour in two species of zooplanktivorous fishes. *Journal of Experimental Biology*, *186*(1), 187–198.

Carleton, K. L., Dalton, B. E., Escobar-Camacho, D., & Nandamuri, S. P. (2016). Proximate and ultimate causes of variable visual sensitivities: Insights from cichlid fish radiations. *Genesis*, *54*(6), 299–325.

Carleton, K. L., & Kocher, T. D. (2001). Cone opsin genes of African cichlid fishes: Tuning spectral sensitivity by differential gene expression. *Molecular Biology and Evolution*, *18*(8), 1540–1550.

Carleton, K. L., Parry, J. W. L., Bowmaker, J. K., Hunt, D. M., & Seehausen, O. (2005). Colour vision and speciation in Lake Victoria cichlids of the genus *Pundamilia*. *Molecular Ecology*, *14*(14), 4341–4353.

Carleton, K. L., Spady, T. C., Streelman, J. T., Kidd, M. R., McFarland, W. N., & Loew, E. R. (2008). Visual sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC Biology*, *6*(1), 22.

Carleton, K. L., & Yourick, M. R. (2020). Axes of visual adaptation in the ecologically diverse family Cichlidae. *Seminars in Cell & Developmental Biology*, *106*, 43–52. https://doi.org/10.1016/j.semcdb.2020.04.015

Castillo Cajas, R. F., Selz, O. M., Ripmeester, E. A. P., Seehausen, O., & Maan, M. E. (2012). Species-specific relationships between water transparency and male coloration within and between two closely related Lake Victoria cichlid species. *International Journal of Evolutionary Biology*, *2012*, 1–12.

Chang, C.-H., Catchen, J., Moran, R. L., Rivera-Colón, A. G., Wang, Y.-C., & Fuller, R. C. (2021). Sequence analysis and ontogenetic expression patterns of cone opsin genes in the bluefin killifish (*Lucania goodei*). *Journal of Heredity*, *112*, 357–366. https://doi.org/10.1093/jhered/esab017

Flamarique, I N. (2013). Opsin switch reveals function of the ultraviolet cone in fish foraging. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1752), 20122490.

Fryer, G., & Iles, T. D. (1972). *Cichlid fishes of the great lakes of Africa: Their biology and evolution*. Oliver and Boyd.

Goldschmidt, T., Witte, F., & De Visser, J. (1990). Ecological segregation in zooplanktivorous haplochromine species (Pisces: Cichlidae) from Lake Victoria. *Oikos*, *58*, 343–355.

Halekoh, U., & Højsgaard, S. (2014). A kenward-roger approximation and parametric bootstrap methods for tests in linear mixed models–The R package pbkrtest. *Journal of Statistical Software*, *59*(9), 1–30.

Halstenberg, S., Lindgren, K. M., Samagh, S. P. S., Nadal-vicens, M., Balt, S., & Fernald, R. D. (2005). Diurnal rhythm of cone opsin expression in the teleost fish *Haplochromis burtoni*. *Visual Neuroscience*, *22*(2), 135–141.

Härer, A., Karagic, N., Meyer, A., & Torres-Dowdall, J. (2019). Reverting ontogeny: Rapid phenotypic plasticity of colour vision in cichlid fish. *Royal Society Open Science*, *6*(7), 190841. https://doi.org/10.1098/rsos.190841

Härer, A., Torres-Dowdall, J., & Meyer, A. (2017). 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.). *Molecular Ecology*, *26*(20), 5582–5593.

Hofmann, C. M., O'Quin, K. E., Marshall, N. J., Cronin, T. W., Seehausen, O., & Carleton, K. L. (2009). The eyes have it: Regulatory and structural changes both underlie cichlid visual pigment diversity. *PLoS Biology*, *7*(12), e1000266.

Kocher, T. D. (2004). Adaptive evolution and explosive speciation: The cichlid fish model. *Nature Reviews Genetics*, *5*, 288–298. https://doi.org/10.1038/nrg1316

Loew, E. R., & McFarland, W. N. (1990). The underwater visual environment. In M. D. E. Douglas (Ed.), *The visual system of fish* (pp. 1–43). Chapman and Hall.

Loew, E. R., & Wahl, C. M. (1991). A short-wavelength sensitive cone mechanism in juvenile yellow perch, *Perca flavescens*. *Vision Research*, *31*(3), 353–360.

Maan, M. E., Seehausen, O., & Groothuis, T. G. G. (2017). Differential survival between visual environments supports a role of divergent sensory drive in cichlid fish speciation. *The American Naturalist*, *189*(1), 78–85.

McKinney, M. L., & McNamara, K. J. (1991). *Heterochrony: The evolution of ontogeny*. Plenum Press.

Meier, J. I., Marques, D. A., Wagner, C. E., Excoffier, L., & Seehausen, O. (2018). Genomics of parallel ecological speciation in Lake Victoria cichlids. *Molecular Biology and Evolution*, *35*(6), 1489–1506. https://doi.org/10.1093/molbev/msy051

Meier, J. I., Sousa, V. C., Marques, D. A., Selz, O. M., Wagner, C. E., Excoffier, L., & Seehausen, O. (2017). Demographic modelling with whole-genome data reveals parallel origin of similar *Pundamilia* cichlid species after hybridization. *Molecular Ecology*, *26*(1), 123–141. https://doi.org/10.1111/mec.13838

O'quin, K. E., Smith, A. R., Sharma, A., & Carleton, K. L. (2011). New evidence for the role of heterochrony in the repeated evolution of cichlid opsin expression. *Evolution & Development*, *13*(2), 193–203.

Parry, J. W. L., Carleton, K. L., Spady, T., Carboo, A., Hunt, D. M., & Bowmaker, J. K. (2005). Mix and match color vision: Tuning spectral sensitivity by differential opsin gene expression in Lake Malawi cichlids. *Current Biology*, *15*(19), 1734–1739.

Ramakers, C., Ruijter, J. M., Deprez, R. H. L., & Moorman, A. F. M. (2003). Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data. *Neuroscience Letters*, *339*(1), 62–66. https://doi.org/10.1016/s0304-3940(02)01423-4

Salzburger, W. (2018). Understanding explosive diversification through cichlid fish genomics. *Nature Reviews Genetics*, *19*, 705–717. https://doi.org/10.1038/s41576-018-0043-9

Sandkam, B. A., Campello, L., O'Brien, C., Nandamuri, S. P., Gammerdinger, W. J., Conte, M. A., Swaroop, A., & Carleton, K. L. (2020). Tbx2a modulates switching of RH2 and *LWS* opsin gene expression. *Molecular Biology and Evolution*, *37*(7), 2002–2014. https://doi.org/10.1093/molbev/msaa062

Seehausen, O. (2006). African cichlid fish: A model system in adaptive radiation research. *Proceedings of the Royal Society B: Biological Sciences*, *273*, 1987–1998. https://doi.org/10.1098/rspb.2006.3539

Seehausen, O., Terai, Y., Magalhaes, I. S., Carleton, K. L., Mrosso, H. D. J., Miyagi, R., Van Der Sluijs, I., Schneider, M. V., Maan, M. E., Tachida, H., Imai, H., & Okada, N. (2008). Speciation through sensory drive in cichlid fish. *Nature*, *455*(7213), 620–626.

Stevens, M. (2013). *Sensory ecology, behaviour, and evolution*. Oxford University Press.

Veilleux, C. C., & Kirk, E. C. (2014). Visual acuity in mammals: Effects of eye size and ecology. *Brain, Behavior and Evolution*, *83*(1), 43–53. https://doi.org/10.1159/000357830

Venables, W., & Ripley, B. (2002). Random and mixed effects. In J. Chambers, W. Eddy, W. Härdle, S. Sheather, & L. Tierney (Eds.), *Modern applied statistics with S* (pp. 271–300). Springer.

West-Eberhard, M. J. (2003). *Developmental plasticity and evolution*. Oxford University Press.

Wright, D. S., Demandt, N., Alkema, J. T., Seehausen, O., Groothuis, T. G. G., & Maan, M. E. (2017). Developmental effects of visual environment on species-assortative mating preferences in Lake Victoria cichlid fish. *Journal of Evolutionary Biology*, *30*(2), 289–299.

Wright, D. S., van Eijk, R., Schuart, L., Seehausen, O., Groothuis, T. G. G., & Maan, M. E. (2020). Testing sensory drive speciation in cichlid fish: Linking light conditions to opsin expression, opsin genotype and female mate preference. *Journal of Evolutionary Biology*, *33*(4), 422–434. https://doi.org/10.1111/jeb.13577

Wright, D. S., Meijer, R., van Eijk, R., Vos, W., Seehausen, O., & Maan, M. E. (2019). Geographic variation in opsin expression does not align with opsin genotype in Lake Victoria cichlid populations. *Ecology and Evolution*, *9*(15), 8676–8689.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Irazábal-González, L., Wright, D. S., & Maan, M. E. (2024). Developmental and environmental plasticity in opsin gene expression in Lake Victoria cichlid fish. *Evolution & Development*, *26*, e12465. https://doi.org/10.1111/ede.12465