

Article

Reviewing guppy color vision: integrating the molecular and physiological variation in visual tuning of a classic system for sensory drive

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Abstract

Sensory drive predicts coevolution of mate choice signals with the sensory systems detecting those signals. Guppies are a classic model for sensory drive as mate preferences based on coloration differ across individuals and populations. A large body of work has identified variation in color vision, yet we lack a direct tie between how such variation in color vision influences variation in color preference. Here we bring together studies that have investigated guppy vision over the past 40 years to: (1) highlight our current understanding of where variation occurs in the guppy color vision pathway and (2) suggest future avenues of research into sources of visual system variation that could influence guppy color preference. This will allow researchers to design careful studies that couple measures of color preference with measures of visual system variation from the same individual or population. Such studies will finally provide important answers as to what sets the direction and speed of mate preference evolution via sensory drive.

Key words: colour vision, mate choice, opsin, *Poecilia reticulata*, sensory bias, visual ecology

Introduction: Sensory Drive and Guppies

Twenty five years ago the sensory drive model was put forward to both explain and predict, the evolution of traits involved in mate choice (Endler 1992). Sensory drive synthesized what were previously separate evolutionary hypotheses and laid out three central tenets: (1) the environment shapes sensory systems, (2) signals from potential mates exploit the biases of the chooser's sensory system, and (3) the environment constrains the signal properties of the traits (Endler 1992; Boughman 2002; Ryan and Cummings 2013; Price 2017). With these three foundations, sensory drive set up a framework to explain how covariation in traits and sensory systems can drive mate choice. As the tuning of sensory systems adapt to local environments, the traits on which mate choice decisions are based also change to maximally stimulate the chooser. The sensory drive framework has been used to explain covariation in mate

choice and traits observed across populations in a broad range of taxa including frogs (Feng et al. 2006), birds (Mockford and Marshall 2009), and fish (Seehausen et al. 2008). Although most studies have compared long-established populations, sensory drive could also drive rapid evolution any time populations suddenly differ in their transmission properties (e.g., colonizing new environments, ecosystem shifts). This is especially possible if sensory systems either: (1) have variation present in the starting populations, or (2) can adapt plastically to their new environments. Therefore a thorough understanding of both standing variation and plasticity of sensory systems is required to evaluate the role of sensory drive in rapidly evolving systems.

In his seminal paper introducing sensory drive, Endler presented female preferences for male coloration in guppies *Poecilia reticulata* as a possible example of sensory drive (Endler 1992). Populations of guppies

are well known to undergo rapid evolution in color preferences (Breden and Stoner 1987; Endler and Houde 1995; Houde 1997), which are mediated by their color vision. Color vision is the sensory system that detects and discriminates different wavelengths of light, this depends strongly on the tuning of the visual system (i.e., the strength and range of light detected). One of the primary components underlying the tuning of color vision is the sensitivity of the cone cells in the retina. Archer et al. (1987) found that guppy cone cell sensitivities vary between individuals. The identification of such variation in visual tuning played a strong role in the formation of the theory of Sensory Drive (Endler 1992). Yet 25 years later, we are still stuck with a “black box” as we lack a direct tie between visual system variation and variation in color preference (Price 2017). Understanding such a link between the physiology of the visual system and color preference behavior will require experiments directly linking variation in color preference and variation in color vision. However, such studies are made difficult by the fact that there could be variation in several components responsible for the processes between light striking the eye and signals being interpreted by the brain, which we will call the color vision pathway.

Here we bring together studies that have investigated guppy vision over the past 40 years to: (1) highlight our current understanding of where variation occurs in the guppy color vision pathway and (2) suggest future avenues of research into sources of visual system variation

that could influence guppy color preference. To do this we will follow the color vision pathway from light striking the eye to electrical signals being sent to the brain (Figure 1), and highlight where either variation is known or the potential for variation exists. A great deal of variation in the visual system can occur before electrical signals begin to be processed. Therefore, we focus here on the stages of color vision that lead up to the initiation of the phototransduction cascade, which we refer to as the peripheral sensory system.

Light Transmission through the Eye

The photoreceptors detect light signals, which begins the process of turning such signals into electrical signals that can be interpreted by the brain. However, the photoreceptors are situated at the back of the retina and the retina itself is positioned at the back of the eye. Therefore, when light first arrives at the eye it must pass through a series of layers before it reaches the photoreceptors. Each of these layers has its own transmission properties which can vary by wavelength, thus altering the signal available to the photoreceptors.

Cornea, lens, and vitreous transmission

When light first reaches the eye, it passes through the mostly transparent outer layer called the cornea, then the lens, and finally

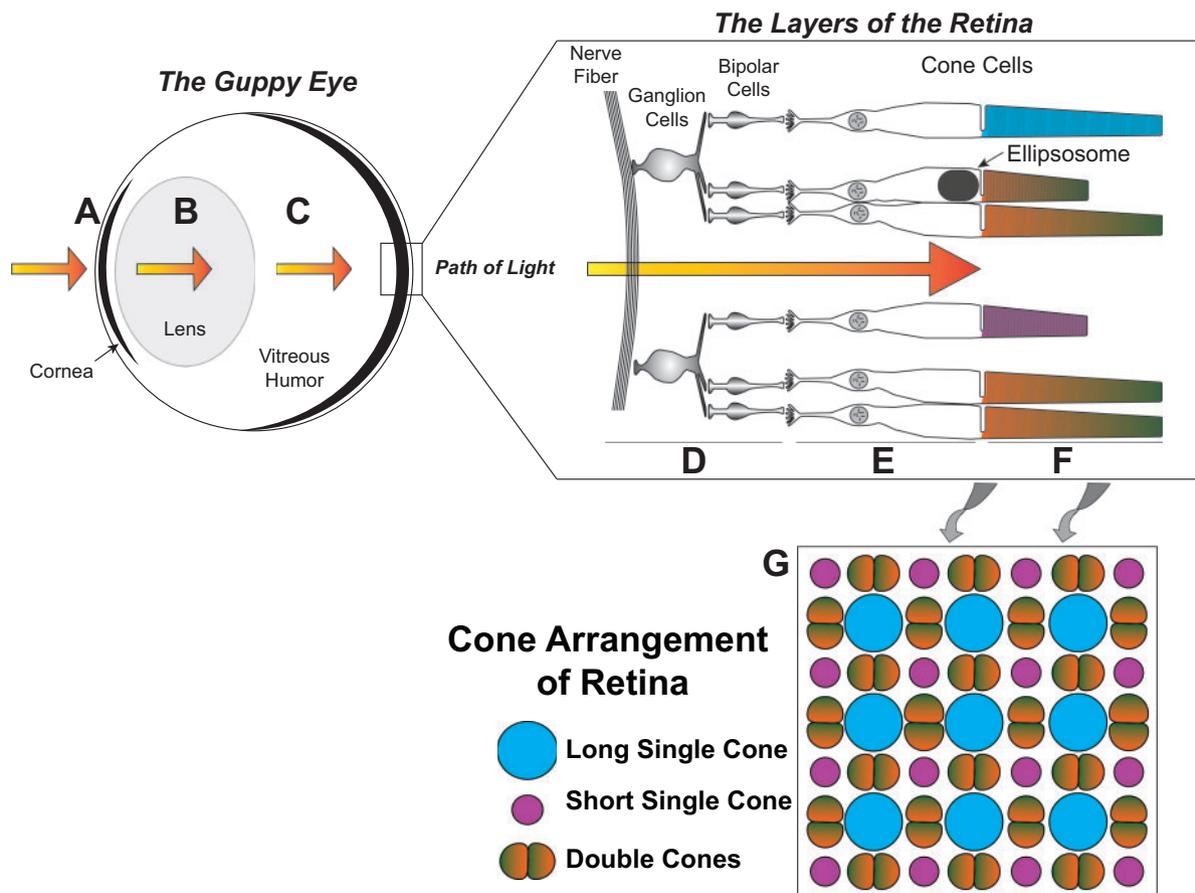


Figure 1. Guppy vision pathway. Light strikes the eye then passes through the (A) cornea, (B) lens, (C) vitreous humor, (D) inner layers of the retina, (E) cone cell body and finally reaches the photoreceptors in the (F) outer segment of the cone cells. The short member of double cones possess dense mitochondrial bodies called “ellipsosomes” which act as band pass filters (see text). The cone cells are arranged in a square mosaic (G) with a long single cone surrounded by four pairs of double cones, each separated by a short single cone (a.k.a. corner cones). The long single cones likely express *SWS2B*, short single cones express *SWS1* and the double cones express *RH2-1*, *Rh2-2*, *LWS-1*, and/or *LWS-3*.

through the clear “jelly-like” vitreous before actually reaching the retina (Figure 1; stages A–C). Together these layers are often referred to as the “ocular media.” Harmful ultraviolet (UV) light (≤ 315 nm) can damage the delicate retina, therefore many species have evolved the ability to selectively block UV light at the level of the cornea and/or the lens (Thorpe et al. 1993; Hofmann et al. 2010). Although Douglas and Djamgoz (1990) report that the ocular media is UV transparent in guppies, they did not publish these transmission values. Nor do they state which components of the ocular media nor how many individuals were examined. Thus, there may be unidentified variation in each layer of the ocular media. Corneal transmission generally blocks only the lower range wavelength of visible light, acting to filter out UV, but the cut of wavelength can be quite variable across teleost species (Siebeck and Marshall 2001). However, guppy cornea transmission and its variation across populations or individuals remains to be tested.

Like the cornea, lens transmission has a classic stepwise function, allowing it to block shorter wavelengths of light while transmitting longer wavelengths equally well (Thorpe et al. 1993). The transmission of a lens is categorized by finding what wavelength is transmitted through the lens at a rate of 50%, and for guppies this has been reported as a strikingly short 315 nm (Thorpe et al. 1993). However, this was measured in only one individual from a single population. Lens transmission has been shown to vary not only across species, but also across individuals, and may even change with age in some species (Thorpe and Douglas 1993). Differences in lens transmission have been shown to play a strong role in the tuning of color vision (Hofmann et al. 2010) and such differences in UV transmission due to a component of the ocular media may play an important role in guppy mate choice. UV signals are thought to act as “private signals” because the predators in some populations cannot detect UV light (Endler 1991; Kemp et al. 2008; Weadick et al. 2012). Guppy females from some populations have been reported as having strong preferences for males with UV signal (Smith et al. 2002). However, studies on other populations have found a lack of such UV preferences (White et al. 2003; Millar and Hendry 2011). If there is indeed variation in UV preference between populations, this could be explained by sensory drive in the peripheral sensory system if there is variation in the properties of the ocular media. This could be easily tested by describing the transmission characteristics of the cornea, lens and vitreous between individuals with UV preferences and individuals without UV preferences. Interestingly, a recent paper reported that guppy ocular media may vary in transmission. Guppies with the “Pb” color gene possess chromatophores and crystalline platelets in the ocular media that are thought to alter transmission properties and possibly block UV (Bias and Squire 2017). Although their findings need to be verified by measures of differences in the transmission properties, it does further support the notion that light transmission through the ocular media may be a previously unexplored source of variation in guppy color vision that could impact mate choice via sensory drive.

Retinal transmission

Once light reaches the retinal layer it must pass through several layers of cells, including the nerve fibers, ganglion cells, bipolar cells, horizontal cells, and amacrine cells before even reaching the cone cells (Figure 1; stage D). To our knowledge, there have been no studies examining the variation in light transmission through the retinal layers in any species, making this stage possible, but unlikely, to influence guppy color vision.

However, light detection only occurs in the outer segment of the cone cells, therefore light must pass through the cell body of the very cone cell that will ultimately detect it (Figure 1; stage E). Although

most cone cell bodies are transparent, guppies are one of a few species that possess dense, colorless, organelles called ellipsosomes adjacent to the outer segment of some cone cells (MacNichol et al. 1978). In guppies, ellipsosomes act as bandpass filters, absorbing light at three peak wavelengths; the strongest at 417.7 nm, with minor peaks also at 520.4 nm and 549.2 nm. It has been proposed that ellipsosomes are acting to protect the outer segments from harmful UV light (Nag and Bhattacharjee 1995), yet this seems unlikely as they do not block the light below ~ 350 nm, which is far more harmful than light above that range. Furthermore, they only occur in one cone type, the shorter members of double cones (the smaller of two adjoined cone cells) (Kunz and Wise 1978; MacNichol et al. 1978). All shorter members with ellipsosomes have been reported as having visual pigments that maximally detect light at 476 nm (corresponding to the RH2-2 opsin, see below). However, it is unclear if the ellipsosomes are also present in the shorter member of red–red double cones. Either way, the lack of ellipsosomes in the longer member of double cones suggests they are not acting to protect the outer segment from harmful UV light. MacNichol et al. (1978) proposed that ellipsosomes may act to increase contrast in the blue–violet region by blocking light to the double cone with the closest sensitivity, this would limit signal from light in this region to the single cones. However, because ellipsosomes are highly dense mitochondrial structures, it is also possible that the filtering effect is simply a byproduct of heavily used mitochondria (Kunz and Wise 1978). Answering this question could be accomplished by examining ellipsosome presence in guppies under shifted or narrow light environments that would only stimulate the longer member of the double cones. Surprisingly, a close guppy relative, *Poecilia latipinna*, has ellipsosomes not only in the shorter member of the double cones, but in both members (MacNichol et al. 1978). Presuming that ellipsosome regulatory mechanisms are conserved between guppy and *P. latipinna*, it is curious that guppy only has them in the shorter member. Because ellipsosomes have been studied in very few guppy populations, it is possible that some populations more closely resemble *P. latipinna* and possess ellipsosomes in both double cones. However, it should be noted that the light blocked by ellipsosomes (minor peaks at 520.4 nm and 549.2 nm) would be expected to directly interfere with the λ_{\max} of the longer member of guppy double cones.

Early work on guppy ellipsosomes has shown that the structure of ellipsosomes respond to their light environment; with the normally “matrix-type” conformation transitioning to a “cristate-type” when individuals are exposed to bright light (Kunz and Wise 1978). This is especially interesting as ellipsosomes in bright light are 25–50% denser than those that are dark adapted (MacNichol et al. 1978). This observed conformational change is likely due to an increase in cellular activity as ellipsosomes are packed with mitochondria (Kunz and Wise 1978). This may function to keep the light levels in check to prevent light from overpowering the outer segment. Variation in the abundance of ellipsosomes or their transmission properties could provide a previously unexamined source of visual system variation. It is also interesting to note that studies relying on models of guppy color vision have not yet included the effect of ellipsosomes (e.g., Endler 1991; Kemp et al. 2009; Cole and Endler 2015b). If light intensity itself shifts visual sensitivity, then sensory drive could explain differences in color preference without shifts to the spectrum of the available light.

Light Detection by the Cone Cells

Cone cells are made up of a small cell body, connected to a highly folded membrane in the shape of a cone called the outer segment

(Figure 1; stages E and F). Guppies possess three types of cone cells; short single cones, long single cones (outer segments $\sim 2.5 \mu\text{m}$ wide), and double cones (outer segments $\sim 3.6 \mu\text{m}$ wide) (Archer and Lythgoe 1990).

The visual pigments, made up of an opsin protein bound to a chromophore, detect light and are packed into the outer segments of the cone cell (Figure 1; stage F). The range of wavelengths detected by a cone cell are centered around a peak of maximum sensitivity (λ_{max}). The λ_{max} of a cone cell is largely determined by two factors: the amino acid sequence of the opsin protein expressed by that cone cell, and the chromophore bound by that opsin (Sharpe and Gegenfurtner 1999). Each opsin protein crosses the cell membrane of the outer segment at seven “transmembrane domains”; these domains form the “retinal binding pocket” where the opsin protein binds to either 11-*cis*-retinal (A1) or 11-*cis*-3, 4-dehydroretinal (A2) to form a visual pigment (Sharpe and Gegenfurtner 1999). The amino acid sequence of the opsin protein, especially those along the retinal binding pocket, act to determine the λ_{max} of a visual pigment. Thereby the λ_{max} of a cone cell is determined by which opsin gene that cell expresses, and which chromophore is bound to the opsin proteins.

Retinal cone cell mosaic

The cone cells in the guppy retina are arranged in a square mosaic, with each long single cone surrounded by four pairs of “double cones” and each double cone set separated by a short single “corner” cone (Figure 1; stage G). Maintaining such an arrangement stabilizes the ratio of cone cell types throughout the retina and matches the reports of Kunz and Wise (1978) who found roughly 73% of all cones in the dorsal–ventral region to be double cones. Short single cones have been suggested to be absent over most of the ventral retina (Kunz 1980). However, *in situ* expression of the opsin expressed in short cones revealed even expression across the dorsal–ventral axis, suggesting that the square mosaic may in fact be retained (Rennison et al. 2011). It is also possible that there is variation in short cone abundance across individuals or populations. Surprisingly, guppies have been shown to change from a square mosaic to a row mosaic when dark adapted (Kunz 1980; Kunz 1983; Kunz and Ennis 1983). This is due to the fact that under bright conditions, rods withdraw into the layer of retinal pigment epithelium to help protect the delicate scotopic (non-color vision) photodetectors, but in dim conditions rods move out of the pigment epithelium (Menger et al. 2005). Kunz (1980) hypothesized that such a movement may impact visual perception, although it is unclear how this would occur since rods and cones are expected to maintain their connection to their ganglions cells. Although there is no reason to think there would be variation in this photoresponse to light environment, it is interesting to note that guppy males increase display rates around dawn and dusk (Houde 1997), when the row mosaic may be in a state of flux. This raises the possibility that mate preferences are made on the combined signals received from rods and cones, to our knowledge this has not yet been explored and presents an interesting future line of research.

Opsin gene naming and organization

The visual opsin genes are organized into five main classes, forming five monophyletic clades that arose early in vertebrate evolution (Ebrey and Koutalos 2000; Yokoyama 2000; Rennison et al. 2012). The RH1 class is used exclusively in rod cells, which act in scotopic (dim-light) vision and do not play a role in color vision (Yokoyama 2008). The remaining four classes are expressed in the cone cells:

short wavelength sensitive 1 (SWS1) detects UV; short wavelength sensitive 2 (SWS2) detects blues and violets; rhodopsin-like (RH2) detects greens and Long Wavelength Sensitive (LWS) detects reds and oranges. Guppies possess a total of nine cone-opsin loci that encompass all four gene families: one SWS1 (*SWS1*), two SWS2 (*SWS2A* and *SWS2B*), two RH2 (*RH2-1* and *RH2-2*), and four LWS. The naming convention of the four LWS opsin loci has varied across publications beginning with Ward et al. (2008) calling each opsin locus by the amino acid present at the position corresponding to the 180th position in the human M/LWS opsins. This site is one of six amino acid positions that are thought to strongly influence the λ_{max} of guppy LWS genes, the other key sites being 194, 197, 277, 285, and 308 (Yokoyama and Radlwimmer 1998; Ward et al. 2008; Kawamura et al. 2016). It should be noted that site 194, put forward by Kawamura et al. (2016) (reported as site 178 using the bovine numbering system) has not yet been tested with mutagenesis experiments as the other five sites have.

The discovery of two alleles at the same locus with different “180 site” amino acids (180 *Ala* and 180 *Ser*) (Tezuka et al. 2014) made the original nomenclature difficult because two other loci also have a *Ser* at the 180 site. Using BAC clone sequencing, Watson et al. (2011) determined that three of the LWS opsins occur in a tandem array, whereas the fourth was a retrotransposed gene and found on another chromosome. We therefore introduced a new naming scheme for Poeciliidae LWS opsins based on the order of the LWS loci in proximity to the LWS Locus Control Region (LCR): *LWS-1* (previously *LWS A180*), *LWS-2* (previously *LWS P180*), *LWS-3* (previously *LWS S180*), and *LWS-R* (previously *LWS S180r*) (Sandkam et al. 2013). This naming convention has largely been taken up with the modification of *LWS-R* occasionally being referred to as *LWS-4* (Tezuka et al. 2014; Kawamura et al. 2016; Sakai et al. 2016). Here we will use the *LWS-R* designation as we feel it simultaneously provides information about both the genomic organization and differences in regulation because the LWS LCR occurs 4.6 kb upstream of *LWS-1*.

With the release of the guppy reference genome by Künstner et al. (2016) we now know that the RH2, SWS2, and LWS genes all occur on linkage group (LG) 5. The exception is the retrotransposed *LWS-R*, which is located in the intron of the *GPHN* gene on LG21 (Künstner et al. 2016). However, the genomic location of the *SWS1* gene is still unknown as it fell on an unanchored contig in the current version of the guppy genome (Figure 2).

The genomic organization of the opsin genes likely plays an important role in generating variation in guppy color vision. The tandem nature and inverted orientation of *LWS-1* and *LWS-3* is shared across the family Poeciliidae (Rennison et al. 2012), and such a genomic arrangement promotes gene conversion between these genes (Sandkam et al. 2017). Gene conversion results in all or part of one locus overwriting another, which can rapidly change the amino acid sequence of the opsins, acting to homogenize or diversify opsin loci in just a couple of generations depending on how much of the locus undergoes conversion. Such conversion may explain the findings of Hoffmann et al. (2007) where at least one individual from the Quare drainage had at least two LWS genomic sequences that were identical at all amino acid sites. The frequency of gene conversion between LWS loci within and across guppy populations remains unknown, but may be a source of variation and divergence in guppy color vision. Such differences in visual tuning could drive differences in mate choice through sensory drive, suggesting genomic processes could provide a previously unidentified component to the sensory drive framework.

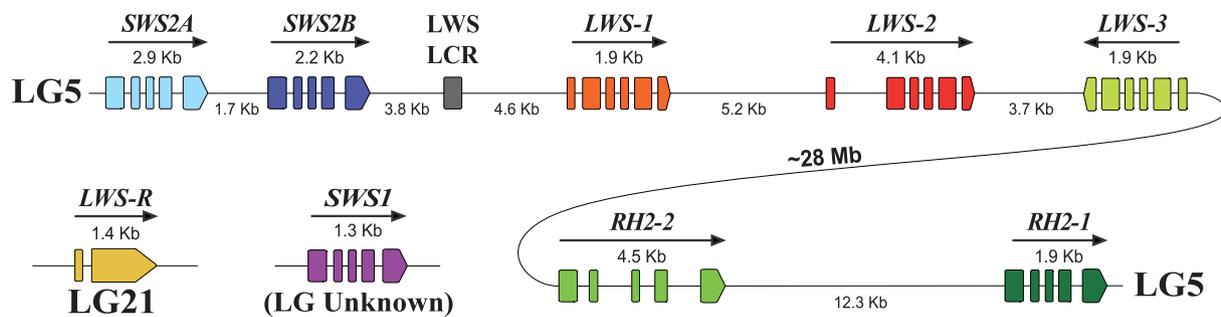


Figure 2. The genomic organization of the guppy cone opsin genes. Colored blocks denote coding sequence, spaces between same colored blocks denote introns. Arrows above gene names show the directionality of the genes relative to one another. Numbers below gene names denote total length of introns and exons for that gene. Numbers below intergenic regions denote distance between loci.

LWS-1 allele variation

For several years, there was much debate about the precise λ_{\max} values of the various guppy opsins, especially the LWS opsins. This was recently resolved when Kawamura et al. (2016) expressed each of the guppy opsin genes using in vitro expression vectors, followed by binding the protein to A1 chromophore. By measuring the wavelength absorbed by the resulting visual pigment they determined the λ_{\max} values for each of the guppy opsin genes (summarized in Figure 3). They found that the 180 *Ala* and 180 *Ser* alleles of *LWS-1* do indeed have different λ_{\max} values (562 versus 571 nm respectively). This is especially interesting because the frequency of the 180 *Ala* and 180 *Ser* alleles have been shown to vary across populations both on the island of Trinidad (Tezuka et al. 2014; Sandkam et al. 2015a) and on mainland South America (Sandkam et al. 2015b). On Trinidad, “low predation” populations were found to have a higher frequency of the 180 *Ser* allele than corresponding “high predation” populations in the same river (Tezuka et al. 2014; Sandkam et al. 2015a). Low predation populations are typically covered by more trees which tend to shift available light toward longer wavelengths (Endler 1993). Because the 180 *Ser* allele has a longer λ_{\max} than the 180 *Ala*, it is possible that the change in allele frequency is the result of visual systems evolving to better utilize the available light spectrum. Tezuka et al. (2014) found a strong correlation between dissolved oxygen and *LWS-1* 180 *Ser* versus 180 *Ala* allele frequency across watersheds. However, we found no such relationship when we tested dissolved oxygen and allele frequency across populations within watersheds (Sandkam et al. 2015a). Differences in *LWS-1* alleles are easily assessed, making it a prime candidate to study how variation in color vision could impact color preferences.

Opsin expression in cone types

Microspectrophotometry (MSP) is a method used to assess the λ_{\max} of the outer segment of a cone cell. By tying together MSP measures of the different cone types (MacNichol et al. 1978; Levine and MacNichol 1979; Levine et al. 1979; Archer et al. 1987; Archer and Lythgoe 1990; Watson et al. 2011), in vitro expression measures of the individual opsins (Kawamura et al. 2016), and in situ characterization of opsin expression in the retina (Rennison et al. 2011), we are close to knowing which cone type expresses which opsin (Table 1).

Guppies have two single cone classes. The λ_{\max} of the shortest single cones was reported as 389 nm by Archer and Lythgoe (1990) but 359 nm by Watson et al. (2011). This discrepancy is likely due to the technological limitations of MSP at the time of Archer and Lythgoe’s study (which they described in their paper), as their

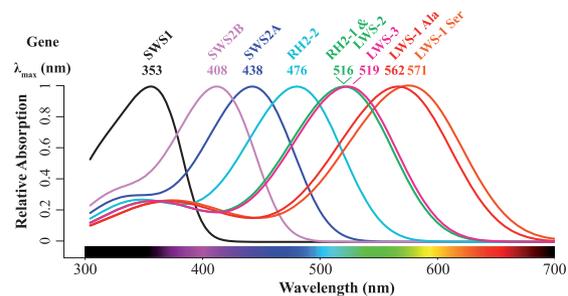


Figure 3. The absorption curves of the guppy opsins based on λ_{\max} values determined by in vitro pigment reconstitution (Kawamura et al. 2016) and modeled assuming 100% A1 chromophore usage (Govardovskii et al. 2000). The corresponding human visible light spectrum runs along the horizontal axis.

machine was only able to measure wavelengths starting at 371 nm (Archer and Lythgoe 1990). Therefore, the true shortest single cone class (359 nm) matches the λ_{\max} of the in vitro expressed *SWS1* opsin (353 nm) (Kawamura et al. 2016). Furthermore, the *SWS1* opsin is expressed at appreciable levels (Laver and Taylor 2011; Ehlman et al. 2015; Sandkam et al. 2015a; Sandkam et al. 2015b; Corral-Lopez et al. 2017), and is expressed throughout the retina (Rennison et al. 2011). Therefore, we have high confidence assigning the *SWS1* opsin to the shorter single cone class.

The second single cone class has been identified by six studies, which have reported λ_{\max} values between 406 nm and 411 nm (MacNichol et al. 1978; Levine and MacNichol 1979; Levine et al. 1979; Archer et al. 1987; Archer and Lythgoe 1990; Watson et al. 2011). This matches the λ_{\max} of the *SWS2B* opsin (408 nm) when expressed in vitro (Kawamura et al. 2016). *SWS2B* is expressed at appreciable levels (Laver and Taylor 2011; Ehlman et al. 2015; Sandkam et al. 2015a; Sandkam et al. 2015b; Sandkam et al. 2016; Corral-Lopez et al. 2017), and is expressed throughout the retina (Rennison et al. 2011). Therefore, we have high confidence in assigning the *SWS2B* opsin to the longer single cone class.

Interestingly, no MSP study has found cones corresponding to the anticipated *SWS2A* λ_{\max} (438 nm), this may not be surprising as *SWS2A* has not been found to be expressed at biologically meaningful levels in any guppy opsin quantitative PCR (qPCR) study to date (Laver and Taylor 2011; Ehlman et al. 2015; Sandkam et al. 2015a; Sandkam et al. 2015b; Sandkam et al. 2016; Corral-Lopez et al. 2017). Nor was *SWS2A* identified in cDNA libraries when the guppy opsins were first being described (Hoffmann et al. 2007). However, using *in situ* hybridization Rennison et al. (2011) found

Table 1. The λ_{\max} values of cone cell classes identified from MSP studies followed by their standard deviation (if presented in the original study) below is the putative opsin gene expressed by that cone class, and the λ_{\max} of that opsin when expressed in vitro (Kawamura *et al.* 2016).

MSP Study	Single cones		Double cones			
	Short	Long	Accessory	Primary		
MacNichol <i>et al.</i> (1978)	NA	~408.5	~468.5		~546.5	
Levine <i>et al.</i> (1979)	NA	408	468		546	
Levine and MacNichol (1979)	NA	411 (± 5)	472 (± 3)		551 (± 9)	
Archer <i>et al.</i> (1987)	NA	410	465		529–579	
Archer and Lythgoe (1990)	389	408	464	533 (± 2.7)	548 (± 7.5)	572 (± 3.1)
Watson <i>et al.</i> (2011)	358.6 (± 2.7)	406.4 (± 1.8)	464.7 (± 2.4)	525.4 (± 3.6)	540.7 (± 3.7)	560 (± 0.06)
Putative Opsin	<i>SWS1</i>	<i>SWS2B</i>	<i>RH2-2</i>	<i>RH2-1</i> or <i>LWS-3</i>	<i>LWS-1</i> with <i>RH2-1</i> or <i>LWS-3</i>	<i>LWS-1</i>
Opsin in vitro λ_{\max}	353 (± 2)	408 (± 1.3)	476 (± 2)	516 (± 1) or 519 (± 2)		562 (± 0.6) (<i>Ala</i>) 571 (± 0.6) (<i>Ser</i>)

SWS2A to be expressed at a high level throughout the entire retina of guppies from Cumaná, Venezuela. It is possible that there are differences in *SWS2A* expression between populations, or even between mainland South America and Trinidad. However, Laver and Taylor (2011) performed qPCR on the same population of guppies used by Rennison *et al.* and also found *SWS2A* to be expressed at biologically insignificant levels. Rather than variation in *SWS2A* use, it seems more probable that the *SWS2A* probe used for the in situ experiments was binding to either *SWS1* or *SWS2B*. We have also observed strong cross binding between in situ probes for the *SWS2* opsins in cichlids.

Guppies have four double-cone classes. The shortest sensitive is always found as the shorter member of a double cone (a.k.a. the accessory cone), and has been reported as having a λ_{\max} value between 464 nm and 472 nm (Table 1) (MacNichol *et al.* 1978; Levine and MacNichol 1979; Levine *et al.* 1979; Archer *et al.* 1987; Archer and Lythgoe 1990; Watson *et al.* 2011). The opsin with the closest in vitro expressed λ_{\max} value is *RH2-2* (476 nm) (Kawamura *et al.* 2016), which is expressed at appreciable levels (Laver and Taylor 2011; Ehlman *et al.* 2015; Sandkam *et al.* 2015a; Sandkam *et al.* 2015b; Sandkam *et al.* 2016; Corral-Lopez *et al.* 2017). It is interesting to note that while *RH2-2* was found to be expressed throughout the retina, it was expressed by a lower frequency of cone cells than the other opsins (Rennison *et al.* 2011). This matches the MSP data, which has shown that double cones have either one 464–472 nm λ_{\max} cone paired with a longer sensitive cone; or “twin cones” (two equally sensitive longer cones), but never two 464–472 nm λ_{\max} cones (Levine *et al.* 1979; Archer *et al.* 1987). Therefore we have high confidence in assigning the *RH2-2* opsin to the shortest double cone class.

Archer and Lythgoe (1990) found the other three double cone classes to have λ_{\max} values at 533 nm, 548 nm and 572 nm and individuals were found to possess one, two or even all three cone types. The in vitro λ_{\max} values of Kawamura *et al.* (2016) correspond perfectly with the longest sensitive cone class which is clearly the *LWS-1 Ser* allele (571 nm). Furthermore, MSP on individuals from another population which is known to have the *LWS-1 Ala* allele (Watson *et al.* 2011) found double cones with λ_{\max} at 525 nm, 540 nm, and 560 nm, the longest sensitive cone class matching the in vitro *LWS-1 Ala* allele (562 nm). The *LWS-1* opsin is expressed at appreciable levels (Laver and Taylor 2011; Ehlman *et al.* 2015; Sandkam *et al.* 2015a; Sandkam *et al.* 2015b; Sandkam *et al.* 2016; Corral-Lopez *et al.* 2017), and is expressed throughout the retina

(Rennison *et al.* 2011). Therefore, we have high confidence that the 560 nm double cone class expresses *LWS-1 Ala*, whereas the 572 nm double cone class expresses *LWS-1 Ser*.

Archer and Lythgoe (1990) proposed the middle cone class is a co-expression of two opsins. Indeed, using the visual pigment models put forth by Govardovskii *et al.* (2000) with a 50: 50 co-expression ratio of the *LWS-1 Ala* with the 525 nm cone class identified in Watson *et al.* (2011) predicts the middle cone class to be 539 nm—almost precisely matching the 540 nm cone class they found. However, modeling a 50: 50 co-expression of *LWS-1 Ser* with the 533 nm cone class identified by Archer and Lythgoe (1990) predicts a middle cone class at 548 nm precisely matching their 548 nm cone class. This suggests the variation in the λ_{\max} of the 525/533 nm cone class may be due to either population variation in opsin sequence or differences in gene expression. It also suggests the λ_{\max} values determined by in vitro expression may be off for either *RH2-1*, *LWS-2*, or *LWS-3* as none of these opsins match the shorter cone class (Archer and Lythgoe 1990; Watson *et al.* 2011; Kawamura *et al.* 2016).

Which short wavelength opsin is co-expressed with *LWS-1* to generate the middle cone class is unclear, as it could be either *RH2-1*, *LWS-2* or *LWS-3*. We can rule out *LWS-2* because MSP measures show the middle cone class is abundant (Archer and Lythgoe 1990; Watson *et al.* 2011) yet qPCR studies have shown *LWS-2* is expressed below biologically significant levels (Laver and Taylor 2011; Ehlman *et al.* 2015; Sandkam *et al.* 2015a; Sandkam *et al.* 2015b; Sandkam *et al.* 2016; Corral-Lopez *et al.* 2017). This leaves *RH2-1* or *LWS-3* as the potential contributor to co-expressed opsins. In the closely related *Xiphophorus helleri*, the *LWS-1* and *LWS-3* loci are highly homogenized, differing by only one amino acid, and MSP revealed only two long cone classes at 534 nm and 568 nm, without the middle class (Watson *et al.* 2010). Kawamura *et al.* (2016) hypothesized that if guppies and *X. helleri* share regulatory mechanisms then the middle cone class observed in guppies could be due to the co-expression of the diverged *LWS-1* and *LWS-3* loci. However, co-expression of these nearly identical *LWS-1* and *LWS-3* in *X. helleri* would not change the λ_{\max} of the cone cell relative to either being expressed alone.

Nonetheless, following the methods of Dalton *et al.* (2014), we have conducted preliminary assays using *fluorescent in situ hybridization* (*FISH*) on whole mount guppy retinas to determine whether *RH2-1* is co-expressed with *LWS* opsins. Unfortunately, we were not able to assess co-expression of the individual *LWS* alleles due to

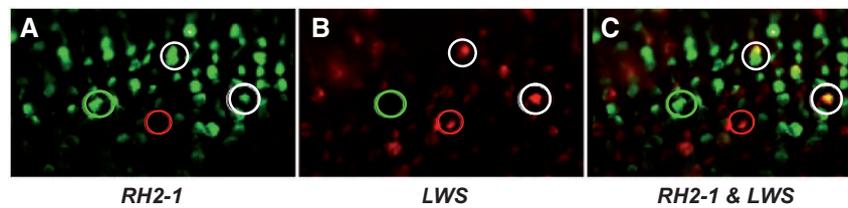


Figure 4. FISH of whole mount guppy retina with *RH2-1* stained green (A and C) and *LWS* stained red (B and C). Some cells are expressing only *RH2-1* (e.g. circled in green), some expressing only *LWS* (e.g. circled in red), and some co-expressing *RH2-1* and *LWS* (e.g. circled in white). When co-expression does occur it appears to only occur in one member of the double cone.

the high sequence similarity of the *LWS* alleles, the short (~120 bp) lengths of the untranslated regions (UTRs), and the long probe length required for such *FISH* assays. For this reason, we designed one probe to bind to all *LWS* opsins equally well and one probe to bind to the *RH2-1* allele. We found *RH2-1* is expressed with *LWS* in some cells (Figure 4). Further work is needed to determine (1) which *LWS* opsin *RH2-1* is co-expressed with (co-expression with *LWS-3* would not change the cone λ_{\max}), (2) whether there is co-expression of *LWS-1* and *LWS-3*, and (3) the frequency of cells co-expressing *RH2-1/LWS* versus *LWS-1/LWS-3* opsins. In light of the limitations in distinguishing opsin alleles with *FISH* probes, we suggest future work use single cell based methods for quantifying gene expression in the retina, such as single cell qPCR or single cell transcriptomics (Enright et al. 2015a; Macosko et al. 2015; Laboissonniere et al. 2017).

A1 versus A2 chromophore

To form a visual pigment, an opsin is bound to either an A1 or A2 chromophore (Sharpe and Gegenfurtner 1999). Using an A2 chromophore shifts the λ_{\max} of the visual pigment toward longer wavelengths; these shifts can be as large as 53 nm (Seehausen et al. 2008; Terai et al. 2017). Although chromophore usage has been found to vary across species, populations, and even cone types in the nine-spine stickleback (Saarinen et al. 2012), it seems unlikely that adult guppies vary in chromophore usage for three reasons: (1) MSP peak widths, (2) the tight match of MSP with in vitro expression, and (3) lack of extra-long wavelength sensitive cones. All MSP studies on adult guppies have reported 100% A1 usage (Schwanzara 1967; MacNichol et al. 1978; Levine and MacNichol 1979; Archer and Lythgoe 1990; Watson et al. 2011) which can be distinguished from A2 usage by having slightly narrower absorption curves. Furthermore, the λ_{\max} of the longest cone cells as measured by MSP precisely matched the λ_{\max} of the in vitro expression work of Kawamura et al. (2016), who exclusively used A1 chromophore to reconstitute the visual pigment. Finally, A2 chromophore usage would be expected to shift *LWS-1 Ala* to 642 nm (Govardovskii et al. 2000), yet MSP has been conducted on several populations and no study has found cone cells beyond the A1 coupled λ_{\max} . Taken together it seems likely that guppies use only the A1 chromophore.

However, this is somewhat surprising because freshwater fishes generally use either A2 or a mix of A1 and A2 chromophores, in fact, another member in the family Poeciliidae *Gambusia affinis* is known to use both A1 and A2 chromophores (Toyama et al. 2008). Furthermore, the gene thought to be responsible for mediating the switch from A1 to A2 chromophore usage, *Cyp27c1* (Enright et al. 2015b), is present and intact in the guppy genome (GenBank accession XM_008404425). Some species of fish only use A2 chromophore at certain stages of development or in certain environments

(Temple et al. 2006). Therefore, although it seems reasonable that adult guppies strictly use A1 chromophore, it is possible that they use A2 chromophore in an as yet unexamined life history stage or environmental condition (e.g. diet).

Cone expression distribution

The arrangement of the cone cells expressing the different opsins is not uniform throughout the retina. Rennison et al. (2011) used in situ hybridization to show that while *SWS1*, *SWS2A*, *SWS2B*, and *RH2-2* were expressed relatively evenly throughout the retina, the *LWS* and *RH2-1* expressing cone cells are primarily located in the dorsal and ventral retina respectively. Because the λ_{\max} of *RH2-1* is considerably lower than that of *LWS-1*, spatial distribution of *LWS* and *RH2-1* could result in differently tuned color vision for the individual's upper and lower field of vision (Levine and MacNichol 1979). However, this possibility depends heavily on the spatial organization of the different *LWS* proteins. Rennison et al. (2011) was unable to distinguish *LWS-1* from *LWS-3*, which has a λ_{\max} value quite similar to *RH2-1*. In a cichlid fish, the dorsal-ventral pattern of opsin expression can vary depending on the light environment (Dalton et al. 2014; Dalton et al. 2015). Thus, regardless of whether the *LWS* spatial organization contributes to variation in color vision, it is also possible that spatial patterning differs across individuals and/or populations. Consistent with individual variation in the spatial organization, Rennison et al. also observed individual variation in the abundance of cones expressing the *LWS-1/3* and *LWS-2* opsins.

Variation in opsin expression

Studies comparing MSP and opsin gene expression in other species have shown that whole retinal measures of opsin gene expression made by qPCR provide a fairly reliable approximation for relative cone cell abundance (Carleton and Kocher 2001; Fuller et al. 2004; Carleton et al. 2008; Shand et al. 2008; Fuller and Claricoates 2011). Opsin expression in cichlids is known to have both a genetic and plastic component (Carleton and Kocher 2001; Carleton et al. 2016; Nandamuri et al. 2017). Guppies are well known for genetic differences across populations as a result of rapid evolution in some traits (Endler 1980; Reznick and Bryga 1987), whereas other traits have been shown to vary across populations due to plasticity (Torres-Dowdall et al. 2012; Ruell et al. 2013). We found opsin gene expression can vary substantially across populations in the field (Sandkam et al. 2015a; Sandkam et al. 2015b), suggesting these differences are likely due to both genetic and plastic influences. Understanding population variation in color vision will require measures of opsin expression in the field. It is important to note that while the sensory drive model only requires visual systems adapt to local environments, this can occur through either genetic changes and/or plastic adaptations. Lab studies have begun to tease apart the

sources of environmental variation that influence opsin gene expression through plasticity, including the light spectrum (Sakai et al. 2016), total irradiance (Ehlman et al. 2015), and the level of dietary carotenoids (Sandkam et al. 2016).

Population variation

By collecting tissue in the field, we have found guppy opsin gene expression can differ substantially across populations (Sandkam et al. 2015a; Sandkam et al. 2015b). We found that differences in opsin expression covary with mate preferences across populations in two watersheds of Trinidad, such that populations characterized by stronger female preferences for orange males had higher expression of both *LWS-1* and *LWS-3* (Sandkam et al. 2015a). Although these results do support sensory drive, it is interesting to note that it may not be differences in the light environment itself driving selection on the guppy visual system. Expressing higher levels of LWS opsin would be expected to better utilize light environments that are shifted toward longer wavelengths, yet we found no difference in the light environments as measured by λ_{p50} . Thus we proposed an alternative hypothesis that the populations with higher LWS expression may be the result of decreased pressures from predation. The rational being that the balance between sexual selection and natural selection would be shifted to stronger pressures from sexual selection as noted in other guppy life history traits (Endler 1988). However, this study needs to be expanded and utilize full spectral data (rather than just λ_{p50}) to test for effects of finer variation in light environments.

All populations in Guyana experience very high predation pressure. However, we found opsin expression varies substantially across these populations, with more variation occurring across populations, than across sympatric species (Sandkam et al. 2015b). Such convergence of opsin expression across species in the same location suggests shared selection pressures on visual tuning and requires a thorough characterization of the ecological pressures in these populations. Whether the observed population differences in opsin expression are due to evolved changes in gene regulation, plastic responses to environmental conditions, or a combination of the two remain to be determined. However, it is likely that plasticity explains at least some of these differences.

Lighting environment

Sakai et al. (2016) raised guppies under either green or orange light, then measured both their sensitivity to different wavelengths (532 nm, 546 nm, 570 nm, and 600 nm) and their opsin expression. By modeling quantum catch of the guppy visual system they showed that the total irradiance available to the guppy visual system was the same in both treatments and only the wavelength distribution changed. This means that the magnitude of visual stimulation was the same and the relative stimulation of the cone cells differed. They found that guppies raised under the orange light had higher expression of *LWS-3*. Interestingly, guppies raised under orange light also had a stronger sensitivity in optomotor tests under 600 nm light than guppies raised under green light. The top three principal components explaining the difference in optomotor behavior had positive correlations with *LWS-1* and *LWS-3* expression but negative correlations with all other opsins. This fits with the findings of Anstis et al. (1998), where they found that only the double cones play a role in optomotor response behavior; however, it is surprising that *RH2* expression (also double cone opsins) did not have an effect. Remarkably, Smith et al. (2012) also found that expression of

only the *LWS* opsin correlated with optomotor response in cichlid fishes. These results suggest there can be important effects of plasticity on behavior, even if it only drives differences in *LWS* expression between populations.

Ehlman et al. (2015) raised guppies in either clear water or turbid water. Bentonite clay was used to generate the turbid treatment, which lowers the total irradiance without shifting the wavelength distribution. Guppies raised under turbid conditions expressed much higher levels of *LWS-3* and much lower levels of *RH2-1* than did guppies raised in clear conditions. Therefore, *RH2-1* was found to change expression with changes in total irradiance but not light spectrum, whereas *LWS-3* was found to change both with total irradiance and spectrum (Ehlman et al. 2015; Sakai et al. 2016). This suggests the mechanisms underlying plasticity in opsin gene expression may differ between the opsins. It is interesting that *LWS-3* and *RH2-1* are the two opsins responding plastically to light because they have identical λ_{max} and are expected to play the same function in cone cell tuning.

Effect of diet

In Sandkam et al. (2016), we found that opsin gene expression also responds to levels of dietary carotenoids. Dietary carotenoids are well known to vary across guppy populations, are a major component of orange male coloration, and even have a direct correlation with female preferences for such male coloration (Grether et al. 2001; Grether et al. 2005). In animals carotenoids can be broken down into retinoic acid (Nagao 2004), which has been shown to bind to the Poeciliid LWS LCR and dramatically upregulates LWS gene expression (Tam et al. 2011). Guppies raised on diets containing trace levels of carotenoids expressed significantly lower levels of LWS opsins than do those raised on low or high carotenoid diets (Sandkam et al. 2016). Unfortunately, these gene expression data were generated before the LWS alleles were resolved and so we have only a broad measure of all LWS expression. Future work is needed with allele-specific assays to determine which opsins are responding to dietary carotenoids, and the magnitude of those effects (because large changes in one LWS could have been hampered by small or no changes in another).

Effect of age and sex

Laver and Taylor (2011) found no difference in opsin expression between left and right eyes. They examined opsin expression during development and found 1-month and 2-month-old juveniles express almost entirely *SWS2B* and *RH2-2*. Such a difference may indicate differences in microhabitat between life history stages as juveniles are almost exclusively found at the edges of water, less than 1–2 cm deep, whereas adults can be found throughout the water column. Surprisingly they also found *LWS-1* and *LWS-3* expression differed between adult males and females, despite there being no differences observed in frequency of cone cells as measured with MSP (Archer and Lythgoe 1990). Nor has a sex difference been observed in any population where tissue was taken in the field (Sandkam et al. 2015a; Sandkam et al. 2015b). However, a sex difference in expression of the *RH2-1* opsin was found in two studies in the lab, but there was no sex difference in expression of the *LWS-1* or *LWS-3* opsins (Ehlman et al. 2015; Sandkam et al. 2016). It is interesting to note that Laver and Taylor used a population of guppies that has been described as a different species (Poesser et al. 2005; Pollux et al. 2014), and therefore the sex difference in LWS may be unique to guppies from Cumaná, Venezuela.

Alternatively, sex differences in opsin expression are often predicted under conditions of strong sexual conflict (Price 2017), yet the other roles of color vision (e.g., survival, navigation, and foraging) are shared between the sexes. It is possible that sex differences in particular opsins could differ by population if there are differences in the strength or direction of sexual conflict. Measures of opsin expression in both males and females from a greater number of populations are required before we can rule out a sex difference in guppy opsin expression.

Plasticity versus heritability

Additional sources of variation in guppy opsin expression are likely, because opsin expression has been suggested to differ between seasons in both damselfish (Stieb et al. 2016) and medaka (Shimmura et al. 2017). Also, length of day has been shown to change LWS opsin expression in stickleback (Shao et al. 2014). However, opsin expression is not an entirely plastic trait, as there is likely a strong genetic component as well. Amazingly, Endler et al. (2001) artificially selected lines of guppies for their optomotor response under a red versus blue light and got a response to selection in less than eight generations. Meanwhile, Cole and Endler (2015a) also found there to be a rapid response to selection, this time on attraction to different color food items. If such differences in optomotor ability and color preference are due to differences in opsin gene expression (as it was in Sakai et al. (2016)), this suggests there is a strong genetic component to opsin expression that is amenable to selection. Further studies are needed to examine opsin expression across a wider range of environments to clarify what are the plastic, versus genetic, drivers shaping variation in opsin gene expression.

Moving forward with sensory drive

The theory of sensory drive was originally put forward to both explain and predict trait evolution (Endler 1992). According to the sensory drive model; the environment shapes sensory systems, signals from potential mates exploit the biases of the chooser's sensory system, and the environment constrains trait evolution (Endler 1992; Boughman 2002; Ryan and Cummings 2013; Price 2017). Although such a framework has been used successfully to explain observed trait preferences in many systems (reviewed in Cummings and Endler 2018), there are two outstanding problems with sensory drive that need to be addressed: (1) there are systems where sensory drive fails to explain trait evolution, (2) it is still not possible to use the sensory drive framework to predict the magnitude or speed of trait evolution. Both of these problems have a shared cause; sensory drive relies on a phenotypic gambit approach to both trait and sensory system evolution. Such an approach assumes that evolution can create any phenotype and given "enough time" the model's prediction will occur (Rubin 2016). However, we now know that evolution can be constrained by molecular evolution both in the short term and over long periods of time (Springer et al. 2011). We now need more predictive models for trait evolution under the sensory drive framework that ties together the advances in genomics, spectroscopy, and molecular evolution that have been made over the last 25 years. Such models will require understanding the standing variation, evolutionary limits, and plasticity of each component of both sensory systems and traits. It is only with such models that we can finally evoke the second aim of sensory drive; predicting trait evolution.

Conclusions

Guppies played a pivotal role in the formation of the theory of sensory drive, which predicts that variation in sensory systems can drive the evolution of mate preferences (Endler 1992). Guppies are perhaps the best-documented species with variable female preferences for male coloration (Houde 1997; Magurran 2005; Breden 2006). Although variation in guppy color vision was first identified over 30 years ago (Archer et al. 1987), the effect of such variation on color preference remains a "black box." Opening this box will require careful studies that couple measures of color preference with measures of visual system variation from the same individual or population. Here we have identified a number of variables underlying visual sensitivities that need further study, both between individuals and populations. These include quantifying: transmission through the ocular media, presence and transmission of ellipsosomes, differences in opsin alleles, expression of opsin genes, λ_{\max} of cones, the relationship of opsin expression and cone types, and what are the roles of plasticity in visual tuning. Finally, mate choice assays need to be conducted while varying each of the components of visual system tuning to examine the role of peripheral sensory tuning on mate choice evolution. Such studies will finally provide important answers as to what sets the direction and speed of mate preference evolution.

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