Genetic and plastic variation in opsin gene expression, light sensitivity, and female response to visual signals in the guppy

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According to the sensory drive model, variation in visual properties can lead to diverse female preferences, which in turn results in a range of male nuptial colors by way of sexual selection. However, the cause of variation in visual properties and the mechanism by which variation drives female response to visual signals remain unclear. Here, we demonstrate that both differences in the long-wavelength–sensitive 1 (*LWS-1*) opsin genotype and the light environment during rearing lead to variation in opsin gene expression. Opsin expression variation affects the visual sensitivity threshold to long wavelengths of light. Moreover, a behavioral assay using digitally modified video images showed that the expression of multiple opsin genes is positively correlated with the female responsiveness to images of males with luminous orange spots. The findings suggest that genetic polymorphisms and light environment in habitats induce variations in opsin gene expression levels. The variations may facilitate variations in visual sensitivity and female responsiveness to male body colors within and among populations.

visual pigment | sexual selection | color perception | *Poecilia* | female preference

In a broad range of animals, visual signals such as nuptial colors are used to appeal to potential mates in the context of a mate choice. These visual signals are perceived by the receivers' vision, and thus the characteristics of the receivers' visual systems can determine the evolutionary direction of these signals. Thus, the conceptual model of sensory drive suggests an integrated evolution of visual signals, visual systems, and communication behaviors under a given light environment (1). The guppy, *Poecilia reticulata*, has been a model organism for investigating the evolution of sexual signals influenced by sensory drive (1, 2). Male guppies have extreme body color polymorphisms, and females exhibit preferences for some components of these male color patterns, which also vary within and among populations (3, 4). Moreover, microspectrophotometry studies have demonstrated that the spectral sensitivity of cone cells in the long-wavelength range is highly variable among individual guppies (5, 6), suggesting that this may affect color perception and contribute to differences in female preferences for male color signals (1).

Mate choice based on color signals requires color vision, which is enabled by at least two types of cone visual pigments, having the different spectral sensitivities. Each visual pigment is composed of an opsin and a chromophore (7), and these are primarily responsible for variation in the spectral sensitivity of the visual pigments. The guppy carries nine cone opsin genes: a UV-sensitive gene (*SWS1*), two subtypes of blue-sensitive genes (*SWS2-A* and *SWS2-B*), two subtypes of green-sensitive genes (*RH2-1* and *RH2-2*), and four subtypes of red-sensitive genes (*LWS-1*, *LWS-2*, *LWS-3*, and *LWS-4*) (8–11). A population genetic study on guppies from Trinidad and Tobago showed that variations in the amino acid sequences of *LWS-1* and *LWS-3* among populations are maintained under natural selection (11). Kawamura et al. (12) demonstrated that two of the *LWS-1* alleles have differing spectral sensitivities (*LWS-1*/180_{Ser}, λ_{max} = 571 nm; *LWS-1*/180_{Ala}, λ_{max} = 562 nm). Moreover, the expression levels of opsin genes have also been reported to vary among populations or individuals within a population. For instance, Sandkam et al. (13) showed that individuals inhabiting low-predation environments express higher levels of *LWS* opsin genes than those inhabiting high-predation environments. In a laboratory experiment, plastic variation in the expression of *LWS* opsin genes has been observed under different light environments, which affects the light sensitivity as measured by optomotor responses (14). Therefore, in addition to differences in the amino acid sequences of the opsin protein, genetic or plastic variation in opsin gene expression could result in diverse visual characteristics, potentially leading to subsequent variations in female mate preferences. However, the mechanism by which genetic and environmental factors influence variation in opsin gene expression and the correlation between the variation in opsin expression and the variation in visual sensitivity and behavior remain unexplored.

Here, we evaluate genetic and plastic variation in cone opsin gene expression in guppies and determine the effects of these variations on visual light sensitivity and female response to male images with different sexual colors. We compared opsin expression levels in individuals with different *LWS-1* genotypes (AA type, homozygous for the *LWS-1*/180_{Ala} allele, and SS type, homozygous for the *LWS-1*/180_{Ser} allele) that were reared under

Significance

High diversity in sexual color signaling in animals has attracted considerable and sustained interest from evolution researchers. It has been suggested that variations in visual properties in guppies result in diverse female preference for sexual color signals, leading to genetic variation based on male body colors. Here, we report that opsin expression varies because of allelic differences as well as the different rearing light environments. The variation in opsin expression influences the diversity in visual light sensitivity. Moreover, the expression of multiple opsin genes influences female responsiveness to the luminous orange colors. Consequently, genetic and environmental variation in opsin gene expression could affect female responsiveness and preference for male sexual colors, facilitating male color polymorphisms.

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different light environments. Subsequently, to evaluate the relationship between variation in the opsin gene expression and visual light sensitivity at the behavioral level, we observed their optomotor responses under monochromatic light stimuli. Moreover, we assessed female response to male sexual colors by mate choice tests using digitally modified video images of a male. In these assessments, we focused on female response to carotenoid-based orange colors, which are regarded as a major criteria for mate choice by females in some populations (4, 15).

Results

Individuals with a homozygous *LWS-1* allele (SS type, $180_{Ser}/180_{Ser}$; AA type, $180_{Ala}/180_{Ala}$) were obtained through random crossing between the offspring from wild-caught pregnant females in the well-established feral wild population in Okinawa, Japan. The details for producing the genetic line based on the *LWS-1* genotype are summarized in *SI Appendix*, *SI Materials and Methods*. In brief, wild-caught female guppies were individually reared to give birth, and their offspring were isolated until reaching sexual maturity to create the first-generation line. The partial sequences of *LWS-1* were determined for the first-generation offspring (F_1) from each brood, and the *LWS-1* genotypes were defined as AA type (homozygous for the *LWS-1*/180_{Ala} allele with Ala at 180), AS type (heterozygous), or SS type (homozygous for the *LWS-1*/180_{Ser} allele with Ser at 180). Next, we crossed a homozygous female F_1 offspring (SS or AA type) with a male of the same *LWS-1* genotype (SS or AA type) to obtain homozygous offspring (F_2; *SI Appendix*, *SI Materials and Methods*). These individuals were reared under white, green, or orange light produced using acetate filters (see *SI Appendix*, Fig. S1 for irradiance spectra of the three light environments) and were maintained under these conditions until they reached sexual maturity.

Variations in the Expression of Nine Cone Opsin Genes. To measure the opsin expression levels, qPCR assays were conducted on nine cone opsin genes, and the expression value of each opsin gene was normalized against *β-actin*. *LWS-1*, *LWS-2*, and *SWS2-B* expression levels were different between SS-type and AA-type individuals, with SS-type individuals exhibiting higher expression levels of these genes than AA-type individuals [generalized linear mixed model (GLMM): *LWS-1* genotype: $P = 0.0013$, $P < 0.0001$, and $P = 0.0002$, for *LWS-1*, *LWS-2*, and *SWS2-B* expression, respectively; Fig. 1 and *SI Appendix*, Table S1]. In addition, the expression levels of two short-wavelength–sensitive opsins (*SWS1* and *SWS2-A*) were significantly influenced by the rearing light environment; individuals reared under white light had higher *SWS1* and *SWS2-A* expression levels than in those reared under green or orange light (GLMM: light environment: $P = 0.0419$ and $P < 0.0001$ for *SWS2-A* and *SWS1* expression, respectively; Fig. 1 and *SI Appendix*, Table S1). The effect of sex was also significant; *LWS-1* expression was significantly higher in males than in females (GLMM: sex: $P = 0.0054$; *SI Appendix*, Fig. S2 and Table S1). The interaction between sex and the rearing light environment was significant with regard to *SWS2-B* expression (GLMM: sex × light environment: $P = 0.0137$; *SI Appendix*, Table S1). In addition to opsin expression levels normalized against *β-actin*, we calculated the relative expression levels among opsin genes (i.e., individual opsin expression/total opsin expression). The effects of *LWS-1* genotype, rearing light environment, and sex on relative opsin expression levels were similar to those on the expression levels normalized against *β-actin*, although additional significant effects were observed (*SI Appendix*, Fig. S3 and Table S2). The relative expression levels of *LWS-1* and *LWS-2* in SS-type individuals were higher than those observed in AA-type individuals. In contrast, the relative *SWS-1* expression levels were higher in AA-type individuals than in SS-type individuals. The relative expression of *RH2-2* (blue-light–sensitive opsin, $\lambda_{max} = 476$ nm) in individuals reared under orange light was significantly higher than that observed in individuals reared under white and green light.

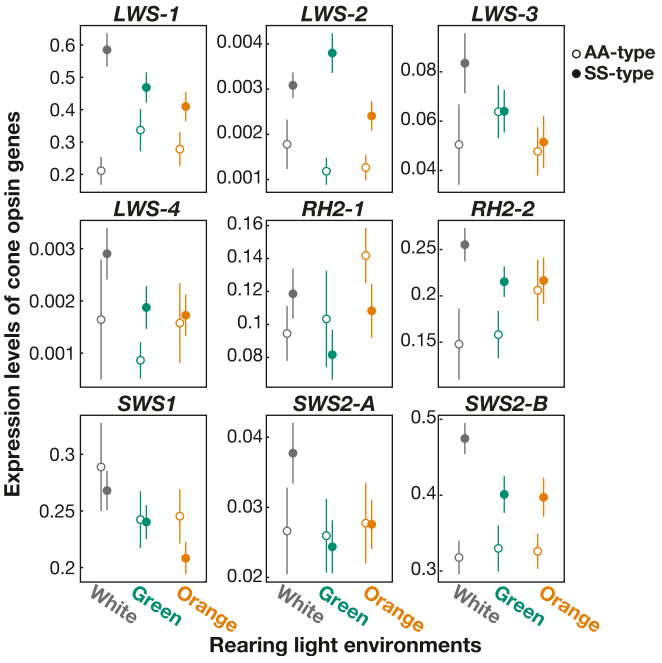

Fig. 1. Gene expression levels of nine cone opsins. The expression values are normalized against that of *β-actin*, a housekeeping gene. The open and solid circles indicate the means ± SEMs for AA-type *LWS-1* individuals (white, $n = 5$; green, $n = 8$; orange, $n = 10$) and SS-type *LWS-1* individuals (white, $n = 19$; green, $n = 18$; orange, $n = 14$), respectively.

Sensitivity to Long Wavelengths of Light Measured by Optomotor Response. The optomotor response is an innate orientation behavior in animals that is responsible for the involuntary tracking of moving visual patterns and has been used to investigate the visual sensitivity threshold to light stimuli in various teleost fish species, including the guppy (16–18). To evaluate whether variation in opsin expression correlates with variation in light sensitivity at behavioral level, we performed optomotor experiments and measured visual sensitivity to two monochromatic light stimuli (532 and 600 nm) of individual fish. These two light stimuli are within the absorbance spectrum of LWS opsins and are dominant wavelengths in green and orange light environments, respectively (*SI Appendix*, Fig. S1). We observed a significant interaction between the *LWS-1* genotype and the rearing light environment on visual light sensitivity (Table 1). SS-type individuals reared under white light displayed higher sensitivities to 532- and 600-nm light than AA-type individuals. However, the effect of the *LWS-1* genotype was not clearly demonstrated in individuals reared under green or orange light (Fig. 2 and *SI Appendix*, Fig. S4). The optomotor response was reportedly mediated by LWS cone opsin (14, 19), and the spectra of light stimuli in the optomotor experiment were within the absorbance spectra of LWS opsins. Therefore, we investigated the relationship between the expression level of each of the four *LWS* opsin genes and visual sensitivity to monochromatic light stimuli (532- and 600-nm light). The results of a multiple-regression analysis revealed that *LWS-1* expression level was positively correlated with sensitivity to 532- and 600-nm light (Fig. 3 and Table 2). The other three *LWS* opsins expression had no significant effect on light sensitivity at either of the two wavelengths (Table 2).

Table 1. Results of a GLMM analysis for the light sensitivity measured by optomotor response

Explanatory variables	df	χ^2	*P*
Light environment (Env.)	2	0.72	0.6973
LWS-1 genotype (Gen.)	1	0.60	0.4425
Wavelength (Wave.)	1	2.62	0.1057
Env. × Gen.	**2**	**8.33**	**0.0155**
Env. × Wave.	2	4.53	0.1039
Gen. × Wave.	1	0.04	0.8384

A GLMM was fitted that included the light sensitivity to 532- and 600-nm light as a response variable, and individual identifications as a random effect. The values highlighted in bold are statistically significant ($P < 0.05$).

Female Response to Digital Image of a Male. We evaluated female response to male sexual colors using digitally modified video images of a male. Digital video techniques for the mate choice test permitted the control of factors other than body color, such as courtship behavior (20–22). We displayed two videos of a single male guppy with differently modified orange spots: a high-orange (HO) male image with large/colorful orange spots and a low-orange (LO) male image with small/drab orange spots (see *SI Appendix*, Fig. S5 for screenshots of HO and LO male video images and the radiance spectra of light emitted from the orange spots of the male in the images). These HO and LO male video images were simultaneously displayed on one side of the test chamber. This experimental setup allowed the females to compare these two video images. The visual perception of a digital video image of a male may be different from the visual perception of a real male. Thus, we estimated how the visual system of the guppy perceives video images of orange colors of males by developing a receptor noise-limited model (23, 24). Before constituting the model, we conducted a von Kries transformation so that the photon catches from orange colors in digital images of males were normalized by catches of the illuminant photons in the tank. Using the model, we calculated the chromatic (ΔS) and luminance (ΔL) contrasts of orange spots against the background body colors (see *SI Appendix*, *SI Materials and Methods* for the detailed model equation for the calculation of ΔS and ΔL). Both chromatic and luminance (often described as brightness) contrasts of the HO male guppy were found to be higher than those of the LO male (*SI Appendix*, Fig. S6). Particularly, the luminance contrast of orange spots on the HO male was approximately fourfold higher than that of orange spots on the LO male. Therefore, female response to the HO male image could be interpreted as a response to objects with higher luminance (and potentially chromatic) contrast. The orange spots on the real male guppy had a high chromatic contrast against the background body color, whereas the luminance contrast was relatively low compared with that of the HO/LO digital images of the male (*SI Appendix*, Fig. S6).

The combination of all of the nine opsin expressions could be involved in visual perception, and the expression levels of the opsin genes for females were highly correlated (*SI Appendix*, Table S4). Therefore, we conducted partial least-squares regression (PLSR) to assess the relationship between the expression level of each of nine cone opsin gene and female response to the HO and LO images of a male. PLSR is more suitable than multiple regression analysis when explanatory variables are highly correlated and sample size is comparatively small (25). Table 3 shows the regression coefficients of variables derived from the two-component PLSR models. The results showed that *LWS-1*, *LWS-3*, *SWS2-A*, and *SWS2-B* expression levels were significantly correlated with the time spent by females near the HO image of the male (hereafter, female response to the HO male image). Thus, females with higher expression of these opsins exhibited strong responses to the HO male image. In contrast, the opsin expression levels did not influence female response to the LO male image. Moreover, we analyzed the effects of the expression of each opsin gene on female preference, defined as the time spent by the female on the side of the tank displaying the HO male image divided by the sum of the time spent on the sides of both the HO and LO male images. *SWS2-B* expression was positively correlated with female preference for the HO orange male (*SI Appendix*, Table S4). The component loadings for each variable of the PLSR model for female response and preference are summarized in *SI Appendix*, Table S5.

Discussion

In the present study, *SWS2-B*, *LWS-1*, and *LWS-2* expression levels varied between individuals with different *LWS-1* genotypes (SS type and AA type). *LWS-1* and *LWS-2* are located downstream of *SWS2-B* and are tightly linked (see *SI Appendix*, Fig. S7 for a physical map of the opsin genes *SWS2* and *LWS*) (2, 10). The present results suggest that polymorphisms at putative regulatory regions that are linked to a substitution at the 180th amino acid residue of *LWS-1* are responsible for different gene expression patterns in *SWS2* and *LWS* opsin clusters. A regulatory region that regulates the multiple opsin genes and is located in the intergenic region between the *SWS2* and *LWS* genes has been reported in several teleost fish species (26–28). In poeciliid fishes, including the guppy, two highly conserved candidate opsin regulatory regions have been identified within the intergenic sequence between *SWS2-B* and *LWS-1* (10, 29) (represented by black boxes in *SI Appendix*, Fig. S7). Furthermore, the finding that expression differs according to both the *LWS-1* genotype and the light environment during rearing implies that *LWS* and *SWS2* gene expression is affected by allele-dependent environmental effects, although the interaction between these was not statistically significant. Thus, the sequences of the different alleles appear to be controlled by different types of epigenetic regulation, such as DNA methylation. Therefore, it will be important to compare epigenetic modifications at these intergenic regions among different alleles and different light environments in the future.

SWS1 and *SWS2-A* expression levels decreased when individuals were grown under green or orange light, where the spectrum composition shifted toward longer wavelengths. In these

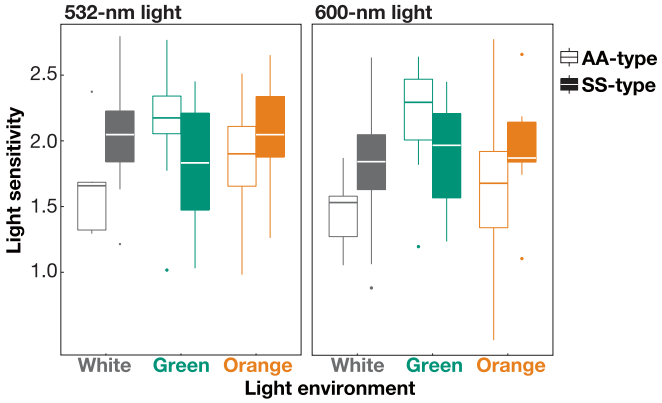

Fig. 2. The sensitivities of individuals to long-wavelength light stimuli measured by optomotor response. Light sensitivity was calculated as the negative logarithm of the threshold of detectable light intensities (in micromoles per square centimeter per second) measured by the optomotor response of individuals (*SI Appendix*, *SI Materials and Methods*). Box plots indicate the light sensitivity of AA-type *LWS-1* individuals (white, $n = 5$; green, $n = 8$; orange, $n = 10$; open boxes) and SS-type *LWS-1* individuals (white, $n = 19$; green, $n = 14$; orange, $n = 11$; solid boxes), respectively.

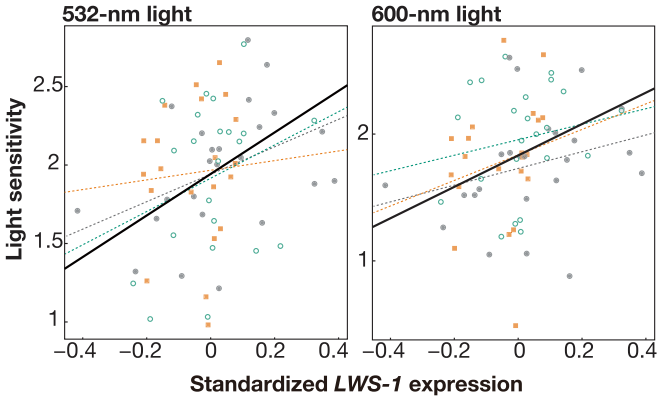

Fig. 3. The relationship between the *LWS-1* expression and sensitivity to 532-nm (*Right*) and 600-nm (*Left*) light. The standardized expression levels of *LWS-1* were the residuals from linear regression of the *LWS-1* expression level normalized against *β-actin* on the difference of experimental groups in qPCR (i.e., group of samples on the same PCR plate in qPCR run). Colors and shapes of plots indicate the rearing light environments (gray/solid circles, green/open rectangles, and orange/solid rectangles represent white, green, and orange light environment, respectively). The solid bold black lines indicate the linear regression lines (for 532-nm light: $y = 1.94 + 1.32x$, $P = 0.0165$; for 600-nm light: $y = 1.83 + 1.23x$, $P = 0.0324$). The dashed colored thin lines are the linear regression lines estimated through linear regression for each light environment (gray, green, and orange lines represent white, green, and orange light environments, respectively).

environments, RH2 and LWS cones are strongly stimulated, while SWS cones receive less stimulation. Thus, *SWS1* and *SWS2-A* can be down-regulated in short-wavelength–reduced light environments. It has been shown previously that *SWS1* expression is most sensitive to variable light environments (30, 31). For example, Fuller and Claricoates (30) have reported highly reduced *SWS1* expression levels in bluefin killifish (*Lucania goodei*) in individuals inhabiting swamps with red-shifted water color and a low transmission of UV and blue wavelengths. In guppies, it has previously been shown that individuals reared under orange light exhibit higher *LWS-1* and *LWS-3* expression than those reared under green light (14). However, such a difference was not observed here. In the present study, only individuals homozygous for *LWS-1* (AA type or SS type) were used, whereas a majority of the individuals used in the previous study were heterozygous for *LWS-1* (AS type) (14). When gene expression levels among genotypes are affected by the environment, heterozygous individuals could exhibit a different response to a particular stimulus than that exhibited by homozygous individuals [e.g., see Champoux et al. (32)]. Therefore, the different opsin gene expression responses to light environments observed in these studies may have resulted from differences in *LWS-1* genotypes.

Our results indicate that *LWS-1* expression differs between the sexes. It has previously been shown that androgen, which controls the development and maintenance of male characteristics in vertebrates, increases *LWS* opsin expression and red light sensitivity in the three-spined stickleback *Gasterosteus aculeatus* (33). In the guppy, the region upstream of *LWS-1* contains several hormone response elements (10, 29). Therefore, the sexual dimorphism observed in the present study may result from gene expression responses to differences in the amount of sex steroids, including androgen. Consistent data are lacking for sex-based differences in expression of *LWS-1* and other opsin genes in the guppy (34–36); thus, further studies are required to identify factors that drive sex differences in opsin expression in guppies.

In addition to the opsin expression normalized against *β-actin*, the relative opsin expression levels among opsin genes (individual opsin expression/the sum of opsin expression) were affected by the light environment and *LWS-1* genotype. The two measurements of opsin expression had different meanings: opsin expression normalized against housekeeping genes reflected absolute abundance of the opsin expression and thus sensitivity to the light intensity in a spectral range to which the particular opsin gene is sensitive, whereas relative opsin expression reflected relative abundance among the opsin genes and thus color discrimination sensitivity with a given combination of two opsin genes. The present results showed that the relative expression level of *LWS-1* and *SWS2-B* in SS-type individuals was higher than that observed in AA-type individuals. This suggests that SS-type individuals may have the higher color discriminability of long- vs. short wavelength of light. Moreover, the relative expression of *RH2-2* (blue-light–sensitive opsin, $\lambda_{max} = 476$ nm) in individuals reared under orange light was higher than that observed in individuals reared under white and green light, suggesting that the former may become sensitive to color discrimination of ∼476-nm light from other light.

Different *LWS-1* expression levels were correlated with sensitivity to 532- and 600-nm light at the behavioral level. Similarly, Sakai et al. (14) observed a positive correlation between sensitivity to 600-nm light and *LWS-1* and *LWS-3* expression levels. Therefore, variation in the *LWS* opsin expression (particularly *LWS-1*) seems to largely influence visual sensitivity to achromatic light stimuli. In the present study, SS-type individuals with the *LWS-1* genotype exhibited a higher sensitivity to 532- and 600-nm light than AA-type individuals with the *LWS-1* genotype when reared under white light. However, the differences in sensitivity to 532- and 600-nm light were not observed in individuals reared under green or orange light. These results reflect potential differences between the two *LWS-1* genotypes in their response to light environments (*SI Appendix*, Fig. S4). SS-type individuals expressed *LWS-1* at higher levels when reared under white light, but the difference was not significant for those reared under green or orange light. Therefore, greater *LWS-1* expression may lead to higher sensitivity in SS-type individuals only

Table 2. The effects of *LWS* opsin expression affecting light sensitivity to 532- and 600-nm light

Response variable	Explanatory variables	Estimate	SEM	*t* value	*P*
Sensitivity to 532-nm light	***LWS-1* expression**	**1.32**	**0.54**	**2.47**	**0.0165**
	LWS-2 expression	−104.62	54.64	−1.92	0.0601
	LWS-3 expression	−1.71	2.66	−0.64	0.5224
	LWS-4 expression	33.40	52.31	0.64	0.5256
Sensitivity to 600-nm light	***LWS-1* expression**	**1.24**	**0.57**	**2.19**	**0.0324**
	LWS-2 expression	−56.85	55.58	−1.02	0.3103
	LWS-2 expression	−3.76	2.80	−1.34	0.1838
	LWS-2 expression	47.29	55.82	0.85	0.4001

A generalized linear model (GLM) was constructed using the expression levels of LWS opsins as explanatory variables. The values highlighted in bold are statistically significant ($P < 0.05$).

Table 3. Results of PLSR for the effect of the expression of nine cone opsin genes on female response to HO and LO male images

	Response to the HO male image					Response to the LO male image				
Variables	Estimate	SE	df	*t* value	*P*	Estimate	SE	df	*t* value	*P*
LWS-1 expression	0.904	0.373	38	2.421	**0.020**	1.176	1.595	38	0.737	0.466
LWS-2 expression	−0.002	0.007	38	−0.375	0.710	−0.014	0.036	38	−0.388	0.700
LWS-3 expression	0.294	0.111	38	2.648	**0.012**	0.552	0.596	38	0.926	0.360
LWS-4 expression	0.019	0.010	38	1.840	0.074	−0.006	0.028	38	−0.199	0.843
RH2-1 expression	0.325	0.342	38	0.950	0.348	−1.284	1.709	38	−0.752	0.457
RH2-2 expression	0.503	0.334	38	1.505	0.141	−1.248	2.810	38	−0.444	0.660
SWS2-A expression	0.162	0.071	38	2.282	**0.028**	0.049	0.209	38	0.233	0.817
SWS2-B expression	0.984	0.451	38	2.182	**0.035**	−0.456	1.493	38	−0.306	0.762
SWS1 expression	0.711	0.372	38	1.912	0.063	0.436	1.280	38	0.341	0.735
LWS-1 genotype	−0.065	0.262	38	−0.250	0.804	−0.117	0.353	38	−0.3311	0.742

A PLSR with two components was conducted to evaluate the effects of the expression of each opsin on the total time spent by the female on the side of the tank displaying the HO or LO male. A logarithmic transformation was performed on the response variables before constituting the PLSR models. *P* values were obtained through a jackknife test. The values highlighted in bold are statistically significant ($P < 0.05$).

under white light, reflecting the significant interactions between genotype and light environment observed in the optomotor experiment. The findings suggest that the variation in opsin expression levels (particularly *LWS-1* expression levels) influenced by both genetic polymorphisms at regulatory regions linked to a substitution at the 180th amino acid residue of *LWS-1* and plastic responses to changes in the rearing light environments facilitate variations in behavioral light sensitivity. Moreover, the variation may cause the diversity in color vision and response to color signals. Our optomotor experiments examined a behavioral response to moving achromatic luminance patterns rather than color vision. Consequently, further behavioral and theoretical neural science studies combined with existing knowledge about relative cone opsin gene expression are required to investigate the color vision of guppies.

The present study demonstrated that alleles with different absorbance spectra (*LWS-1* Ala/Ser alleles) affected the linked opsin gene expression. We have previously demonstrated that divergent selection for the *LWS-1* Ala/Ser alleles among Trinidadian guppy populations corresponds with differences in the level of dissolved oxygen among populations, which could be considered a eutrophication index (11). Moreover, Sandkam et al. (13) have reported that the frequency of *LWS-1* Ala/Ser alleles varied across populations and the divergence might be correlated with differences in light environments associated with canopy closure (2). In addition to such divergent selection among different populations, individual guppy populations can occupy heterogeneous and mosaic light environments; therefore, the opsin gene expression levels may be plastic depending on the rearing light environment. These findings suggest that both genetic variation (linked to the *LWS-1* Ala/Ser polymorphism) and plasticity in response to changes in the environment could facilitate variation in opsin gene expression levels within and among populations, leading to variation in visual properties. Additionally, visual sensitivity induced by differences in absorbance spectra could be adjusted by changes of several opsin gene expressions to facilitate adaptation to local light environments. This may lead to coevolution of genetic changes in absorbance spectra and genetic and environmental regulation of opsin gene expression. A locus control region between the *SWS* and *LWS* genes has been conserved in fishes, birds, reptiles, and mammals (37). The tight linkage between control regions regulating the expression levels and the coding regions determining absorption spectra of several opsin genes may facilitate interaction effects between genetic and plastic changes in visual sensitivity. This linkage may have been conserved through natural selection. Future studies are required to test this hypothesis.

The variations in the opsin gene expression levels may lead to female responsiveness to male sexual colors. In the present study, females showing high *LWS-1*, *LWS-3*, *SWS2-A*, and *SWS2-B* expression levels exhibited strong responses to the HO male image but not to the LO male image. The orange spots in the image of the HO male guppy were larger and potentially more "luminous" for female guppies than those in the image of the LO male. Thus, we inferred that the combination of increased expression of multiple cone opsins may amplify the intensity of response to light entering the eyes of the female guppy, leading to increased responsiveness to luminous targets, including male sexual colors. *LWS-1*, *SWS2-A*, and *SWS2-B* expression levels varied depending on the rearing light environments and/or *LWS-1* genotypes. Therefore, variations in expression induced by different light environments and/or genotypes may facilitate variations in female responses. In fact, female responses following rearing under white light were significantly more pronounced than those observed following rearing under green light (*SI Appendix*, Fig. S8). The result was reflected by the higher expression of multiple opsins under white light compared with those reported under green light, although opsin expression in AA-type individuals under white light was relatively lower. Our results also showed that female preference for the HO male image was correlated with *SWS2-B* expression. Higher expression of both long- and short-wavelength–sensitive opsins enables better color discrimination ability of long vs. short wavelength of light, possibly leading to the higher female mate preference based on sexual color signals. Female preference for orange spots and chromatic and luminance contrasts of male color patterns vary among populations and individuals within populations (4, 38, 39). This variation may be partially explained by variations in the expression of the opsin genes. However, for the vision of the female guppy, the perception of color from a digital image of a male is different from that of a real male. The orange spots on real male guppies are more chromatic, and female guppies may select mates based on the combination of chroma, luminance, and color patterns. The mechanism by which the chromatic contrast of HO/LO males influences female behavior remains unclear. Similarly, the mechanism of response of females with different opsin expression profiles to males with high chromatic body color remains to be determined. Further behavioral studies to unravel the response and preference to various components of the visual body colors are warranted.

Materials and Methods

We collected wild guppies from well-established feral populations in Okinawa, Japan, and individuals homozygous for the *LWS-1* allele (SS type, $180_{Ser}/180_{Ser}$; AA type, $180_{Ala}/180_{Ala}$) were obtained from their second descendants. The observation of optomotor response was conducted for measuring visual light sensitivity to long wavelengths of light. After the optomotor experiment, we observed female response to digitally modified video images of a male guppy. To estimate how female guppies perceived the male images, we developed a receptor noise-limited model. For measuring the expression levels of nine opsin genes, a real-time qPCR was performed. See *SI Appendix, SI Materials and Methods* for more detailed methods for animal maintenance, light treatments, optomotor experiments, mate choice tests, and real-time qPCR assays.

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