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Intraspecific variation in retinal cone distribution in the bluefin killifish, *Lucania goodei*

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Abstract Studies of visual ecology have typically focused on differences among species while paying less attention to variation among populations and/or individuals. Here, we show that the relative abundance of UV, violet, yellow, and red cones varies between two populations of bluefin killifish, *Lucania goodei*. Animals from a spring population (high-transmission UV/blue light) have a higher frequency of UV and violet cones and a lower frequency of yellow and red cones than animals from a swamp population (low-transmission UV/blue light). Visual sensitivity does not vary significantly between the populations, but spring animals tend to be more sensitive in the UV/blue wavelengths (360–440 nm) and less sensitive in longer wavelengths (560–600 nm) than swamp animals. The results have two important implications. First, the tight conservation of functional regions of opsin genes across taxa does not imply that visual systems are constrained in their evolution; differential sensitivity can arise through differential expression of cone classes within the retina. Second, intraspecific visual signals in this species may evolve to maximize contrast between the signaler and the background (as opposed to brightness); males with blue anal fins are most abundant in swamp habitats where animals express fewer UV and violet cones.

Keywords Fundulidae · Microspectrophotometry · Sensory drive · Ultraviolet vision · Visual ecology

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Introduction

Sensory drive predicts that natural selection favors adaptations of the sensory system and/or signal design to habitat conditions for efficiency of the communication system (Endler 1992, 1993). Within the field of visual ecology, this hypothesis has been addressed through comparative studies that seek to link differences in the visual properties of animals from different populations (or species) with differences in the environmental conditions of their habitats (Partridge and Cummings 1999; Cronin et al. 2001; Cummings and Partridge 2001) and/or differences in their behavior (Boughman 2001). However, what constitutes an efficient system of communication is not always clear. This has lead to both studies showing a positive correlation between visual sensitivity at a given wavelength of light with the abundance of that wavelength (Lythgoe et al. 1994; McDonald and Hawryshyn 1995) and studies showing a negative correlation (Boughman 2001) being taken as evidence in support of sensory drive. How should visual systems covary with environmental conditions?

The literature offers evidence for three patterns. First, a positive correlation will be generated between visual sensitivity and lighting conditions if selection favors visual systems that match the available wavelengths of light in the environment. In a number of aquatic animals, the spectral sensitivities of rods and some classes of cones in the retina are greatest for the wavelengths of light that are transmitted best in the habitat (Lythgoe 1984; Lythgoe et al. 1994; Hunt et al. 1996; Partridge and Cummings 1999; Yokoyama et al. 1999; Shand et al. 2002). There is also evidence that the intraocular filters

animals use vary among habitats so that visual sensitivity is maximized for the available wavelengths of light (Cronin et al. 2001; Cronin and Caldwell 2002). Furthermore, in some cases there is evidence of differences in overall spectral sensitivity that correlate with habitat light conditions (McDonald and Hawryshyn 1995; Leal and Fleishman 2002).

Second, a negative correlation between environmental conditions and visual sensitivity will arise if selection had molded visual systems to compensate for reduced intensities in particular wavelengths. Laboratory studies support this idea by indicating that animals can adjust cones to maintain a constant rate of photon-catch (i.e., photostasis, reviewed by Penn 1998). Penn and Williams (1986) demonstrated that albino rats raised under high light conditions had less rhodopsin and shorter rod outer segments than rats raised under low light conditions. Similarly, Kröger et al. (1999) showed that *Aequidens pulcher* (Cichlidae) raised in monochromatic blue light had a lower proportion of blue sensitive cones in their retina than animals raised in red or green monochromatic light, suggesting decreased sensitivity to blue when reared in a predominantly blue environment. Boughman (2001) found that female sticklebacks from red-shifted lighting environments were less sensitive to red light (as measured by the optomotor response) than females from more blue-shifted lighting environments.

Third, visual systems in some groups exhibit little variation despite the fact that species within the group occupy a diversity of lighting environments, suggesting that certain types of visual systems do not evolve easily (but see Nilsson and Pelger 1994). Recent comparative reviews of retinal photoreceptor classes in insects (Briscoe and Chittka 2001) and lizards (Loew et al. 2002) found little or no evidence of evolutionary divergence in the spectral sensitivity of individual photoreceptor classes across species occupying distinctly different lighting habitats. More variation is found in aquatic organisms, but even here there is evidence that photoreceptors do not evolve easily within some groups (Cronin et al. 2002). For example, large regions of opsins are strongly conserved (Archer 1999; but see Shimmin et al. 1997) and the plasticity of cone spectral sensitivity is limited to shifts induced by chromophore usage (Archer 1999; Partridge and Cummings 1999). In an experiment, Kröger et al. (1999) found no appreciable plasticity in the spectral sensitivities of cones in a cichlid (although the relative frequency of cones was variable). Animals were reared under two different lighting environments, and no differences were found in the normalized spectral sensitivities of cones between environments. Similar results have been found in mantis shrimp (Cronin et al. 2002). These studies lend some credence to the idea that there is low variation in some aspects of vision physiology.

The goal of this study is to determine whether there is variation among populations in vision physiology in the bluefin killifish, *Lucania goodei*, and, if there is variation, whether visual sensitivity is positively or negatively correlated with lighting conditions. *Lucania goodei* is a freshwater fundulid found in a variety of lighting environments throughout Florida (Page and Burr 1991) ranging from crystal clear springs to tea-stained, turbid swamps (Fuller 2001, 2002). Males are highly polymorphic in coloration, and the relative abundance of the anal fin color morphs varies predictably with the lighting environment (Fuller 2002). Males with blue anal fins are more abundant in populations with low transmission of UV and blue wavelengths. In contrast, males with red anal fins (and to a lesser extent, males with yellow anal fins) are more abundant in populations with high transmission of UV and blue wavelengths. The correlation of male color patterns with lighting conditions suggests that there might well be correlations between the visual system and lighting conditions.

In this study we ask whether vision physiology varies concordantly across lighting environments. We address this question by comparing the spectral sensitivity of individuals and the types and abundances of retinal cones between animals from a spring population (high light, high UV/blue wavelength transmission) and animals from a swamp population (low light, low UV/blue wavelength transmission). Specifically, we test the following three hypotheses: (1) there is no variation in vision physiology across habitat type; vision physiology is fixed; (2) there is a positive correlation between lighting transmission and vision physiology; increased UV/blue wavelength transmission leads to increased visual sensitivity at UV/blue wavelengths; and (3) there is a negative correlation between lighting transmission and vision physiology; decreased UV/blue wavelength transmission leads to increased sensitivity at UV/blue wavelengths in order to maintain a constant photon-catch.

Materials and methods

Spectral sensitivity based on electroretinographic flicker photometry

Animals were collected by RCF from a swamp population (26-Mile Bend, Everglades, Broward, Fla., USA) and from a spring population (Wakulla Upper Bridge, Wakulla River, Wakulla, Fla., USA) in February 2001 and transported to Union College, Schenectady, N.Y., USA. All animals were adults. Animals were maintained on a 12L:12D light schedule and fed frozen brine shrimp twice daily. All electroretinogram (ERG) readings were recorded in February–March 2001.

ERG flicker photometry methodology is detailed elsewhere (Fleishman et al. 1997; Jacobs et al. 1996). Animals were immobilized with an intramuscular injection of curare. Each individual was placed in a small holder with a wet sponge and was intubated so that aerated water flowed over its gills. ERGs were recorded differentially. The active electrode consisted of a small stainless steel tube placed in contact with the cornea after application of xylocaine gel to the surface of the eye. The indifferent electrode was a platinum wire placed around the nape. The active electrode was mounted at the tip of a quartz fiber-optic light guide through which the stimulus light was delivered, such that the stimulus light passed through the small stainless steel tube. The fiber optic was bifurcated and received input from two different source lights: a colored test light and a broad-band white control. The stimulus consisted of

alternating flashes of equal duration of the colored test light and the control, with an equal period of dark (no stimulus) between each flash. The test light stimulus consisted of a monochromatic light the intensity of which could be varied over 5 log units using a linearly-variable optical quartz density neutral-density filter. The colored test stimuli were created by passing the focused output from a 300 W xenon arc lamp through a 1/8 m monochrometer resulting in monochromatic stimuli (10 nm 1/2-energy pass-band). The control consisted of a dim light from a 50-W QTH fiber-optic illuminator passed through a neutral-density filter. The test and control stimuli were passed through a spinning chopper wheel and into the two ends of the bifurcated fiber optic leading to the eye. These were positioned so that they created the alternating control-off-stimulus-off pattern described above. The entire alternating pattern was presented at a frequency of 4 Hz.

Spectral sensitivity was measured by determining the test light intensity for a given wavelength that elicited a response equal in magnitude to that of the control stimulus. To determine the test intensity at which the response to test and control light were equal, the output was passed through a narrow electronic bandpass filter and centered on the stimulus frequency (4 Hz). The output from the filter was a nearly sinusoidal signal with a frequency equal to the stimulus frequency. The phase of the sinusoid depended on whether the ERG response was greater to the control or to the test. By adjusting the intensity of the test light stimulus, we could adjust the ERG output until the phase was intermediate and amplitude was minimal. This occurred when responses to the test and control stimulus flashes were equal. In order to find this equal response point, we repeatedly collected and digitized a buffer of four complete stimulus cycles in duration, which was an average of 20 repetitions. This entire process was performed for each wavelength in 40-nm steps from 360 to 640 nm.

Absolute sensitivity at each wavelength was measured as the inverse of the radiance of the test light when responses to test and control light were equal (1/radiance at criterion, with radiance measured in units of $\mu mol\ m^{-2}\ sr^{-1}\ s^{-1}$, where $1\ \mu mol = 6.02 \times 10^{17}$ quanta. The absolute sensitivity was computed for each subject for light from 360–640 nm. We calculated the overall absolute sensitivity by averaging absolute sensitivity across all wavelengths. We also calculated the relative sensitivity at each wavelength by dividing all values in the absolute sensitivity curves by the maximum value for each individual. Hence, the wavelength where each individual is maximally sensitive is scored as 1. We compared the absolute sensitivity between the two populations provided that there were no statistically significant differences in overall sensitivity. We also examined the relative sensitivities to ascertain whether this variable produced the same pattern across populations.

We compared the curves between two populations using analysis of variance provided that variances were not significantly heteroscedastic as indicated by a Barttlett's test of homogeneity. Otherwise, we used a non-parametric Kruskal-Wallis test. We present the unadjusted P values, but also consider the P values after a sequential bonferroni adjustment (Sokal and Rohlf 1995) to control for the accumulation of type 1 error, where we controlled for 16 tests (8 wavelengths, 2 variables).

Microspectrophotometry

Animals from a swamp population (26-Mile Bend) and a spring population (Wakulla) were collected and transported to Cornell University, Ithaca, N.Y. in October 2001. Animals were maintained on a 12L:12D ratio at 21C and fed twice daily. Microspectrophotometry (MSP) readings were taken in October 2001. All animals were adults. In addition, one swamp and two spring animals were sent to Cornell for MSP analysis March 2001.

MSP measurements were performed using methods identical to those described in Loew (1994) and Provencio et al. (1992). All procedures were carried out under infrared illumination using appropriate image converters and video cameras. Animals were dark adapted for a minimum of 1 h after which they were euthanized. Enucleated eyes were hemisected and pieces of retina were immersed in a simple Sorensen's phosphate buffer (pH 7.2) with 6% sucrose or dextran added. The retinas were carefully teased from the retinal pigment epithelium and macerated using razor blade fragments and tungsten needles. A drop of the dispersed retina was sandwiched between two cover slips and transferred to the stage of the MSP. The MSP itself has been described in detail elsewhere (Loew 1994; Loew et al. 2002). A 100-W tungsten-halogen lamp together with quartz and mirror optics allowed for accurate absorbance measurement down to 340 nm with a rectangular measuring aperture as small as 1.5 μm^2.

We used template fitting to determine λ_{max} (the wavelength at maximum absorbance for a template-derived visual pigment best fitting the experimental data). The rationale for using template fitting (as opposed to simply using the peak absorbance as a measure of λ_{max}) is that the entire absorbance curve is informative as to the true λ_{max}. Hence, we can increase our precision by using all of the data. Determination of λ_{max} involves six steps: (1) smooth the data, (2) determine the peak absorbance (X_{max}), (3) normalize the absorbance curve, (4) fit the templates, (5) calculate the standard deviation (SD) of λ_{max}, and (6) compare with the actual data and choose the best fit.

The raw data were first smoothed using a digital filter routine (''smooft'' Press et al. 1989). The smoothed spectrum was overlaid on the raw data and checked by eye to make sure that over-filtering or spurious data points had not shifted the apparent maximum. If this was the case, then the unsmoothed data were used. Next, the peak absorbance was determined and used in the normalization. The peak absorbance (X_{max}) was the calculated maximum of the best fit Gaussian to the data points 20 nm either side of the estimated-by-eye absorbance maximum of the alpha band. Using X_{max}, the data were then normalized using the method of Mansfield (1985) as presented by MacNichol (1986). Normalized data were then fit using the A1 and A2 templates of Lipetz and Cronin (1988). These templates allowed us to generate 40 estimates of λ_{max} from the long-wavelength limb (absorbance data for wavelengths slightly greater than λ_{max}) and 30 estimates of λ_{max} from the short-wavelength limb (absorbance data for wavelengths slightly less than λ_{max}). Mean $\lambda_{max} \pm$ standard deviation (SD) was then determined using the short-wavelength limb estimates, the long-wavelength estimates, and the combined estimates. For each of these three λ_{max} values, a template curve was fit to the original data. A decision as to which fitted best was made by visual examination. The template fit having the lowest SD usually had the best visual fit. The curve was discarded if the SD of λ_{max} was greater than 7.5 nm (see Sillman et al. 1999, 2001 for similar criteria). This process was repeated for each microspectrophotometer curve, after which the λ_{max} values for each curve of a spectral class were averaged to yield a final estimate of mean $\lambda_{max} \pm$ SD.

We compared the presence and absence of cone types between individuals of the two populations using X^2 tests. For each individual, we calculated the relative frequencies of all cone types (i.e., no. of UV cones recorded/no. of total cones recorded), the mean $\lambda_{max} \pm$ SD for each cone class, and the coefficient of variation in λ_{max} (CV λ_{max}). We used analysis of variance to compare populations provided that variances were not significantly heteroscedastic as indicated by a Barttlett's test of homogeneity. Otherwise, we used a non-parametric Kruskal-Wallis test. All probabilities are two-tailed and considered significant at $P < 0.05$. We also present results after considering a sequential bonferroni adjustment for 15 comparisons (5 cone classes, 3 variables, Table 1, Table 2). All analyses were performed with SAS V.8 statistical software (SAS Institute, Cary, N.C., USA).

Results

ERG flicker photometry

Absolute sensitivity to UV and blue wavelengths tended to be higher for spring animals than for swamp animals

Table 1 Mean and coefficient of variation (CV) of λ_{max} calculated across individuals for the spring and swamp populations

Opsin class	Spring mean λ_{max} (SD)	Swamp mean λ_{max} (SD)	Spring CV λ_{max} (SD)	Swamp CV λ_{max} (SD)
Ultraviolet	359.38 (2.39)	359.45 (7.14)	1.072 (0.408)	–
Violet	405.13 (1.56)	404.73 (2.28)	0.716 (0.167)	0.806 (0.249)
Blue	453.59 (3.25)	456.38 (7.95)	0.910 (0.313)	0.744 (0.620)
Yellow	537.51 (2.71)	540.92 (3.80)	0.940 (0.369)	1.046 (0.240)
Red	572.58 (2.43)	573.17 (1.96)	0.949 (0.234)	1.149 (0.364)

$n = 11$ for spring; $n = 10$ for swamp
Note that CV λ_{max} is approximately 1% for all opsin classes

Table 2 Mean frequencies for each opsin class in each of the two populations

Opsin class	Spring mean frequency (SD)	Swamp mean frequency (SD)	*F*	*P*
Ultraviolet	0.111 (0.054)	0.006 (0.013)	35.61	< 0.001
Violet	0.450 (0.072)	0.267 (0.102)	23.00	< 0.001
Blue	0.080 (0.037)	0.088 (0.053)	0.17	0.6823
Yellow	0.177 (0.033)	0.279 (0.044)	36.60	< 0.001
Red	0.182 (0.044)	0.360 (0.081)	40.62	< 0.001

$n = 11$ for spring; $n = 10$ for swamp
Values in bold indicate statistically significant differences after Bonferonni adjustment. *F*-values and unadjusted probability values are shown. For all *F*-tests, numerator $df = 1$, and denominator $df = 19$

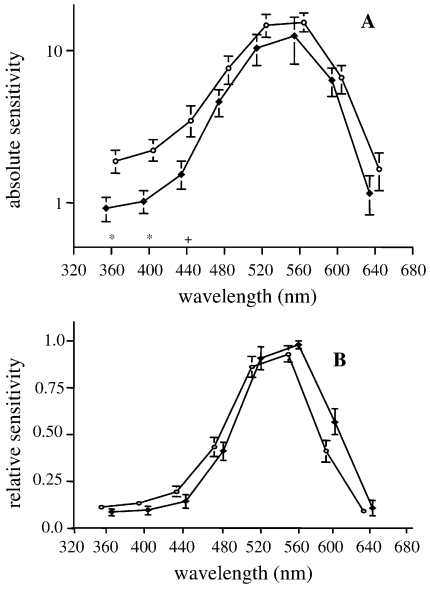

Fig. 1A Absolute sensitivity for the spring and swamp populations. Units are 1/radiance at criterion with radiance in units of μmol m^{-2} sr^{-1} s^{-1}. **B** Relative sensitivity for the spring and swamp populations. Means and standard errors are shown. *Open symbols* denote spring values; *filled symbols* denote swamp values. $n = 8$ for all spring values; $n = 5$ for all swamp values except for wavelength 640 nm where $n = 4$. *Significantly different with *ANOVA*, $P < 0.05$; + Significantly different with Kruskal-Wallis, $P < 0.05$. No comparisons are statistically significant after a sequential Bonferroni adjustment. Points are jittered for the purpose of display

(Fig. 1A). Animals from the spring population were more sensitive at 360 nm ($F_{1,11} = 4.99$, unadjusted $P = 0.0473$), 400 nm ($F_{1,11} = 6.09$, unadjusted $P = 0.0312$), and 440 nm (Kruskal-Wallis $X^2 = 3.927$, $df = 1$, unadjusted $P = 0.0475$). However, none of the comparisons were statistically significant after a sequential Bonferroni adjustment. There were no significant differences between populations in overall absolute sensitivity ($F_{1,11} = 1.45$, $P = 0.2545$). Examination of relative sensitivity shows that spring animals tended to be more sensitive in the UV/blue wavelengths (360–440 nm) and swamp animals were more sensitive in long wavelengths (560–600 nm), although these differences were not statistically significant (Fig. 1B, unadjusted $P > 0.12$ in all tests).

Microspectrophotometry

Five cone classes were present in both populations (UV $\lambda_{max} = 359 \pm 4$ nm, $n = 68$, violet $\lambda_{max} = 405 \pm 3$ nm, $n = 382$, blue $\lambda_{max} = 455 \pm 6$ nm, $n = 86$, yellow $\lambda_{max} = 539 \pm 6$ nm, $n = 226$, red $\lambda_{max} = 573 \pm 7$ nm, $n = 262$). Most cones best fit an A1 template (88.6%) while a smaller proportion best fit A2 (11.43%) (see electronic appendix for individual data). Neither average λ_{max} nor the coefficient of variation in λ_{max} (CV λ_{max}) differed between the two populations (Table 1, average λ_{max}: population×cone class $F_{4,86} = 1.02$, $P < 0.403$; CV λ_{max}: population×cone class $F_{4,86} = 1.00$, $P < 0.399$).

All violet and UV cones were single cones. All red cones were members of double cones. The vast majority of yellow cones were members of double cones. We detected one single, yellow cone that we presume was a double cone that became detached from its complementary cone. Similarly, the vast majority of blue cones were members of double cones. We detected 11 single blue cones that we presume were actually detached elements of double cones. There were no statistically significant differences between λ_{max} of single and double blue cones (single blue $\lambda_{max} = 454 \pm 7$, double blue $\lambda_{max} = 454 \pm 5$). Finally, we also measured λ_{max} on 10 detached fragment tips (5 blue, 3 red, 2 violet). Obviously, we could not assign a cone type to these pigments. Blue fragment tips had a slightly higher λ_{max} than either the single blue or double blue cones ($F_{2,83} = 3.69$, $P < 0.029$, blue fragment tip $\lambda_{max} = 462 \pm 11$). Similarly, red fragment tips had a slightly higher λ_{max} than red double cones ($F_{1,260} = 10.92$, $P < 0.0011$, red double cone $\lambda_{max} = 573 \pm 7$, red fragment tip $\lambda_{max} = 586 \pm 4$).

All animals had double cones. We obtained MSP readings for both cones from 146 sets of double cones. There were three distinctly different types of double cones. We found 121 double cones where a yellow cone was paired with a red cone. We also found 23 double cones where a blue cone was paired with a yellow cone. We found two twin double cones. One involved a pair of red cones (λ_{max} 566/568 nm). Another involved a pair of far-red sensitive cones (λ_{max} 584/586). In addition to the 146 sets of double cones (where we measured

both cones), we also measured 262 cones (47 blue, 81 yellow, 134 red) that were members of double cones for which we were unable to measure the complementary cone.

UV cones were found much more readily in animals from the spring population (11/11) than they were in animals from the swamp population (2/10) (Fig. 2, Pearson $X^2 = 14.22$, $df = 1$, $P = 0.0002$). This result was not attributable to the fact that we measured more cones per animal for the spring population than for the swamp population (total cones per animal: $F_{1,19} = 6.33$, $P = 0.0210$, spring: 55 ± 11, swamp: 41 ± 13). Assuming that the frequency of UV cones in the swamp is 0.11 (i.e. the same as in the spring), the probability of not finding an UV cone in a single individual where a minimum of 25 cones were measured was 0.054 [(1–0.11)×25]. Out of 10 animals, the expected number of animals in which no UV cones would be 0.54 (0.054×10). Hence, we would expect to find UV cones in at least 9 individuals. This conservative analysis demonstrates that these results cannot be attributed to differences in sampling.

Finally, an analysis of the mean cone frequencies indicates that UV and violet cones were more abundant in the spring population and that yellow and red cones were more abundant in the swamp population (Table 2, Fig. 3). The relative abundance of blue cones did not differ between the two populations (Table 2, Fig. 3).

Discussion

The MSP analysis indicated differences in the numbers of different photoreceptor types between the two populations. The ERG-based spectral sensitivity curves did not differ between the populations, although all trends were in the direction predicted by the MSP data. Low sample sizes restricted our power to detect any differences between the two populations in relative sensitivity.

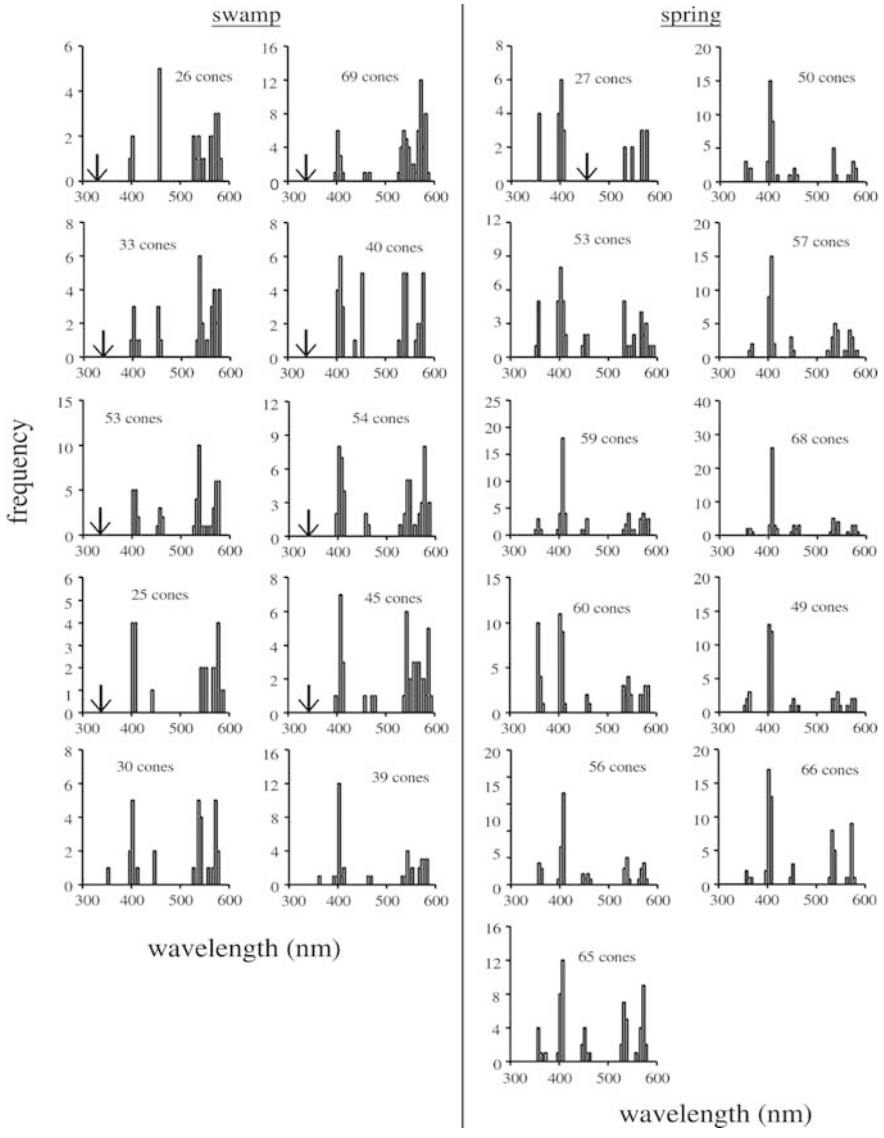

Fig. 2 Cone profiles for animals from the swamp and spring population. Each graph is a histogram of wavelength at maximum absorbance (λ_{max}) cone values from a single animal. *Arrows* indicate missing cone classes. The total number of measured cones is indicated in each graph

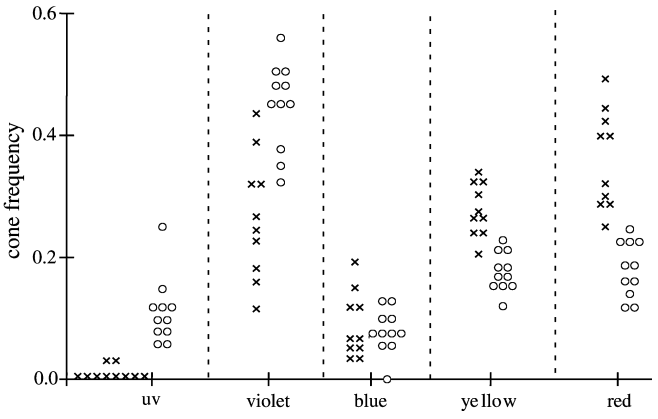

Fig. 3 The relative frequency of UV, violet, blue, yellow, and red cones for both the spring population (*open circles*) and the swamp population (*x's*). For each opsin class, each data point represents a single individual

Still, the overall direction of the curves supports the interpretation that higher expression of UV and violet cones in the spring population leads to a blue shifted sensitivity curve with higher sensitivity in the UV and blue range (360–440 nm). Similarly, higher expression of yellow and red cones in the swamp populations leads to a red shifted curve with higher sensitivity from 560 to 600 nm.

The match between the ERG patterns and the quantitative differences detected by the MSP analysis is less precise. In the spring population, the most abundant photoreceptor was the single, violet cone. Yet, according to ERG, spring animals were most sensitive to light at 560 nm. We cannot explain this discrepancy. We believe the qualitative pattern found in this study is robust (i.e., more UV and violet cones in spring animals, more yellow and red cones in swamp animals). However, whether MSP is a precise tool for measuring the exact retinal make-up of various cone-types has yet to be resolved.

The main conclusion from this study is that there is intraspecific variation in the relative abundance of UV, violet, yellow and red cones in *L. goodei*. These results provide evidence for a positive matching between the available wavelengths of light and the relative abundance of photoreceptors. The relative abundance of cones appears to be much more plastic than the λ_{max} of the photopigments which did not vary between the two populations.

In some fish, UV cones are expressed in juveniles but then lost in the adult stage (Bowmaker and Kunz 1987; Bowmaker 1990; reviewed in Beaudet and Hawryshyn 1997). We doubt that the differences among *L. goodei* populations in this study were due to differential effects of ontogeny. First, all of our experimental animals were adults. Second, animals from the spring population (which had more UV cones) are larger than animals from the swamp (R.C. Fuller, unpublished data). Although the manner in which body size translates into age is unknown in *L. goodei*, in other freshwater fish in these habitats larger animals are older animals (unpublished otolith data of J. Travis and R. Allman). Therefore, it is unlikely that the larger animals from the spring are significantly younger than the smaller animals from the swamp. Whether the differences in UV cone expression are due to genetic differences or differences in the ontogenetic lighting environment is currently under investigation.

The presence of both UV and violet cones in the same individual is striking because usually only one short-wavelength-sensitive I (UV) opsin is expressed in fish. Many fish possess UV cones and lack violet ones (Cichlidae: van der Meer and Bowmaker 1995; Carleton and Kocher 2001; Adrianichthyidae: Hisatomi et al. 1997; Cyprinidae: Palacios et al. 1998; Hisatomi et al. 1996; Pleuronectidae: Helvik et al. 2001). In addition to *L. goodei*, the mummichog, *Fundulus heteroclitus*, the guppy, *Poecilia reticulata,* and a cyprinid, *Danio aequipinnatus,* have both ultraviolet cones (λ_{max} 363, 389, 358 nm, respectively) and violet cones (λ_{max} 400, 408, 408 nm, respectively) (Archer et al. 1987; Archer and Lythgoe 1990; Flamarique and Hárosi 2000; Palacios et al. 1996). Molecular analyses suggest that violet cones in chicken and frog evolved from UV-sensitive opsins (SWS1) found in fish (Hisatomi et al. 1996; Yokoyama and Yokoyama 1996; Hisatomi et al. 1997; Yokoyama 1997; Hunt et al. 2001). Either a gene duplication of the SWS1 opsin has occurred with subsequent divergence in function or the violet opsin in these fish has evolved de novo from a different opsin class.

Variation in visual capabilities appears to exist both within and among species. Carleton and Kocher (2001) have recently shown in cichlids that closely related species expressing nearly identical opsins can vary by more than tenfold in relative expression levels of opsins (and, therefore cones). The preponderance of variation in vision physiology and visual sensitivity across multiple populations and habitat types (McDonald and Hawryshwyn 1995; Boughmann 2001; Cronin et al. 2001; Rodd et al. 2002, this study), along with the knowledge that closely related species can vary greatly in opsin expression (Carleton and Kocher 2001) means that the possibility of readily available genetically based variation in vision systems should not be neglected. We are currently investigating the genetic and environmental contributions to variation among animals within populations.

A curious pattern is emerging with respect to the male color patterns employed by *L. goodei*. There is an inverse relationship between UV and violet cone abundance and the abundance of blue morphs within a population. Males with blue anal fins are most common in populations where UV/blue wavelengths attenuate quickly (Fuller 2002) and where animals possess fewer UV and violet cones (this study). Perceived brightness of blue anal fins can clearly not be greater in swamp populations. The most likely scenario is that blue males create high contrast with the water column or with other color elements on the body. If indeed no photons are being

detected from the anal fin, then it will appear black, which will produce high contrast with the body and visual background. Such contrast is detected most effectively by those photoreceptors whose sensitivity matches the background illumination. Thus, the blue and UV fins in the swamp habitats may be effectively stimulating the long wavelength photoreceptors by contrasting with the bright background. On the other hand, spring populations have high transmission of UV/blue wavelengths causing the water column to have a bluish tint. This environment should create high contrast for yellow and red color morphs, but lower contrast for blue morphs, and in this case the short wavelength photoreceptors (whose sensitivity matches the backlight) may be effectively stimulated by the contrast between the backlight and animal color patterns. Similar patterns have been found across species in Anolis lizards (Leal and Fleishman 2002). *Anolis cristatellus* males are found in habitats with less UV background radiance yet have dewlaps that reflect strongly in the UV, while *A. cooki* males are found in habitats with higher UV background radiance and have dewlaps that lack UV reflectance. Males appear to be optimizing contrast with background lighting conditions even if this results in a decrease in overall brightness.

Another possibility is that blue males are quite conspicuous to conspecific animals in clear, spring water (due to the high amounts of light at all wavelengths), but that they suffer high predation. We are currently using our data on the visual properties of *L. goodei* to analyze reflectance spectra and compute actual brightness and contrast of blue anal fins in tea-stained and in clear water using the methods of Endler (1990).

In conclusion, *L. goodei* populations vary in the relative abundance of UV, violet, yellow, and red cones. The results match previously described patterns of interspecific variation (more sensitivity to more commonly encountered wavelengths) (Lythgoe 1984; Lythgoe et al. 1994; Hunt et al. 1996; Partridge and Cummings 1999; Yokoyama et al. 1999) better than they match patterns of compensation found in other studies (wherein increases in sensitivity occur in response to reductions in specific wavelengths) (Penn and Williams 1986; Kröger et al. 1999; Boughman 2001). Our results imply that the tight conservation of opsins and photoreceptor spectral sensitivity across taxa does not imply that visual systems are tightly constrained in their evolution. We are currently determining the degree to which variation in cone expression is determined by environmental and/or genetic factors.

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