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RESEARCH ARTICLE

Spectral sensitivity in ray-finned fishes: diversity, ecology and shared descent

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ABSTRACT

A major goal of sensory ecology is to identify factors that underlie sensory-trait variation. One open question centers on why fishes show the greatest diversity among vertebrates in their capacity to detect color (i.e. spectral sensitivity). Over the past several decades, λ_{max} values (photoreceptor class peak sensitivity) and chromacy (photoreceptor class number) have been cataloged for hundreds of fish species, yet the ecological basis of this diversity and the functional significance of high chromacy levels (e.g. tetra- and pentachromacy) remain unclear. In this study, we examined phylogenetic, physiological and ecological patterns of spectral sensitivity of ray-finned fishes (Actinoptergyii) via a meta-analysis of data compiled from 213 species. Across the fishes sampled, our results indicate that trichromacy is most common, ultraviolet λ_{max} values are not found in monochromatic or dichromatic species, and increasing chromacy, including from tetra- to pentachromacy, significantly increases spectral sensitivity range. In an ecological analysis, multivariate phylogenetic latent liability modeling was performed to analyze correlations between chromacy and five hypothesized predictors (depth, habitat, diet, body coloration, body size). In a model not accounting for phylogenetic relatedness, each predictor with the exception of habitat significantly correlated with chromacy: a positive relationship in body color and negative relationships with body size, diet and depth. However, after phylogenetic correction, the only remaining correlated predictor was depth. The findings of this study indicate that phyletic heritage and depth are important factors in fish spectral sensitivity and impart caution about excluding phylogenetic comparative methods in studies of sensory trait variation.

KEY WORDS: Chromacy, Cone photoreceptor, Depth, Light, Vision, Visual ecology

INTRODUCTION

How animals acquire information about the environment is variable, both in the sensory systems they employ and how those systems operate. Studying this variation has given rise to the field of sensory ecology, which aims to understand the adaptive relationship, if any, between ecological variables and sensory traits (Dangles et al., 2009). Fishes, in particular, provide an excellent system for studying sensory variation due to their diverse ecologies, sensory abilities and phylogenetic history. Of all vertebrates, fishes show the greatest diversity in spectral sensitivity (Cronin et al., 2014) – the capacity of the retina to discriminate light of different spectral compositions. Spectral sensitivity has been documented for hundreds of fish species, yet determining the underlying ecology of this trait has proven difficult. Studies of fish spectral sensitivity often are restricted to specific clades (e.g. Parry et al., 2005; Lythgoe et al., 1994) and have found varying relationships between visual ability and ecology (e.g. Dartnall and Lythgoe, 1965; Lythgoe, 1966). The current wealth of published spectral sensitivity data has permitted us to conduct the largest ever examination of spectral sensitivity diversity of any animal clade. Using a recently published phylogeny of actinopterygian fishes (Rabosky et al., 2013), we incorporate phylogenetic comparative methods to examine how the relationship of phylogenetic history and ecological variables may underlie variation in this trait.

Two major aspects of spectral sensitivity, λ_{max} and chromacy, are determined by the differential expression of opsins (light-sensitive proteins) and vitamin-A-derived chromophores to which they are bound. The term λ_{max} refers to the wavelength of peak sensitivity of a given photoreceptor class (MacNichol, 1986), and chromacy describes the number of distinct photoreceptor classes within a given retina (e.g. Bowmaker, 1983; Cronin and Hariyama, 2002). In vertebrates, chromacy generally ranges from monochromacy, where there is only one cone photoreceptor class and likely achromatic vision, to pentachromacy, with five cone classes and possibly acute chromatic vision. Higher chromacy, can – in theory – improve both the retina's wavelength range of sensitivity and its capacity for spectral discrimination (Vorobyev, 1997a). Stepwise increases in chromacy, however, may not always predict enhanced color vision, as cone signals can contribute to other visual tasks (e.g. luminance detection; Osorio and Vorobyev, 2005) and color perception can be modified by downstream processing from the retina (Kelber et al., 2003). The purpose of the present study was to examine the great diversity of photoreceptor classes observed across this clade.

The capacity for spectral discrimination by the retina, and perhaps color vision, is thought to have arisen early in vertebrate evolutionary history (Collin et al., 2009). The most basal vertebrates, the jawless fishes (hagfish and lamprey), have four spectrally distinct cone classes (Bowmaker and Hunt, 2006), providing the basis for spectral discrimination for all extant vertebrates. In the subsequent evolution of fishes, opsin gene duplication and diversification gave rise to extensive variation in photoreceptor λ_{max} values and levels of chromacy (Hofmann and Carleton, 2009). The ecological basis of this variation remains unclear, but is largely attributed to the visual challenges imparted by the underwater light field (e.g. Lythgoe, 1979; Bowmaker, 1990; Sabbah et al., 2013).

Relative to studies of photoreceptor λ_{max} values, variation in fish chromacy remains understudied, although five major ecological predictors of this trait have been hypothesized. First, it is thought that chromacy is inversely proportional to depth, because the

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spectrum of underwater light narrows with depth, reducing the range of wavelengths that are available for cone activation (Lythgoe, 1972; Levine and MacNichol, 1982). The second hypothesis posits that chromacy is associated with the presence of dissolved and particulate matter that, via absorption, reduce the spectral range of the underwater light field (Levine and MacNichol, 1979). For example, fishes that occupy clear waters (as found offshore) are predicted to have higher chromacy than fishes that occupy the more monochromatic turbid or productive waters typical of many inshore and freshwater habitats (Lythgoe et al., 1994; Bowmaker, 1990). In the third hypothesis, chromacy varies with the appearance of visual targets such as prey (Munz and McFarland, 1973). Visual detection of prey is partially dependent on the contrast of that target to the background environment. In such a scenario, visual detection can potentially be improved by higher levels of chromacy (Lythgoe, 1979) depending on viewing angle of the predator and the spectral reflectance of the prey. The fourth hypothesis states that chromacy is influenced by the color complexity of intraspecific visual signals, in particular those used for mate selection/recognition and same-sex competition. Higher chromacy may improve intraspecific identification and the detection of signals from colorful conspecifics (Ward et al., 2008; Sabbah et al., 2013). The final hypothesis proposes that chromacy varies with body size because it influences the sighting distance of targets such as prey (Cronin et al., 2014). Large predators often see prey over long distances. This narrows the spectrum of light reflected by prey, via wavelength-dependent attenuation, thus reducing chromatic contrast of prey against background. For larger fishes, which typically have longer sighting distances, target detection may be improved by lower levels of chromacy, by trading additional photoreceptor classes for increased spatial acuity and achromatic contrast sensitivity.

The work cited above is a small example of a larger field aimed at determining the ecological variables that influence sensory variation. Despite this large body of work, few studies control for the role of shared evolutionary history in driving patterns of sensory ability among species (Dangles et al., 2009). The failure to account for phylogenetic relatedness can have critical consequences for evolutionary interpretations, and as such, there have been calls for sensory ecologists to take a more systematic, evolutionary approach in studies of trait variation (e.g. Northcutt, 1988; Chittka and Briscoe, 2001; Kemp et al., 2015). In the present study, we compiled published data for 213 species of actinopterygian fishes to investigate: (1) patterns and performance of cone-based spectral sensitivity; (2) relationships between certain ecological predictors (depth, habitat, diet, body coloration and body size) and chromacy after correcting for shared descent; and (3) the effects of excluding phylogenetic correction on these relationships. Together, this work reveals phylogenetic, physiological and ecological patterns of cone spectral sensitivity across actinopterygian fishes and helps elucidate the effects of shared phylogenetic history on our ecological understanding of spectral sensitivity.

MATERIALS AND METHODS

Compilation of the spectral sensitivity database

Known rod λ_{max} values, cone λ_{max} values and chromacy levels were compiled from the literature for ray-finned fishes (n=277; Table S1). As photoreceptor sensitivity and other aspects of vision can change over ontogeny, only data collected from adults were included. For each recorded cone λ_{max} value, we also recorded cone morphological type, which in part, is defined by the cell's number of light-sensitive outer segments (i.e. specialized stacks of membrane containing opsin). As such, λ_{max} values were assigned to either single cone (which have one outer segment), double cone (which have two outer segments with differing λ_{max}) or twin cone types (which have two outer segments with identical λ_{max}; Lyall, 1957). In cases where values were associated with two or more morphological types in a given retina, cone λ_{max} values were assigned as 'mixed'. In addition, the two λ_{max} values from double cones were counted individually toward chromacy, as each can aid in spectral discrimination (Pignatelli et al., 2010).

The retinas of four species contained either triple cone morphologies (*Marosatherina ladigesi* and *Melanotaenia maccullochi*; Reckel et al., 2002) or cone classes serving hexachromatic vision (*Hemitripterus villosus* and *Engraulis encrasicolus*; Kondrashev, 2010; Kondrashev et al., 2012). For the purpose of our analysis, these outlying values were binned into the closest common assignment: double cone morphology and pentachromacy, respectively. Lastly, we only included data that had been collected by using microspectrophotometry (MSP), a method that yields single-cell measurements of photoreceptor absorption spectra (Bowmaker, 1984).

A large amount of MSP data has been collected across fishes, and unfortunately, can be difficult to compare to data from other methods that assess spectral sensitivity. For example, λ_{max} estimation from electroretinography (i.e. electrophysiology of retinal responses to light; ERG) is susceptible to error as photoreceptor sensitivity curves are interpolated from broader datasets, and behavioral assessments of color vision are affected by processing that occurs downstream from the retina (e.g. Simpson et al., 2016). Furthermore, visual pigment extraction methods are not a reliable measure of cone class number and sensitivity because, at least in fish, several pigments can be expressed in a single photoreceptor type (e.g. Dalton et al., 2014). It is important to note that the present results may be susceptible to sampling error in MSP, including the accidental exclusion of photoreceptor classes during manual cell by cell analysis of the retina. Fortunately, the large sample size of animals included in the study helps obscure this source of error, allowing us to uncover patterns of spectral sensitivity diversity across fishes.

Phylogenetic signal and corrected analysis of sensitivity range

In order to control for phylogenetic relatedness, we restricted our analyses to species in a recently published phylogeny of 7822 ray-finned fishes (Rabosky et al., 2013). Of the 277 species included in the cone spectral sensitivity database, 213 were present in the phylogeny, thereby allowing branch length information to be extracted to create a sub-tree for analysis. Prior to ecological analysis of the data, the degree of phylogenetic signal in fish chromacy was estimated by calculating Pagel's lambda (Pagel, 1999; Freckleton et al., 2002) using the *phytools* v0.6 package (Revell, 2012) in R v3.4.3. Pagel's lambda represents a branch length transformation that maximizes the likelihood of the observed data. Expressed as a value ranging from 0 to 1, this parameter denotes the degree of covariance of trait variation to phylogenetic structure, respectively. A likelihood ratio test was used to determine significance against the null hypothesis that lambda=0.

Initially, cone λ_{max} values were analyzed to examine patterns of spectral sensitivity across cone morphologies and levels of chromacy. In particular, we examined whether higher chromacy levels increase the overall range of wavelengths to which the eye is sensitive. The sensitivity ranges of opsins typically span ~200 nm surrounding the λ_{max} (MacNichol, 1986); therefore, a value of 100 nm was added to and subtracted from each species' highest and lowest λ_{max} values, respectively. The differences in these values were taken, resulting in a range of sensitivity for each species. We then calculated and compared the mean range for each chromacy level. After rank transformation, we performed a simulation-based phylogenetic ANOVA (Garland et al., 1993) to compare mean ranges, again using the *phytools* package. Pairwise *t*-test *post hoc* analyses were conducted and the alpha level was adjusted for multiple testing (n=5 comparisons; α=0.01) using the Bonferroni correction (Dunn, 1961).

Assignment of ecological variables

Five ecological variables (depth, habitat, diet, body coloration and body size) were selected to address the five existing hypotheses of chromacy variation in fishes (see Introduction). Similar to an approach taken by Caves and colleagues (2017) in a study of fish visual acuity, each ecological variable was segmented into three bins based on criteria established *a priori* using FishBase (www.fishbase.org). The classification of each variable into three bins facilitated the detection of spectral sensitivity patterns despite extensive variation in fish ecology. These bins were assigned to each species according to the life history of adults. For the depth variable, bins were set to encompass major spectral transitions that can occur over depth, with shallow (0–49 m), moderate (50–199 m) and deep (200 m+) water ranges. Fish habitats were binned to serve as a general proxy of water color, including freshwater (lakes and rivers; brown water), inshore (estuarine and coasts; green water), and offshore (pelagic and coral reef; blue water) habitats. The diet predictor was organized relative to trophic level, encompassing differences in prey appearance, position in the water column and motility, with fishes consuming mostly: (1) planktonic or non-motile prey (e.g. larvae, coral, detritus and algae); (2) crustaceans and other small invertebrates; or (3) cephalopods and fish. Body color complexity was categorized by hue diversity over the body, assigned as either low (one hue), medium (two hue) or high (three or more hues). In cases of color-based sexual dimorphism, the more colorful phenotype was used (typically of males). Lastly, size was binned across observed fish size classes (Gust et al., 2001), with fish total lengths identified as either small (<10 cm), medium (10–19 cm) or large (≥20 cm).

Latent liability models of ecology and chromacy

Using the multivariate phylogenetic latent liability model described by Cybis et al. (2015), we examined the pairwise correlations among chromacy and all assigned ecological predictors. This model is an extension of Felsenstein's (2012) latent liability model, capable of handling discrete multistate data types, such as chromacy. The latent liability model is a Bayesian, multivariate phylogenetic model that assesses the correlation among traits, while simultaneously accounting for the uncertainty in their shared evolutionary history. The model was run on our sub-tree of species (from Rabosky et al., 2013), using BEAST v.1.8.0 (Drummond and Rambaut, 2007; Lemey et al., 2010; Drummond et al., 2012).

A total of four independent Markov-chain Monte Carlo (MCMC) simulations, or chains, were run for 2×10^8 steps, thinning every 10^4 steps, for a total of 2×10^4 samples from the posterior distribution per chain. Marginal likelihoods were also estimated in BEAST using a stepping stone method of 100 path steps run for 10^7 generations sampled every 1000th generation (Beale et al., 2012, 2013). The performance of each chain was assessed using TRACER v.1.7 (http://beast.community/tracer) to ensure that the effective sample size of each parameter was ≥200 and stationarity (convergence) was reached. We further examined for convergence of Σ using the R package BOA v.1.1.8 (Smith, 2007). We first converted each sample of Σ to a pairwise correlation matrix using the 'cov2cor' function in R v.3.4.3 and discarded the first 10% of samples as burn in – resulting in 18,000 posterior samples from each chain. Then, we ensured that each chain and its correlations independently converged using the corrected scale reduction factors (CSRFs; Gelman and Rubin, 1992; Brooks and Gelman, 1998). A chain is suggested to have converged when the CSRF is 1, or more specifically, when the 97.5% upper confidence limit of the estimate is ≤1.254. Furthermore, we also examined for convergence for all parameters across chains simultaneously using the multivariate potential scale reduction factor (MPSRF; Gelman and Rubin, 1992; Brooks and Gelman, 1998). The MPSRF converges to 1 upon reaching stationarity when the number of steps is reasonably large. After convergence was verified, all four chains were merged and each correlation tested independently for convergence using Geweke's statistic (Geweke, 1992) and corrected for multiple comparisons using the Bonferroni method. The posterior mean and 95% Bayesian confidence interval (BCI) were estimated for the correlations. Example code to run the multivariate phylogenetic latent liability model in BEAST v.1.8.0 (e.g. Drummond et al., 2012) can be found on Github (https://github.com/LorianSchweikert).

One notable challenge in latent liability modeling is an issue of limited power (Felsenstein, 2012; Cybis et al., 2015). Felsenstein (2012) argues that for moderately sized datasets, large confidence intervals hinder the detection of significantly non-zero BCI trait relationships. To contend with this issue, and to elucidate the effect of shared phylogenetic history on ecological correlations with fish chromacy, we repeated the analysis above, but replaced the tree with a single-node star phylogeny to recreate equal phylogenetic relationships among all taxa. The results from this model should be similar to those obtained by contingency table testing without accounting for phylogenetic signal. However, we were unable to perform these tests because the minimum expected cell count was often less than 5, violating assumptions of both the Chi-square and Fisher's exact tests in contingency table analyses (McHugh, 2013). Lastly, we compared the full latent liability model with that from the star phylogeny using the Bayes factor (eqn 14 from Cybis et al., 2015) calculated from the posterior mean log marginal likelihoods across chains. A Bayes factor ≥20 or ≥150 is considered strong or very strong evidence for a model relative to another model, respectively (Kass and Raftery, 1995).

RESULTS

Physiological and phylogenetic patterns of spectral sensitivity

Across the 213 species included in the analysis, cone λ_{max} values averaged 500±56 nm (mean±s.d.) and ranged between 347 and 625 nm (Fig. 1). Patterns of cone sensitivity across morphological types indicated that single cones on average have shorter-wavelength λ_{max} values (455±50 nm) and appear more variable than twin (529±27 nm), double (532±34 nm) or mixed cone types (532±39 nm; Fig. 1). Between species at each level of chromacy, however, cone λ_{max} averages were nearly identical, centering around 497 nm (Fig. 2 and Table S2). As for the distribution of chromacy among species, trichromacy (having three cone λ_{max} values) was the most common (n=86, 40%), followed by dichromacy (n=76, 36%), whereas monochromacy was relatively rare (n=10, 5%; Fig. 2). With the exception of monochromacy, the distribution of cone λ_{max} values across polychromats was multimodal, typically revealing a number of peaks that coincided

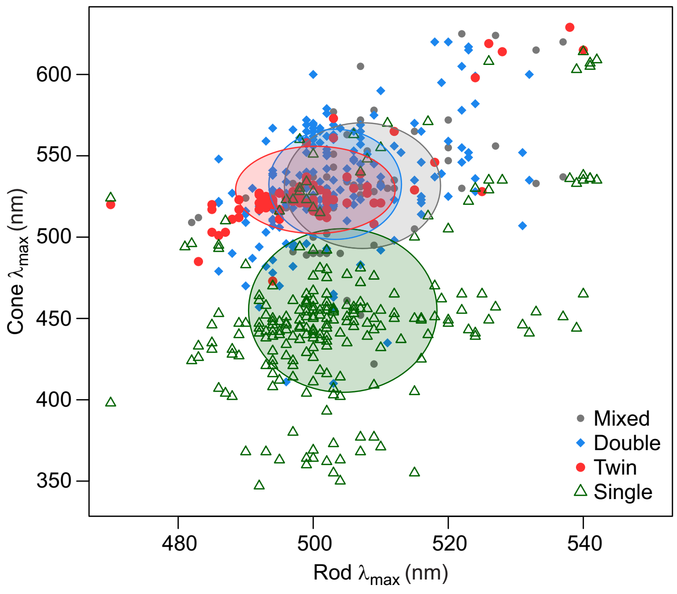

Fig. 1. Photoreceptor λ_{max} values across cone morphological types in ray-finned fishes. Cone photoreceptor λ_{max} values are plotted as a function of rod λ_{max} for 213 species. Cone type is indicated by symbol color and shape (see legend). The category of 'mixed' indicates λ_{max} values assigned to more than one cone morphological type. Shaded ellipses indicate one standard deviation from the mean.

with chromacy level (e.g. five peaks in the distribution of λ_{max} values for pentachromacy).

Estimation of phylogenetic signal indicated that chromacy significantly covaries with phylogenetic structure in ray-finned fishes (Pagel's lambda=0.946, *P*<0.001; Fig. 3). After accounting for this relatedness, mean differences in cone sensitivity range (nm) conferred by the different levels of chromacy were observed (*F*=53.6, *P*=0.001). Across increasing levels of chromacy, the mean range of sensitivity over light wavelengths appeared to follow a logarithmic trend (Fig. 4 and Table S2), significant improvement in range occurred between dichromacy (271±20 nm) and trichromacy (311±37 nm; *t*=9.94, *P*<0.01) and again at pentachromacy (363±46 nm; *t*=3.55; *P*<0.01; Fig. 4). The subsequent addition of peaks across chromacy levels appeared to improve short-wavelength sensitivity more than long-wavelength sensitivity, with the presence of ultraviolet λ_{max} values first occurring in trichromatic species.

Trait correlations in the phylogenetically corrected model

First, the convergence of the four chains and the parameters within those chains were assessed. The effective sample size for all model parameters was >200, indicating a sufficient number of independent samples from the posterior. The CSRF of all estimated correlation coefficients was ∼1 (upper 97.5% confidence limit <1.254; Table 1) and the MPSRF across all parameters of all four chains converged upon 1 (Table S3). No correlation coefficient significantly differed from a stationary distribution after correction for multiple testing (Geweke's statistic, *P*<0.05; Table S3). As a result, the four chains were merged and the resulting correlation coefficients were also consistent with stationarity (Geweke's statistic, *P*<0.05; Table S3). For the latent liability model run on the star phylogeny, again, the four chains showed evidence of convergence and were subsequently merged (Table 1, Table S3).

The relationships between fish chromacy and five hypothesized ecological predictors were examined (Fig. 5). Using the latent liability model (Cybis et al., 2015), pairwise correlation analyses for all combinations of the six traits of interest were performed (Table 1). In the model controlling for phylogenetic relatedness, only depth was significantly correlated with chromacy, having the only significantly non-zero BCI (posterior mean=−0.29, 95% BCI=−0.47 to −0.11; Table 1). In this model, the predictors of size, diet, habitat and body coloration were not significantly correlated to chromacy. Comparisons among the ecological predictors indicated five significant correlations, with the closest correlation occurring between size and diet (posterior mean=0.78, 95% BCI=0.67–0.88; Table 1). In fact, body size had the greatest number of ecological correlations. Aside from diet, fish size also correlated with habitat type (posterior mean=0.32, 95% BCI=0.11–0.53), as well as depth (posterior mean=0.31, 95% BCI=0.10–0.5; Table 1).

Trait correlations in the phylogenetically uncorrected model

To determine the effect of shared descent on the output of the latent liability model and to further assess phylogenetic signal among trait relationships, we repeated the model without phylogenetic signal (i.e. a tree with all taxa derived from a single polytomy) and compared it with the corrected model using a Bayes factor analysis. In contrast to the model accounting for phylogeny, all predictors except habitat significantly correlated with chromacy (Table 1). Like depth, body size was negatively correlated with chromacy (posterior mean=−0.25, 95% BCI=−0.4 to −0.09), as was diet (posterior mean=−0.21, 95% BCI=−0.36 to −0.05; Table 1). Body coloration, however, positively correlated with chromacy (posterior mean=0.31, 95% BCI=0.10–0.5; Table 1), indicating that more colorful fishes have higher levels of chromacy.

In this model, all pairwise correlations of the ecological predictors were found to be different from zero, except for the relationships between body coloration and diet, and between coloration and habitat (Table 1). Lastly, when comparing the marginal likelihoods (Table S4) between both models, the Bayes factor was 345.6, which is consistent with very strong evidence in favor of a model accounting for phylogeny.

DISCUSSION

The phylogenetic and ecological diversity of actinopterygian fishes provides an excellent system in which to identify the factors that

Table 1. Pairwise posterior mean correlation coefficients from the full model (lower triangle) and the model from the star phylogeny (upper triangle)

	Chromacy	Size	Diet	Color	Depth	Habitat
Chromacy	**1**	**−0.25** (−0.40 to −0.09)	**−0.21** (−0.36 to −0.05)	**0.22** (0.06–0.38)	**−0.35** (−0.50 to −0.21)	−0.08 (−0.23 to 0.08)
Size	−0.14 (−0.34 to 0.06)	**1**	**0.78** (0.69–0.86)	**−0.26** (−0.43 to −0.09)	**0.36** (0.20–0.51)	**0.43** (0.28–0.57)
Diet	−0.11 (−0.30 to 0.09)	**0.78 (0.67–0.88)**	**1**	−0.16 (−0.32 to 0.01)	**0.36** (0.21–0.51)	**0.30** (0.15–0.46)
Color	0.17 (−0.03 to 0.37)	−0.16 (−0.38 to 0.06)	0.01 (−0.21 to 0.22)	**1**	**−0.34** (−0.51 to −0.18)	0.03 (−0.15 to 0.20)
Depth	**−0.29** (−0.47 to −0.11)	**0.31** (0.10–0.50)	**0.28** (0.08–0.47)	−0.19 (−0.39 to 0.03)	**1**	**0.31** (0.15–0.47)
Habitat	−0.11 (−0.33 to 0.13)	**0.32** (0.11–0.53)	**0.23** (0.01–0.44)	−0.03 (−0.25 to 0.22)	0.41 (0.18–0.64)	**1**

The posterior 95% highest probability density intervals are shown in brackets. Significantly non-zero correlations are shown in bold.

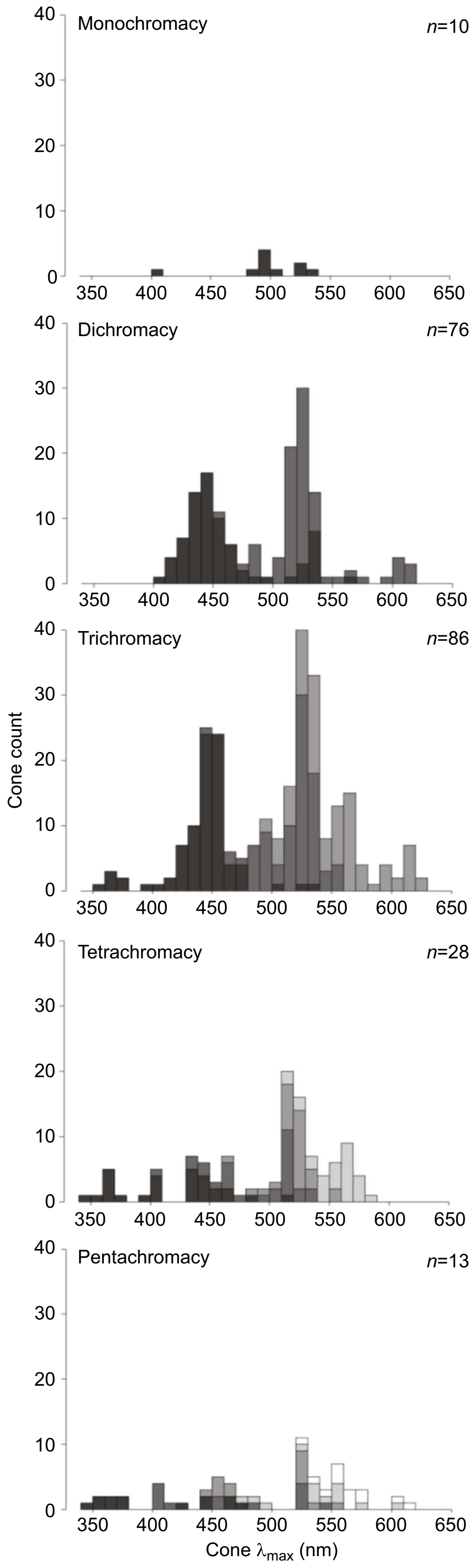

Fig. 2. Cone λ_{max} distribution across each level of chromacy. All cone λ_{max} values, for every species, across each level of chromacy are represented. Grayscale shading of bars indicates the order of cone λ_{max} within a chromacy level. For example, the single λ_{max} values of monochromacy are shown in dark gray. By comparison, the shortest wavelength λ_{max} values of pentachromacy are shown in dark gray, while the long wavelength λ_{max} values are shown in white.

underlie variation in spectral sensitivity. Over the past several decades, λ_{max} and chromacy estimates have been cataloged for hundreds of fish species, which we compiled into the largest meta-analysis of spectral sensitivity to date. Across the fishes sampled, our findings indicated that trichromacy is most common, ultraviolet λ_{max} values are exclusive to trichromatic species or those with higher chromacy levels, and increasing chromacy, including from tetra- to pentachromacy, significantly increases spectral sensitivity range. Moreover, this work demonstrated that shared evolutionary history is an important factor in fish chromacy and that several variables (excluding habitat type) may underlie chromacy variation, but depth and chromacy is the only relationship robust to the correction of shared descent.

Patterns of spectral sensitivity in ray-finned fishes

The mean cone λ_{max} values calculated here – within each level of chromacy, across all species together and in relation to the mean λ_{max} of rods (503 nm) – were nearly identical at ~500 nm, tuning to blue-green light. This unifying trend in fish spectral sensitivity likely reflects a selective advantage to blue-green sensitivity, as light of these wavelengths is maximally transmitted by oceanic water (Jerlov, 1976). In other vertebrates, photoreceptor λ_{max} values at least of rods also center around 500 nm (Munz and McFarland, 1973); together suggesting that blue-green sensitivity was perhaps the ancestral state of an early vertebrate opsin (Bowmaker, 1998) and that λ_{max} values have subsequently diverged over time.

Examining λ_{max} values across morphological types of cones confirmed a pattern previously observed in other studies, namely that single cones, on average, have shorter wavelength sensitivity than either double or twin cone types (Loew and Lythgoe, 1978; Lythgoe et al., 1994). These differences in sensitivity are predicted, at least for some species, to aid spectral discrimination in varied visual scenarios in the underwater light field (Lythgoe et al., 1994). That is, having both short- and long-wavelength λ_{max} values in a given retina simultaneously permits sensitivity to color of the background light field, as well as to visual targets with offset coloration. Similar arguments have been made for the utility of different levels of chromacy. For example, trichromacy has been argued by Lythgoe (1979) to underlie the most efficient spectral discrimination in underwater vision, as it permits one λ_{max} value to match the background spectral environment, while the others are shifted to longer and shorter wavelengths. Here, trichromacy was the most common level among the fishes sampled, standing in contrast to the monochromatic vision typical of marine mammals (Meredith et al., 2013) and dichromatic vision of most other marine fauna (Marshall et al., 2015).

Among the fishes sampled, trichromacy was not only most common, but represented a threshold for the presence of ultraviolet photoreceptor sensitivity. As also observed in previous studies (Losey et al., 1999), λ_{max} values tuned to ultraviolet light ($\leq$400 nm) appeared specific to fishes with at least trichromatic vision. Aside from increasing the dimensionality of spectral sensitivity, ultraviolet sensitivity is thought to aid detection of prey in planktivory (Losey et al., 1999; Utne-Palm, 2002) and potentially, serve in mate choice

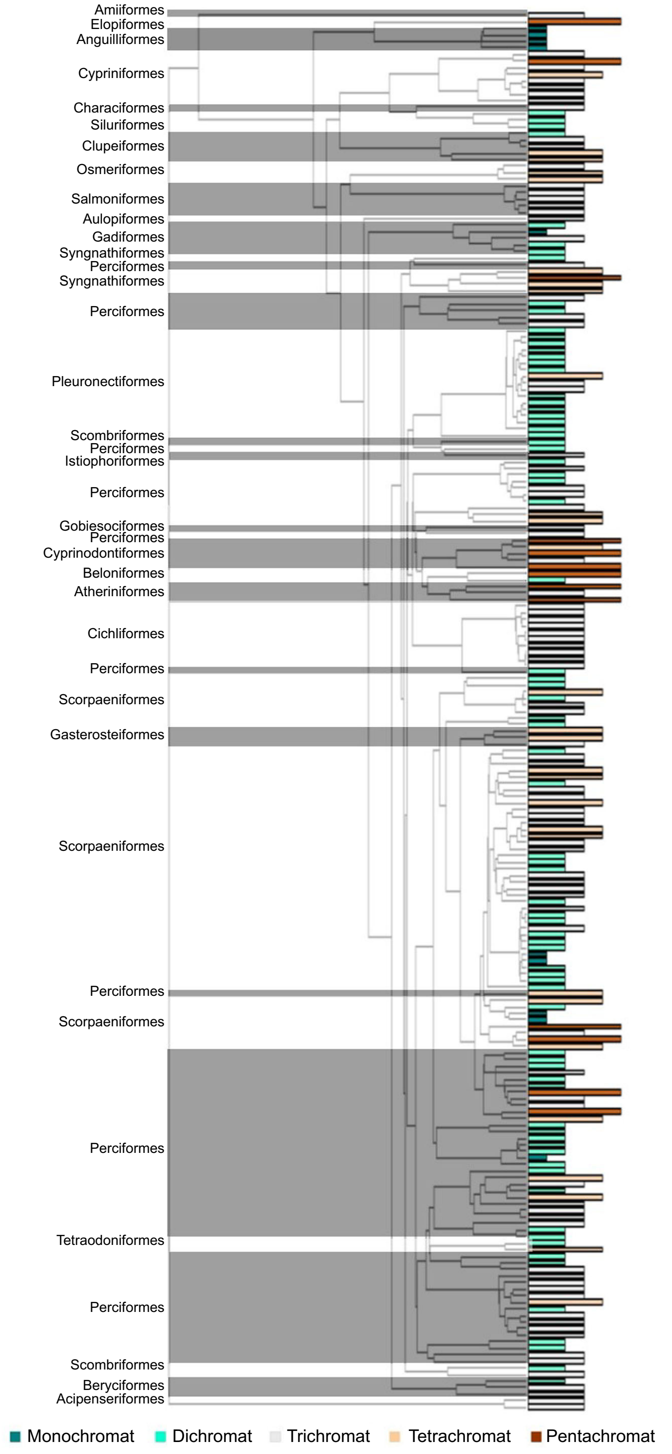

Fig. 3. Levels of chromacy across a subset of the actinopterygian. Chromacy is indicated by bar length and color (see key). Fish orders are demarcated by alternating white and gray shading. Original phylogeny from Rabosky et al. (2013).

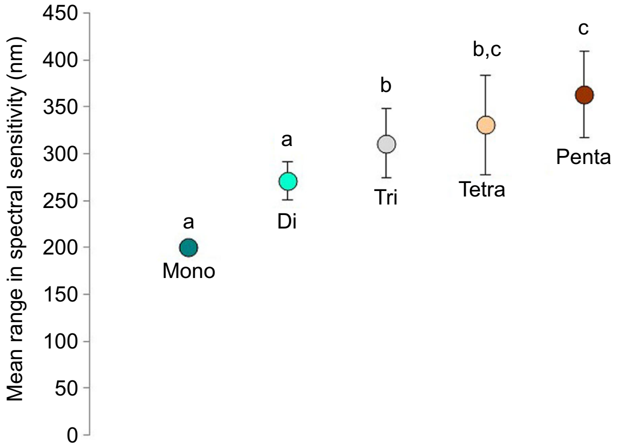

Fig. 4. Spectral sensitivity across each level of chromacy. The mean range (nm) between the most extreme cone λ_{max} values for each level of chromacy is given. Raw values are increased by 200 nm and plotted here to reflect the full wavelength range of photoreceptor sensitivity (i.e. the addition and subtraction of 100 nm at each extreme end). As such, the single λ_{max} value of monochromats result in a mean range of 200 nm without variation. Chromacy is indicated by abbreviations, values are means±s.d. and letters indicate statistical significance (α=0.01).

and social signaling (Losey et al., 1999; Garcia and de Perera, 2002). These predictions are supported by trends in the present work, as species that feed on planktonic (or non-motile) prey had proportionally higher levels of chromacy (Fig. 5). In these species, ultraviolet transmission through the cornea and lens makes the retina vulnerable to photo-oxidative damage (Siebeck and Marshall, 2001). Determining how these species contend with this potential cost of ultraviolet spectral sensitivity would be an interesting area of study. In addition, why mono- and dichromatic fish species, over a range of ecologies, lack ultraviolet photoreceptor λ_{max} remains unclear.

In general, higher chromacy is thought to increase the wavelength range of sensitivity and improve spectral discrimination; however, physiological costs of additional cone classes, and perhaps marginal gains in visual performance (Vorobyev, 1997b; Osorio and Vorobyev, 2008), make the advantage of the highest known vertebrate chromacy levels unclear. Specifically, the costs of chromacy include the need for more sophisticated downstream neural processing (Attneave, 1954) and reduced spatial acuity of vision conferred by each cone class (Young, 1802; Williams et al., 1993; Osorio and Vorobyev, 2005). Improvements in spectral discrimination may offset these costs, but perhaps only until trichromacy, as several analyses of natural spectra have shown that just three appropriately tuned classes can recover almost all spectral information within natural scenes (Barlow, 1982; Maloney, 1986; Chiao et al., 2000). Although the adaptive significance of high chromacy levels remains unknown, approximately one-fifth of the fish species examined here were either tetra- or pentachromatic. These additional cone classes are likely maintained by selection, rather than by neutral drift, because photoreceptors and their downstream neural circuits are metabolically costly to sustain (Niven and Laughlin, 2008).

As an alternative to improved spectral discrimination, additional cone classes may serve in the identification of colored objects in conditions of changing illumination (color constancy). A model developed by Vorobyev (1997b) suggested that higher chromacy is only advantageous to color constancy when additional λ_{max} values increase the range of wavelength sensitivity. Congruently in fishes, we found that significant increases in range occurred both at trichromacy and pentachromacy, providing support for broader spectral sensitivity even at an upper-most limit of vertebrate chromacy. Therefore, we must consider that the diverse spectral sensitivities observed among fishes may not only serve tasks of spectral discrimination, but may enable color-constant vision in the variable lighting conditions that occur in aquatic environments.

Ecology and evolution in fish chromacy

Recent works have advocated for sensory ecologists to better integrate evolutionary perspectives in their study of sensory variation (Dangles et al., 2009; Kemp et al., 2015). The incorporation of phylogenetic comparative methods has been encouraged, as phylogenetic relatedness may underlie patterns of trait variation that otherwise could be falsely interpreted as ecological adaptation (Northcutt, 1988; Chittka and Briscoe, 2001). Here, owing to recent advances in phylogenetic reconstruction and increased availability of spectral sensitivity data, we were able to correct for phylogenetic signal in our analyses. To our knowledge, the latent liability model described by Cybis and colleagues (2015) is the only phylogenetic comparative method capable of handling discrete multistate data (such as chromacy), although unfortunately, it is susceptible to issues of power, particularly when examining categorical traits.

In the model correcting for phylogenetic signal, the only ecological predictor to significantly correlate with chromacy was depth. As water and other ecological properties cause narrowing of the light spectrum in aquatic environments, changes in wavelength availability and/or light intensity over depth may be the greatest limiting factor to fish spectral sensitivity. Perhaps also true for other aspects of vision, past studies have demonstrated that depth largely predicts rod λ_{max} values among fishes (Munz and McFarland, 1973) and is an important factor in sighting distance performance in the pelagic environment (Nilsson et al., 2014).

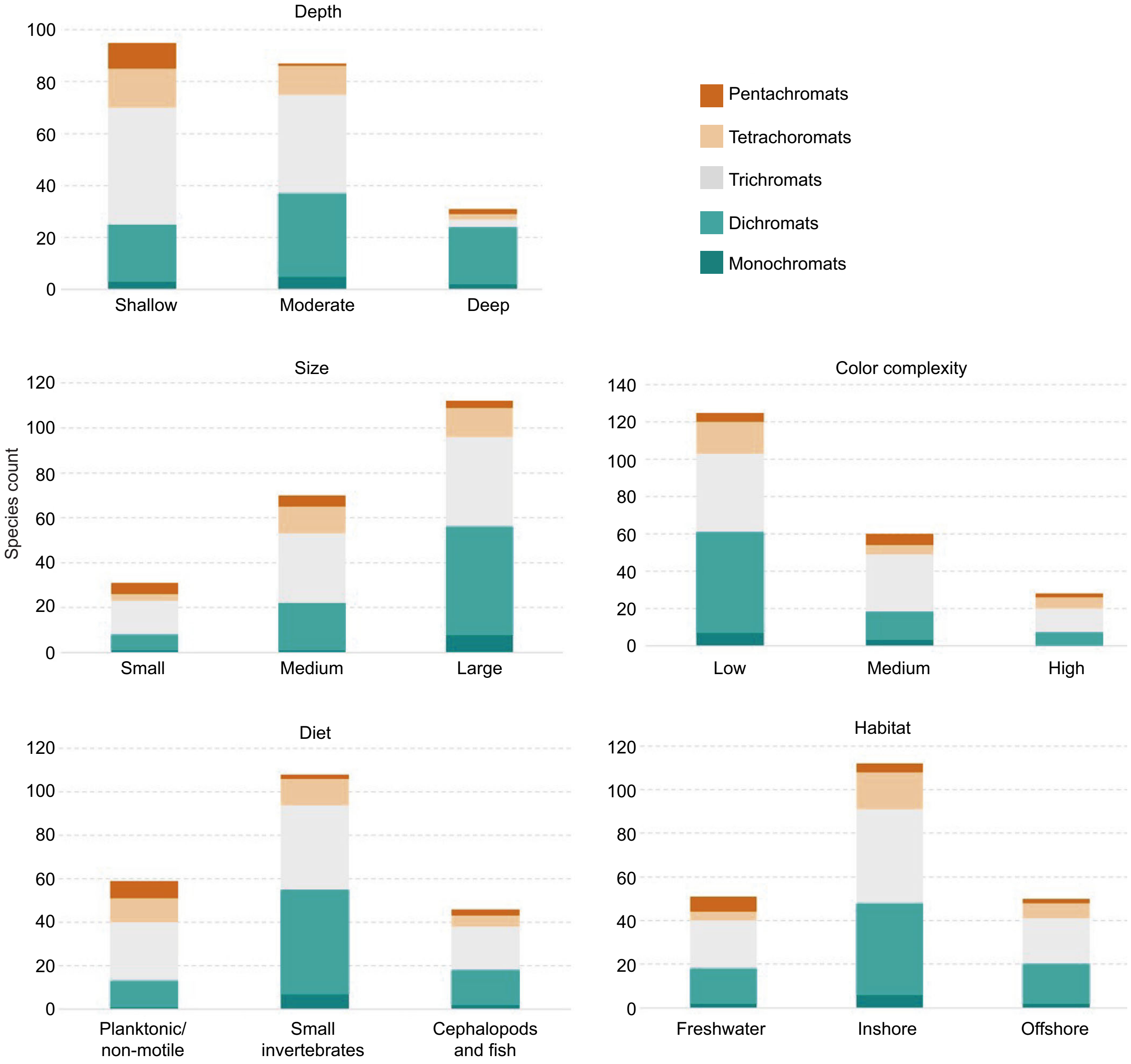

Fig. 5. Stacked bar plots of chromacy distribution by ecological category. Chromacy is indicated by color (see legend). *y*-axis shows the number of cones, across all 213 species that have λ_{max} in each wavelength sensitivity bin.

It is unlikely, however, that depth is the only valid ecological predictor of chromacy, as broad spectra in shallow waters do support, but not necessarily require, polychromatic vision in fishes. The ecology of fish size, diet and body coloration may be predictors of fish chromacy, which is supported by trends between these traits in the uncorrected model. However, the strong covariation of these traits with phylogenetic structure makes their relationship to chromacy indistinguishable from variation that may occur simply from shared descent (Gould and Lewontin, 1979; Northcutt, 1988; Chittka and Briscoe, 2001; Dangles et al., 2009).

Here, a strong phylogenetic signal was evidenced in chromacy variation both by a high Pagel's lambda value and the Bayes factor support for the phylogenetically corrected model. Not only can a strong phylogenetic signal obscure ecological relationships, but so can co-variation of ecological variables themselves. For example, in the uncorrected model, the close relationship of chromacy and fish size may in fact be driven by the relationship of chromacy and fish coloration. This significant correlation of size and coloration make it difficult to parse clear evolutionary relationships between these ecologies and fish chromacy. Taken together, depth and chromacy is the only relationship in this study robust to phylogenetic correction and free of confounds by ecological covariation. In the future, it is possible that additional robust ecological relationships with chromacy may be detected by increasing species sampling as this field continues to grow.

Although we examined a large number of species (*n*=213), this only represents a small fraction of known actinopterygian fishes (~27,000 spp.). Specifically, the majority of fishes examined were large, inshore, shallow species. Future sampling of fish spectral sensitivity should focus on species from a broader range of ecological conditions, as well as across underrepresented portions of the phylogeny, including the orders of Characiformes, Lophiiformes and Siluriformes. This limitation aside, this work represents the most comprehensive analysis of spectral sensitivity conducted to date, revealing phylogenetic, physiological and ecological patterns of spectral sensitivity across sampled fishes.

Conclusion

The ecological basis of cone spectral sensitivity variation in fishes has remained a central question in the field of sensory ecology. Patterns of spectral sensitivity previously observed across fish species have been attributed both to selective forces due to ecology (Munz and McFarland, 1973; Lythgoe, 1979; Bowmaker, 1990) and drift due to the process of descent (Wald, 1960; Bridges and Yoshikami, 1970; Cronin et al., 2014; Marshall et al., 2015). The results presented here lend credibility to both an ecological and evolutionary perspective, indicating that phyletic heritage is a major factor in chromacy variation among fishes and that depth has played an important role in shaping variation of this trait.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: L.E.S., S.J.; Methodology: L.E.S., R.R.F., E.M.C., S.J.; Validation: L.E.S., E.M.C., T.T.S., S.J.; Formal analysis: L.E.S., R.R.F.; Investigation: L.E.S.; Resources: L.E.S.; Data curation: L.E.S., R.R.F., E.M.C., T.T.S.; Writing - original draft: L.E.S.; Writing - review & editing: L.E.S., R.R.F., E.M.C., T.T.S., S.J.; Visualization: L.E.S., E.M.C.; Supervision: L.E.S., S.J.; Project administration: S.J.; Funding acquisition: L.E.S.

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Supplementary information

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Table S1. Meta-table of spectral sensitivity data and ecological assignments of adult ray-finned fishes. Bolded λ_{max} values have appeared at least twice across different cone morphological types for a given species. † designations indicate λ_{max} values for which cone morphological type had not been identified. § indicate λ_{max} values that are associated with a rare morphological 'triple' cone type. ‡ indicate species whose synonymized names are listed in the published phylogeny (Rabosky, 2013). Species listed without ecological assignments had adult spectral sensitivity data available, but were not present in the phylogeny. See supplemental .xls file.

Click here to Download Table S1

Table S2. Cone λ_{max} (nm) averages across morphological types of cones and levels of chromacy for the 213 species included in analysis. In cases where λ_{max} values were associated with two or more morphological types in a given retina, cone λ_{max} were assigned as "mixed."

Cone Morphological Type	Mean Cone λ_{max} (nm) ± s.d.
Single	455 ± 50
Double	532 ± 34
Twin	529 ± 27
Mixed	532 ± 39
Chromacy Level	
Monochromacy	496 ± 37
Dichromacy	494 ± 49
Trichromacy	505 ± 54
Tetrachromacy	496 ± 59
Pentachromacy	496 ± 70

Table S3. Summary of the MCMC chains from the multivariate phylogenetic latent liability models produced in BEAST.

Chain	**Phylogeny**	**Steps ($x10^6$)**	**Samples**	**Samples (Post burn-in)**	**MPSRF**	**Log Marginal Likelihood**
1	normal	200	20000	18000	1.010798	-2981.03
2	normal	200	20000	18000		-3224.91
3	normal	200	20000	18000		-3125.70
4	normal	200	20000	18000		-3100.92
merged	normal	800	80000	72000	N/A	-2957.76
5	star	200	20000	18000	1.001739	-3546.85
6	star	200	20000	18000		-3563.12
7	star	200	20000	18000		-3588.41
8	star	200	20000	18000		-3294.80
merged	star	800	80000	72000	N/A	-3303.40

Table S4. Pairwise Bayes factors analysis of model likelihood.

	Chain 1	**Chain 2**	**Chain 3**	**Chain 4**	**Merged (normal)**
Chain 5	565.8	321.9	421.2	445.9	-
Chain 6	582.1	338.2	437.4	462.2	-
Chain 7	607.4	363.5	462.7	487.5	-
Chain 8	313.8	69.9	169.1	193.9	-
Merged (star)	-	-	-	-	345.6