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The Evolution of Color Pattern in Butterflyfishes (Chaetodontidae)

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Synopsis Coral reef fishes constitute one of the most diverse assemblages of vertebrates on the planet. Color patterns are known to serve a number of functions including intra- and inter-specific signaling, camouflage, mimicry, and defense. However, the relative importance of these and other factors in shaping color pattern evolution is poorly understood. Here we conduct a comparative phylogenetic analysis of color pattern evolution in the butterflyfishes (Chaetodontidae). Using recently developed tools for quantifying color pattern geometry as well as machine learning approaches, we investigate the tempo of evolution of color pattern elements and test whether ecological variables relating to defense, depth, and social behavior predict color pattern evolution. Butterflyfishes exhibit high diversity in measures of chromatic conspicuousness and the degrees of fine versus gross scale color patterning. Surprisingly, most diversity in color pattern was not predicted by any of the measures of ecology in our study, although we did find a significant but weak relationship between the level of fine scale patterning and some aspects of defensive morphology. We find that the tempo of color pattern diversification in butterflyfishes has increased toward the present and suggest that rapid evolution, presumably in response to evolutionary pressures surrounding speciation and lineage divergence, has effectively decoupled color pattern geometry from some aspects of ecology. Machine learning classification of color pattern appears to rely on a set of features that are weakly correlated with current color pattern geometry descriptors, but that may be better suited for the detection of discrete components of color pattern. A key challenge for future studies lies in determining whether rapid evolution has generally decoupled color patterns from ecology, or whether convergence in function produces convergence in color pattern at phylogenetic scales.

Introduction

Reef fishes constitute one of the most colorful assemblages of vertebrates on the planet. This astonishing biodiversity of color and pattern is associated with diversification in visual systems, signaling, development, and ecology of reef fish lineages (Marshall 2000; Losey et al. 2003; Marshall et al. 2003; Cheney et al. 2009; Salis et al. 2018). For these reasons, reef fishes have been identified as a key group for understanding the evolution of color pattern diversity (Salis et al. 2019). Key color pattern elements, including, bars, stripes, and false eyespots, are thought to play roles in signaling and defensive behavior (Barlow 1972; Neudecker 1989; Domeier and Colin 1997; Marshall 2000; Randall 2005). Color pattern divergence is also important for speciational processes (Salis et al. 2018; Hemingson et al. 2019) and has been suggested to be more important than diversification along ecological axes for the recent macroevolution of the reef fish fauna (Bellwood et al. 2015, 2017). However, despite the opportunities afforded by the repeated acquisition of similar patterns and the increasing availability of phylogenetic frameworks for marine fishes (Rabosky et al. 2018), few phylogenetic comparative studies have been applied to reef fish color evolution. As a result, we have a poor understanding of the relative importance of hypothesized drivers of color pattern diversity.

Here we conduct a comparative analysis of color pattern evolution across butterflyfishes (Chaetodontidae). We focused on the butterflyfishes as visual communication is known to play an important role in their evolution and divergence. Members of this group typically exhibit strongly contrasting colors including yellows,

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blacks, and whites, and ornate color patterns hypothesized to be tied to different selective pressures, including predation and sexual selection (Neudecker 1989; McMillan et al. 1999; Stevens 2007; Kjernsmo and Merilaita 2013; Lönnstedt et al. 2013). Butterflyfishes exhibit conspicuous interspecific variation in color pattern that exceeds intraspecific variation (Hemingson et al. 2019), making this family ideal for cross-species comparisons. Prior comparative studies of color pattern evolution in butterflyfishes have revealed that although body striping is associated with ecology, habitat, and social behavior, many other aspects of pattern evolution, including eye stripes, and false eye spots, are highly evolutionarily labile (Kelley et al. 2013; Hemingson et al. 2019). We sought to identify whether recently developed tools for quantifying geometry and similarity could help reveal the tempo of color pattern evolution as well as the importance of ecology in shaping functional features of color pattern diversity.

Methods

Image sources

High quality images of fishes were aggregated from Internet databases, with the majority of the images in this dataset acquired through a database of J.E. Randall's images accessible through the Bishop Museum (http://pbs.bishopmuseum.org/images/JER/). Images from fishbase.org and the FishWise Professional Database were also used. Butterflyfishes are sexually monomorphic. We selected images of adults and excluded uncommon color morphs, such as melanistic individuals for a total of 116 species (Supplementary Table S1). We standardized images using a custom-written interface that removed the background (source code available at https://github.com/ShawnTylerSchwartz/FishBGRemoval_Interface) and by orienting images so that the fish was in left lateral view, parallel to the horizontal axis (Supplementary Fig. S1).

Color pattern analysis

We characterized color pattern geometry following approaches developed by Endler and colleagues (Endler 2012; Endler et al. 2018). The first step in this pipeline is to classify colors present in an image into k categories. The dominant colors across butterflyfishes are yellows, blacks, and whites (Marshall et al. 2003; Hemingson et al. 2019) and we set $k=4$ to capture this variation and while accommodating some of the diversity of more complex color patterns. Following classification, we subsampled the images using a 100×100 pixel grid. Color geometry statistics are based upon the colors of the sampled pixels, the frequency of color transitions to adjacently sampled pixels, and the color distance between adjacent pixels (Endler 2012; Endler et al. 2018). We calculated the overall transition density (m), the aspect ratio (ratio of row-wise to column-wise transitions, A), the scaled Simpson color class diversity (Jc), the scaled Simpson transition diversity (Jt), and the mean chromatic and achromatic boundary strength (m_dS and m_dL) using "pavo" (Version 2.0.0; Maia et al. 2019).

We use RGB values to calculate color distances to capture aspects of the overall color pattern. For each color region, we calculated two color measurements $(R - G)/(R+G)$ and $(G - B)/(G+B)$ and one measurement of luminance $(R+G+B)$ (Endler 2012). We calculated color distances between regions by taking the Euclidean distance between the two color measurements (dS), and separately calculated the luminance distance by taking the Euclidean distance between the luminance measurements for each region (dL). Ideally, boundary strength analyses would be calculated from color distances calculated by the photoreceptor outputs of the appropriate observer (Endler 2012). These distances can be calculated by obtaining data on reflectance per wavelength for each color region and applying a visual model, either by using a reflectance spectrometer or a color and luminance calibrated digital photograph (Endler 2012). Unfortunately, we could not perform these calculations in this way for two reasons. First, our data were not collected with a calibrated camera, so we could not obtain objective calculations of reflectance per wavelength. Second, while some species from this family have had their visual systems described with three cones (two based in the short wavelengths and one in the yellow/green spectrum; Losey et al. 2003; Marshall 2017), these data do not exist for all species and may vary. Furthermore, the evolution of these color patterns may be the product of selection from both conspecifics and other organisms with different visual systems (e.g. predators). Although the image data used in our study does not necessarily incorporate information about the visual system of a particular aquatic viewer, we argue that they describe empirical aspects of the color patterns.

Comparative analyses

We sought to test evolutionary hypotheses related to the tempo of color pattern evolution and ecological drivers of these patterns. To conduct comparative analyses, we used a previously published time-calibrated phylogeny (Cowman and Bellwood 2011) and data on the social behavior and defensive morphology (Hodge et al. 2018). To reduce the dimensionality of our color geometry dataset, we conducted a phylogenetic principal component analysis (PCA) (Revell 2009) on the log transformed color pattern variables (*m*, *A*, *Jc*, *Jt*, *m_dS*, *m_dL*) using the "phytools" package in R (Revell 2012). We used Hodge et al. (2018) defensive trait measure data and followed their procedures for size correction and principal components analysis. We coded species social behavior (social or solitary) and foraging strategy (benthic hunting, facultative hunting and grazing, obligate coral grazing, and pelagic hunters) following Hodge et al. (2018).

The tempo of color pattern evolution

Butterflyfishes have been shown to exhibit rapid divergence in color pattern between closely related species (Hemingson et al. 2019). To examine how different components of color pattern have evolved, we calculated Blomberg's *K* for PCs 1–3. Blomberg's *K* describes the variance in evolution of a trait across a clade relative to expectations under Brownian motion (BM). *K* values that are near 1 indicate that variation amongst individuals is similar to expectations under Brownian evolution while values <1 indicate that closely related individuals are more variable than expected under BM. We also calculated the mean disparity through time (DTT) and the morphological disparity index (MDI) statistic (Harmon et al. 2003; Slater et al. 2010) to test whether butterflyfishes show greater than expected disparity within subclades. DTT measures the average diversity within subclades compared to the diversity expected under BM. The MDI (Slater et al. 2010) uses simulation to test whether observed patterns of DTT differ from Brownian expectations. A significant MDI statistic means that that observed subclade diversity is unlikely to have been produced by Brownian evolution. We calculated DTT using phylogenetic PCs 1–3 of the color geometry variables using the pruned Cowman et al. tree (Cowman and Bellwood 2011). We performed the MDI test using 1000 simulated trees. To avoid biases that can be created by incomplete taxonomic sampling, we excluded the last 5% of the tree in calculating the MDI statistic. Finally, we tested whether color pattern evolution is more rapid at the tips of the tree using the node heights test (Freckleton and Harvey 2006) for PCs 1–3. The node heights test examines the absolute magnitude of independent contrasts in a trait as a function of the "height" or distance of the contrast from the root of the tree. A significant node heights test indicates that the rate of evolution has increased toward the present.

Ecological and social drivers of color pattern evolution

We tested the fit of BM versus Ornstein–Uhlenbeck (OU) models, using the "geiger" package in R (Pennell et al. 2014), to determine which model would be the best fit for our comparative analyses. To test the relationship between pattern geometry and anti-predator defenses, we ran a Phylogenetic Generalized Least Squares (PGLS) regression on PCs 1–3 of the defensive morphology traits against the color pattern PCs 1–3 using the "nmle" package in R (Pinheiro et al. 2018). Additionally, we tested PCs 1–3 of the color pattern variables against average depth. Average depth (Supplementary Table S2) was calculated from the reported minimum and maximum depth for each species in fishbase.org (Froese and Pauly 2019) via the "rfishbase" package in R (Boettiger et al. 2012). To evaluate the contribution of ecology and social behavior on pattern geometry, we ran A PGLS regression on PCs 1–3 of the color pattern variables against categorical assignments of foraging strategy and social behavior, both coded following Hodge et al. (2018). We also ran PGLS regressions of color pattern variable PCs 1–3 against depth with interactions of foraging strategy and social behavior.

Machine learning

Coral reef fishes exhibit startling diversity in their color patterns. When patterns are simple, such as solid blocks of color or repeated stripes along the body, similarity can be readily identified. However, the sheer number of permutations of hue and arrangement of color patches present in even a relatively small family like butterflyfishes defies easy categorization. Machine learning has been shown to outperform human beings in identifying pattern categories under some conditions (Pang et al. 2002) and we explored the utility of machine learning in classifying fish color pattern as part of this study.

Currently, the best image processing algorithms are convolutional neural networks (CNNs), deep learning networks modeled after the neural connectivity in the animal visual cortex that can handle high levels of complexity (Krizhevsky et al. 2012). Some of these networks have shown to be equivalent or superior to the performance of human experts (Cireşan et al. 2012). We utilized the VGG-16 CNN (Simonyan and Zisserman 2014), which consists of a uniform architecture of 16 convolutional layers and allows for feature extraction from images. Through the implementation of an unsupervised machine learning algorithm with the VGG-16 pre-weighted CNN using the Keras API (Version 2.2.4) with a TensorFlow backend (Version 1.11.0) written in Python (Version 3.6.5), we extracted 1000 feature layer activation proportions per image. We then used this matrix of fish images and feature layers to perform hierarchical agglomerative (bottom-up) clustering through an unsupervised machine learning algorithm (Pedregosa et al. 2011) with Euclidean distance and ward linkage.

We performed unsupervised machine learning on the image feature activation matrix for the background-removed image sets. We partitioned these data into a range of 2–20 clusters for the hierarchical agglomerative clustering algorithm, and measured the classifiers' Caliński and Harabasz (Variance Ratio Criterion) score (Caliński and Harabasz 1974), for each of the 2–20 hierarchical clusters. This metric allowed us to both quantify the performance of the machine learning algorithm, as well as to aid us in deciding on the optimal number of clusters for our dataset. This was necessary since an unsupervised machine learning algorithm does not have associated truth labels, as compared to a supervised algorithm which can assess its performance accuracy by comparing the classification labels to the truth labels. The Caliński and Harabasz score for each of the 2–20 clusters allowed us to understand the within-cluster and between-cluster dispersion ratio (Caliński and Harabasz 1974), which helped us decide on the number of clusters to visualize our dataset. Finally, we orthogonally reduced the 1000 feature layer activations of each fish image from the VGG-16 CNN to two-dimensions through a PCA. This provided us with a two-coordinate system to visualize the relative distances in image feature similarity and relatedness between the Chaetodontidae images and their assigned cluster. We then regressed PCs 1 and 2 from the machine learning against the color pattern geometry variables to compare the machine learning clustering to features identified by analysis of color pattern geometry.

Results

Color pattern geometry

Species with high transition density (m) tended to possess fine patterning at the level of individual scales such as *Chaetodon reticulatus* (Supplementary Table S3) while low transition density species tended to possess color patterns dominated by a single or few large body patches. As expected, high aspect ratio (A) species typically exhibited prominent vertical stripes while low aspect ratio species had horizontal stripes. Species with high transition diversity (Jt) tended to show significant texture and fine patterning around individual scales, while low Jt species did not. High color diversity (Jc) species also tended to have color patches distributed widely across the body while low Jc species were characterized by thick, two tone vertical stripes. Chaetodontids varied widely in their chromatic boundary strength (Supplementary Table S4) with high m_dS species showing conspicuous patches of black against yellows and whites and low m_dS species tending toward monochromatic with few large patches of contrasting colors. Achromatic boundary strength was less variable (Supplementary Table S4). Chromatic and achromatic boundary strengths were significantly but weakly correlated ($R^2 = 0.1914$, $F = 20.82$, df $= 82$, $P < 0.0001$).

Phylogenetic PCA analysis

PCs 1–3 color pattern variables explained 90% of the cumulative variation in the dataset (Tables 1 and 2). Chromatic boundary strength (m_dS) exhibited an effectively perfect correlation with PC1 with achromatic boundary strength (m_dL) and transition density (m) also showing strong loadings. This axis ordered similarly colored taxa with high transitions between them to taxa with highly dissimilar colors and few transitions. Loadings on PC2 were dominated by m, Jc (color diversity), and m_dL. This axis ordered taxa with high frequency of color transitions and relatively even distribution of color to species with few color transitions and a dominant color. Transition diversity (Jt), aspect ratio (A), and m_dL loaded heavily on PC3. This axis ordered taxa from vertically striped species with most transitions between a small number of colors to horizontally striped species with a more even number of color transitions. Visualization of color pattern PC space is shown in Fig. 1.

Tempo of color pattern evolution

Blomberg K values for PCs 1–3 were significantly lower than 1 (PC1 $k = 0.122$, $P = 0.005$; PC2 $k = 0.192$, $P = 0.001$; PC3 $k = 0.136$, $P = 0.006$) indicating that color geometry evolution in closely related species is faster than expected under BM. DTT plots for PCs 1–3 showed among clade diversity near the present that was higher than expected under BM (Fig. 2, DTT). These differences were significant for PC1 ($P = 0.0238$), PC2 ($P = 0.0476$), and PC3 ($P = 0.0476$). The node heights test similarly revealed a significant increase in the rate of evolution of color pattern traits toward the present (Fig. 3 node heights test), for PCs 1–3 (PC1 $P = 0.0066$; PC2 $P = 0.00163$, PC3 $P = 0.0001$).

Ecological and social drivers of color pattern evolution

All continuous traits tended to fit the OU model better than the BM model (Table 3). The PGLS analyses of PCs 1–3 of the defensive morphology traits against color pattern variables revealed a significant relationship between anti-predator morphology and some measures of pattern geometry (Tables 4–6). PC1 was not correlated with any of the variables tested. PC2 of color pattern variables was found to be negatively predicted by both PC1 (an axis strongly influenced by dorsal and anal fin spine length, Table 2) and PC3 (an axis strongly influenced by body depth), of antipredator morphology variables. PC2 of color pattern variables was also found to be predicted by foraging strategy for facultative hunting and grazing and obligate coral grazing (Table 6), as well as negatively associated with average depth (Tables 5). Color pattern geometry was not predicted by social behavior.

Table 1 Loadings, variance, and cumulative variance for phylogenetic PCA analysis of color pattern geometry

Descriptor	PC1	PC2	PC3
m	0.33	0.90	−0.13
A	0.16	−0.31	0.63
Jc	−0.02	0.44	0.06
Jt	−0.29	−0.33	−0.77
m_dS	−1.00	0.09	0.04
m_dL	−0.49	−0.47	−0.46
Variance explained	**0.68**	**0.16**	**0.06**
Cumulative variance	**0.68**	**0.83**	**0.90**

Table 2 Loadings, variance, and cumulative variance for phylogenetic PCA analysis of antipredator morphology

Descriptor	PC1	PC2	PC3
Anal fin spine length	0.49	−0.01	−0.33
Body depth	0.16	0.31	0.94
Caudal fin shape	−0.05	−0.09	0.21
Dorsal anal fin spine offset	0.47	0.85	−0.23
Dorsal fin spine length	0.93	−0.35	0.02
Eye diameter	0.31	−0.12	−0.01
Pelvic fin spine length	0.50	0.09	0.23
Maximum body size	0.09	−0.05	0.06
Variance explained	**0.44**	**0.29**	**0.21**
Cumulative variance	**0.44**	**0.73**	**0.94**

Machine learning

Our analysis identified $k = 4$ as the optimal number of clusters, as suggested by both the Caliński and Harabasz score (Caliński and Harabasz 1974) and feature dendrogram output from the hierarchical clustering algorithm (Pedregosa et al. 2011) (Supplementary Fig. S2). Within this feature space, Clusters 0 and 2 showed strong overlap with Cluster 3 along PC1 with Cluster 1 showing the greatest degree of separation (Fig. 4). With respect to color pattern geometry, machine learning PC1 was significantly correlated with transition density (m), color diversity (Jc), transition diversity (Jt), and chromatic (m_dS) and achromatic (m_dL) boundary strength, but not aspect ratio (Table 7). However, these correlations tended to be weak (R^2 = 0.7–0.8). Along machine learning PC2, all four clusters exhibited substantial overlap. PC2 was most significantly correlated with color pattern diversity and chromatic and achromatic boundary strength, but again the overall relationship was weak (R^2 = 0.06–0.08).

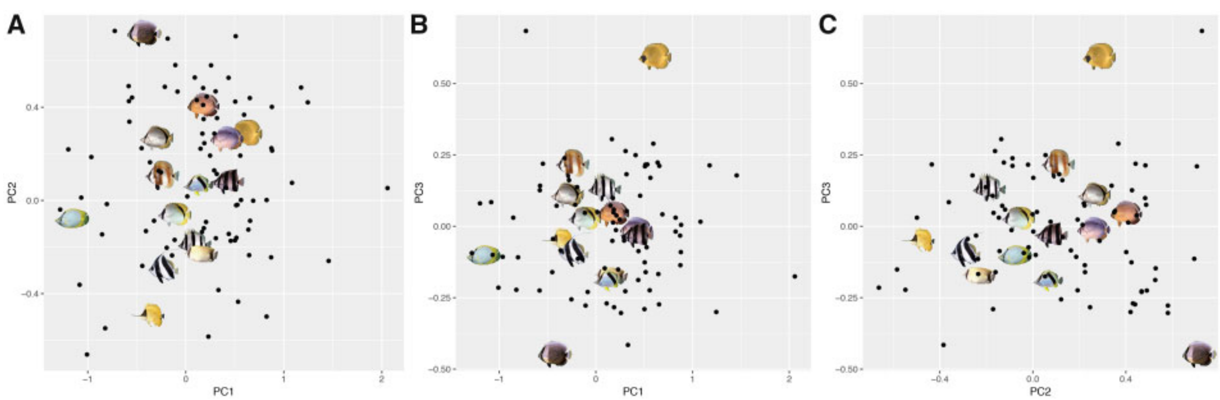

Fig. 1 Phylogenetic principal components analysis of color geometry descriptors. (**A**) PC1 versus PC2. (**B**) PC1 versus PC3. (**C**) PC2 versus PC3. Color patterns in PC space shown for a randomly chosen subset of species for visualization.

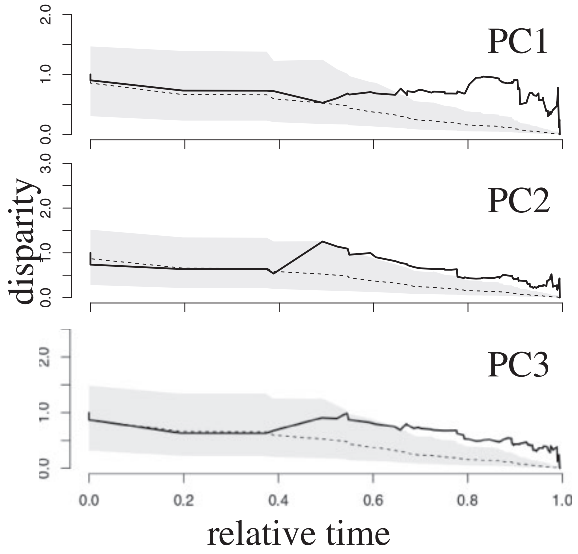

Fig. 2 DTT analysis of principal components 1–3. Subclade disparity for all three PCs was significantly higher than expected under BM indicating that clades tended to vary more in color pattern more within clades than across clades. Departure from Brownian expectation increased toward the present.

Discussion

Butterflyfishes exhibit high diversity in each of the measures of color pattern geometry considered in this study, and the tempo of diversification of these elements has increased toward the present. Species especially vary in their chromatic boundary strength which is one measure of pattern conspicuousness. Analysis of the tempo of color pattern evolution reveals a strong signal of increasingly rapid evolution toward the present for all aspects of color pattern geometry. Although color patterns are thought to play a role in defense and social behavior and the perception of pattern is strongly affected by available light, we found few aspects of defensive morphology, sociality, or depth that predicted patterns of color evolution. Machine learning based clustering identified four groups with similar features. Although the machine learning feature space was significantly correlated with other measures of color pattern geometry, these relationships were weak.

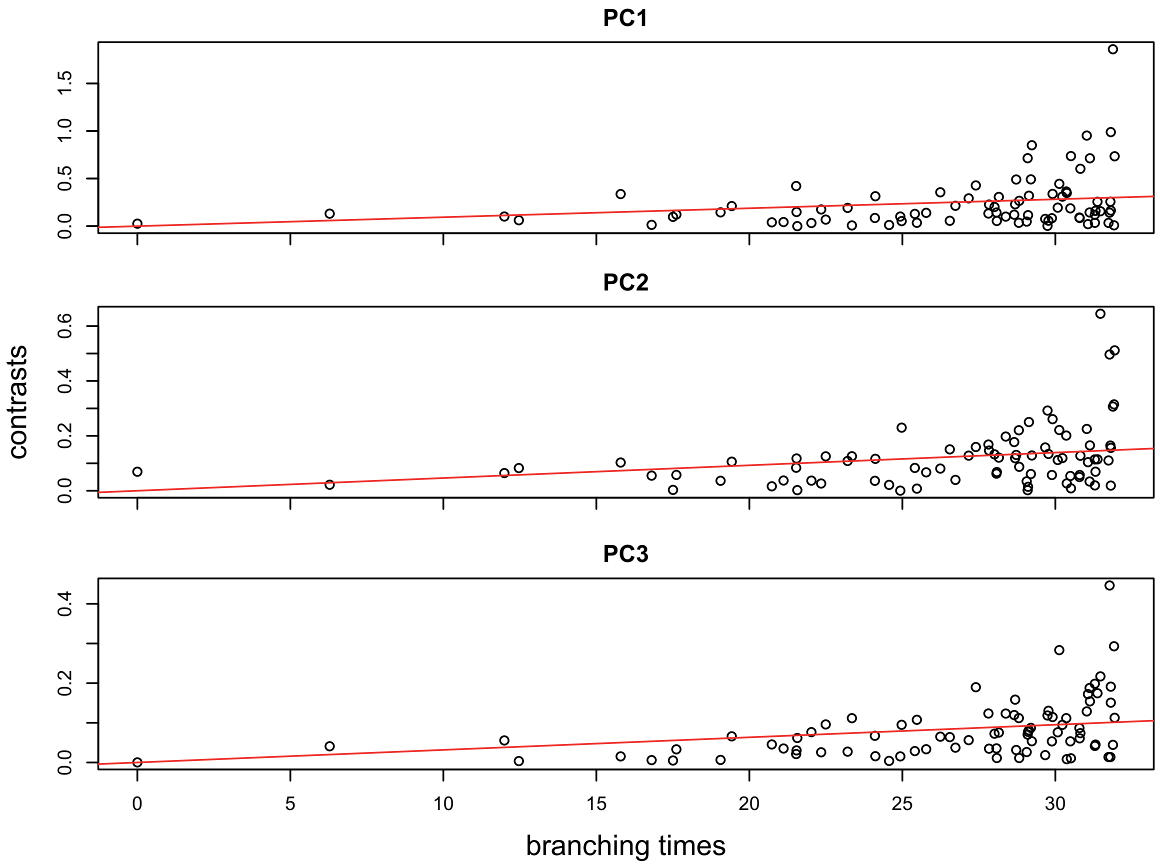

Fig. 3 Absolute value of standardized independent contrasts ordered by branching times for principal components 1–3. The slope of contrasts (red line) was significantly different than 0 for all three PCs indicating that rates of evolution in color pattern have tended to increase toward the present.

The tempo of color pattern evolution

Our comparative analyses considered diverse aspects of color pattern including the total diversity of colors, the frequency of transitions to other colors, the general orientation of color patches, and the expected chromatic and achromatic conspicuousness of the overall pattern. We found that at the shallowest time scales, divergences in these pattern elements were rapid. Diversity within young subclades tends to be high and tends to capture a large fraction of the total diversity present within the family (Fig. 2) and tempo of diversification of all pattern elements shows a strong trend of increasing toward the present (Fig. 3). Although analyses of pairs of sister species reveal that time of divergence is a poor predictor of color pattern diversity (Hemingson et al. 2019), our results show that time-dependence of rates emerges with a deeper phylogenetic perspective. This increase in the tempo of color pattern diversification may help explain the pattern of relationships observed between ecology and color pattern geometry (see below).

Table 3 Model fits of PCs 1–3 of color pattern geometry and average depth for comparative analyses (91 species)

Trait	Model	LnL	AIC	Δ AIC
PC1	BM	−113.45	230.89	54.02
	OU	**−85.43**	**176.87**	**0.00**
PC2	BM	−37.62	79.24	36.97
	OU	**−18.14**	**42.27**	**0.00**
PC3	BM	4.40	−4.80	37.42
	OU	**24.11**	**−42.22**	**0.00**
Average depth	BM	−478.36	960.72	65.37
	OU	**−444.68**	**895.35**	**0.00**

For each model, we report the log-likelihood (LnL), Akaike information criterion (AIC), and the model's mean AIC minus the min. AIC (ΔAIC). The best fit models, determined by lowest AIC score, are bolded.

Color pattern evolution and ecology

Analysis of color pattern geometry revealed that butterflyfishes have diversified along all of the axes measured in our study. Across the family, species vary 3×–6× in the amount of transition density, aspect ratio, color and transition diversity, and achromatic boundary strength. However, species vary 25× in their chromatic boundary strength. Phylogenetic PCA analysis reflected this high diversity of chromatic boundary strength with PC1 being perfectly correlated with *m_dS* (Tables 1 and 2). Thus, the major axis of butterflyfish color pattern diversification has been with respect to a measure of color pattern conspicuousness and species within this group range from highly inconspicuous to highly conspicuous with respect to color. Diversification along this axis suggests that color patterns have evolved across the group in response to pressures relating to crypsis and communication. The second major axes of diversification relates to transition density, with *m* showing a very high loading on PC2. High transition density indicates that the colors change quickly across the fish while low *m* indicates a low degree of turnover in color across the pattern. Thus butterflyfishes also vary widely in whether they possess fine-scale patterning to diverse colors or whether color patterns tend to be concentrated in patches.

Diversification along an axes of color conspicuousness, might suggest that depth, social behavior, and defensive morphology would predict color pattern evolution. Social species might be expected to rely on more conspicuous patterns than asocial species. Shallow-water habitats would allow conspicuous color patterns to be more easily perceived than deep water habitats. And highly defended species might be able to afford greater conspicuousness by virtue of being less vulnerable to predation. However, in our analyses, none of these factors

Table 4 PGLS results of adjacency PCs 1–3 against antipredator morphology PCs 1–3, estimated under an OU model (84 species)

Adjacency	Morphology	Estimate	SE	*t*-value	*P*-value
PC1	PC1	-4.70×10^{-3}	7.16×10^{-3}	−0.66	0.51
PC1	PC2	2.04×10^{-3}	0.01	0.19	0.85
PC1	PC3	0.02	0.01	1.35	0.18
PC2	PC1	-8.58×10^{-3}	4.21×10^{-3}	−2.04	**0.04**
PC2	PC2	4.3194E-05	5.60×10^{-3}	7.72×10^{-3}	0.99
PC2	PC3	−0.01	6.24×10^{-3}	−2.34	**0.02**
PC3	PC1	-1.47×10^{-3}	2.25×10^{-3}	−0.65	0.52
PC3	PC2	3.94×10^{-3}	3.26×10^{-3}	1.21	0.23
PC3	PC3	5.52×10^{-3}	3.98×10^{-3}	1.39	0.17

Phylogenetic signal was optimized using maximum likelihood as Pagel's λ. Degrees of freedom for all regressions were 84. Significant *P*-values bolded.

Table 5 PGLS results of adjacency PCs 1–3 against average depth (91 species)

PC	Estimate	SE	*t*-value	*P*-value
PC1	-1.59×10^{-3}	2.04×10^{-3}	−0.78	0.44
PC2	-2.01×10^{-3}	9.76×10^{-4}	−2.06	**0.04**
PC3	-7.91×10^{-4}	6.30×10^{-4}	−1.26	0.21

predicted the diversity in color pattern along PC1. The main color pattern axis predicted by ecological variables was PC2, which is dominated by variation in color pattern transition frequency. Species with the highest *m*, which appears to reflect fine scale patterning at the level of individual scales, also tended to be less physically defended and to inhabit shallower depths. The ecological significance of this

Table 6 PGLS results of adjacency PCs 1–3 against foraging strategy—benthic invert., facultative, obligate, and planktivore—as well as solitary vs. paired social behavior (84 species)

Model	Trait/interaction	Estimate	SE	*t*-value	*P*-value
PC1 ~ Foraging	(Intercept)	−0.05	0.12	−0.40	0.69
	Facultative	0.11	0.18	0.60	0.55
	Obligate	0.37	0.19	1.98	0.05
	Planktivore	0.07	0.26	0.28	0.78
PC2 ~ Foraging	(Intercept)	−0.07	0.06	−1.28	0.21
	Facultative	0.28	0.08	3.36	$\mathbf{1.2 \times 10^{-3}}$
	Obligate	0.35	0.09	4.05	$\mathbf{1 \times 10^{-4}}$
	Planktivore	0.08	0.12	0.66	0.51
PC3 ~ Foraging	(Intercept)	−0.02	0.04	−0.49	0.63
	Facultative	0.02	0.06	0.36	0.72
	Obligate	1.66×10^{-3}	0.06	0.03	0.98
	Planktivore	−0.04	0.08	−0.43	0.67
PC1 ~ Social Behavior	(Intercept)	0.05	0.09	0.60	0.55
	Solitary	0.10	0.14	0.69	0.49
PC2 ~ Social Behavior	(Intercept)	0.10	0.05	1.98	0.05
	Solitary	0.07	0.07	0.97	0.34
PC3 ~ Social Behavior	(Intercept)	−0.04	0.03	−1.42	0.16
	Solitary	0.07	0.04	1.54	0.13

Table 7 Linear regressions of color geometry statistics for overall transition density (*m*), aspect ratio (*A*), scaled Simpson color class diversity (*Jc*), scaled Simpson transition diversity (*Jt*), and mean chromatic and achromatic boundary strength (*m_dS* and *m_dL*) with machine learning PC1 and PC2 from the PCA of the VGG-16 image classifications for fish images of the family Chaetodontidae

	m	*A*	*Jc*	*Jt*	*m_dS*	*m_dL*
(Intercept)	**−428.00****	441.60†	367.50	**872.30****	**227.99****	**842.60****
	(3.05)	(1.93)	(1.58)	**(3.28)**	**(2.92)**	**(3.32)**
PC1	**2995.30****	−389.60†	−543.20	**−1391.20****	**−1616.12*****	**−884.40****
	(3.20)	(1.97)	(1.61)	**(3.32)**	**(3.45)**	**(3.36)**
R^2	**0.08**	0.03	0.02	**0.09**	**0.09**	**0.09**
Adj. R^2	**0.07**	0.02	0.01	**0.08**	**0.09**	**0.08**
(Intercept)	95.23	−31.09	**−599.70*****	−360.20†	**−147.79***	**−556.70****
	(0.83)	(0.17)	**(3.43)**	(1.67)	**(2.38)**	**(2.76)**
PC2	−666.63	27.41	**886.50*****	574.40†	**1047.46****	**584.30****
	(0.87)	(0.17)	**(3.49)**	(1.69)	**(2.82)**	**(2.80)**
R^2	0.01	0.0002	**0.10**	0.02	**0.07**	**0.06**
Adj. R^2	−0.002	−0.01	**0.09**	0.02	**0.06**	**0.06**
Num. obs.	116	116	116	116	116	116

$^{\dagger}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *t* statistics in parentheses.

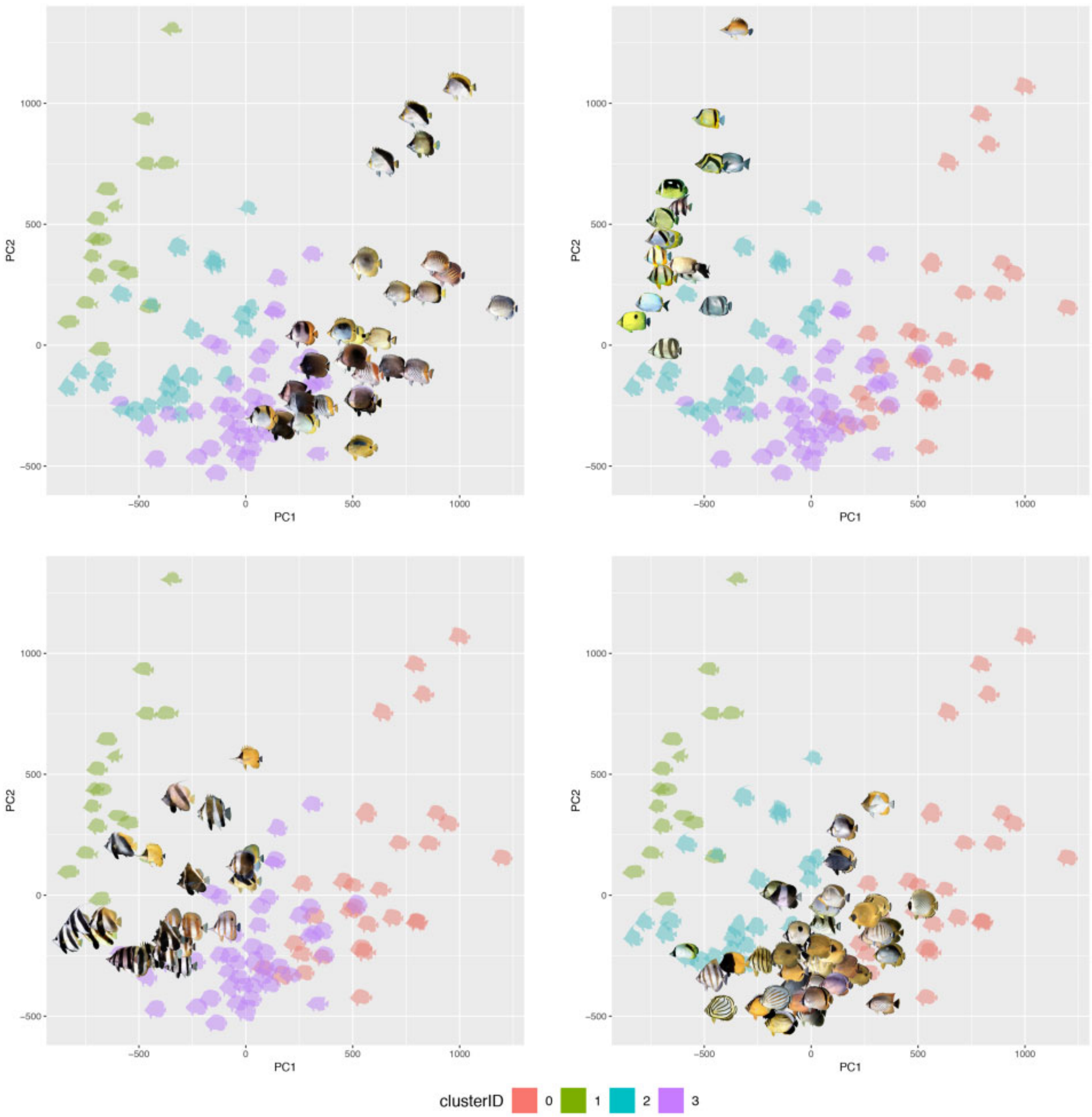

Fig. 4 A visualization of the first two principal components, and their cluster assignment, from the two-dimensional PCA based on the

relationship is not clear. If high transition-based color patterns are important to intraspecific signaling then perception of these patterns would be most effective at shallower depths with more light. However, high transition patterns are not more conspicuous with respect to chromatic boundary strength as might be expected if they evolved under selection for intraspecific communication. Species at shallower depths do tend to have lower achromatic boundary strength values (Tables 1 and 5), making them less visible at distance (Endler et al. 2018). This raises the possibility that high transition patterns have evolved to be less visible to shallow-water predators.

The general lack of relationship between color pattern and ecology, although surprising, is consistent with prior work on body, eyestripe, and eyespot evolution (Kelley et al. 2013). Color patterns are presumed to play a key role in multiple aspects of butterfly fish ecology including intraspecific communication as well as predator avoidance and deception (Marshall 2000). The widespread prevalence of yellows, blacks, and whites across this group (Hemingson et al. 2019) should produce high-contrast patterns that should be relatively conspicuous (Marshall 2000; Endler et al. 2018). Furthermore, many species inhabit shallow water (Supplementary Table S2) and possess eyes with spectral sensitivity appropriate for perceiving patterns of other members of the family (Losey et al. 2003; Marshall 2017). Yet we found weak to nonexistent or relationships between almost all ecological predictors and color pattern. Social species do not possess color patterns that are distinctive from solitary species despite the expectation that sociality might favor patterns that are more conspicuous. Heavily defended species, which might be expected to advertise their defenses or at least be able to possess more conspicuous patterns dues to decreased vulnerability to predation (Hodge et al. 2018) do not significantly differ in boundary strength from less defended species. And despite the attenuation of light with depth in marine environments, we found little evidence that color pattern systematically varies with mean depth.

One possible exception to this lack of a trend between ecology and coloration may be the distribution of high transition patterning. Species with high m and low achromatic boundary strength tend to i) occur in shallow water, ii) have reduced physical defenses, and iii) be more associated with corals (Tables 1–6). These results suggest that the the trend towards a low defense, shallow-bodied phentoype identified by Hodge et al. (2018) as characteristic of species that forage and seek refuge furthermore includes a color pattern with high color transitions and low achromatic boundary strength. It is possible that this color pattern enhances camouflage within corals by breaking up the bpdy outline and by reducing conspicuousness at distance to predators (Endler et al. 2018).

One possibility for the lack of a strong relationship between ecology and color pattern is that we have not included key aspects of pattern in our analysis. A possible example could be false eyespots which occur across chaetodontids (Kelley et al. 2013). This color pattern trait presumably functions to confuse predators and thus might be strongly favored under conditions where species are vulnerable to predation (Kjernsmo and Merilaita 2013; Kjernsmo et al. 2016). However, this trait shows high variability with respect to the size, position, and number of spots (Kelley et al. 2013) and might thus be poorly captured by the methods employed here. It is possible that comparative analyses that discretize key pattern elements might prove more useful in identifying relationships between ecology and color pattern (Salis et al. 2018, 2019). However, we note that Kelley et al. (2013) also found a generally weak relationship between ecology and a set of discretely coded pattern elements. Since our analysis was restricted to digital photographs, it is also possible that more ecologically relevant aspects of color pattern would emerge from an analysis that included the entire visual spectrum, including ultraviolet. However, given that most members of this family are diurnally active, inhabit shallow water, and do not appear to have visual sensitivity to short wavelengths (Losey et al. 2003), we believe that is unlikely.

An alternative possibility is that color pattern evolution evolves so rapidly under pressures related to reproductive isolation and species maintenance that it becomes effectively decoupled from ecology. Our analyses of the tempo of color pattern evolution are consistent with rapid diversification under such a scenario. Indeed, some species pairs of butterflyfishes have been shown to develop substantial color differences in as little as 300,000 years (Hemingson et al. 2019). Paleontological and comparative studies have revealed that almost all innovation in functional traits related to feeding and ecology in modern reef fish communities has taken place between 5 and 50 million years ago (Bellwood et al. 2015, 2017; Floeter et al. 2018) and suggest that species diversification over the last 5 million years is dominated by changes in coloration without functional innovation (Bellwood et al. 2017). Our analyses provide support for scenarios of rapid changes in color pattern over this time frame and furthermore, that color pattern may be so plastic that convergences in pattern elements across species with diverse ecologies are common. Hemingson et al. (2019) suggested that color pattern evolution is an extreme example of many-to-one mapping (Wainwright et al. 2005) such that pressures for lineage diversification relating to species recognition can be met through nearly unconstrained diversification in pattern. This idea is intriguing and consistent with the lack of relationship between ecology and pattern found in our study, and such a relationship is broadly consistent with some predictions of macroevolutionary "radiation in stages" models (Streelman and Danlel 2003; Sallan and Friedman 2012). Yet it is difficult to reconcile with the wide range of studies demonstrating the ecological importance of color pattern variation (Marshall 2000, 2017; Losey et al. 2003; Endler et al. 2018; Salis et al. 2018). Disentangling the relative importance of the drivers of pattern evolution remains a key frontier in understanding color pattern diversity in reef fishes (Salis et al. 2019).

Machine learning and identification of fish color patterns

Our application of machine learning to the problem of color pattern identification suggests that chaetodontids can be divided into four categories. Although some color pattern elements within these grouping are obvious (Clusters 0 and 2, Fig. 4), patterns appear to be highly diverse within others (Cluster 3). Features identified by machine learning as important to explaining variation in the dataset are related to color pattern geometry but the correlation between them is weak. This is not surprising and reflects both the strengths and weaknesses of machine learning approaches in general. Unlike our analysis of color pattern geometry, which subsamples both the color diversity and number of pixels within the image, ML clustering considers the entire image in searching for features. On one hand, this may provide ML an advantage in identifying pattern elements that are restricted to parts of a fish such as the strong dorsal banding seen in most members of Cluster 1 (Fig. 4). However, ML is also likely weighting image features relating to body shape more heavily than our analysis of color pattern geometry. The relationship between body shape and color pattern has yet to be explored within fishes, so whether this is an advantage or disadvantage for this approach is not known. Although a more restricted sampling of the image area in our dataset could reduce or eliminate the contribution of body shape to clustering, the problem of identifying the key elements of pattern that contribute to clustering is difficult with current ML implementations. The general area of feature extraction in ML is a major frontier in the field and so solutions to this problem may emerge in the near future. With respect to pattern identification, another consideration of this study is the size of our data. Our clustering analysis is based upon 116 images. This is an exceedingly small sample size from a machine learning perspective. Although the utility of pattern recognition within chaetodontids appears somewhat limited, feature identification is expected to improve substantially with increasing dataset size. Thus, ML may provide an important tool for identifying color patterns at the scale of all reef fishes (Salis et al. 2019), or alternatively, in nested studies that include multiple images per species.

Conclusions and future directions

The study of color pattern evolution across coral reef fishes is just emerging. The development of new tools for quantifying pattern (Endler 2012; Van Belleghem et al. 2018; Maia et al. 2019) combined with the extraordinary diversity of fish color patterns on reefs (Salis et al. 2019) makes this a promising system for understanding the factors responsible for this conspicuous aspect of biodiversity. A key challenge is to identify the relative importance of ecology versus species identification in driving color pattern evolution. Although studies on the history of fishes on reefs suggest that color is decoupled from functional innovations, the evidence for ecological relevant diversity in reef fish color and visual systems is widespread. Further comparative study at broader phylogenetic scales, in conjunction with new machine learning approaches for identifying pattern similarities, will help illuminate how temporal scale, ecology, and phylogeny relate to understanding color pattern evolution.

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Supplementary data

Supplementary data available at *ICB* online.

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