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PERSPECTIVE

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Diving into the diversity of colour patterns in reef fishes

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Colours and associated patterns are probably some of the most obvious phenotypic traits in animals and reef teleost fishes are often cited as a textbook example for illustrating this type of diversity. Even if it is well established that colour patterns play a central role in the ecology and evolution of reef fishes, we still lack the necessary toolkits to fully grasp the mechanisms driving the diversification of this obvious phenotypic trait. On the one hand, genotyping power seems now limitless thanks to current DNA sequencing technologies. Today, entire genomes of fishes can be easily produced for large sets of species. On the other hand, the description of colour patterns and the quantification of their variation across reef fishes might be highly challenging. In a cover manuscript in this issue of Molecular Ecology, Coulmance et al. (2023) introduced an innovative approach for extracting and quantifying the major colour pattern elements present in the hamlets (*Hypoplectrus* spp., Serranidae), a recent reef fish radiation from the Caribbean. Then, they intelligently used the quantified colour pattern variation as a phenotypic trait for a genome-wide association study (GWAS). Interestingly, using a method that required no a priori knowledge, they were able to recover well-established marks (e.g., vertical bars) and to highlight less expected colour pattern elements (e.g., dark to light gradient on ventral part as well as caudal and anal fins), which show strong association peaks on linkage group (LG) 12 and 04. Beyond the demonstration of the potential of their new quantitative analysis of colour pattern variation in reef fishes combined with GWAS, their findings offer new perspectives on our understanding of the intrinsic and extrinsic factors generating this outstanding diversity of the fish world.

We are living in a period during which advances in technologies provide thrilling perspectives in our understanding of ecological and evolutionary processes driving biodiversity. Alongside the development of cutting-edge methods allowing the collection of large amounts of molecular data, we still need new approaches to effectively quantify some aspects of the phenotype. Colours and associated patterns are a major component of phenotypic diversity in vertebrates. In addition to birds (e.g., Eliason et al., 2023; Hidalgo et al., 2022), reef teleost fishes appear as a key group to expand our understanding of development, ecological and evolutionary mechanisms driving this astonishing phenotypic biodiversity (Salis et al., 2019). Indeed, reef fishes are ecologically and phylogenetically diverse, and they present the largest diversity of pigment cell types (chromatophores,) providing very interesting developmental perspectives (Salis et al., 2019).

The functionality of fish colours and associated patterns is diverse, including camouflage, mimicry and/or communication (Marshall, 2000). The study of reef fish colour pattern allows the investigation of fundamental ecological and evolutionary processes that include speciation, ecological adaptation and sexual selection. However, colour pattern is a highly complex trait, including all the aspects of colours (hue, saturation, etc.) and the diversity of markings such as bars, stripes, lines, spots, blotches and many more that occur in seemingly unlimited sets of combinations. The development of new approaches allowing a precise quantification of colour patterns is fundamental for their study in reef fishes.

A simple procedure would be the coding of the presence/absence of discrete colour motifs on regular images but it would only rely in elements defined a priori (Figure 1). During the last 10 years, multiple effective methods were developed to quantify and compare the geometry of colour patterns (e.g., Endler et al., 2018; Van Belleghem et al., 2018; Weller & Westneat, 2019). Non-standardized images could be processed along this method (Figure 1) but, with such type of images, you need to restrict your analyses on dominant colours (e.g., Alfaro et al., 2019; Hemingson et al., 2019). On the one hand, these two methodologies provide a valuable way to collect information about colours and associated patterns in a very large number of reef teleosts. On the other hand, it has to be admitted that

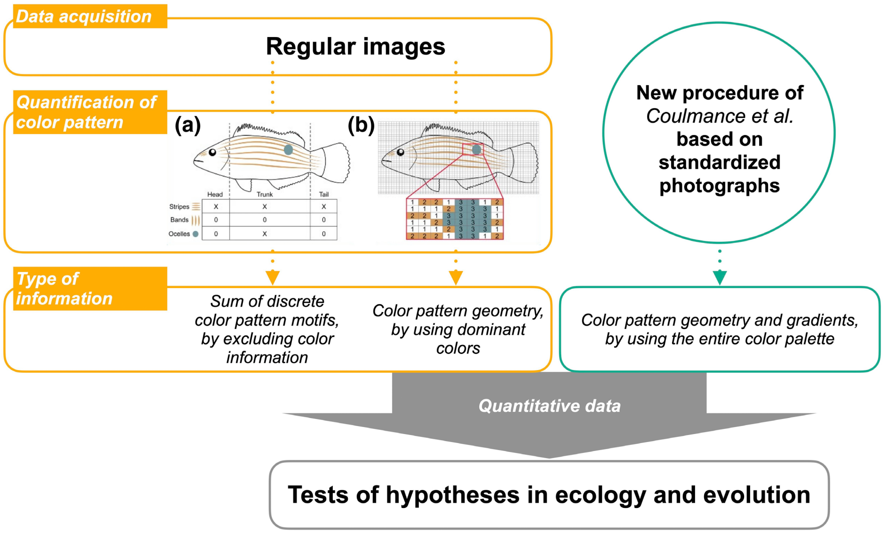

FIGURE 1 Overview of different ways to produce traits characterizing colour patterns and associated colours. All the published methods devoted to the quantification of colour patterns are not illustrated. Here are shown (a) a method of quantification by coding the presence/absence of predefined markings in specific body regions as well as (b) a quantitative method through clustering of each pixel based on colour and luminance similarity, hence allowing the estimation of geometric parameters based on the distribution and number of transitions between adjacent clusters (Endler et al., 2018). The procedure introduced by Coulmance et al. (2023) relies on the acquisition of standardized photographs obtained in situ and it complements the available toolkits devoted to the characterization of colour patterns in fishes. Outputs of these methods are quantitative traits, which can be summarized in principal components analyses (or similar ordination methods) and then used to test hypotheses in ecology and evolution.

phenotypic characterization would be mainly built on obvious markings, but tenuous information based on colours restricted to few markings could be lacking. Here, Coulmance et al. (2023) introduce and describe a very promising procedure to extract colour data by including all the range of colours visible by human eye and quantify colour pattern variation without any a priori definition of markings. They provide a clear protocol to take standardized photographs of live fish specimens in situ and then they detail steps of colour standardization, fish alignment (use of landmarks and Bézier curves) and image background suppression. The output results in image vectors to be used as input for principal components analysis (PCA).

First, Coulmance et al. (2023) applied their innovative protocol on a data set of 13 hamlet species (*Hypoplectrus*) to characterize the diversification of their colour patterns. Very interestingly, some PC axes retrieved well-identified markings such as vertical bars and peduncle mark while some other PCs recovered continuous variation which would be more difficult to code (e.g., dark vs light coloured species). Variation of the yellow and the blue colour components across species, two very frequently used colours in reef fishes (Marshall, 2000), was also highlighted. By applying the same methodology in other reef fish groups, it would be easily possible to check if the major axes of colour pattern variation are similar and recurrent across taxa.

In a second step, they used this quantified colour pattern variation as trait for a GWAS. These analyses revealed (1) the presence of association peak between a genomic region and a colour pattern element, as well as (2) the plural effect of some genomic regions on several colour pattern elements. Further similar studies in other reef fish groups will certainly help to expand our catalogue of genes associated with colour pattern variation. These genetic tools will then become very helpful in understanding the evolution and the development of colour patterns in reef fishes (Salis et al., 2019). An additional promising avenue for the use of these continuous traits defining colour pattern variation among species would be the combination of these phenotypic data with time-calibrated phylogenies and the toolkit of phylogenetic comparative methods (Felsenstein, 1985; Revell & Harmon, 2022). Indeed, multiple hypotheses concerning the tempo and the mode of colour pattern diversification could be tested and would help us to decipher the main factors driving this astonishing phenotypic diversity of reef fishes.

By aiming to identify genes associated with colour diversity in reef fishes, Coulmance et al. (2023) introduce a new promising procedure to collect and produce continuous traits associated with colour pattern variation. This method is here illustrated on the acquisition of colours visible by the human eye, but it is well established that reef fishes can also possess colour patterns that contain ultraviolet (UV) components invisible to the human eye (Marshall, 2000; Siebeck, 2004). By associating the protocol of Coulmance et al. (2023) with a photographic method to make the UV patterns visible to the human eye (e.g., Siebeck, 2004), it would also be feasible to describe and study the diversification of UV patterns in groups such as damselfishes, well known for their use of UV vision.

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CONFLICT OF INTEREST STATEMENT

The authors declare no competing interests.

DATA AVAILABILITY STATEMENT

Not applicable.

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