Colour patterns are less variable in highly unpalatable nudibranchs

Cedric P. van den Berg[1†], John A. Endler[2], Leon Drummond[1], Bethany Dawson[1], Carl Santiago[1], Nathaly Weber[3], Karen L. Cheney[1]

[1]Visual Ecology Laboratory, School of Biological Sciences, The University of Queensland, 4072 Brisbane, Australia

[2]Centre for Integrative Ecology, School of Life and Environmental Sciences, Deakin University, Geelong, Victoria 3216, Australia

[3]Faculty of Biology and Medicine, School of Biological Sciences, The University of Lausanne, 1015 Lausanne, Switzerland

Corresponding author: c.vandenberg@bristol.ac.uk

† Current address: School of Biological Sciences, The University of Bristol, BS81TQ, United Kingdom

Abstract

Predators learn and memorise the association between conspicuous colour patterns of aposematic prey and their underlying chemical or secondary defences. Consequently, variation in signal design within a species should be selected against, because it can decrease the rate of predator learning and enhance the rates of predator errors. However, quantitative assessments have not been made on whether the strength of chemical defences influences colour pattern variation. We examined this by quantifying colour pattern variation using Quantitative Colour Pattern Analysis (QCPA) in 12 Dorid nudibranch species (Infraorder: Doridoidei) that varied in their unpalatability. We accounted for the physiological limitations of a potential predator's visual system (a triggerfish, *Rhinecanthus aculeatus*), modelling animal appearance along an escalating predation sequence. We found that various colour pattern statistics were less variable in highly unpalatable species, with pattern statistics being up to 72% less variable than those of more palatable species. No correlations indicating the opposite were found across 157 colour pattern statistics. However, the strength and number of correlations depended on viewing distance. Our results suggest that low colour pattern variability could be favourable for aposematic signalling in Dorid nudibranchs. We provide evidence for distance-dependent signalling facilitating context-specific feature selection in multicomponent visual signals.

Keywords: Signal honesty, aposematism, crypsis, predator psychology, purifying selection, defensive animal colouration

1. Introduction

Many animals use aposematic colour patterns to warn potential predators of underlying defences (Poulton, 1890). Once aposematism has evolved, purifying selection is expected to constrain colour pattern diversity within a species and/or populations as predators learn to associate a visual signal with unprofitability (Turner, 1984; Endler & Greenwood, 1988; Mallet & Barton, 1989; Endler & Mappes, 2004; Sherratt, Speed, & Ruxton, 2004; Borer, van Noort, Rahier, & Naisbit, 2010). A consistent appearance across aposematic individuals may facilitate predator learning and memorisation, whereas variation in signal design may cause predators to make errors when attacking prey and decrease rates of predator learning (Endler, 1991; Endler & Mappes, 2004; Karpestam et al., 2014; Mappes et al., 2005). Yet some degree of colour

pattern diversity within aposematic species is ubiquitous and is thought to be driven by countervailing evolutionary factors such as genetic drift, gene flow, sexual selection, or variation in resource abundance (reviewed in Briolat et al., 2019; also see Speed & Turner, 1999; Turner, 1981).

Colour pattern variation in aposematic species depends on the absolute and relative abundance of individual species in a given environment and the strength of their secondary defences (Clarke & O'Donald, 1964; Greenwood, 1985; Mappes et al., 2005). For strongly defended and abundant species, anti-apostatic selection is expected to be common (Greenwood, 1985). This is because, in strongly defended species, rare and unfamiliar morphs, proportional to the strength of associated secondary defences, are increasingly predated on (Endler & Greenwood, 1988). Such correlations between reduced colour pattern variability and increased strength of secondary defences may further be accelerated in aposematic species with aggregated occurrence as inclusive fitness through kin selection is argued to enhance anti-apostatic selection (Fisher, 1930; Guilford, 1985).

Cumulatively, such mechanisms explain variable colour patterns in aposematic species across heterogeneous environments while explaining reduced variability within aposematic species at a local scale (e.g. Barnett et al., 2021; Rönkä et al., 2020). Specifically, aposematism in a homogeneous and stable environment is expected to coincide with selection towards reduced colour pattern variability within a population (Sherratt, 2002; Summers et al., 2015). However, whether the strength of chemical defences influences pattern variability is unclear, and quantitative assessments are lacking (see Summers et al. 2015 and White & Umbers 2021 for review). Highly defended species should exhibit more uniform colour patterns to strengthen predator learning of the aposematic signal. However, attacking well-defended prey is costly; therefore, predators may generalise more broadly between colour patterns of previously attacked prey and prey they subsequently encounter (e.g. Banich & Caccamise, 2010; Gamberale & Tullberg, 1996; Rowe, 1999). This could relax selection pressures on uniform colour patterns within a population resulting in higher variability. Selection for or against colour pattern variability could also only occur on individual colour pattern elements, depending on which elements of the signal predators learn or pay attention to (Winters et al. 2017).

When interpreting the ecological relevance of phenotypic variation, it is important to consider how the appearance of an organism changes as a function of observer acuity and

viewing distance (Endler, 1978). For example, colour patterns may be cryptic when viewed from a distance but may become aposematic as a predator approaches (J. B. Barnett et al., 2016; Endler, 1978). Consequently, predator learning of associations between primary and secondary prey defences, or the subsequent retrieval of formed associations from memory upon encounters with aposematic prey, might happen at a specific range of viewing distances. Animals also detect objects and make decisions on their identity and quality based on complex combinations of spatiochromatic features (Endler & Houde, 1995; Rosselli et al., 2015; Sibeaux et al., 2019; van den Berg, Endler, Papinczak et al., 2022). However, empirical evidence on the ecological significance of colour pattern variability in aposematic animals remains largely restricted to investigations of colour alone (e.g. Nokelainen et al., 2022; Rönkä et al., 2018).

In this study, we examined whether highly defended aposematic nudibranch species exhibited less variable colour patterns than less defended species. We further hypothesized that distance-dependent signalling might also affect any correlations between colour pattern variability and level of defence. To do this, we modelled the visual appearance of 12 sympatric Dorid nudibranch species, for which we have previously published information on their levels and type of chemical defences (Winters et al. 2022). We did this across viewing distances corresponding to an escalating predation sequence (Endler, 1986, 1991). We quantified the perception of within-species colour pattern variability using the Quantitative Colour pattern Analysis (QCPA) (van den Berg, Troscianko, et al., 2020), allowing for the consideration of colour, luminance and spatial vision of triggerfish (*Rhinecanthus aculeatus*). We then correlated the perceived variability of colour patterns with our previously published unpalatability data for each species (Winters et al., 2022).

2. Materials and Methods

(a) Study species

We took digital photographs of 353 Dorid nudibranchs using a calibrated Olympus EPL-5 with a 60mm macro lens. These individuals belonged to 12 species: *Goniobranchus collingwoodi* (N=20); *Phyllidiella pustulosa* (N=40); *Glossodoris vespa* (N=32); *Goniobranchus splendidus* (N=37); F) *Doriprismatica atromarginata* (N=35); *Hypselodoris bennetti* (N=13); *Chromodoris kuiteri* (N=49); *Chromodoris magnifica* (N=14); *Phyllidia ocellata* (N=32); *Chromodoris elisabethina* (N=31); *Discodoris* sp. (N=19); *Aphelodoris varia* (N=31) (Fig. 1) from five locations on the east coast of Australia: Mackay (QLD), Sunshine

Coast (SE Queensland, QLD), Gold Coast (SE QLD), Cook Island (New South Wales, NSW) and Nelson Bay (NSW) between March 2016 and February 2021. Two individuals of *Chromodoris magnifica* were provided by an aquarium supplier (Cairns Marine, Pty Ltd, Cairns, QLD). A sub-sample of these images were previously used to investigate background habitat variability of nudibranch molluscs (van den Berg, Endler, & Cheney, 2022a) and distance-dependent signalling in terms of colour pattern detectability and boldness (van den Berg, Endler, & Cheney, 2022b). These species were selected as they were relatively abundant at our study sites and cover a broad range of visual appearance and strength of chemical defences. Specifically, we have previously provided a detailed investigation into the strength and identity of chemical defences in these species sampled from the same locations as individuals from this study (Winters et al., 2022).

Two out of 12 species (*Doriprismatica atromarginata, Goniobranchus splendidus*) were sampled across sites in QLD and NSW in high numbers, whereas the other species were only sampled in either NSW or QLD or with highly uneven numbers between sites (Table S2). Nudibranchs were either photographed underwater against their natural habitat (n = 216) with the camera in an Olympus PT-EP10 underwater housing and using white LED illumination from a combination of VK6r and PV62 Scubalamp video lights. Alternatively, nudibranchs (n = 137) were collected, taken back to the laboratory, submerged in water in a petri dish and photographed against a white background with the same camera. In the laboratory, nudibranchs were illuminated with 300nm-700nm full-spectrum white LED lights. Details on collection sites and dates are provided in the Supplementary Information, camera and illumination spectra are provided in van den Berg, Troscianko, et al., (2020). Nudibranchs were collected under the Queensland General Fisheries Permits 183990, 205961 and NSW Scientific Collection Permit P16/0052-1.0.

(b) Image analysis

We used ImageJ (Schneider et al., 2012) and the MICA toolbox (Troscianko & Stevens, 2015), to manually segment the images into regions of interest (ROI), selecting the animal from its background and defining a size standard. All nudibranch were aligned vertically prior to analysis using QCPA (van den Berg, Troscianko, et al., 2020). To analyse the nudibranch colour patterns, we used the visual system parameters of a trichromatic triggerfish, *Rhinecanthus aculatus*, a common shallow reef inhabitant found throughout the Indo-Pacific,

which feeds on invertebrates, algae, and detritus (Randall et al., 1997). Their visual system has been well described (Champ et al., 2014, 2016; Cheney et al., 2013, 2022; Green et al., 2022; Pignatelli et al., 2010).

We analysed colour patterns for viewing distances of 2cm, 5cm, 10cm and 30cm assuming the spatial acuity of the triggerfish was 3 cycles per degree (Champ et al., 2014; Cheney et al., 2022). Following acuity modelling, the images were processed with a Receptor Noise Limited (RNL) ranked filter (falloff: 3, radius: 5, repetition: 5) and clustered using RNL clustering with a colour threshold of 2 ΔS (Cheney et al., 2019; Green et al., 2022) and a luminance contrast threshold of 4 ΔS (van den Berg, Hollenkamp, et al., 2020) for all analyses except the local edge intensity analysis (LEIA) which does not require image segmentation but is recommended to be subjected to RNL ranked filtering. We calculated Weber fractions based on a relative photoreceptor abundance of 1:2:2:2 (sw:mw:lw:dbl) and photoreceptor noise of 0.05, resulting in 0.07:0.05:0.05:0.05.

QCPA analysis was achieved using a custom batch script running on high performance computing (HPC) infrastructure. We analysed each animal's values for: 1) colour adjacency analysis (CAA), which describes pattern geometry in a segmented image; 2) visual contrast analysis (VCA), which describes pattern boldness based on chromatic and spatial pattern element properties in a clustered image; 3) boundary strength analysis (BSA), which describes the colour and luminance contrast of boundaries between pattern elements at the scale of an animal in an unclustered image; and 4) local edge intensity analysis (LEIA) which describes the strength of colour and luminance contrast at the scale of an edge-detecting receptive field in an unclustered image. This resulted in a highly descriptive array of 157 colour pattern statistics for each animal. A full detailed description of each pattern statistic is found in van den Berg, Troscianko, et al. (2020). Here we use CAA, VCA, BSA, and LEIA as prefixes for each type of analysis. All pattern analyses used a segmented image and measured transitions between pixels along vertical (along body axis) and horizontal (perpendicular to body axis) sampling transects in a transition matrix. Statistics ending with 'vrt' or 'hrz' are the vertical and horizontal version (analysing the respective transition matrix only) of their respective statistic (analysing the full transition matrix) and are able to represent differential directionality sensitivity in the visual system of an observer (Endler, 2012; Endler et al., 2018; Hubel & Wiesel, 1962). LEIA does not use a transition matrix, but equally discriminates between horizontal and vertical edge contrast (van den Berg, Troscianko, et al., 2020).

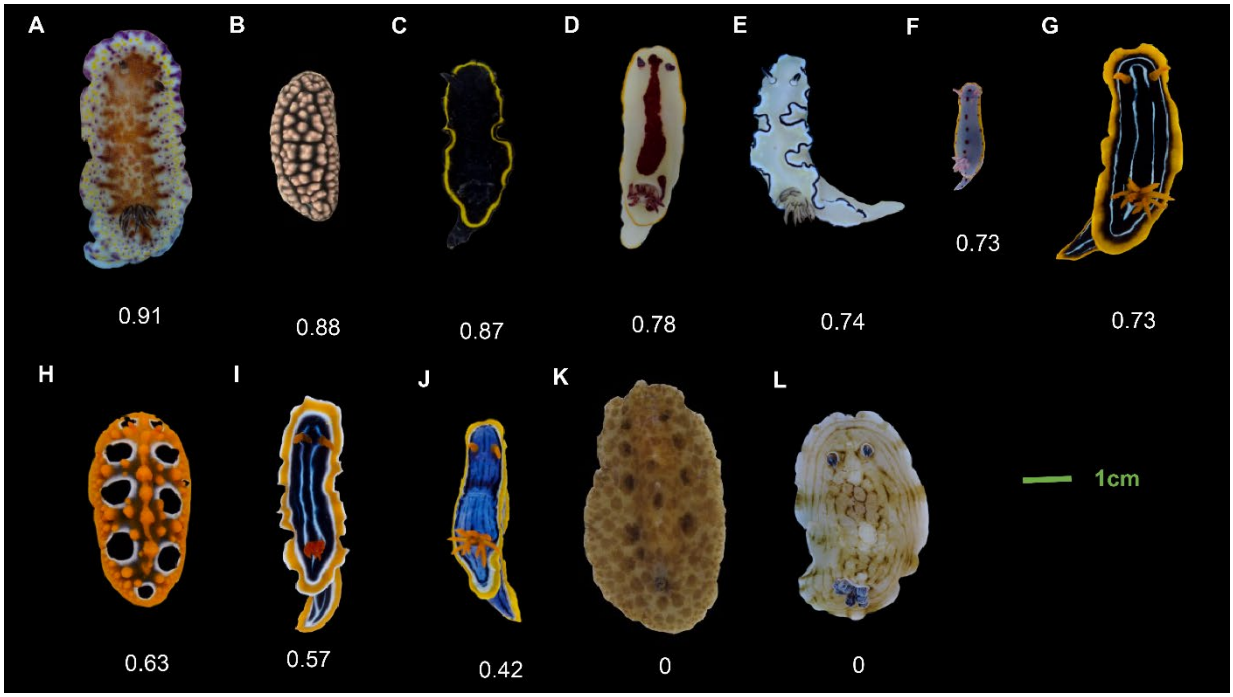

Figure 1. Representative photographs of the 12 species used in this study, ordered from most unpalatable to least unpalatable with the strength of chemical defences reported as 1-ED_{50} (Winters et al., 2022). A) *Goniobranchus collingwoodi;* B) *Phyllidiella pustulosa;* C) *Glossodoris vespa*; D) *Goniobranchus splendidus*; E) *Doriprismatica atromarginata;* F) *Hypselodoris bennetti;* G) *Chromodoris kuiteri;* H) *Phyllidia ocellata*; I) *Chromodoris magnifica*; J) *Chromodoris elisabethina;* K) *Discodoris sp.*; L) *Aphelodoris varia.* Level of chemical defence (1-ED_{50}) indiated underneath each species.

To determine the level of chemical defences for each species (i.e. the 'palatability spectrum', Brower et al., (1968)), we used previously published data on the deterrent properties from feeding rejection assays with rockpool shrimp (*Palaemon serenus*) that are similar to assays performed with triggerfish and toadfish (Winters et al., 2022). Here, we use unpalatability as opposed to toxicity, as the former is more likely reflective of a repellent property of chemical defences (Chan et al., 2021; Winters et al., 2022). Assays were conducted by adding extracted nudibranch compounds at increasing concentrations to food pellets made from squid mantle. Effective dose (ED_{50}) values were calculated based on the concentration that elicited a rejection response in at least 50% of the shrimp. For the purpose of this study, ED_{50} values from Winters et al., (2022) were averaged when multiple extracts from the same species were tested. We then subtracted these values from 1 so that values close to 0 were the least defended/most palatable and values close to 1 highly defended/least palatable) (Table S2). Importantly, specimens in Winters et al., (2022) were collected from the same locations as in this study. Although *Chromodoris magnifica* was not included in Winters et al., (2022), Cheney et al., (2016) demonstrated that this species also stores latrunculin A as the sole defensive

compound in the mantle rim. We have also recently shown that this is at concentrations in between those found in *Chromodoris elisabethina* and *Chromodoris kuiteri* (Chan, 2022). We, therefore, set unpalatable ED_{50} values as the average from these two sister species. Lastly, assay data for *Glossodoris vespa* is presented in Winters et al., (2018).

(d) Statistical analysis

To quantify colour pattern variability, we calculated the inter-quartile range (IQR) for each parameter of each species. All pattern statistics were then scaled from 0 to 1 using the 'caret' package (Kuhn, 2021), with 1 being most variable. We assessed whether there were colour pattern differences between sites for those species that were sampled roughly equally across locations (*G. splendidus, D. atromarginata*); however, we found no significant differences between locations in the IQR across all 157 parameters (lmer: $F_{17} = 2.12$, $p = 0.16$). Due to the small number of species and variable colour pattern statistics, it was not possible to estimate the phylogenetic signal reliably using phylogenetic least square regression (pgls), with lambda estimates being inconclusive. Therefore, in line with previous studies (Cheney et al., 2014; Cortesi & Cheney, 2010), we assumed a phylogenetic signal of 0 for all viewing distances when analysing the median IQR for each species across all 157 pattern statistics using a Pearson product moment correlation test in the 'stats' package (R Core Team, 2021). Prior to analysis, the IQR values were normalised using the 'bestNormalize' package (Peterson & Cavanaugh, 2020). As all colour pattern statistics essentially describe the same three core properties (colour, luminance and pattern), we applied a conservative Bonferroni correction of n = 3 to avoid an excessive type II error (for discussion see Moran, 2003), resulting in an adjusted significance threshold of $p = 0.017$.

We present the results from the largest viewing distance to the smallest to replicate an escalating predation sequence (Endler, 1991). Detailed reporting of test statistics for all 157 pattern statistics at each viewing distance are presented in the supplementary material.

3. Results

The species-specific interquartile range (IQR) of various colour pattern statistics was negatively correlated with increasing unpalatability levels at each viewing distance (Pearson correlation: $r < -0.63$, $p < 0.017$, Fig. 2), indicating that highly unpalatable species showed less less among-individual variation in visual appearance than more palatable ones. Importantly, we

did not find any significant positive correlations in our data (Tables S3) nor any positive correlations of moderate strength ($r > 0.58$, $p < 0.05$) which would have indicated the opposite.

At 30 cm, the colour pattern regularity measured as the Shannon diversity of the synonymous transitions in the transition matrix (*CAA.Hc*) is 71% less variable in highly defended species ($r = -0.84$, $p = 0.001$). This is the case for both, looking at the horizontal (*CAA.Hc.Hrz*, $r = -0.85$, $p < 0.001$) and the vertical axis (CAA.Hc.Vrt, $r = -0.84$, $p = 0.001$) of colour patterns with variability (IQR) in highly defended species being 72% (Hrz) and 71% (Vrt) more reduced (Fig. 2A, Table 1). Additionally, nine other pattern statistics showed moderately strong negative correlations between chemical defences and colour pattern variability ($r < -0.58$, $p < 0.05$, Table S2).

At 10 cm, six out of 157 pattern statistics were significantly negatively correlated with unpalatability (Fig. 2 B, Table 1). The more unpalatable a species was, the less variable the colour pattern boldness elicited by the mean abundance weighted saturation contrast between colour pattern elements. This was the case for both, saturation measured as the distance from the achromatic point in the RNL colour space (Hempel de Ibarra et al., 2001), as well as maximum possible pairwise contrast between photoreceptors irrespective of relative abundances and receptor noise (Endler & Mielke, 2005). These are denoted as *Ssat* and *Dmax* respectively (see van den Berg, Troscianko, et al., 2020 for details). The mean abundance-weighted saturation contrast measured as *Dmax* saturation (*VCA.MDmax*) between colour pattern elements was 52% ($r = -0.72$, $p = 0.008$) less variable in highly defended species than it was in undefended ones, irrespective of the horizontal or vertical axis (Fig. 2 B, Table 1). When measuring saturation as RNL saturation (*Ssat*), the mean abundance-weighted saturation contrast (*VCA.MSsat*) was 50% ($r = -0.71$, $p = 0.010$) less variable in highly defended species than in undefended ones.

At a closer viewing distance of 5 cm, three out of 157 pattern statistics were significantly negatively correlated with unpalatability (Fig. 2 C, Table 1). The mean saturation contrast (*VCA.MDmax*, $r = -0.70$, $p = 0.011$) was 51% less variable in highly defended species as opposed to undefended ones. This was true for both, the vertical (*VCA.MDmax.Vrt*, $r = -0.71$, $p = 0.010$) and horizontal axis (*VCA.MDmax.Vrt*, $r = -0.71$, $p = 0.010$). Additionally, we found six colour pattern statistics showing moderately strong negative correlations ($r < -0.58$, $p < 0.05$, Table S2).

Lastly, at 2 cm, the same three out of 157 statistics were significantly negatively correlated with unpalatability (Fig. 2 D, Table 1). This distance most likely reflects immediate contact between predator and prey. The more unpalatable a species was, the mean abundance weighted saturation contrast between colour pattern elements was 49% less variable (*VCA.MDmax*, $r = -0.70$, $p = 0.011$). However, saturation contrast variation along the horizontal (*VCA.MDmax.Hrz*, $r = -0.75$, $p = 0.005$) and vertical axis (*VCA.MDmax.Vrt*, $r = -0.72$, $p = 0.008$) was slightly more reduced in highly defended species when looked at separately. We also found three other pattern statistics to show a moderately strong negative correlation ($r < -0.58$, $p < 0.05$, Table S2).

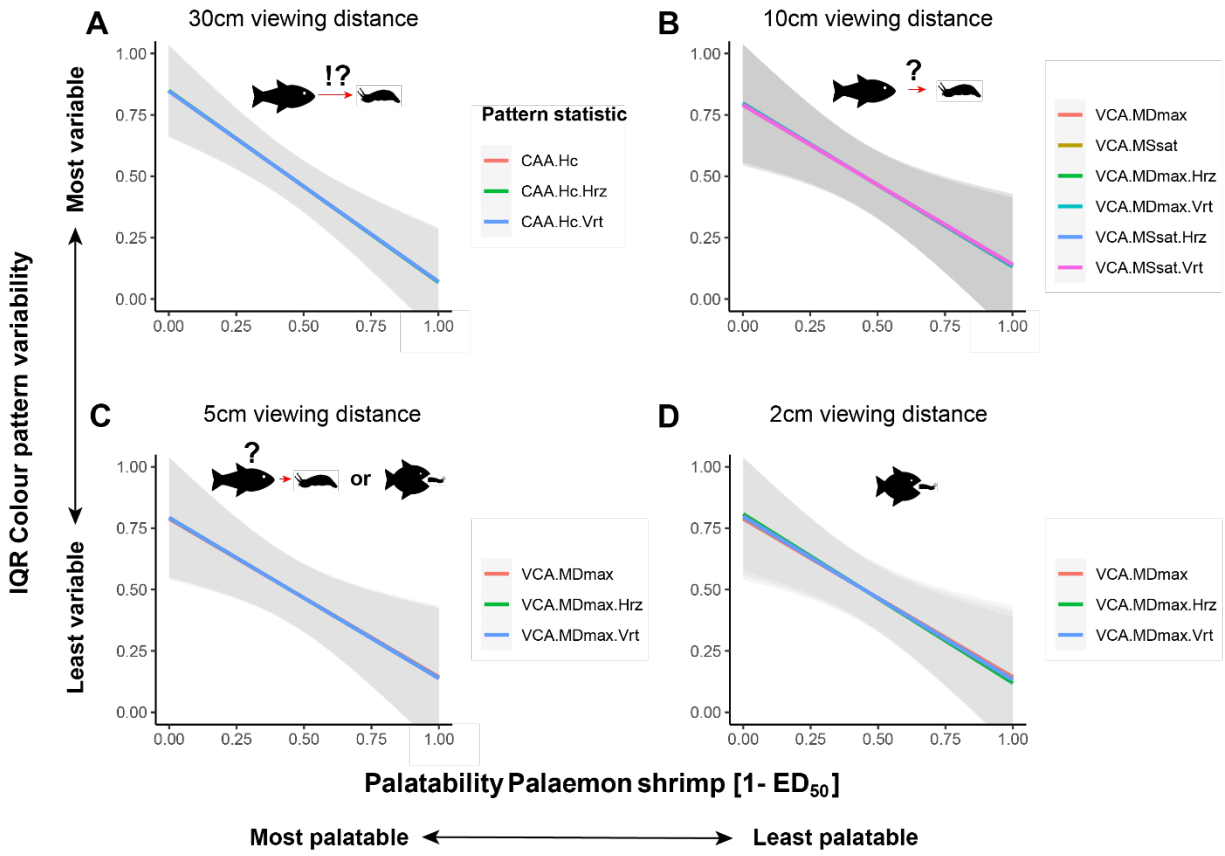

Figure 2. All significant correlations between colour pattern variability (interquartile range, IQR) and level of unpalatability (ED_{50}) out of 157 assessed colour pattern statistics across all 12 species (Pearson correlation: $r < -0.5$, $p < 0.017$) for each viewing distance: A) 30 cm, B) 10 cm, C) 5 cm, D) 2cm. Grey shading indicates 95% confidence intervals of the linear regressions. The most likely corresponding step of the predation sequence is indicated with schematics: exclamation mark signifies encounter and detection; the question mark signifies identification and approach, and finally subjugation and consumption. The x-axis shows the normalised 1-ED_{50} from palatability assays with Palaemon shrimp where 0 refers to the most palatable species

and 1 refers to the most unpalatable species. The y-axis shows the variability of colour pattern statistics expressed as the normalised IQR (interquartile range) for each species where 0 refers to the least variable species and 1 to the most variable species. D) Summary table showing the Pearson correlation (r), percentage of change (r^2 x 100) and significance (p).

Table 1. All significantly correlated pattern statistics for each viewing distance with a Bonferroni adjusted Pearson correlation significance of $p < 0.017$. Statistics are ranked according to their significance at each viewing distance. Pearson correlations (r) are also expressed as a percentage.

Viewing distance	Stage in Predation Sequence	Pattern statistic	Pearson Correlation [r] / % [r^2*100]	P value
30cm	Detection / Identification	CAA.Hc.Hrz	-0.85 / 72	<0.001
		CAA.Hc	-0.84 / 71	<0.001
		CAA.Hc.Vrt	-0.84 / 71	<0.001
10cm	Identification / Approach	VCA.MDmax	-0.72 / 52	0.008
		VCA.MDmax.Hrz	-0.72 / 52	0.008
		VCA.MDmax.Vrt	-0.72 / 52	0.008
		VCA.MSsat	-0.71 / 50	0.010
		VCA.MSsat.Hrz	-0.71 / 50	0.010
		VCA.MSsat.Vrt	-0.71 / 50	0.010
5cm	Approach / Subjugation	VCA.MDmax.Hrz	-0.71 / 51	0.010
		VCA.MDmax.Vrt	-0.71 / 51	0.010
		VCA.MDmax	-0.70 / 49	0.011
2cm	Subjugation / Consumption	VCA.MDmax.Hrz	-0.75 / 56	0.005
		VCA.MDmax.Vrt	-0.72 / 52	0.008
		VCA.MDmax	-0.70 / 49	0.011

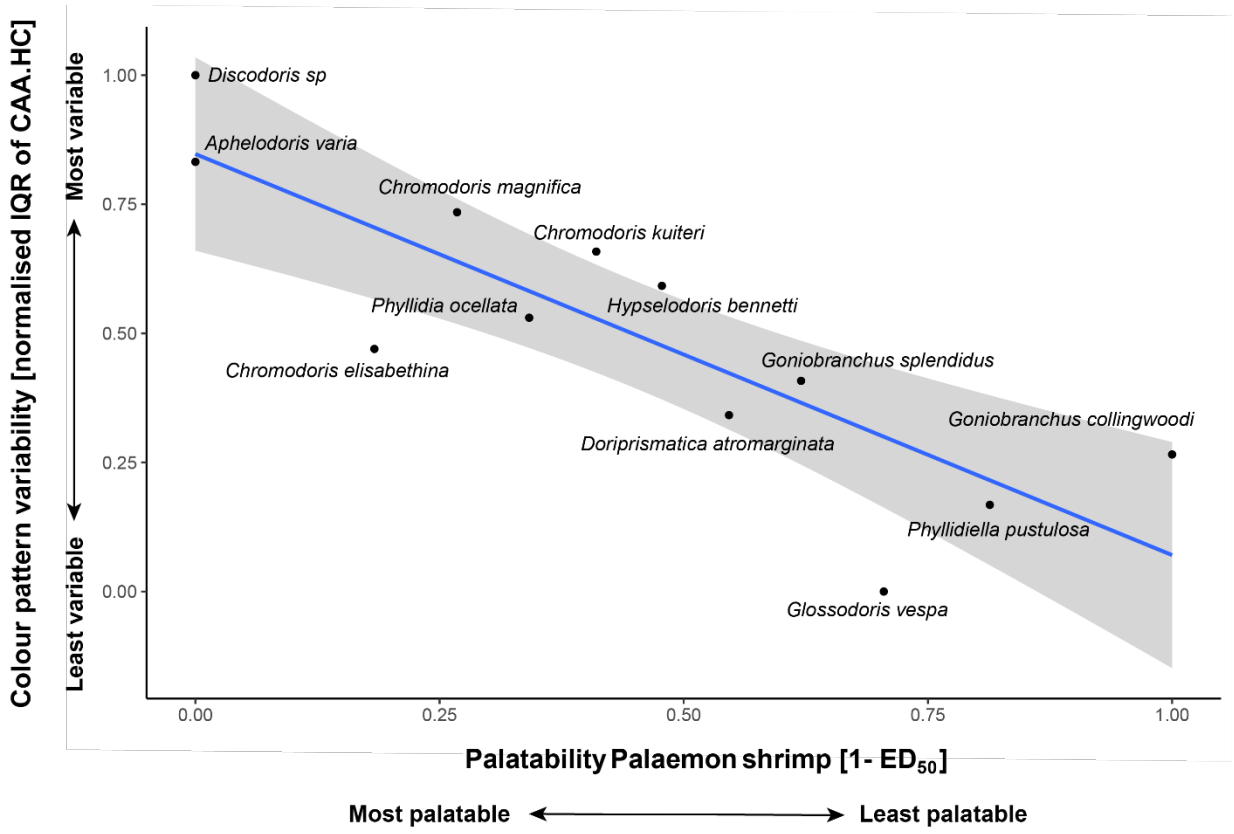

Figure 3. Pattern variability measured as the variability of the Shannon colour diversity index (*CAA.HC*) at 30 cm viewing distance (Pearson correlation: r = -0.84, p = < 0.001). *CAA.HC* calculates the Shannon diversity index of the synonymous (off-diagonal) transitions in the transition matrix, *i.e.* the evenness of colour pattern elements in a pattern (electronic Supplement in van den Berg, Troscianko, et al., 2020).

4. Discussion

Our results indicate that highly defended species exhibit less variable colour patterns than moderately or undefended species. We found that different measures of colour pattern variation, specifically parameters measuring chromatic boldness (*VCA.MSsat & VCA.MDmax*) and colour pattern regularity (*CAA.Hc*), were correlated with unpalatability at different viewing distances. This is intriguing, as chromatic contrast and colour pattern regularity are generally regarded as indicators of potent aposematic signalling (Kenward et al., 2004; Ruxton et al., 2018). Our data does not show any differential importance of the pattern information contained in the horizontal or vertical dimension along the body axis at any viewing distance (Table 1). This suggests that selective pressure imposed on colour pattern variability by differential orientation sensitivity of predatory visual systems (e.g. Endler et al., 2018; Hubel & Wiesel, 1962) may be negligible. In fact, the strongest correlations between colour pattern variability and the strength of chemical defences were found at 30cm where colour pattern regularity (*CAA.Hc*). *CAA.Hc* was 72% less variable in species with strong chemical defences.

The second most significant correlation occurred at a viewing distance of 10cm, where prey identification and discrimination are likely possible. Indeed, colour pattern boldness through internal colour contrast, such as that measured by *VCA.MSsat* and *VCA.MDmax* is a crucial component of the effectiveness of aposematic signalling (Aronsson & Gamberale-Stille, 2009; J. B. Barnett et al., 2016). Given that variability in colour, not luminance, contrast is significantly correlated with the strength of secondary defences further aligns with the importance of colour for object identification and assessment (Osorio & Vorobyev, 2008), a crucial aspect of aposematic signalling. Interestingly, the maximum possible colour contrast between colour pattern elements (*Dmax*) by a triggerfish (*Rhincenathus aculeatus*) almost identically matches the colour contrast calculated using the RNL model (*Ssat*). This suggests that the visual system of *R. aculeatus* is indeed very well adapted to perceive colour pattern contrast in nudibranch molluscs.

We also highlight how distance-dependent signalling in Dorid nudibranchs can facilitate selection on specific aspects of the design and function of aposematic colour patterns (J. B. Barnett & Cuthill, 2015; Endler, 1978; Marshall, 2000; van den Berg, Endler, Papinczak, et al., 2022). Aposematic colour patterns in nudibranchs might have evolved to be cryptic at a distance but bold when viewed up close or bold upon successful detection (J. B. Barnett & Cuthill, 2015; Sherratt, 2002). For example, we found the largest number of correlations

between pattern variation and unpalatability at 10 cm, a distance where fine colour pattern details become apparent (Fig. 2 & 3). Our findings align with the idea of purifying selection shaping the regularity-specific colour pattern elements (as opposed to the entirety of an animal's visual appearance) as context-specific aposematic signals in Dorid nudibranchs (e.g. Winters et al., 2017). Specifically, we show negative correlations between colour pattern variability and the strength of secondary defences to occur for separate colour pattern properties at distinct viewing distances. Thus, our results suggest colour patterns of nudibranch molluscs to comprise true multicomponent signals (Hebets and Papaj, 2005) with distance-dependent functionality.

Our study is unable to assess how and if the observed correlations between traits are heritable or the consequence of phenotypic plasticity, due to the absence of reliable estimates of signal heritability for each of the measured traits. Without such estimates, conclusions of natural selection are complicated as selection affects the phenotypes, not the genotypes of species in a given community (Endler & Greenwood, 1988; Falconer & Mackay, 1996; Lande & Arnold, 1983). Therefore, how and if the observed phenotypes are indeed confounded by the evolutionary history between species remains unknown. However, high degrees of phenotypic plasticity and rapid radiation of species morphology in Dorid nudibranchs are common (e.g. Layton et al., 2018) and the general presence of gene flow across populations in the study area is likely (Winters et al., 2017). For example, *Phyllidia ocellata* is known to be highly variable in appearance across its range (Debelius & Kuiter, 2007), but is found to be very uniform in appearance within our study area (Fig. 3), thus further supporting our assumptions of significant connectivity between sampling locations. Furthermore, it is reasonable to assume similar visual and cognitive abilities of the predator community across the studied area (Losey et al., 2003), as well as the overall similarity of the visual appearance of rocky reef habitats across the area (van den Berg, Endler, & Cheney, 2022a) . Together, these factors indicate our study to quantify variability between species with low levels of phylogenetic confound, consisting of well-connected meta-populations in a comparably homogeneous landscape of selective pressures across the study area.

Existing literature predicts highly defended species to display uniform colour patterns, as consistent appearance across individuals should enhance learning and memorisation of aposematic signals by predators. However, it is not easy to explain why moderately defended species have moderate levels of pattern variation. Moderately defended nudibranchs may have different predators compared to highly defended species. These predators (e.g. crabs) may not

be as reliant on visual signals when attacking prey, so selection for uniform patterns may be relaxed for these species (e.g. Stevens, 2007). Alternatively, attacking moderately defended species is less costly for predators and may occur more frequently, particularly in areas or times where alternative food sources are unavailable, which could weaken predator learning. Finally, moderately defenced species could be found on particularly complex backgrounds, making a uniform colour pattern less effective, again weakening selection on uniformity.

However, reasoning for specific causes underlying the observed correlation is difficult. For example, unlike in other organisms considered in the study of aposematic colouration (e.g. Nokelainen, Valkonen, Lindstedt, & Mappes, 2014; Rönkä et al., 2020) predator communities and their interactions with local populations of nudibranchs beyond anecdotal observations remain poorly understood. Furthermore, many predictions on the direction of selection for or against colour pattern variability in aposematic signals (Endler & Greenwood, 1988; Endler & Mappes, 2004) rely on assumptions on the genetic makeup and thus mechanisms underlying the traits under selection. However, it remains unknown which genes or how many of them are involved in the expression of colour pattern morphology in nudibranchs; a complex trait under multiple selective pressures (e.g. Hebets & Papaj, 2005; Postema, Lippey, & Armstrong-Ingram, 2022). Indeed, genetic mechanisms underlying adaptive processes in defensive colouration remain largely elusive, even for individual components of colour patterns such as spot size or colour variability of a distinct pattern element (but see De Pasqual et al., 2022). Furthermore, how and if environmental factors, such as turbidity, temperature and variability in spatial and seasonal processes (e.g. Cadena, Rankin, Smith, Endler, & Stuart-Fox, 2018; Larkin, Smith, Willan, & Davis, 2018; Smith & Davis, 2019; Schubert & Smith, 2020) interact with such selection pressures and thus variability in phenotype, remains unknown as well. Lastly, the defensive properties of secondary metabolites can be highly context and predator specific (C. A. Barnett et al., 2007; Rojas et al., 2017; Winters et al., 2022). Such context-dependent or predator specific insights do not exist for the study of secondary metabolites in nudibranchs, despite the growing number of identified compounds and generalisable assays.

Therefore, drawing conclusions the specific mechanisms underlying our observation is difficult, especially given the complicated and poorly resolved phylogenetic relations between many nudibranch species discussed previously. Importantly, our understanding of both, human and animal visual perception and cognition are constantly evolving, and with it, our understanding of the ecological significance of signal variation in the context of aposematism. For example, Raviv et al., (2022) recently showed that, in humans, variability in visual stimuli

is actually likely to enhance learning under certain conditions. This is a highly relevant finding for our understanding of selective pressures shaping diversity in defensive animal colouration which is currently not considered in the literature. In fact, perceiving variability as a factor enhancing (rather than inhibiting) animal learning diametrically opposes the general understanding in the existing literature. Importantly, such differentiated views on the complexity of visual perception and cognition, in combination with the known complexity of factors underlying phenotypic variation in aposematic animals, make it rather unsurprising that the strength of secondary defences and colour pattern morphology share a continuous rather than a discrete relationship.

Nudibranchs only have rudimentary visual abilities enabling basic phototaxis (Eakin et al., 1967) and the species observed in this study live in thermally comparably stable aquatic habitats (i.e. no species were collected in rockpools or at shallow depths <2m) experiencing only gradual seasonal fluctuations. Given the multitude of alternative selective pressures such as intraspecific signalling or thermoregulation acting on colour pattern morphology (and thus variability) in traditionally considered study organisms such as poison dart frogs (e.g. Yeager & Barnett, 2022), makes our findings particularly valuable for understanding the potential effects of visual predation on colour pattern variability in aposematic species.

Our study has important implications for quantifying colour pattern variation in complex natural environments by addressing the importance of ecologically relevant observers and their perception of complex, multicomponent visual signals. Colour pattern variability can be observed and quantified in several ways; however, metrics that are perceptually relevant and contributing to the cognitive processes should be used to help inform our knowledge regarding the ecology and evolution of animal colouration (Endler, 1978; Endler & Mappes, 2017; Kemp et al., 2015). To date, few studies investigating colour pattern variability make this distinction beyond the modality of colour (e.g. Olsson et al., 2018; Silvasti et al., 2021; van den Berg, Hollenkamp, et al., 2020b). In the context of a steadily growing array of tools available for the study of animal colour patterns, we emphasize the need for careful consideration of the physiological limits of animal visual systems and the ecologically relevant perceptual context studies investigating quantitative and qualitative aspects of the ecology and evolution of aposematism and defensive animal colouration more broadly. Importantly, we test each colour pattern statistic individually. However, considering colour pattern statistics cumulatively, allowing for interactions between multiple descriptors, is of great interest in future studies (see van den Berg et al., 2022 for discussion).

In summary, we showed that colour pattern variability in aposematic nudibranchs is negatively correlated with the strength of their secondary defences, thus being indicative of quantitative and qualitative signal honesty (Blount et al., 2009; Mappes et al., 2005; Summers et al., 2015; White & Umbers, 2021) in local populations of Dorid nudibranchs belonging to substantially different genera and families. Our findings align with broader indications of quantitative signalling honesty in nudibranchs from existing literature (Cortesi and Cheney, 2010). In fact, the strength of the association between reduced intraspecific colour pattern variability and increased secondary defences is among the strongest indications of quantitative signalling honesty in any aposematic organism observed to date (see White & Umbers, (2021) for review).

Data accessibility

The data can be accessed on UQ's e-space: https://doi.org/10.48610/a596710

Authors' contributions

CPvdB: Primary concept of study, coding, investigation, data analysis, original draft, manuscript writing. JAE: project funding, manuscript writing, review and editing. BD, CS, BD: Image analysis. KLC: concept of study, sample collection, project funding, manuscript writing, review and editing.

Competing interests

We declare we have no competing interests.

Acknowledgements

We would like to thank Dr Nicholas Condon for his assistance with the creation of automated QCPA scripts; various volunteers for assistance with image analysis; the High-Performance Computing (HPC) infrastructure at UQ (Wiener & Awoonga) and infrastructure provided by Simone Blomberg which contributed to the computing of image statistics.

Funding

This work was funded by the Australian Research Council (FT190199313 awarded to KLC and DP180102363 awarded to JAE), Holsworth Wildlife Research Endowment (two grants awarded to CPvdB) and the Australasian Society for the Study of Animal Behaviour (research grant awarded to CPvdB).

References

Aronsson, M., & Gamberale-Stille, G. (2009). Importance of internal pattern contrast and contrast against the background in aposematic signals. *Behavioral Ecology*, *20*(6), 1356–1362. https://doi.org/10.1093/beheco/arp141

Banich, M. T., & Caccamise, D. (2010). Generalization of knowledge : multidisciplinary perspectives / Marie T. Banich and Donna Caccamise, editors. In *Generalization of knowledge : multidisciplinary perspectives*. Psychology Press.

Barnett, C. A., Bateson, M., & Rowe, C. (2007). State-dependent decision making: Educated predators strategically trade off the costs and benefits of consuming aposematic prey. *Behavioral Ecology*, *18*(4), 645–651. https://doi.org/10.1093/beheco/arm027

Barnett, J. B., & Cuthill, I. C. (2015). Distance-dependent defensive coloration. *Current Biology*, *24*(24), R1157–R1158. https://doi.org/10.1016/j.cub.2014.11.015

Barnett, J. B., Michalis, C., Scott-Samuel, N. E., & Cuthill, I. C. (2021). Colour pattern variation forms local background matching camouflage in a leaf-mimicking toad. *Journal of Evolutionary Biology*, *34*(10), 1531–1540. https://doi.org/10.1111/jeb.13923

Barnett, J. B., Scott-Samuel, N. E., & Cuthill, I. C. (2016). Aposematism: balancing salience and camouflage. *Biology Letters*, *12*(8), 20160335. https://doi.org/10.1098/rsbl.2016.0335

Blount, J. D., Speed, M. P., Ruxton, G. D., & Stephens, P. a. (2009). Warning displays may function as honest signals of toxicity. *Proceedings. Biological Sciences / The Royal Society*, *276*(November 2008), 871–877. https://doi.org/10.1098/rspb.2008.1407

Borer, M., Van Noort, T., Rahier, M., & Naisbit, R. E. (2010). Positive frequency-dependent selection on warning color in alpine leaf beetles. *Evolution*, *64*(12), 3629–3633. https://doi.org/10.1111/j.1558-5646.2010.01137.x

Briolat, E. S., Burdfield-Steel, E. R., Paul, S. C., Katja, H. R., Seymoure, B. M., Stankowich, T., & Stuckert, A. M. M. (2019). Diversity in warning coloration: selective paradox or the norm? *Biological Reviews*, *94*(2), 388–414. https://doi.org/10.1111/brv.12460

Brower, L. P., Ryerson, W. N., Coppinger, L. L., & Glazier, S. C. (1968). Ecological chemistry and the palatability spectrum. *Science*, *161*(3848), 1349–1351. https://doi.org/10.1126/science.161.3848.1349

Cadena, V., Rankin, K., Smith, K. R., Endler, J. A., & Stuart-Fox, D. (2018). Temperature-induced colour change varies seasonally in bearded dragon lizards. *Biological Journal of the Linnean Society*, *123*(2), 422–430. https://doi.org/10.1093/biolinnean/blx152

Champ, C. M., Vorobyev, M., & Marshall, N. J. (2016). Colour thresholds in a coral reef fish. *Royal Society Open Science*, *3*(9), 160399. https://doi.org/10.1098/rsos.160399

Champ, C. M., Wallis, G., Vorobyev, M., Siebeck, U., & Marshall, J. (2014). Visual acuity in a species of coral reef fish: *Rhinecanthus aculeatus*. *Brain, Behavior and Evolution*, *83*(1), 31–42. https://doi.org/10.1159/000356977

Chan, W. (2022). The Chemical Ecology of Nudibranchs. In *UQ espace*.

Chan, W., Shaughnessy, A. E. P., van den Berg, C. P., Garson, M. J., & Cheney, K. L. (2021). The Validity of Brine Shrimp (Artemia Sp.) Toxicity Assays to Assess the Ecological Function of Marine Natural Products. *Journal of Chemical Ecology*, *47*(10–11), 834–846. https://doi.org/10.1007/s10886-021-01264-z

Cheney, K. L., Cortesi, F., How, M. J., Wilson, N. G., Blomberg, S. P., Winters, A. E., Umanzör, S., & Marshall, N. J. (2014). Conspicuous visual signals do not coevolve with increased body size in marine sea slugs. *Journal of Evolutionary Biology*, *27*(4), 676–687. https://doi.org/10.1111/jeb.12348

Cheney, K. L., Green, N. F., Vibert, A. P., Vorobyev, M., Marshall, N. J., Osorio, D. C., & Endler, J. A. (2019). An Ishihara-style test of animal colour vision. *Journal of Experimental Biology*, *222*(1), jeb189787. https://doi.org/10.1242/jeb.189787

Cheney, K. L., Hudson, J., de Busserolles, F., Luehrmann, M., Shaughnessy, A., van den Berg, C., Green, N. F., Marshall, N. J., & Cortesi, F. (2022). Seeing Picasso: an investigation into the visual system of the triggerfish *Rhinecanthus aculeatus*. *Journal of Experimental Biology*, *225*(7), jeb243907. https://doi.org/10.1242/jeb.243907

Cheney, K. L., Newport, C., McClure, E. C., & Marshall, N. J. (2013). Colour vision and response bias in a coral reef fish. *The Journal of Experimental Biology*, *216*(15), 2967–2973. https://doi.org/10.1242/jeb.087932

Cheney, K. L., White, A., Mudianta, I. W., Winters, A. E., Quezada, M., Capon, R. J., Mollo, E., & Garson, M. J. (2016). Choose your weaponry: Selective storage of a single toxic compound, latrunculin a, by closely related nudibranch molluscs. *PLoS ONE*, *11*(1), 1–16. https://doi.org/10.1371/journal.pone.0145134

Clarke, B., & O'Donald, P. (1964). Frequency-dependent selection. *Heredity*, *19*(2), 201–206. https://doi.org/10.1038/hdy.1964.25

Cortesi, F., & Cheney, K. L. (2010). Conspicuousness is correlated with toxicity in marine opisthobranchs. *Journal of Evolutionary Biology*, *23*(7), 1509–1518. https://doi.org/10.1111/j.1420-9101.2010.02018.x

De Pasqual, C., Suisto, K., Kirvesoja, J., Gordon, S., Ketola, T., & Mappes, J. (2022). Heterozygote advantage and pleiotropy contribute to intraspecific color trait variability. *Evolution*, 2389–2403. https://doi.org/10.1111/evo.14597

Debelius, H., & Kuiter, R. H. (2007). *Nudibranchs of the world*. IKAN-Unterwasserarchiv.

Eakin, R. M., Westfall, J. A., & Dennis, M. J. (1967). Fine Structure of the Eye of a Nudibranch Mollusc, *Hermissenda Crassicornis*. *Journal of Cell Science*, *2*(3), 349–358. https://doi.org/10.1242/jcs.2.3.349

Endler, J. A. (1978). A predator's view of animal color patterns. *Evolutionary Biology*, *11*(5), 320–364. https://doi.org/10.1007/978-1-4615-6956-5_5

Endler, J. A. (1986). Defense Against Predators. In *Predator-prey relationships, Perspectives and Approaches from the Study of Lower Vertebrates* (pp. 109–134). University of Chicago Press.

Endler, J. A. (1991). Interactions between predators and prey. In J. R. Krebs & N. B. Davies (Eds.), *Behavioural Ecology* (pp. 169–196). Blackwell Scientific.

Endler, J. A. (2012). A framework for analysing colour pattern geometry: Adjacent colours. *Biological Journal of the Linnean Society*, *107*(2), 233–253. https://doi.org/10.1111/j.1095-8312.2012.01937.x

Endler, J. A., Cole, G. L., & Kranz, A. M. (2018). Boundary strength analysis: Combining colour pattern geometry and coloured patch visual properties for use in predicting behaviour and fitness. *Methods in Ecology and Evolution*, *9*(12), 2334–2348. https://doi.org/10.1111/2041-210X.13073

Endler, J. A., & Greenwood, J. J. D. (1988). Frequency-dependent predation, crypsis and aposematic coloration. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *319*(1196), 505–523. https://doi.org/10.1098/rstb.1988.0062

Endler, J. A., & Houde, A. E. (1995). Geographic variation in female preferences for male traits in *Poecilia reticulata*. *Evolution*, *49*(3), 456. https://doi.org/10.2307/2410270

Endler, J. A., & Mappes, J. (2004). Predator Mixes and the Conspicuousness of Aposematic Signals. *The American Naturalist*, *163*(4), 532–547. https://doi.org/10.1086/382662

Endler, J. A., & Mappes, J. (2017). The current and future state of animal coloration research.

Philosophical Transactions of the Royal Society B: Biological Sciences, *372*(1724), 20160352. https://doi.org/10.1098/rstb.2016.0352

Endler, J. A., & Mielke, P. W. (2005). Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society*, *86*(4), 405–431. https://doi.org/10.1111/j.1095-8312.2005.00540.x

Falconer, D. S., & Mackay, T. F. C. (1996). *Introduction to quantitative genetics.* (4th ed. /). Longman.

Fisher, R. A. (1930). *The genetical theory of natural selection.* Clarendon Press. https://doi.org/10.5962/bhl.title.27468

Gamberale, G., & Tullberg, B. S. (1996). Evidence for a peak-shift in predator generalization among aposematic prey. *Proceedings. Biological Sciences / The Royal Society*, *263*(1375), 1329–1334. https://doi.org/10.1098/rspb.1996.0195

Green, N. F., Guevara, E., Osorio, D. C., Endler, J. A., Marshall, N. J., Vorobyev, M., & Cheney, K. L. (2022). Colour discrimination thresholds vary throughout colour space in a reef fish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, *225*(7), jeb243533. https://doi.org/10.1242/jeb.243533

Greenwood, J. J. D. (1985). *Frequency-Dependent Selection by Seed-Predators*. *44*(1), 17–22.

Guilford, T. (1985). Is Kin Selection Involved in the Evolution of Warning Coloration? *Oikos*, *45*(1), 31. https://doi.org/10.2307/3565219

Hebets, E. A., & Papaj, D. R. (2005). Complex signal function: Developing a framework of testable hypotheses. *Behavioral Ecology and Sociobiology*, *57*(3), 197–214. https://doi.org/10.1007/s00265-004-0865-7

Hempel de Ibarra, N., Giurfa, M., & Vorobyev, M. (2001). Detection of coloured patterns by honeybees through chromatic and achromatic cues. *Journal of Comparative Physiology A: Sensory, Neural, and Behavioral Physiology*, *187*(3), 215–224. https://doi.org/10.1007/s003590100192

Hubel, D. H., & Wiesel, T. N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *The Journal of Physiology*, *160*(1), 106–154. https://doi.org/10.1113/jphysiol.1962.sp006837

Karpestam, E., Merilaita, S., & Forsman, A. (2014). Natural levels of colour polymorphism reduce performance of visual predators searching for camouflaged prey. In *Biological Journal of the Linnean Society* (Vol. 112). https://doi.org/https://doi.org/10.1111/bij.12276

Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T. D., Dyer, A. G., Hart, N. S., Marshall, N. J., & Whiting, M. J. (2015). An integrative framework for the

appraisal of coloration in nature. *The American Naturalist*, *185*(6), 705–724. https://doi.org/10.1086/681021

Kenward, B., Wachtmeister, C. A., Ghirlanda, S., & Enquist, M. (2004). Spots and stripes: The evolution of repetition in visual signal form. *Journal of Theoretical Biology*, *230*, 407–419. https://doi.org/10.1016/j.jtbi.2004.06.008

Kuhn, M. (2021). *caret: Classification and Regression Training. R package version 6.0-90. https://CRAN.R-project.org/package=caret* (6.0-88). https://cran.r-project.org/package=caret

Lande, R., & Arnold, S. J. (1983). The Measurement of Selection on Correlated Characters. *Evolution*, *37*(6), 1210. https://doi.org/10.2307/2408842

Larkin, M. F., Smith, S. D. A., Willan, R. C., & Davis, T. R. (2018). Diel and seasonal variation in heterobranch sea slug assemblages within an embayment in temperate eastern Australia. *Marine Biodiversity*, *48*(3), 1541–1550. https://doi.org/10.1007/s12526-017-0700-9

Mallet, J. L. B., & Barton, N. H. (1989). Strong Natural Selection in a Warning-Color Hybrid Zone. *Evolution*, *43*(2), 421. https://doi.org/10.2307/2409217

Mappes, J., Marples, N., & Endler, J. A. (2005). The complex business of survival by aposematism. *Trends in Ecology and Evolution*, *20*(11), 598–603. https://doi.org/10.1016/j.tree.2005.07.011

Marshall, N. J. (2000). Communication and camouflage with the same 'bright' colours in reef fishes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *355*(1401), 1243–1248. https://doi.org/10.1098/rstb.2000.0676

Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, *100*(2), 403–405. https://doi.org/10.1034/j.1600-0706.2003.12010.x

Nokelainen, O., Galarza, J. A., Kirvesoja, J., Suisto, K., & Mappes, J. (2022). Genetic colour variation visible for predators and conspecifics is concealed from humans in a polymorphic moth. *Journal of Evolutionary Biology*, *January*, 1–12. https://doi.org/10.1111/jeb.13994

Nokelainen, O., Valkonen, J., Lindstedt, C., & Mappes, J. (2014). Changes in predator community structure shifts the efficacy of two warning signals in Arctiid moths. *Journal of Animal Ecology*, *83*(3), 598–605. https://doi.org/10.1111/1365-2656.12169

Olsson, P., Lind, O., & Kelber, A. (2018). Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. *Behavioral Ecology*, *29*(2), 273–282. https://doi.org/10.1093/beheco/arx133

Osorio, D., & Vorobyev, M. (2008). A review of the evolution of animal colour vision and visual communication signals. *Vision Research*, *48*, 2042–2051.

https://doi.org/10.1016/j.visres.2008.06.018

Peterson, R. A., & Cavanaugh, J. E. (2020). Ordered quantile normalization: a semiparametric transformation built for the cross-validation era. *Journal of Applied Statistics*, *47*(13–15), 2312–2327. https://doi.org/10.1080/02664763.2019.1630372

Pignatelli, V., Champ, C. M., Marshall, J., & Vorobyev, M. (2010). Double cones are used for colour discrimination in the reef fish, *Rhinecanthus aculeatus*. *Biology Letters*, *6*(4), 537–539. https://doi.org/10.1098/rsbl.2009.1010

Postema, E. G., Lippey, M. K., & Armstrong-Ingram, T. (2022). Color under pressure: how multiple factors shape defensive coloration. *Behavioral Ecology*, 1–13. https://doi.org/10.1093/beheco/arac056

Poulton, E. B. (1890). *The Colours of Animals*. Kegan Paul, Trench & Trubner.

R Core Team. (2021). *R: A Language and Environment for Statistical Computing* (4.1.). R Foundation for Statistical Computing. http://www.r-project.org/

Randall, J. E., Allen, G. R., & Steene, R. C. (1997). *Fishes of the Great Barrier Reef and Coral Sea*. Crawford House Publishing.

Raviv, L., Lupyan, G., & Green, S. C. (2022). How variability shapes learning and generalization. *Trends in Cognitive Sciences*, *26*(6), 462–483. https://doi.org/10.1016/j.tics.2022.03.007

Rojas, B., Burdfield-Steel, E., Pakkanen, H., Suisto, K., Maczka, M., Schulz, S., & Mappes, J. (2017). How to fight multiple enemies: Target-specific chemical defences in an aposematic moth. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1863). https://doi.org/10.1098/rspb.2017.1424

Rönkä, K., De Pasqual, C., Mappes, J., Gordon, S., & Rojas, B. (2018). Colour alone matters: no predator generalization among morphs of an aposematic moth. *Animal Behaviour*, *135*, 153–163. https://doi.org/10.1016/j.anbehav.2017.11.015

Rönkä, K., Valkonen, J., Nokelainen, O., Rojas, B., Gordon, S., Burdfield-Steel, E., & Mappes, J. (2020). Geographic mosaic of selection by avian predators on hindwing warning colour in a polymorphic aposematic moth. *Ecology Letters*. https://doi.org/10.1101/2020.04.08.032078

Rosselli, F. B., Alemi, A., Ansuini, A., & Zoccolan, D. (2015). Object similarity affects the perceptual strategy underlying invariant visual object recognition in rats. *Frontiers in Neural Circuits*, *9*(MAR), 1–22. https://doi.org/10.3389/fncir.2015.00010

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, *58*(5), 921–931. https://doi.org/10.1006/anbe.1999.1242

Ruxton, G. D., Allen, W. L., Sherratt, T. N., & Speed, M. P. (2018). *Avoiding Attack* (Vol. 1).

Oxford University Press. https://doi.org/10.1093/oso/9780199688678.001.0001

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, *9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Schubert, J., & Smith, S. D. A. (2020). Sea slugs—"rare in space and time"—but not always. *Diversity*, *12*(11), 1–14. https://doi.org/10.3390/d12110423

Sherratt, T. N. (2002). The coevolution of warning signals. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1492), 741–746. https://doi.org/10.1098/rspb.2001.1944

Sherratt, T. N., Speed, M. P., & Ruxton, G. D. (2004). Natural selection on unpalatable species imposed by state-dependent foraging behaviour. *Journal of Theoretical Biology*, *228*(2), 217–226. https://doi.org/10.1016/j.jtbi.2003.12.009

Sibeaux, A., Cole, G. L., & Endler, J. A. (2019). The relative importance of local and global visual contrast in mate choice. *Animal Behaviour*, *154*, 143–159. https://doi.org/10.1016/j.anbehav.2019.06.020

Silvasti, S. A., Valkonen, J. K., & Nokelainen, O. (2021). Behavioural thresholds of blue tit colour vision and the effect of background chromatic complexity. *Vision Research*, *182*(June 2020), 46–57. https://doi.org/10.1016/j.visres.2020.11.013

Smith, S. D. A., & Davis, T. R. (2019). Slugging it out for science: volunteers provide valuable data on the diversity and distribution of heterobranch sea slugs. *Molluscan Research*, *39*(3), 214–223. https://doi.org/10.1080/13235818.2019.1594600

Speed, M. P., & Turner, J. R. G. (1999). Learning and memory in mimicry: II. Do we understand the mimicry spectrum? *Biological Journal of the Linnean Society*, *67*(3), 281–312. https://doi.org/10.1006/bijl.1998.0310

Stevens, M. (2007). Predator perception and the interrelation between different forms of protective coloration. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1617), 1457–1464. https://doi.org/10.1098/rspb.2007.0220

Summers, K., Speed, M. P., Blount, J. D., & Stuckert, A. M. M. (2015). Are aposematic signals honest? A review. *Journal of Evolutionary Biology*, *28*(9), 1583–1599. https://doi.org/10.1111/jeb.12676

Troscianko, J., & Stevens, M. (2015). Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution*, *6*(11), 1320–1331. https://doi.org/10.1111/2041-210X.12439

Turner, J. R. G. (1981). Adaptation and Evolution in Heliconius: A Defense of NeoDarwinism. *Annual Review of Ecology and Systematics*, *12*(1), 99–121. https://doi.org/10.1146/annurev.es.12.110181.000531

Turner, J. R. G. (1984). Mimicry: The Palatability Spectrum and its Consequences. *Symposia of the Royal Entomological Society of London*, 142–161.

van den Berg, C. P., Endler, J. A., & Cheney, K. L. (2022a). Highly defended nudibranchs do not 'escape' to visually diverse background habitats. *BioRxiv*. https://doi.org/https://doi.org/10.1101/2022.12.13.520332

van den Berg, C. P., Endler, J. A., & Cheney, K. L. (2022b). Signal detectability and boldness are not the same: the function of defensive colouration in nudibranchs is distance-dependent. *BioRxiv*. https://doi.org/https://doi.org/10.1101/2022.12.20.521213

van den Berg, C. P., Endler, J. A., Papinczak, D. E. J., & Cheney, K. L. (2022). Using colour pattern edge contrast statistics to predict detection speed and success in triggerfish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.244677

van den Berg, C. P., Hollenkamp, M., Mitchell, L. J., Watson, E. J., Green, N. F., Marshall, N. J., & Cheney, K. L. (2020). More than noise: Context-dependant luminance contrast discrimination in a coral reef fish (*Rhinecanthus aculeatus*). *Journal of Experimental Biology*, *223*(21), jeb.232090. https://doi.org/10.1242/jeb.232090

van den Berg, C. P., Troscianko, J., Endler, J. A., Marshall, N. J., & Cheney, K. L. (2020). Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature. *Methods in Ecology and Evolution*, *11*(2), 316–332. https://doi.org/10.1111/2041-210X.13328

White, T. E., & Umbers, K. D. L. (2021). Meta-analytic evidence for quantitative honesty in aposematic signals. *Proceedings of the Royal Society B: Biological Sciences*, *288*(1949). https://doi.org/10.1098/rspb.2021.0679

Winters, A. E., Chan, W., White, A. M., Berg, C. P., Garson, M. J., & Cheney, K. L. (2022). Weapons or deterrents? Nudibranch molluscs use distinct ecological modes of chemical defence against predators. *Journal of Animal Ecology*, *91*(4), 831–844. https://doi.org/10.1111/1365-2656.13643

Winters, A. E., White, A. M., Dewi, A. S., Mudianta, I. W., Wilson, N. G., Forster, L. C., Garson, M. J., & Cheney, K. L. (2018). Distribution of Defensive Metabolites in Nudibranch Molluscs. *Journal of Chemical Ecology*, *44*(4), 384–396. https://doi.org/10.1007/s10886-018-0941-5

Yeager, J., & Barnett, J. B. (2022). Continuous Variation in an Aposematic Pattern Affects Background Contrast, but Is Not Associated With Differences in Microhabitat Use. *Frontiers in Ecology and Evolution*, *10*(March), 1–10.

https://doi.org/10.3389/fevo.2022.803996