Highly defended nudibranchs do not 'escape' to visually diverse background habitats

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Abstract

1. The 'escape and radiate' hypothesis predicts that once species have evolved aposematism, defended species can utilise more visually diverse background habitats as they 'escape' the need to be well camouflaged. This enables species to explore new ecological niches, resulting in increased diversification rates.
2. To test the 'escape' component of this hypothesis, we examined whether the background habitats of 13 nudibranch mollusc species were more variable for highly defended species (in terms of unpalatability), compared to undefended (palatable) species.
3. We obtained 157 colour pattern statistics from the Quantitative Colour Pattern Analysis (QCPA) framework to analyse backgrounds viewed through the eyes of a potential predator (triggerfish, *Rhinecanthus aculeatus*). This was done at viewing distances simulating an escalating predation sequence.
4. Contrary to the hypothesis, we found no significant positive correlations between the diversity of visual backgrounds and the strength of chemical defences, but instead found numerous significant negative correlations. Highly defended species were viewed against backgrounds up to 56.9% less variable than non-defended species. Furthermore, the strongest correlations were found at viewing distances coinciding with likely prey detection and identification.

5. Our results provide empirical evidence against background generalisation in aposematic nudibranch species. Instead, we suggest that less variable visual backgrounds may strengthen aposematic signalling.

Keywords

Aposematism, camouflage, defensive animal colouration, escape and radiate hypothesis, signal honesty, visual ecology, visual modelling, warning signals

Introduction

Animals and plants use bold aposematic colour patterns for advertising defences to potential predators (Poulton, 1890). One of the hypothesised benefits of an organism evolving aposematism is the potential to 'escape' the costs of crypsis, including limited movement and thus access to resources due to the need to match a specific background habitat or preventing detection through motion contrast for primary defence (Endler, 1984; Merilaita & Tullberg, 2005; Regan & Beverley, 1984). Therefore, Merilaita & Tullberg (2005) suggested that alternative visual defence strategies, such as aposematism, are more likely to evolve in highly variable visual environments, where crypsis is difficult to achieve. The evolution of aposematic signalling then provides increased access to resources across ecological niches as individuals are not restricted to particular habitats to avoid predator detection. This reduced dependency on crypsis may then drive increased rates of speciation and diversification (Arbuckle & Speed, 2015; Ehrlich & Raven, 1964). This process was coined 'escape and radiate' by Thompson (1989) and has since been adopted as a core concept underpinning the importance of chemical defences for adaptive radiations (e.g. Arbuckle & Harris, 2021).

Theoretical modelling supports the conclusion that aposematic species are less constrained by the visual properties of their habitat (habitat generalists) than undefended background-matching species (habitat specialists) (Merilaita et al., 1999; Speed et al., 2010). However, empirical evidence for this in natural systems across multiple species is limited. Here, we tested the hypothesis that more defended species inhabit more variable habitats using 13 species of co-occurring Eastern Australian nudibranch species (see Fig. S1 for images of all species). Nudibranchs are an intriguing system for the study of defensive animal colouration due to the presence of potent chemical defences in many species (Avila, 1995; Winters et al., 2018, 2022), and colour patterns that range from bold aposematic displays to near-perfect camouflage (Debelius & Kuiter, 2007). Indeed, the evolution of specialised glands (mantle dermal formations) for the storage and secretion of defensive chemicals (Carbone et

al., 2013; Wägele et al., 2006), in combination with the evolution of aposematic signalling, may have led to an adaptive radiation in nudibranchs (Gosliner, 2001).

Until recently, studies quantifying visual backgrounds in the context of defensive animal colouration have relied on the independent consideration of spatial and spectral (colour and luminance) properties (e.g. Cortesi & Cheney, 2010; Nokelainen et al., 2021). In this study, we considered these pattern components in combination (van den Berg, Troscianko, et al., 2020) using calibrated digital photography, the multispectral image calibration and analysis toolbox (MICA) (Troscianko & Stevens, 2015) and its' integrated Quantitative Colour Pattern Analysis (QCPA) framework (van den Berg, Troscianko, et al., 2020). This enabled us to assess the spatiochromatic information of natural backgrounds upon which nudibranch individuals (n = 226) were found. QCPA provides a descriptive array of image statistics capturing the spatiochromatic properties of each background. We analysed backgrounds using the intraspecific interquartile range (IQR) of colour pattern parameters, including: 1) colour adjacency analysis (CAA), which describes pattern geometry; 2) visual contrast analysis (VCA), which describes pattern boldness based on pattern element properties; and 3) boundary strength analysis (BSA), which describes the contrast of boundaries between pattern elements. This was performed according to the physiological limitations of an ecologically relevant observer, a triggerfish (*Rhinecanthus aculeatus*). We analysed different viewing distances to model an escalating predation sequence to account for changes in background appearance as a predator approaches.

We then compared these colour pattern parameters to measures of the strength of chemical defence for each species, which ranged from highly unpalatable to palatable using previously published data from antifeedant assays with rockpool shrimp (*Palaemon serenus*) (Winters et al., 2022). We hypothesised that highly defended (unpalatable) species would be found on more variable visual backgrounds, and weakly defended species (palatable) would be found on backgrounds that were less variable. Furthermore, we predicted that we would find a stronger correlation between the level of defence and habitat variability at distances where prey detection and identification are most likely (Endler, 1986, 1991; Ruxton et al., 2018), rather than their subjugation or consumption.

Materials and Methods

Study species. We took calibrated digital photographs of 226 Dorid nudibranchs belonging to 13 species (8-27 individuals per species, median = 16) (Supplementary Information, Fig. S1) from four locations on the east coast of Australia: 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. Species were identified visually using various nudibranch ID guides (Coleman et al., 2015; Debelius & Kuiter, 2007; Gosliner et al., 2018): *Phyllidia varicosa* (n = 8); *Phyllidiella pustulosa* (n = 18); *Phyllidia elegans* (n = 8); *Phyllidia ocellata* (n = 23); *Goniobranchus collingwoodi* (n = 15); *Goniobranchus splendidus* (n = 25); *Glossodoris vespa* (n = 15); *Doriprismatica atromarginata* (n = 27), *Hypselodoris bennetti* (n = 10); *Chromodoris kuiteri* (n = 17); *Chromodoris elisabethina* (N = 21); *Discodoris sp.* (n = 15); *Aphelodoris varia* (n = 24). *Discodoris sp*. in our study may have constituted a mixture of closely related species, including *Sebadoris fragilis*, *Jorunna pantheris, Tayuva lilacina* and undescribed species; however, these species are visually indiscriminable. Therefore, they are named *Discodoris sp.* as they were found in the same locations, have no known chemical defences and are predominantly nocturnal (Larkin et al., 2018). Only three out of the 13 species (*Doriprismatica atromarginata, Goniobranchus splendidus, Hypselodoris obscura*) were found in comparable numbers across sites in NSW and SE QLD, with most species found either in NSW or SE QLD (see Supplementary Information, Table S1). Nudibranchs were photographed underwater against their natural habitat using a calibrated Olympus EPL-5 with a 60mm macro lens in an Olympus PT-EP10 underwater housing using white LED illumination from a combination of VK6r and PV62 Scubalamp video lights (van den Berg, Troscianko, et al., 2020; see Supplementary Information Fig. S2 for spectral sensitivities of camera, spectral output of LED lights, and irradiance spectrum of light environment at 5 m). All pictures were taken at roughly a 90-degree angle relative to each animal and its background. Examples of images are provided in Fig. 1.

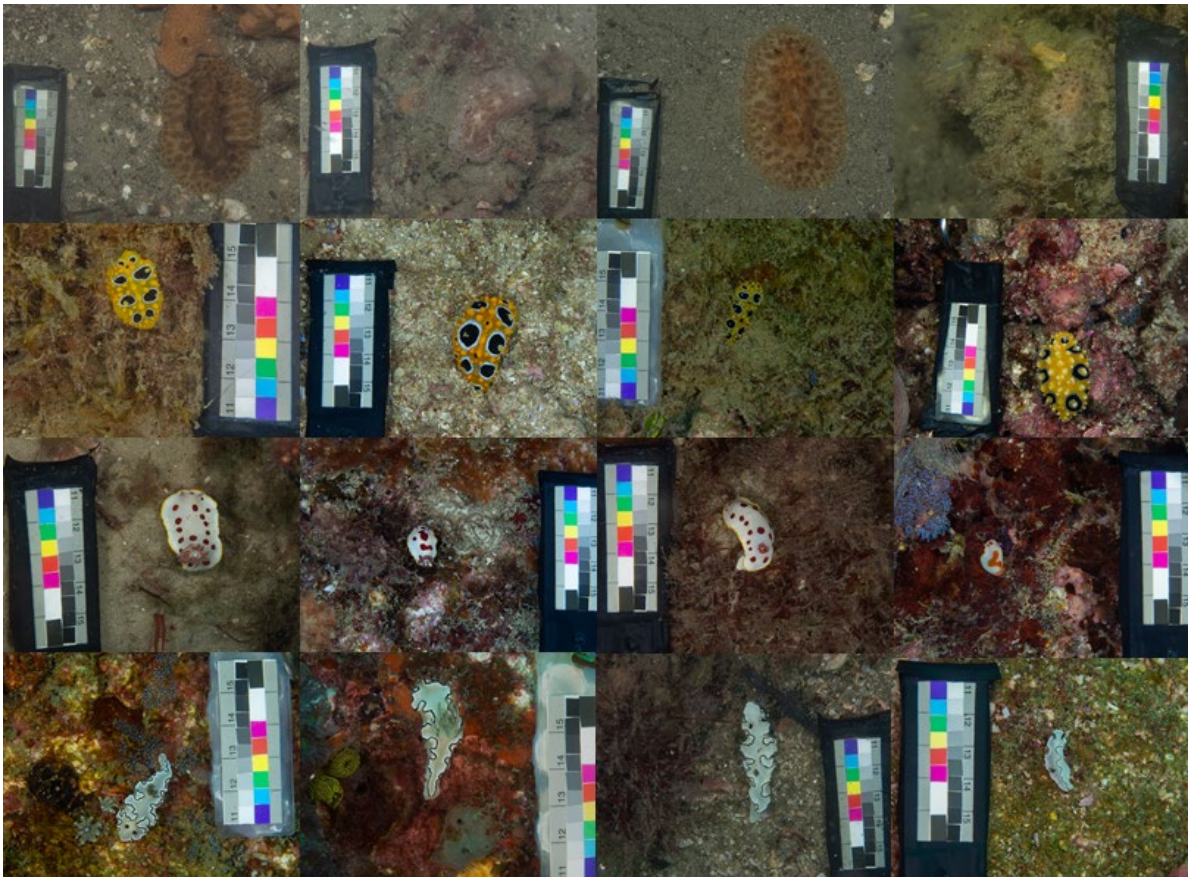

Figure 1. Representative nudibranch and background images for 4 out of the 13 species in this study. From top to bottom: *Discodoris sp., Phyllidia ocellata, Goniobranchus splendidus, Doriprismatica atromarginata.*

Image analysis. Using ImageJ (Schneider et al., 2012) and the MICA toolbox (Troscianko & Stevens, 2015), the images were manually segmented into regions of interest (ROI), selecting the animal from its background and defining a size standard using a custom-made colour and grey standard placed in each image (Fig. 1). All images were aligned, so the nudibranch was aligned vertically before analysis using QCPA (van den Berg, Troscianko, et al., 2020). To analyse the images, we used the visual system parameters of a trichromatic triggerfish, *Rhinecanthus aculeatus* (with spectral sensitivities of 413 nm, 480 nm and 528 nm λ_{max}; Cheney et al. 2022). This species (adult total length, TL ~ 15 cm) is a common shallow reef inhabitant found throughout the Indo-Pacific and feeds on invertebrates, algae, and detritus (Randall et al., 1997) and is deemed a potential predator of nudibranchs. We analysed for viewing distances of 2 cm, 5 cm, 10 cm, 30 cm and 50 cm assuming a triggerfish spatial acuity of 3 cycles per degree (Champ et al., 2014). The viewing distances reflect a range covering the maximal distance at which the largest specimen and coarse background detail would be detectable to the closest possible distance reflecting ultimate contact between predator and prey or visual background.

127 Following acuity modelling, the images were processed with a Receptor Noise Limited (RNL) ranked
128 filter (falloff: 3, radius: 5, repetition: 5) and clustered using RNL clustering with a colour discrimination
129 threshold of 2 ΔS (Cheney et al., 2019) and a luminance contrast threshold of 4 ΔS (van den Berg,
130 Hollenkamp, et al., 2020). We used Weber fractions based on a relative photoreceptor abundance of
131 1:2:2:2 (sw:mw:lw:dbl) and photoreceptor noise of 0.05, resulting in 0.07:0.05:0.05:0.05.

132 QCPA analysis was achieved using a custom batch script (currently available on request; in
133 preparation). This resulted in a highly descriptive array of 157 colour pattern statistics for each animal,
134 named with their respective prefixes. Wherever pattern statistics could not be calculated, Infinite or
135 NA values were treated as 0. All pattern analyses used a segmented image, and measured transitions
136 between pixels along horizontal and vertical sampling transects in a transition matrix (as per (Endler,
137 2012). Statistics ending with 'hrz' or 'vrt' are the horizontal (across body axis) and vertical (along body
138 axis) versions (analysing the respective transition matrix only) of their respective statistic (analysing
139 the full transition matrix). For example, a background with algae or seagrass will likely have an
140 elongated aspect ratio, whereas a uniform sandy background will not. A detailed description of each
141 pattern statistic is found in van den Berg, Troscianko, et al. (2020).

142 **Level of chemical defence**. As a measure of chemical defence for each species, we used our previously
143 published data from antifeedant assays with rockpool shrimp (*Palaemon serenus*) (Winters et al.,
144 2022). Assay data for *Glossodoris vespa* is presented in Winters *et al.* (2018). In summary, the data
145 were obtained from extracting secondary metabolites from nudibranchs and added to food pellets
146 made from squid mantle at increasing concentrations. Effective dose (ED_{50}) values were calculated
147 based on the concentration that elicited a rejection response in at least 50% of the shrimp. For this
148 study, the resulting ED_{50} values were normalised and calculated as 1-ED_{50} to range from 0 (most
149 palatable) to 1 (least palatable). Where multiple estimates of ED_{50} existed for a species due to multiple
150 extracts/assays being performed, the average value was used.

151 **Statistical analysis.** All statistical analyses were conducted using R Software (R Core Team, 2021)
152 (v4.1.2). To quantify background variability, we calculated the interquartile range (IQR) for each
153 pattern statistic of each species. All statistics were then normalised to range from 0 (least variable) to
154 1 (most variable), with all trait data subjected to an ordered quantile normalisation (Peterson &
155 Cavanaugh, 2020) using the bestNormalize package (v1.8.2). We first assessed whether there were
156 colour pattern differences in background between populations for those species that were found
157 across all four locations (*G. splendidus and D. atromarginata*); however, we found no significant
158 differences between Queensland and New South Wales locations in the mean IQR across all 157
159 parameters, using species and viewing distance as a random factor (lmer, $F_{1,17}$ = 1.56, p = 0.22). To

investigate whether there was a phylogenetic correlation between our pattern statistics and palatability, we pruned the phylogeny from Cheney et al. (2014) using the 'ape' package (Paradis & Schliep, 2019) (v5.6-1) with *Aphelodoris varia* added manually according to its taxonomic classification in the World Register of Marine Species (WoRMS) (Appeltans et al., 2012). However, the small number of species made it impossible to estimate the phylogenetic signal reliably using phylogenetic least square regression (pgls). As a result, we estimated a phylogenetic signal close to 0 across all viewing distances when analysing the median IQR across all 157 pattern statistics. This finding is in line with previous studies (Cheney et al., 2014; Cortesi & Cheney, 2010), which have determined a very small phylogenetic signal across various image statistics and Dorid nudibranch species, meaning that closely related species can look considerably different. Therefore, we analysed the data assuming a negligible phylogenetic signal using a Pearson correlation test in the 'caret' package (Kuhn, 2021) (v6.0-90). As all pattern statistics are defined by 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 (Moran, 2003), resulting in an adjusted significance threshold of $p = 0.017$.

Results

The intraspecific Interquartile-Range (IQR) of many visual background colour pattern statistics was negatively correlated with the level of unpalatability at each viewing distance (Table 1), indicating that the visual backgrounds of unpalatable (defended) species were less variable than the background of palatable (undefended) species. We did not find any significant positive correlations in our data. Below, we report both significant values of negative correlations ($r < -0.65$, $p < 0.017$), but also non-significant moderate correlations ($r < -0.4$ or $r > 0.4$, $p = < 0.18$). To reflect the behavioural context of predator-prey interactions, we present the results from the largest viewing distance to the smallest to replicate an escalating predation sequence (Endler, 1991).

At 50 cm, only coarse background details could be resolved by the visual system of our triggerfish predator. We found no significant negative correlations passing the Bonferroni correction (Table 1, Table S2); however, there were moderate negative correlations for 25 pattern statistics with unpalatability (Pearson correlation, $r < -0.4$, $p = < 0.18$). We did not find any significant positive correlations, and there were no moderate positive correlations (all $r < 0.34$, $p > 0.29$) (Table S2).

At 30 cm, four out of 157 pattern statistics were significantly negatively correlated with unpalatability (Pearson correlation: $r < -0.5$, $p < 0.017$; Table 1). All significantly correlated pattern statistics at 30 cm indicated more unpalatable species to be found on backgrounds with lower

variability in their geometric complexity along the horizontal axis relative to an animal (CAA.C.Hrz) as well as reduced variability in the coefficient of variation of the luminance contrast (ΔS) relative to the patch sizes in each background (VCA.CVSL) (Table 1). There were moderate negative correlations for 47 pattern statistics with unpalatability (Pearson correlation, $r < -0.4$, $p = < 0.18$). We did not find any significant positive correlations, and there were no moderate positive correlations (all $r < 0.18$, $p > 0.55$) (Table S2).

At 10 cm, six out of 157 pattern statistics were significantly negatively correlated with unpalatability (Pearson correlation $r < -0.65$, $p < 0.017$; Table 1, Table S2). More unpalatable species were found on less variable visual backgrounds with lower variability of the coefficient of variation of chromaticity contrast between colour pattern elements (VCA.CVS) as well as backgrounds less variable concerning the regularity of the transitions between colour pattern elements overall (CAA.Jt) and in the vertical axis of a background relative to the anterior-posterior axis of an animal (CAA.Jt.Vrt). More unpalatable animals were also found on backgrounds with reduced variability in their Shannon colour diversity (CAA.Qc) (Table 1). There were moderate negative correlations for 47 pattern statistics with unpalatability (Pearson correlation, $r < -0.4$, $p = < 0.18$). We did not find any significant positive correlations, and there were no moderate positive correlations (all $r < 0.26$, $p > 0.40$) (Table S2).

At 5 cm, three out of 157 pattern statistics were significantly negatively correlated with unpalatability ($r < -0.65$; $p < 0.017$; Table 1, Table S2). The strongest correlations were observed at this distance, with the coefficient of variation luminance contrast between colour pattern elements of a background (VCA.CVSL) being negatively correlated with unpalatability (Pearson correlation: $r = -0.75$, $p = 0.003$; Fig. 2). In other words, for VCA.CVSL, highly defended species were viewed against backgrounds that were 56.99% less variable than non-defended species. There were moderate negative correlations for 26 pattern statistics with unpalatability (Pearson correlation, $r < -0.4$, $p = < 0.18$). We did not find any significant positive correlations, and there were no moderate positive correlations (all $r < 0.24$, $p > 0.44$) (Table S2).

At 2 cm, no significant correlations passing the Bonferroni correction could be found (Table 1, Table S2). There were moderate negative correlations for 23 pattern statistics with unpalatability (Pearson correlation, $r < -0.4$, $p = < 0.18$). We did not find any significant positive correlations, and there were no moderate positive correlations (all $r < 0.39$, $p > 0.19$) (Table S2).

Although we found individual pattern parameters were negatively correlated with unpalatability, we did not find that the mean IQR of all 157 pattern parameters for each species was significantly correlated with unpalatability at any viewing distance (50 cm: Pearson correlation $r = -$

0.24, $p = 0.436$; 30 cm: Pearson correlation $r = -0.536$, $p = 0.059$; 10 cm: Pearson correlation $r = -0.51$, $p = 0.078$; 5 cm: Pearson correlation $r = -0.09$, $p = 0.780$; 2 cm: Pearson correlation $r = -0.33$, $p = 0.273$).

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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.

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Viewing distance	Stage in Predation Sequence	Pattern statistic	Pearson Correlation [r] / % [r^2*100]	P value
50cm	Encounter / Detection	None	None	None
30cm	Detection / Identification	CAA.C.Hrz	-0.68 / 46.11	0.011
		VCA.CVSL	-0.66 / 43.88	0.014
		VCA.CVSL.Hrz	-0.66 / 43.88	0.014
		VCA.CVSL.Vrt	-0.66 / 43.88	0.014
10cm	Identification / Approach	VCA.CVS	-0.69 / 48.00	0.009
		VCA.CVS.Hrz	-0.69 / 48.00	0.009
		VCA.CVS.Vrt	-0.69 / 48.00	0.009
		CAA.Jt.Vrt	-0.68 / 46.58	0.010
		CAA.Qc	-0.67 / 45.74	0.011
		CAA.Jt	-0.66 / 42.90	0.015
5cm	Approach / Subjugation	VCA.CVSL	-0.75 / 56.99	0.003
		VCA.CVSL.Hrz	-0.75 / 56.99	0.003
		VCA.CVSL.Vrt	-0.75 / 56.99	0.003
2cm	Subjugation / Consumption	None	None	None

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232 **Figure 2.** An example colour pattern statistic of the background (IQR of luminance contrast; VCA.CVSL) plotted
233 against the normalised unpalatability score of each species, at a 5cm viewing distance, (Pearson correlation: r =
234 -0.75, p = 0.003)

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Discussion

We found several significant and moderately negative correlations between the strength of chemical defences and the variability of individual background statistics at any viewing distance, indicating that specific image statistics of the visual backgrounds inhabited by 13 Dorid nudibranch species are up to 56.99% less variable in species with higher levels of chemical defences (unpalatability). Importantly, we found no significant or moderately positive correlations at any viewing distance. This indicates that, contrary to the predictions derived from the 'escape and radiate' hypothesis (Thompson, 1989), well-defended nudibranch species utilised a smaller range of background habitats, and were less likely to be background generalists than cryptic species. Our study provides the first spatiochromatic quantification of natural background variability for aposematic species beyond the previously separated modalities of colour and spatial frequency. These findings are among the strongest quantitative correlations between primary and secondary defences known to date (White & Umbers, 2021).

Background variability did not vary consistently across all 157 colour pattern statistics and at all distances. This indicates that a distinct set of visual features (rather than all possible colour pattern characteristics) may be ecologically relevant at a given viewing distance. Our data also shows that some of these statistics are relevant for both along and perpendicular to the body long axis of the animal found on each background, whereas others do not (Table 1). This is interesting regarding the potential importance of feature selectivity in the visual perception of prey and their environment by potential predators (Howe & Purves, 2005; Hubel & Wiesel, 1962).

As predicted, the strongest and most correlations between background variability and chemical defences occurred at distances corresponding to prey detection, identification, and approach, with correlations occurring at distances between 5 and 30 cm. From our data, prey detection at distances beyond 30 cm may only be relevant for a few very large species of nudibranchs. In contrast, aspects of object identity and quality are mediated by the intrinsic properties of the animal itself and processed by the predator post-detection and likely independent of distractor features present in the visual background (excluding examples of masquerade) (Endler, 1991; Guilford & Dawkins, 1993; Ruxton et al., 2018). Indeed, the lack of significant correlations at very close distances (2 cm) might suggest that visual backgrounds are more important in object detection rather than discrimination.

However, our modelling choice of 3 cycles per degree (cpd) is likely conservative (Cheney et al., 2022), and feature detection in the visual system of triggerfish could happen at multiple spatial scales simultaneously (Hubel & Wiesel, 1962; van den Berg et al., 2022). Furthermore, nudibranch

species are highly variable in size, and spatial acuity within a predator community is also likely variable. Therefore, if and how specific properties of colour patterns in Dorid nudibranchs correlate with predator cognition will need to be validated with behavioural studies.

Nudibranchs mainly use chemotaxis to move around their environment to find food and mates, with visual input only relevant for basic phototaxis, such as determining daytime or detecting shelter (Eakin et al., 1967). Therefore, unlike many other aposematic species, nudibranchs will unlikely choose resting and foraging microhabitats based on visual cues. Furthermore, to our knowledge, no study has quantified the mobility of different nudibranch species, and the diets of many nudibranch species (however, see Rudman & Bergquist, 2007), and the spatiotemporal distribution of food sources remain poorly known. Some of the most unpalatable nudibranchs in this study are known to forage on sponges containing highly potent secondary metabolites (e.g. latrunculin a) at least at some stage during their lifetime (e.g. *Chromodoris elisabethina*) (Cheney et al., 2016). In contrast, others can synthesise compounds *de novo* (Cimino & Ghiselin, 1999). Nudibranchs with more specialist diets may need to increase mobility to find uncommon food sources. These then allow the animals to maintain functional levels of secondary metabolites or pigments required to maintain salient aposematic colour patterns. Background specialisation might therefore be increased in species dependent on such supplemental food sources. Specifically, colour patterns in aposematic species may be designed to be particularly efficient on certain backgrounds but not others. This might help explain our results while aligning with the abundance of chemically defended but likely cryptic Dorid nudibranch species (Cortesi & Cheney, 2010). Intriguingly, the same mechanism could potentially also lead to reduction in observed background variability. However, our study does not consider species relying on masquerade (Ruxton et al., 2018), which is commonly seen in extreme background specialists (living on one species of prey exclusively) where nudibranchs mimic their food source, such as various species of the genus *Phyllodesmium* (e.g. *Phyllodesmium lizardensis*).

Our study also only considered the degree of unpalatability rather than toxicity (or both) to quantify the level of the chemical defence (Winters et al., 2022). These two mechanisms of defence are not always related (Mappes et al., 2005), but predators are more likely to learn to avoid aposematic prey that is initially aversive through taste, rather than toxicity. Future studies investigating correlations between background variability and additional modalities of prey profitability (such as toxicity or nutritional value) would be of great interest.

When discussing correlations between visual properties of backgrounds and the evolution of aposematic colouration, Merilaita & Tullberg (2005) specifically focused on diurnal lepidopterans as day-active and nocturnal species are expected to be under very different sets of constraints from

visual predation than nudibranchs. Indeed, our study uses species found during both day and (to a much lesser extent) night (Supplementary Information, Table S1); further studies are needed to investigate the correlation between daytime activity and habitat use in nudibranchs (Larkin et al., 2018).

Finally, it is possible that, for some colour pattern statistics, there could be a strong phylogenetic signal, which we have not been able to pick up in this study as we only sampled 13 out of many co-occurring sea slug species at either sampling site (e.g. Schubert & Smith, 2020). To test this, we would need to increase our sample sizes and more systematic sampling across geographical and seasonal ranges. This would allow applying sophisticated comparative phylogenetic analyses necessary for properly investigating coevolutionary processes in Dorid nudibranchs.

In conclusion, while not confirming the escape and radiate hypothesis, our findings are not at odds with existing literature. Furthermore, the mechanisms underlying the 'escape and radiate' hypothesis are often unclear, with little empirical evidence of the process (Suchan & Alvarez, 2015). Nevertheless, our study provides the most thorough investigation of correlations between chemical defences and visual background diversity in aposematic animals to date.

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Conflict of Interest

The authors have no conflict of interest to declare.

Data availability

The data will be uploaded to UQ's digital repository eSpace upon acceptance.

Author contributions

CPvdB conceived the original study with KLC & JAE providing critical input; CPvdB collected and analysed the data; CPvdB and KLC led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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