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RESEARCH ARTICLE

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# Quantitative Colour Pattern Analysis (QCPA): A comprehensive framework for the analysis of colour patterns in nature

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## Abstract

1. To understand the function of colour signals in nature, we require robust quantitative analytical frameworks to enable us to estimate how animal and plant colour patterns appear against their natural background as viewed by ecologically relevant species. Due to the quantitative limitations of existing methods, colour and pattern are rarely analysed in conjunction with one another, despite a large body of literature and decades of research on the importance of spatio-chromatic colour pattern analyses. Furthermore, key physiological limitations of animal visual systems such as spatial acuity, spectral sensitivities, photoreceptor abundances and receptor noise levels are rarely considered together in colour pattern analyses.
2. Here, we present a novel analytical framework, called the Quantitative Colour Pattern Analysis (QCPA). We have overcome many quantitative and qualitative limitations of existing colour pattern analyses by combining calibrated digital photography and visual modelling. We have integrated and updated existing spatio-chromatic colour pattern analyses, including adjacency, visual contrast and boundary strength analysis, to be implemented using calibrated digital photography through the Multispectral Image Analysis and Calibration (MICA) Toolbox.
3. This combination of calibrated photography and spatio-chromatic colour pattern analyses is enabled by the inclusion of psychophysical colour and luminance discrimination thresholds for image segmentation, which we call 'Receptor Noise Limited Clustering'. Furthermore, QCPA provides a novel psycho-physiological approach to the modelling of spatial acuity using convolution in the spatial or frequency domains, followed by 'Receptor Noise Limited Ranked Filtering' to eliminate intermediate edge artefacts and recover sharp boundaries following smoothing. We also present a new type of colour pattern analysis, the 'local edge intensity analysis' as well as a range of novel psycho-physiological approaches to the modelling of spatial acuity in spatio-chromatic data.
4. QCPA combines novel and existing pattern analysis frameworks into what we hope is a unified, free and open source toolbox and introduces a range of novel analytical

Cedric P. van den Berg and Jolyon Troscianko were the joint first author.

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## 2. MATERIALS AND METHODS

### 2.1. Step 1. Acquisition of calibrated digital images

### 2.2. Step 2. Modelling of spatial acuity

### 2.3. Step 3. Image smoothing and edge reconstruction

### 2.4. Step 4. Image segmentation

### 2.5. Step 5. Pattern analysis

### 2.6. Step 6. Data visualisation using

**Abundance Weighted Mean Saturation Contrast:**

$$M_{\Delta S_{Sat}} = \sum_{i=1}^{k} f_i \, \Delta S_{Sat,i}$$

**Number of Colour Pattern Elements:**

$k = 3$

**Relative Abundances Morph A:**

$f_{brown} = 0.34$ $f_{yellow} = 0.44$ $f_{purple} = 0.22$

**Relative Abundances Morph B:**

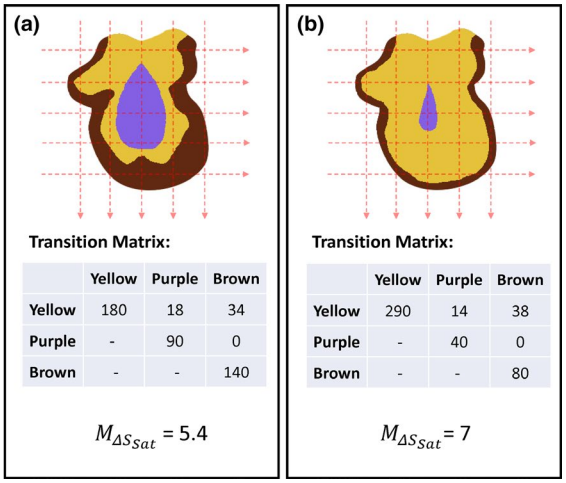
$f_{brown} = 0.20$ $f_{yellow} = 0.70$ $f_{purple} = 0.10$

**Colour Patch Saturation:**

$\Delta S_{Sat,brown} = 1.5$

$\Delta S_{Sat,purple} = 4.3$

$\Delta S_{Sat,yellow} = 8.9$




| | Yellow | Purple | Brown |
|---|---|---|---|
| Yellow | 180 | 18 | 34 |
| Purple | - | 90 | 0 |
| Brown | - | - | 140 |

| | Yellow | Purple | Brown |
|---|---|---|---|
| Yellow | 290 | 14 | 38 |
| Purple | - | 40 | 0 |
| Brown | - | - | 80 |

**FIGURE 6** Using visual contrast analysis to quantify differences in appearance of a hypothetical polymorphism in *Ophrys ciliata* (Figure 5a). Average saturation (distance from the achromatic point in the log-transformed RNL colour space, $\Delta S_{sat}$) in the colour pattern can be expressed as an abundance weighted mean. The relative abundance of each colour pattern element can be calculated as the proportion of diagonal transitions compared to the sum of all diagonal transitions ($f_i$). (a,b) Note how the off-diagonal transitions between morphs change marginally in comparison to Figure 5, however, the level of overall colour pattern saturation for this kind of polymorphism differs substantially due to the increased relative abundance of saturated yellow, calculated as the relative abundance of yellow - yellow transitions in the diagonal of the transition matrix ($f_{yellow}$)

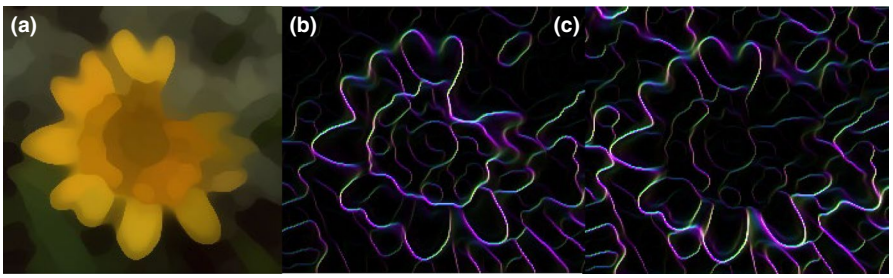



**FIGURE 7** (a) The RNL filtered flower from Figure 4c. (b) Edge intensities of chromatic ΔS contrast. Different colours indicate different angles of hypothetical edge detecting receptive fields, the intensity reflects the contrast. (c) Edge intensities of achromatic (luminance) ΔS contrast. Colours show edge angle whereas intensity shows edge strength

Osorio, 2007; Dalziell & Welbergen, 2016; Mallet & Joron, 1999). In a hypothetical case, CAA could be used to quantify imperfect sexual mimicry of orchids (Figure 5) where the plant mimics the visual and chemical appearance of a potential mate (e.g. Gaskett & Herberstein, 2010; Vereecken, 2008). For further discussion of the biological relevance, worked examples, potential future investigations, and guidance on parameter choices see Endler (2012), Rojas, Devillechabrolle, and Endler (2014), Ligon et al., (2018) and Winters et al. (2018). For details on CAA parameters available in QCPA see the Supplemental Material.

### 2.6.2 | Visual contrast analysis

Visual contrast analysis (VCA) is designed to investigate colour, pattern and luminance simultaneously by providing pattern statistics which combine spatial and chromatic properties of colour patterns such as abundance weighted chromaticity measures (Endler & Mielke, 2005, Supplemental Material). The perception of visual contrast is a combination of spatial (relative size and position of colour pattern elements), chromatic (hue and saturation), and achromatic (luminance) properties of a colour pattern due to lower and higher level neuronal processing of visual information (e.g. Pearson & Kingdom, 2002; Shapley & Hawken, 2011; Simmons & Kingdom, 2002; White et al., 2017; Willis & Anderson, 2002). Furthermore, interactions between the absolute and relative size of colour pattern elements and their chromatic and achromatic properties includes simultaneous colour contrast and colour constancy mechanisms that are understood in very few visual systems (e.g. Simpson, Marshall, & Cheney, 2016). VCA provides a set of metrics that are designed to capture some of these effects. We have adapted some of these metrics to use known or assumed colour opponency mechanisms to measure chromaticity (Supplemental Material). Using the previous orchid example, VCA could be used to investigate how polymorphism in our hypothetical population interacts with pollinator

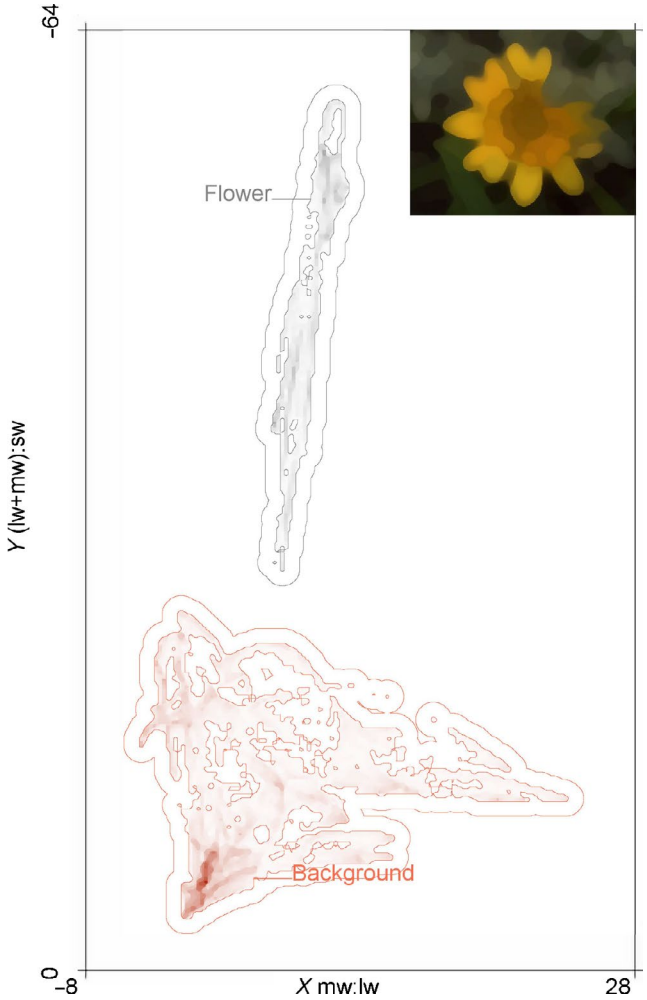



**FIGURE 8** Colour map in a log-transformed Receptor Noise Limited Model (RNL) chromaticity space of the non-UV information in Figure 5c. The axes are automatically labelled based on the names of the receptor channels used to create each dimension of colour, for example the *X*-axis (RNL X dimension) is mw:lw, showing that mw-dominant colours are on the left, and lw-dominant colours are on the right of the plot. *X* and *Y* are defined in equation 4 of Hempel de Ibarra et al. (2001). Darker parts of the cloud indicate more pixels in that ROI are located at that coordinate. The boundary around each ROI pixel cloud reflects 1 ΔS. In this case, the flower and its background do not overlap. For tetra-chromatic colour maps, the *Z*-axis is represented as a stack of *X*&*Y* maps (see Supplemental Material)

learning or differences in attractiveness to pollinators (Figure 6). See the Supplemental Material, original publications (Endler, 1991; Endler & Mielke, 2005) and empirical studies (Endler & Houde, 1995; Sibeaux, Cole, et al., 2019, 2019) for further information.

### 2.6.3 | Boundary strength analysis

Boundary strength analysis (BSA, Endler et al., 2018) is an extension of CAA (Endler, 2012). The transition matrices generated in the process of adjacency analysis can be used to measure properties of boundaries between colour pattern elements. The underlying argument for this type of analysis is that the relative size, abundance, colour, brightness and adjacency of the patches within a colour pattern, and the chromatic or achromatic contrast of the boundaries between adjacent patches, influence its signalling properties (Endler et al., 2018; Green et al., 2018; Shapley & Hawken, 2011). These parameters also define the properties of the edges between and within parts of visual scenes and textures. BSA (as well as CAA and VCA) is also capable of quantifying possible effects of viewer perspective and movement (Endler et al., 2018). For a detailed introduction to BSA, possible future research and guidance on parameter choices, please refer to the original publication (Endler et al., 2018) and empirical studies using BSA (e.g. Sibeaux, Cole, et al., 2019, 2019). For detailed equations and information on modifications of parameters since original publication, see the Supplemental Material.

### 2.6.4 | Local edge intensity analysis and ΔS edge maps

Boundary strength analysis depends on a segmented image with clearly delineated (clustered) colour pattern elements (Endler et al., 2018). However, the segmentation process removes a large degree of subthreshold information, particularly smooth gradients of brightness and colour which the viewer may perceive. For this purpose, we provide LEIA, as a way of quantifying edge properties in an image or ROI (Region of interest) that does not rely on a segmented input. By comparing each pixel to its horizontal, vertical and diagonal neighbours LEIA quantifies edge intensities in terms of colour and luminance contrast in log-linear RNL opponent space (Renoult et al., 2017). The result can be visualized as 'ΔS Edge Images' (Figure 7). BSA weights the strength of boundary classes according to their global (across an entire image or ROI) relative abundance, whereas LEIA provides a local measurement of edge intensity on roughly the scale of an edge detecting receptive field. This approach allows one to consider edge contrast at the scale of the functional units (receptive fields) at which low level edge and feature detection are thought to take place (Marr, 2010; Marr & Hildreth, 1980). While LEIA is suited to the investigation of similar aspects of colour pattern design and function as BSA, it can do this without the need for clustering an image, while using a more neurophysiological approach than BSA. We recommend that LEIA should be used on images which have first been controlled for acuity (to remove imperceptible edge/gradient information) and images which have also been through the RNL ranked filter, so that local chromatic and luminance edges have been reconstructed to their maximal values. LEIA also provides numerical output describing the distribution of edge intensities across an image. These parameters are specifically designed to be robust in the case of non-normally distributed edge intensities in an image (e.g. a small conspicuous object on a homogeneous background). Local edge contrast can be visualized as ΔS edge intensity images (Figure 7b,c).

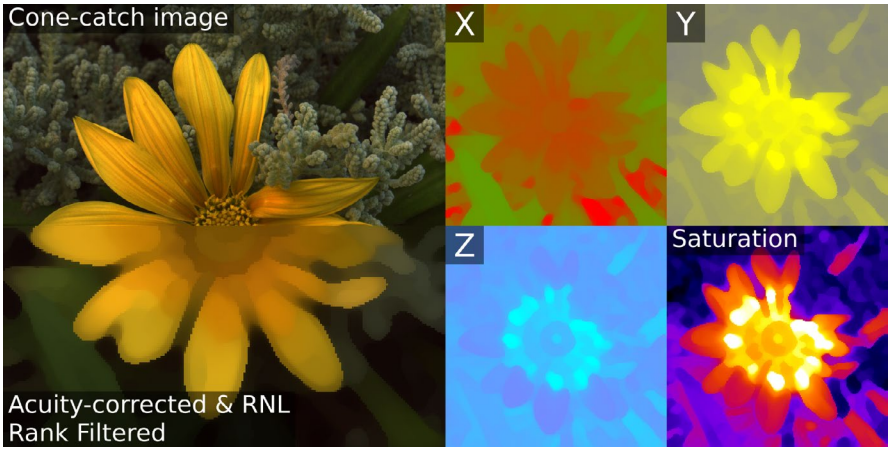



**FIGURE 9** An example of the red-green (lw:mw) opponent channel (*X*), blue-yellow ((lw + mw):sw) channel (*Y*) and the UV channel (*Z*) where the colour indicates the position of a pixel along that axis. The saturation map shows the Euclidean distance of each pixel to the achromatic point

## 2.7 | Step 7: Data visualization

We provide a range of novel approaches for data visualization. Calibrated digital photography and the coupled transformation of image data into psychophysical colour spaces provides a challenge but also an opportunity for visualization. We have already introduced the ΔS edge intensity images and extend that selection with colour maps, XYZ opponency images and saturation images.

### 2.7.1 | 'Colour maps' and 'XYZ chromaticity and saturation images'

The representation of chromatic information in colour spaces is a useful tool for data visualization in visual ecology (Endler & Mielke, 2005; Gawryszewski, 2018; Maia, Eliason, Bitton, Doucet, & Shawkey, 2013; Renoult et al., 2017). To date, most studies present their data as a scattering of points, which are either discrete measurements taken with spectrometers, or the mean centroids of image ROI cone-catch values. Techniques such as area or volume overlap between point clouds, or permutation analysis are then used to determine how dissimilar two colour patches are (e.g. Endler & Mielke, 2005; Kemp et al., 2015; Maia & White, 2018; Stoddard & Prum, 2008).

Colour space data visualizations generally do not incorporate any spatial (colour pattern) information. The use of calibrated digital imaging provides thousands, or even millions of colour measurements within each ROI, capturing the entire range of chromatic gradients present in any natural pattern. Using the log transformed opponent colour space (Hempel de Ibarra et al., 2001; Kelber, Vorobyev, & Osorio, 2003; Renoult et al., 2017), we provide representations of spatio-chromatic information in a perceptually calibrated colour space. 'Colour Maps' allow for the representation of entire visual scenes in a chromaticity diagram, in addition to the abundance of colours across part of the image (Figure 8). Among other purposes, colour maps may be used for visualizations and investigations of chromatic background matching. The overlap of ROIs in colour space can be expressed as an abundance weighted percentage. QCPA integrates tools which enable colour maps to be flexibly combined and compared between image sections, or measurements taken from multiple images.

We also introduce the ability to convert cone-catch images to RNL XYZ chromaticity and saturation images, allowing visualization and measurement of the independent axes of colour in a di- tri- or tetra-chromatic image (showing the Euclidean distance of each pixel's RNL XYZ axes coordinates), in addition to generating a saturation image (Euclidean distance of each pixel to the achromatic point) (Figure 9).

### 2.7.2 | Step 8: Interpreting QCPA output

Quantitative Colour Pattern Analysis provides a huge range of metrics from each image (currently 181 parameters). Some of these parameters likely correlate well with aspects of animal evolution, behaviour and neurophysiology, while others are likely to show no signal. Likewise, some parameters will operate synergistically with each other, while others are independent or antagonistic. Moreover, these relationships could be fundamentally different between taxa, meaning caution should be used when comparing results between highly divergent taxa (such as vertebrate vs. invertebrate systems). QCPA can be used to address specific hypotheses linking one or a small subset of parameters (e.g. mean animal vs. background luminance contrast) to a response variable (e.g. predator attack rates) based on the context of the task. Such experiments require highly calibrated environments and stimuli where confounding influences on the perception of specific spatial, chromatic or temporal properties of a visual stimulus are controlled for (reviewed in Shapley & Hawken, 2011). However, colour patterns can be quantified in a great number of parameters, all of them capturing different aspects of chromatic, achromatic and spatial properties or combinations thereof (of which QCPA only captures a few).

Commonly used terms such as 'Complexity', 'Conspicuousness' or 'Similarity' should be considered as umbrella terms describing perceptual consequences caused by the variation of physical properties of colour patterns and their visual backgrounds (which often cannot be described by a single parameter). The lack of empirical testing of many QCPA parameters (and those of most other pattern analyses) makes it hard to offer broad recommendations for parameter choice

**TABLE 1** A comparison of the Quantitative Colour Pattern Analysis framework to other existing pattern analyses and frameworks. For patternize see van Belleghem et al. (2018). For PAT-GEOM see Chan, Stevens, and Todd (2018). For PAVO see Maia et al. (2019). For NaturePatternMatch see Stoddard et al. (2014). For Colourvision see Gawryszewski (2018). We would also like to point out an approach by Pike (2018) which shares similarities with NaturePatternMatch

| | QCPA | MICA | Patternize | PAT-GEOM | PAVO 2.0 | Colourvision | NaturePatternMatch |
|---|---|---|---|---|---|---|---|
| Modelling of non-human Photoreceptor stimulation | ✓ | ✓ | | | ✓ | ✓ | |
| Modelling of visual acuity | + | | | | | | |
| Visual Contrast Analysis | + | | | | | | |
| Local Edge Intensity Analysis (LEIA) | ★ | | | | | | |
| Adjacency Analysis | + | | | | ✓ | | |
| Boundary Strength Analysis | ✓ | | | | ✓ | | |
| Bandpass based pattern analysis | | + | | | | | |
| Alternative pattern metrics | ★ | ★✓+ | ✓ | + | | | |
| Landmark based pattern analysis | | | + | | | | + |
| Image segementation using perceptual thresholds | ★ | | | | | | |
| Alternative image segmentation tools | + | + | ✓ | | ✓ | | |
| Plotting colours in colour space | + | | | | + | + | |
| Plotting of spatiochromatic information in colour space | ★ | | | | | | |
| Graphical user interface | | | | | | | |
| Software/Language | ImageJ | ImageJ | R | ImageJ | R | R | C++ (Matlab) |

✓ Adapted as per original scientific publications + Substantially modified ★ Novel concept or methodology

across the huge diversity of possible contexts, especially when making observations in natural or nature-like levels of spatio-chromatic complexity. The 'simpler' the research question and the more controlled the experiment, the more adequate it is to consider one or a few pattern parameters in isolation.

Therefore, when there is no a priori reason to choose specific parameters, we recommend the use of multidimensional data analyses, such as principal component analysis (PCA), metric- and non-metric multidimensional scaling (MMDS/NMDS) or similar multivariate approaches such as factor analysis to identify correlations between pattern analysis output and animal behaviour (e.g. Sibeaux, Cole, et al., 2019, 2019) or to distinguish between taxa (e.g. Chan et al., 2019; Ligon et al., 2018; Winters et al., 2018). Doing so can be thought of as operating in a multidimensional pattern space (for discussion see Cuthill, 2019 and Stoddard & Osorio, 2019). Such a pattern space can include categorical data (e.g. presence/absence), data from other pattern analyses (Table 1) as well as environmental data.

Reducing the dimensionality of such data comes with considerable statistical challenges. However, it is possible to avoid many issues such as bias from the structure of datasets or false positives (e.g. Benjamini & Hochberg, 1995; Osborne, Osborne, Costello, & Kellow, 2011). While obvious for the use of QCPA, interpreting many potentially interacting pattern parameters is of increasing importance given a steadily growing diversity of analytical methods (Table 1) and the desire to incorporate effects of higher-level processing of visual information into the analysis of visual signals.

## 3 | DISCUSSION

Quantitative Colour Pattern Analysis is a framework for the analysis of colour patterns in nature at an unprecedented quantitative and qualitative level. At its core, QCPA uses the advantages offered by calibrated digital photography to enable the use of existing

spatio-chromatic colour pattern analyses (Figure 1). It also improves existing methodologies used in visual ecology by introducing a user-friendly and open-source framework which incorporates the ability to contextualize visual scenes according to photoreceptor spectral sensitivities, receptor noise levels and abundances, natural light environments, complex natural backgrounds, spatial acuity and viewing distance (Table 1).

The individual modelling components of QCPA rely on approximations and assumptions, which are based on our best current understanding of the underlying biological processes. As such, it is important to be aware of the limitations and underlying assumptions of the individual components of QCPA, some of which we discuss. QCPA makes extensive use of the receptor noise limited model (RNL) which has been behaviourally validated in various species including: humans, honeybees, birds, lizards, reef fish and freshwater fish (e.g. Champ, Vorobyev, & Marshall, 2016; Escobar-Camacho, Marshall, & Carleton, 2017; Vorobyev et al., 2001; Vorobyev & Osorio, 1998). However, the RNL model and RNL colour space is one of various available visual models and colour spaces that have also considered behavioural context to some degree and which may be considered as alternatives (reviewed in Gawryszewski, 2018; Renoult et al., 2017).

The RNL model (or any other visual model) is unlikely to represent the perceptual complexity of natural visual scenes for all species across all light regimes. To avoid making false assumptions, it is necessary to consider the perceptual context in which it is applied and how this context may violate model assumptions (Kelber, 2019; Lind, 2016; Olsson et al., 2018; Price, Stoddard, Shevell, & Bloch, 2019). For example, behavioural experiments have shown varying sensitivity to differences in colour in specific quadrants of colour space relevant to the behavioural ecology of species (Caves et al., 2018; Sibeaux, Cole, & Endler, 2019; Sibeaux, Keser, Cole, Kranz, & Endler, 2019). Another aspect that needs further investigation is the question of how distances in RNL colour space scale with behavioural thresholds across a wide range of visual systems and perceptual contexts (e.g. Fleishman et al., 2016). QCPA applies the log-transformed RNL colour space to minimize, but not remove, the impact of such threshold distortions (Gawryszewski, 2018; Vorobyev et al., 2001; Vorobyev & Osorio, 1998). Overall, the less validated model parameters are and the more profound assumption violations may be, the more likely deviations from the assumption that 1 $\Delta S$ equates to a behavioural threshold (e.g. a 75% success rate in a pairwise choice paradigm) will occur. Furthermore, the photopic version of the RNL, which is used here was developed to model colour discrimination near the achromatic point under photopic conditions (Vorobyev et al., 2001; Vorobyev & Osorio, 1998). However, when visual systems operate in crepuscular or scotopic conditions, the retinal stimulation to visual information becomes the result of both cone and rod stimulation or rod stimulation only (Kelber, Balkenius, & Warrant, 2002; Olsson et al., 2015; Osorio et al., 2004; Veilleux & Cummings, 2012; Vorobyev & Osorio, 1998). Another example highlighting context-specific threshold modelling is the distinction between detection and discrimination thresholds which has direct implications on the application of the RNL (Lind, 2016; Price et al., 2019).

Quantitative Colour Pattern Analysis enables the application of known sensory limitations to filter the information that is subsequently processed by low-level vision. While a range of parameters provided by the QCPA have been shown to be of importance in some species, many remain to be applied and investigated in a broad range of behavioural contexts and visual systems. To what extent the observed parameterization of visual information bears ecological or behavioural significance subsequently must be inferred and calibrated using behavioural experimentation (Olsson et al., 2018). QCPA provides numerous parameters based on concepts shown to be relevant to a range of natural contexts (Endler, 1991, 2012; Endler et al., 2018; Endler & Houde, 1995; Rojas et al., 2014; Rojas & Endler, 2013; Sibeaux, Cole, et al., 2019, 2019; Winters et al., 2018). However, it also provides parameters which are yet to be validated, particularly on a quantitative scale. This provides great potential for future research as well as parameter calibration using behavioural experiments and highlights the importance and feasibility of a reductionist approach to the quantification of colour patterns and their function (sensu Stoddard & Osorio, 2019). Given the ability to link QCPA parameters and animal behaviour, we encourage the use of QCPA to design carefully calibrated behavioural experiments in the context of complex colour patterns and visual backgrounds.

There is considerable potential to improve QCPA by continuing to refine, test and develop its components. For example, we currently have not considered the loss of spatial and chromatic information due to light scattering or absorption, particularly in aquatic or dusty environments (e.g. Nilsson, Warrant, & Johnsen, 2014). Furthermore, many animal eyes do not have uniform retinas which, in combination with diversity in eye movements and eye shapes, leads to a little investigated diversity of visual perception in addition to the already discussed perceptual diversity in animal visual systems (Daly, How, Partridge, & Roberts, 2018; Hughes, 2018; Land, 1999; Land & Nilsson, 2012; Sibeaux et al., 2019; Willis & Anderson, 2002). QCPA could also be adapted to investigate moving patterns (e.g. Endler, 2012; Endler et al., 2018), given recent advances in the understanding of colour pattern functionality in the context of motion (Cuthill, Matchette, & Scott-Samuel, 2019; Fleishman, 1986; Hughes, Troscianko, & Stevens, 2014; Murali, 2018; Nityananda et al., 2018; Ramos & Peters, 2017; Umeton, Tarawneh, Fezza, Read, & Rowe, 2019). There are types of visual information we have barely begun understanding, such as polarization vision, the use of fluorescence as well as their interaction with an animal's perception of colour and brightness (Foster et al., 2018; Marshall, Cortesi, de Busserolles, Siebeck, & Cheney, 2019; Marshall & Johnsen, 2017; Smithers, Roberts, & How, 2019).

Recent years have seen a growing diversity of colour pattern analyses (Table 1). While some use conceptually similar pattern statistics to QCPA, others provide alternative approaches such as scale invariant feature (SIFT) analysis-based metrics (Lowe, 1999) and combinations with models to describe cognitive aspects of attention (Rosenholtz, Li, Jin, & Mansfield, 2010). The concept of QCPA-based pattern analysis

is entirely compatible with any of these methods. In fact, QCPA does not currently include any computer vision-mediated object recognition or figure-ground segregation. However, QCPA provides a promising platform for future implementations of computational approaches to higher level neuronal processing of visual information (e.g. Serre, 2014).

Quantitative Colour Pattern Analysis provides an unprecedented level of accessibility and user-friendliness by being free, open-source, graphical user interface mediated and accompanied by a vast body of support material. QCPA presents a comprehensive, dynamic and coherent work process starting with the acquisition of calibrated digital images and ending with the extraction of behaviourally and neurophysiologically contextualized pattern space. ImageJ has been the software platform of choice for image analysis for decades. Its architecture minimizes the risk of non-compatibilities due to future patches of co-dependent packages (often seen in R or Matlab) making QCPA (and MICA) well equipped for the future. ImageJ and MICA provide their own, rich, sets of image and pattern analysis and manipulation tools that QCPA profits from and can interact with. For example, GabRat (Troscianko et al., 2017) can be used in combination with QCPA to investigate chromatic aspects of disruptive colouration in the context of spatial acuity. Furthermore, it is possible to use QCPA and MICA with a simple smartphone or cheap digital camera and a colour chart for calibration. While it is advantageous to have access to spectrophotometry for comparison of modelling output, this is no longer a requirement and reduces the cost for equipment drastically.

In conclusion, there are many theories and predictions regarding the design, function and evolution of colour patterns in nature which, if at all, have only been investigated in comparably simplistic or qualitative ways. QCPA provides a powerful framework to investigate these theories in a novel quantitative and qualitative context.

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## AUTHORS' CONTRIBUTIONS

C.P.v.d.B and J.T. conceived and tested the QCPA framework based on an original concept by C.P.v.d.B. J.T. wrote the software code and conceived the original (chromatic) RNL clustering and RNL-ranked filter algorithms, with further testing, debugging and conceptual input and modification by C.P.v.d.B. C.P.v.d.B and K.L.C. conceived the combination of chromatic and achromatic discrimination thresholds for image segmentation. C.P.v.d.B. wrote the MATLAB precursor of the QCPA user interface and pattern analysis which contains the original code by J.A.E. J.A.E. also contributed many of the original concepts, J.A.E., K.L.C and N.J.M. have contributed to conceptual discussions and manuscript review.

## DATA AVAILABILITY STATEMENT

The latest version of QCPA and its open source code (JAVA script) are available for download as part of the MICA toolbox at www.empiricalimaging.com. The website provides detailed manuals, tutorials, FAQs, a dedicated forum and updates. If you intend to use QCPA and/or the micaToolbox, please use the website to familiarise yourself with the latest updates. We do encourage users to use the website and the Supplemental Material as their primary source of information on how to use QCPA. The version of the code used at the time of publication can be found at https://doi.org/10.5281/zenodo.3517896 (troscianko, 2019). A fully functional MATLAB based precursor of QCPA can be accessed at https://doi.org/10.5281/zenodo.3518682 (van den Berg, 2019).

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## REFERENCES

Allen, W. L., & Higham, J. P. (2013). Analyzing visual signals as visual scenes. *American Journal of Primatology*, *75*(7), 664–682. https://doi.org/10.1002/ajp.22129

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Caves, E. M., Frank, T. M., & Johnsen, S. (2016). Spectral sensitivity, spatial resolution, and temporal resolution and their implications for conspecific signalling in cleaner shrimp. *Journal of Experimental Biology*, *219*, 597–608. https://doi.org/10.1242/jeb.122275

Caves, E. M., Green, P. A., Zipple, M. N., Peters, S., Johnsen, S., & Nowicki, S. (2018). Categorical perception of colour signals in a songbird. *Nature*, *560*(7718), 365–367. https://doi.org/10.1038/s41586-018-0377-7

Caves, E. M., & Johnsen, S. (2017). AcuityView: An R package for portraying the effects of visual acuity on scenes observed by an animal. *Journal of Engineering and Applied Sciences*, *12*(10), 3218–3221. https://doi.org/10.1111/ijlh.12426

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, N. J. (2014). Visual acuity in a species of coral reef fish: Rhinecanthus aculeatus. *Brain, Behavior and Evolution*, *83*, 31–42. https://doi.org/10.1159/000356977

Chan, I. Z. W., Chang, J. J. M., Huang, D., & Todd, P. A. (2019). Colour pattern measurements successfully differentiate two cryptic Onchidiidae Rafinesque, 1815 species. *Marine Biodiversity*, *49*(4), 1743–1750. https://doi.org/10.1007/s12526-019-00940-4

Chan, I. Z. W., Stevens, M., & Todd, P. A. (2018). PAT-GEOM: A Software Package for the Analysis of Animal Patterns. *Methods in Ecology and Evolution*, *10*(4), 591–600. https://doi.org/10.1111/2041-210X.13131

Cheney, K. L., Cortesi, F., How, M. J., Wilson, N. G., Blomberg, S. P., Winters, A. E., … 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

Chittka, L., & Osorio, D. (2007). Cognitive dimensions of predator responses to imperfect mimicry? *PLoS Biology*, *5*(12), 2754–2758. https://doi.org/10.1371/journal.pbio.0050339

Clark, R. C., Santer, R. D., & Brebner, J. S. (2017). A generalized equation for the calculation of receptor noise limited colour distances in n-chromatic visual systems. *Royal Society Open Science*, *4*(9), 170712. https://doi.org/10.1098/rsos.170712

Clery, S., Bloj, M., Harris, J. M., Ephane Clery, S., Bloj, M., & Harris, J. M. (2013). Interactions between luminance and color signals: Effects on shape. *Journal of Vision*, *13*(5), 16. https://doi.org/10.1167/13.5.16

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

Cott, H. B. (1940). Adaptive coloration in animals. *Nature*, *146*(3692), 144–145. https://doi.org/10.1038/146144a0

Cronin, T. W., & Bok, M. J. (2016). Photoreception and vision in the ultraviolet. *The Journal of Experimental Biology*, *219*(18), 2790–2801. https://doi.org/10.1242/jeb.128769

Cronin, T. W., Johnsen, S., Marshall, N. J., & Warrant, E. (2014). *Visual ecology*. Journal of Chemical Information and Modeling (Vol. *53*). Princeton, N.J: Princeton University Press. https://doi.org/10.1017/CBO9781107415324.004

Cuthill, I. C. (2019). Camouflage. *Journal of Zoology*, *308*(2), 75–92. https://doi.org/10.1111/jzo.12682

Cuthill, I. C., Allen, W. L., Arbuckle, K., Caspers, B., Chaplin, G., Hauber, M. E., … Caro, T. (2017). The biology of color. *Science*, *357*(6350), 1–7. https://doi.org/10.1126/science.aan0221

Cuthill, I. C., Matchette, S. R., & Scott-Samuel, N. E. (2019). Camouflage in a dynamic world. *Current Opinion in Behavioral Sciences*, *30*, 109–115. https://doi.org/10.1016/j.cobeha.2019.07.007

da Silva Souza, G., Gomes, B. D., & Silveira, L. C. L. (2011). Comparative neurophysiology of spatial luminance contrast sensitivity. *Psychology & Neuroscience*, *4*(1), 29–48. https://doi.org/10.3922/j.psns.2011.1.005

Daly, I. M., How, M. J., Partridge, J. C., & Roberts, N. W. (2018). Complex gaze stabilization in mantis shrimp. *Proceedings of the Royal Society B*, *285*(1878), 20180594. https://doi.org/10.1098/rspb.2018.0594

Dalziell, A. H., & Welbergen, J. A. (2016). Mimicry for all modalities. *Ecology Letters*, *19*(6), 609–619. https://doi.org/10.1111/ele.12602

Day, W. H. E., & Edelsbrunner, H. (1984). Efficient algorithms for agglomerative hierarchical clustering methods. *Journal of Classification*, *1*(1), 7–24. https://doi.org/10.1007/BF01890115

Endler, J. A. (1978). A predator's view of animal color patterns. *Evolutionary Biology*, *11*(5), 320–364.

Endler, J. A. (1984). Progressive background in moths, and a quantitative measure of crypsis. *Biological Journal of the Linnean Society*, *22*(3), 187–231. https://doi.org/10.1111/j.1095-8312.1984.tb01677.x

Endler, J. A. (1990). On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society*, *41*(4), 315–352. https://doi.org/10.1111/j.1095-8312.1990.tb00839.x

Endler, J. A. (1991). Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. *Vision Research*, *31*(3), 587–608. https://doi.org/10.1016/0042-6989(91)90109-I

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., & 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. (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

Escobar-Camacho, D., Marshall, N. J., & Carleton, K. L. (2017). Behavioral color vision in a cichlid fish: Metriaclima benetos. *The Journal of Experimental Biology*, *220*(16), 2887–2899. https://doi.org/10.1242/jeb.160473

Fleishman, L. J. (1986). Motion detection in the presence and absence of background motion in an Anolis lizard. *Journal of Comparative Physiology A*, *159*(5), 711–720. https://doi.org/10.1007/BF00612043

Fleishman, L. J., Perez, C. W., Yeo, A. I., Cummings, K. J., Dick, S., & Almonte, E. (2016). Perceptual distance between colored stimuli in the lizard Anolis sagrei: Comparing visual system models to empirical results. *Behavioral Ecology and Sociobiology*, *70*(4), 541–555. https://doi.org/10.1007/s00265-016-2072-8

Foster, J. J., Temple, S. E., How, M. J., Daly, I. M., Sharkey, C. R., Wilby, D., & Roberts, N. W. (2018). Polarisation vision: overcoming challenges of working with a property of light we barely see. *The Science of Nature*, *105*(5-6), 32. https://doi.org/10.1007/s00114-018-1559-8

Gaskett, A. C., & Herberstein, M. E. (2010). Colour mimicry and sexual deception by *Tongue orchids* (Cryptostylis). *Naturwissenschaften*, *97*(1), 97–102. https://doi.org/10.1007/s00114-009-0611-0

Gawryszewski, F. M. (2018). Color vision models: Some simulations, a general n-dimensional model, and the colourvision R package. *Ecology and Evolution*, *8*(16), 8159–8170. https://doi.org/10.1002/ece3.4288

Green, N. F., Urquhart, H. H., van den Berg, C. P., Marshall, N. J., & Cheney, K. L. (2018). Pattern edges improve predator learning of aposematic signals. *Behavioral Ecology*, *July*, 1–6. https://doi.org/10.1093/beheco/ary089

Hart, N. S., Partridge, J. C., Cuthill, I. C., & Bennett, A. T. (2000). Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: The blue tit (*Parus caeruleus L.*) and the blackbird (*Turdus merula L.*). *Journal of Comparative Physiology A*, *186*(4), 375–387.

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

Hughes, A. E. (2018). Dissociation between perception and smooth pursuit eye movements in speed judgments of moving Gabor targets. *Journal of Vision*, *18*(4), 4. https://doi.org/10.1167/18.4.4

Hughes, A. E., Troscianko, J., & Stevens, M. (2014). Motion dazzle and the effects of target patterning on capture success. *BMC Evolutionary Biology*, *14*(1), 1–10. https://doi.org/10.1186/s12862-014-0201-4

Isaac, L. A., & Gregory, P. T. (2013). Can snakes hide in plain view? Chromatic and achromatic crypsis of two colour forms of the Western Terrestrial Garter Snake (*Thamnophis elegans*). *Biological Journal of the Linnean Society*, *108*(4), 756–772. https://doi.org/10.1111/bij.12020

Kelber, A. (2019). Bird colour vision – From cones to perception. *Current Opinion in Behavioral Sciences*, *30*, 34–40. https://doi.org/10.1016/j.cobeha.2019.05.003

Kelber, A., Balkenius, A., & Warrant, E. J. (2002). Scotopic colour vision in nocturnal hawkmoths. *Nature*, *419*(6910), 922–925. https://doi.org/10.1038/nature01065

Kelber, A., Vorobyev, M., & Osorio, D. (2003). Animal colour vision–behavioural tests and physiological concepts. *Biological Reviews of the Cambridge Philosophical Society*, *78*, 81–118. https://doi.org/10.1017/S1464793102005985

Kemp, D. J., Herberstein, M. E., Fleishman, L. J., Endler, J. A., Bennett, A. T. D., Dyer, A. G., … 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

Koleček, J., Šulc, M., Piálková, R., Troscianko, J., Požgayová, M., Honza, M., & Procházka, P. (2019). Rufous Common Cuckoo chicks are not always female. *Journal of Ornithology*, *160*(1), 155–163. https://doi.org/10.1007/s10336-018-1591-7

Land, M. F. (1999). Motion and vision: Why animals move their eyes. *Journal of Comparative Physiology – A Sensory, Neural, and Behavioral Physiology*, *185*(4), 341–352. https://doi.org/10.1007/s003590050393

Land, M. F., & Nilsson, D. E. (2012). *Animal eyes*. Oxford: OUP.

Ligon, R. A., Diaz, C. D., Morano, J. L., Troscianko, J., Stevens, M., Moskeland, A., … Scholes, E. (2018). Evolution of correlated complexity in the radically different courtship signals of birds-of-paradise. *PLOS Biology*, *16*, e2006962. https://doi.org/10.1371/2006962

Lind, O. (2016). Colour vision and background adaptation in a passerine bird, the zebra finch (*Taeniopygia guttata*). *Royal Society Open Science*, *3*(9), 160383. https://doi.org/10.1098/rsos.160383

Long, C., & Sweet, J. (2006). Hyperspectral imaging of cuttlefish camouflage indicates good color match in the eyes of fish predators. *South East Asia Research*, *14*(3), 445–469. https://doi.org/10.1073/pnas

Lowe, D. G. (1999). Object recognition from local scale-invariant features. In Computer vision, 1999. The proceedings of the Seventh IEEE International Conference on Computer Vision (Vol. 2, pp. 1150–1157).

Lythgoe, J. N. (1979). *The ecology of vision*. Oxford University Press.

Maia, R., Eliason, C. M., Bitton, P. P., Doucet, S. M., & Shawkey, M. D. (2013). PAVO: An R package for the analysis, visualization and organization of spectral data. *Methods in Ecology and Evolution*, *4*(10), 906–913. https://doi.org/10.1111/2041-210X.12069

Maia, R., Gruson, H., Endler, J. A., & White, T. E. (2019). PAVO 2: New tools for the spectral and spatial analysis of colour in R. *Methods in Ecology and Evolution*, *10*(7), 1097–1107. https://doi.org/10.1111/2041-210X.13174

Maia, R., & White, T. E. (2018). Comparing colors using visual models. *Behavioral Ecology*, *29*(3), 649–659. https://doi.org/10.1093/beheco/ary017

Mallet, J., & Joron, M. (1999). Evolution of diversity in warning color and mimicry: Polymorphisms, shifting balance, and speciation. *Annual Review of Ecology and Systematics*, *30*(30), 201–233. https://doi.org/10.1146/annurev.ecolsys.30.1.201

Marr, D. (2010). *Vision: A computational investigation into the human representation and processing of visual information*. Cambridge, MA: The MIT Press. https://doi.org/10.7551/mitpress/9780262514620.001.0001

Marr, D., & Hildreth, E. (1980). Theory of edge detection. *Proceedings of the Royal Society of London B: Biological Sciences*, *207*(1167), 187–217. https://doi.org/10.1098/rspb.1980.0020

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

Marshall, N. J., Cortesi, F., de Busserolles, F., Siebeck, U. E., & Cheney, K. L. (2019). Colours and colour vision in reef fishes: Past, present and future research directions. *Journal of Fish Biology*, *95*(1), 5–38. https://doi.org/10.1111/jfb.13849

Marshall, N. J., & Johnsen, S. (2017). Fluorescence as a means of colour signal enhancement. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *372*(1724), 20160335. https://doi.org/10.1098/rstb.2016.0335

Marshall, N. J., Vorobyev, M., & Siebeck, U. (2006). What does a reef fish see when it sees a reef fish? Eating Nemo. In F. Laddich, S. Collin, P. Moller, & B. Kapoor (Eds.), *Communication in fishes* (pp. 393–422). Plymouth, UK: Science Publishers Inc.

McGarigal, K., & Marks, B. J. (1994). FRAGSTATS: spatial pattern analysis program for quantifying landscape structure. USDA Forest Service General Technical Report PNW, 97331(503).

Merilaita, S., Lyytinen, A., & Mappes, J. (2001). Selection for cryptic coloration in a visually heterogeneous habitat. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *268*(1479), 1925–1929. https://doi.org/10.1098/rspb.2001.1747

Miquilini, L., Walker, N. A., Odigie, E. A., Guimarães, D. L., Salomão, R. C., Lacerda, E. M. C. B., … Souza, G. S. (2017). Influence of spatial and chromatic noise on luminance discrimination. *Scientific Reports*, *7*(1), 1–11. https://doi.org/10.1038/s41598-017-16817-0

Murali, G. (2018). Now you see me, now you don't: Dynamic flash coloration as an antipredator strategy in motion. *Animal Behaviour*, *142*, 207–220. https://doi.org/10.1016/j.anbehav.2018.06.017

Ng, L., Garcia, J. E., & Dyer, A. G. (2018). Why colour is complex: Evidence that bees perceive neither brightness nor green contrast in colour signal processing. *FACETS*, *3*(1), 800–817. https://doi.org/10.1139/facets-2017-0116

Nilsson, D., Warrant, E., & Johnsen, S. (2014). Computational visual ecology in the pelagic realm. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1636), 20130038. https://doi.org/10.1098/rstb.2013.0038

Nityananda, V., Tarawneh, G., Henriksen, S., Umeton, D., Simmons, A., & Read, J. C. A. (2018). A novel form of stereo vision in the praying mantis. *Current Biology*, *28*(4), 588–593.e4. https://doi.org/10.1016/j.cub.2018.01.012

Nock, R., & Nielsen, F. (2004). Statistical region merging. *IEEE Transactions on Pattern Analysis and Machine Intelligence*, *26*(11), 1452–1458. https://doi.org/10.1109/TPAMI.2004.110

Olsson, P., Lind, O., & Kelber, A. (2015). Bird colour vision: Behavioural thresholds reveal receptor noise. *Journal of Experimental Biology*, *218*(2), 184–193. https://doi.org/10.1242/jeb.111187

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

Osborne, J., Osborne, J. W., Costello, A. B., & Kellow, J. T. (2011). Best practices in exploratory factor analysis. *Best Practices in Quantitative Methods*, *4*, 86–99. https://doi.org/10.4135/9781412995627.d8

Osorio, D., Smith, A. C., Vorobyev, M., & Buchanan-Smith, H. M. (2004). Detection of fruit and the selection of primate visual pigments for color vision. *The American Naturalist*, *164*(6), 696–708. https://doi.org/10.1086/425332

Osorio, D., & Vorobyev, M. (2005). Photoreceptor spectral sensitivities in terrestrial animals: Adaptations for luminance and colour vision. *The Royal Society*, *272*(1574), 1745–1752.

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

Pearson, P. M., & Kingdom, F. A. A. (2002). Texture-orientation mechanisms pool colour and luminance contrast. *Vision Research*, *42*(12), 1547–1558. https://doi.org/10.1016/S0042-6989(02)00067-6

Pike, T. W. (2018). Quantifying camouflage and conspicuousness using visual salience. *Methods in Ecology and Evolution*, *9*(8), 1883–1895. https://doi.org/10.1111/2041-210X.13019.

Price, T. D., Stoddard, M. C., Shevell, S. K., & Bloch, N. I. (2019). Understanding how neural responses contribute to the diversity of avian colour vision. *Animal Behaviour*, *155*, 297–305. https://doi.org/10.1016/j.anbehav.2019.05.009

Ramos, J. A., & Peters, R. A. (2017). Motion-based signaling in sympatric species of Australian agamid lizards. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, *203*(8), 661–671. https://doi.org/10.1007/s00359-017-1185-5

Renoult, J. P., Kelber, A., & Schaefer, H. M. (2017). Colour spaces in ecology and evolutionary biology. *Biological Reviews*, *92*(1), 292–315. https://doi.org/10.1111/brv.12230

Rocha, F. A. F., Saito, C. A., Silveira, L. C. L., De Souza, J. M., & Ventura, D. F. (2008). Twelve chromatically opponent ganglion cell types in turtle retina. *Visual Neuroscience*, *25*(3), 307–315. https://doi.org/10.1017/S0952523808080516

Rojas, B., Devillechabrolle, J., & Endler, J. A. (2014). Paradox lost: Variable colour-pattern geometry is associated with differences in movement in aposematic frogs. *Biology Letters*, *10*(6), 20140193. https://doi.org/10.1098/rsbl.2014.0193

Rojas, B., & Endler, J. A. (2013). Sexual dimorphism and intra-populational colour pattern variation in the aposematic frog *Dendrobates tinctorius*. *Evolutionary Ecology*, *27*(4), 739–753. https://doi.org/10.1007/s10682-013-9640-4

Rosenholtz, R., Li, Y., Jin, Z., & Mansfield, J. (2010). Feature congestion: A measure of visual clutter. *Journal of Vision*, *6*(6), 827–827. https://doi.org/10.1167/6.6.827

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

Rowe, C. (2013). Receiver psychology: A receiver's perspective. *Animal Behaviour*, *85*(3), 517–523. https://doi.org/10.1016/j.anbehav.2013.01.004

Rowe, C., & Guilford, T. (1999). The evolution of multimodal warning displays. *Evolutionary Ecology*, 655–672. Retrieved from http://link.springer.com/article/10.1023/A:1011021630244.

Russell, B. J., & Dierssen, H. M. (2015). Use of hyperspectral imagery to assess cryptic color matching in Sargassum associated crabs. *PLoS ONE*, *10*(9), 4–11. https://doi.org/10.1371/journal.pone.0136260

Ruxton, G. D., Allen, W. L., Sherratt, T. N., & Speed, M. P. (2018). *Avoiding attack*. New York: Oxford University Press.

Serre, T. (2014). Hierarchical models of the visual system. In D. Jaeger, & R. Jung (Eds.), *Encyclopedia of computational neuroscience*, Living Edition (Vol. *6*, pp. 1–12). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4614-7320-6_345-1

Shapley, R., & Hawken, M. J. (2011). Color in the cortex: Single- and double-opponent cells. *Vision Research*, *51*(7), 701–717. https://doi.org/10.1016/j.visres.2011.02.012

Shevell, S. K., & Kingdom, F. A. A. (2008). Color in complex scenes. *Annual Review of Psychology*, *59*(1), 143–166. https://doi.org/10.1146/annurev.psych.59.103006.093619

Sibeaux, A., Cole, G. L., & Endler, J. A. (2019). Success of the receptor noise model in predicting colour discrimination in guppies depends upon the colours tested. *Vision Research*, *159*(December 2018), 86–95. https://doi.org/10.1016/j.visres.2019.04.002

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

Sibeaux, A., Keser, M. L., Cole, G. L., Kranz, A. M., & Endler, J. A. (2019). How viewing objects with the dorsal or ventral retina affects colour-related behaviour in guppies (*Poecilia reticulata*). *Vision Research*, *158*(February), 78–89. https://doi.org/10.1016/j.visres.2019.02.007

Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M., & Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *The Journal of Experimental Biology*, *207*(14), 2471–2485. https://doi.org/10.1242/jeb.01047

Simmons, D. R., & Kingdom, F. A. A. (2002). Interactions between chromatic- and luminance-contrast-sensitive stereopsis mechanisms. *Vision Research*, *42*(12), 1535–1545. https://doi.org/10.1016/S0042-6989(02)00080-9

Simpson, E. E., Marshall, N. J., & Cheney, K. L. (2016). Coral reef fish perceive lightness illusions. *Scientific Reports*, *6*(October), 35335. https://doi.org/10.1038/srep35335

Smithers, S. P., Roberts, N. W., & How, M. J. (2019). Parallel processing of polarization and intensity information in fiddler crab vision. *Science Advances*, *5*(8), eaax3572. https://doi.org/10.1126/sciadv.aax3572

Stevens, M., & Merilaita, S. (2011). *Animal camouflage*. Cambridge: Cambridge University Press.

Stevens, M., Parraga, C. A., Cuthill, I. C., Partridge, J. C., & Troscianko, T. S. (2007). Using digital photography to study animal coloration. *Biological Journal of the Linnean Society*, *90*(2), 211–237. https://doi.org/10.1111/j.1095-8312.2007.00725.x

Stoddard, M. C., Kilner, R. M., & Town, C. (2014). Pattern recognition algorithm reveals how birds evolve individual egg pattern signatures. *Nature Communications*, *5*(May), 1–10. https://doi.org/10.1038/ncomms5117

Stoddard, M. C., & Osorio, D. (2019). Animal coloration patterns: Linking spatial vision to quantitative analysis. *The American Naturalist*, *193*(2), 164–186. https://doi.org/10.1086/701300

Stoddard, M. C., & Prum, R. O. (2008). Evolution of avian plumage color in a tetrahedral color space: A phylogenetic analysis of new world buntings. *The American Naturalist*, *171*(6), 755–776. https://doi.org/10.1086/587526

Stoddard, M. C., & Stevens, M. (2010). Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proceedings of the Royal Society B: Biological Sciences*, *277*(January), 1387–1393. https://doi.org/10.1098/rspb.2009.2018

Switkes, E., Mayer, M. J., & Sloan, J. A. (1978). Spatial frequency analysis of the visual environment: Anisotropy and the carpentered environment hypothesis. *Vision Research*, *18*(10), 1393–1399. https://doi.org/10.1016/0042-6989(78)90232-8

Thoen, H. H., How, M. J., Chiou, T.-H., & Marshall, N. J. (2014). A different form of color vision in mantis shrimp. *Science*, *343*(January), 411–413. https://doi.org/10.1126/science.1245824

Troscianko (2019). troscianko/micaToolbox: micaToolbox with QCPA (Version v2.0.1a). Zenodo. https://doi.org/10.5281/zenodo.3517897

Troscianko, J., Skelhorn, J., & Stevens, M. (2017). Quantifying camouflage: How to predict detectability from appearance. *BMC Evolutionary Biology*, *17*(1), 7. https://doi.org/10.1186/s12862-016-0854-2

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

Umeton, D., Tarawneh, G., Fezza, E., Read, J. C., & Rowe, C. (2019). Pattern and speed interact to hide moving prey. *Current Biology*, *29*(18), 3109–3113.e3. https://doi.org/10.1016/j.cub.2019.07.072

Van Belleghem, S. M., Papa, R., Ortiz-Zuazaga, H., Hendrickx, F., Jiggins, C. D., Mcmillan, W. O., & Counterman, B. A. (2018). PATTERNIZE: An R package for quantifying color pattern variation. *Methods in Ecology and Evolution*, *9*(2), 390–398. https://doi.org/10.1111/2041-210X.12853

van den Berg, C. (2019). Quantitative Colour Pattern Analysis (QCPA) Matlab prototype. Zenodo. https://doi.org/10.5281/zenodo.3518682

Veilleux, C. C., & Cummings, M. E. (2012). Nocturnal light environments and species ecology: Implications for nocturnal color vision in forests. *Journal of Experimental Biology*, *215*(23), 4085–4096. https://doi.org/10.1242/jeb.071415

Vereecken, N. J. (2008). Pollinator-mediated selection, reproductive isolation and floral evolution in *Ophrys orchids*. *Proceedings of the Netherlands Entomological Society Meeting*, *19*(January 2008), 9–22.

Vorobyev, M., Brandt, R., Peitsch, D., Laughlin, S. B., & Menzel, R. (2001). Colour thresholds and receptor noise: Behaviour and physiology

compared. *Vision Research*, *41*, 639–653. https://doi.org/10.1016/S0042-6989(00)00288-1

Vorobyev, M., & Osorio, D. (1998). Receptor noise as a determinant of colour thresholds. *Proceedings of the Royal Society B: Biological Sciences*, *265*(1394), 351–358. https://doi.org/10.1098/rspb.1998.0302

White, T. E., Rojas, B., Mappes, J., Rautiala, P., Kemp, D. J., & White, T. E. (2017). Colour and luminance contrasts predict the human detection of natural stimuli in complex visual environments. *Biology Letters*, *13*, 20170375. https://doi.org/10.1098/rsbl.2017.0375

Wickham, J. D., Riitters, K. H., O'Neill, R. V., Jones, K. B., & Wade, T. G. (1996). Landscape 'contagion' in raster and vector environments. *International Journal of Geographical Information Systems*, *10*(7), 891–899. https://doi.org/10.1080/02693799608902115

Willis, A., & Anderson, S. J. (2002). Colour and luminance interactions in the visual perception of motion. *Royal Society*, *269*(1495), 1011–1016. Retrieved from http://www.jstor.org/stable/3068182.

Winters, A. E., Green, N. F., Wilson, N. G., How, M. J., Garson, M. J., Marshall, N. J., & Cheney, K. L. (2017). Stabilizing selection on individual pattern elements of aposematic signals. *Proceedings of the Royal Society B*, *284*(1861), 20170926. https://doi.org/10.1098/rspb.2017.0926

Winters, A. E., Wilson, N. G., van den Berg, C. P., How, M. J., Endler, J. A., Marshall, N. J., … Cheney, K. L. (2018). Toxicity and taste: Unequal chemical defences in a mimicry ring. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1880), 20180457. https://doi.org/10.1098/rspb.2018.0457

Xiao, F., & Cuthill, I. C. (2016). Background complexity and the detectability of camouflaged targets by birds and humans. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1838), 20161527. https://doi.org/10.1098/rspb.2016.1527

Zylinski, S., How, M. J., Osorio, D., Hanlon, R. T., & Marshall, N. J. (2011). To be seen or to hide: Visual characteristics of body patterns for camouflage and communication in the Australian Giant Cuttlefish *Sepia apama*. *The American Naturalist*, *177*(5), 681–690. https://doi.org/10.1086/659626

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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