

1 APPLICATION

2 Running head: PAVO 2.0

3 pavo 2.0: new tools for the spectral and spatial
4 analysis of colour in R

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17 Abstract

- 18 1. Biological colouration presents a canvas for the study of ecological and
19 evolutionary processes. Enduring interest in colour-based phenotypes has
20 driven, and been driven by, improved techniques for quantifying colour pat-
21 terns in ever-more relevant ways, yet the need for flexible, open frameworks
22 for data processing and analysis persists.
- 23 2. Here we introduce pavo 2.0, the latest iteration of the R package pavo. This
24 release represents the extensive refinement and expansion of existing meth-
25 ods, as well as a suite of new tools for the cohesive analysis of the spectral
26 and (now) spatial structure of colour patterns and perception. At its core,
27 the package retains a broad focus on (a) the organisation and processing of
28 spectral and spatial data, and tools for the alternating (b) visualisation, and
29 (c) analysis of data. Significantly, pavo 2.0 introduces image-analysis ca-
30 pabilities, providing a cohesive workflow for the comprehensive analysis of
31 colour patterns.
- 32 3. We demonstrate the utility of pavo with a brief example centred on mimicry
33 in *Heliconius* butterflies. Drawing on visual modelling, adjacency, and bound-
34 ary strength analyses, we show that the combined spectral (colour and lu-
35 minance) and spatial (pattern element distribution and boundary salience)
36 features of putative models and mimics are closely aligned.
- 37 4. pavo 2.0 offers a flexible and reproducible environment for the analysis of
38 colour, with renewed potential to assist researchers in answering fundamen-
39 tal questions in sensory ecology and evolution.

40 Introduction

41 The study of colour in nature continues to generate fundamental knowledge:
 42 from the neurobiology and ecology of information processing (Caves *et al.*, 2018;
 43 Schnaitmann *et al.*, 2018; Thoen *et al.*, 2014; White & Kemp, 2017), to the evolution-
 44 ary drivers of life's diversity (Dalrymple *et al.*, 2015, 2018; Endler, 1980; Maia *et al.*,
 45 2013b). Colour is a subjective perceptual experience, however, so our understand-
 46 ing of the function and evolution of this conspicuous facet of variation depends
 47 on our ability to analyse phenotypes in meaningful ways. Excellent progress con-
 48 tinues to be made in this area, with emerging techniques now able to quantify and
 49 integrate both the spectral (i.e. colour and luminance) and spatial (i.e. the dis-
 50 tribution of pattern elements) properties of colour patterns (Endler, 2012; Endler
 51 *et al.*, 2018; Kemp *et al.*, 2015; Renoult *et al.*, 2015; Troscianko *et al.*, 2017). The need
 52 remains, however, for tools that integrate these complex methods into clear, open,
 53 and reproducible workflows (White *et al.*, 2015), allowing researchers to retain
 54 focus on the exploration of interesting questions.

55 Here we introduce pavo 2.0, a major revision and update of the R package
 56 pavo (Maia *et al.*, 2013a). Since its initial release, the package has provided a
 57 cohesive framework for the processing and analysis of spectral data, yet the inter-
 58 ceding years have seen the advent of novel analytical methods and the refinement
 59 of existing ones. As detailed below, pavo 2.0 has been extensively expanded to
 60 incorporate a suite of new tools, with the most significant advance being the in-
 61 clusion of geometry-based analyses. This allows for the quantification of spectral
 62 and spatial properties of colour patterns within a single workflow, thereby min-
 63 imising the computational and cognitive overhead associated with their otherwise
 64 fragmented analysis.

65 **The pavo package, version 2.0**

66 The conceptual focus of pavo remains centred on three components: (1) data
 67 importing and processing, and ongoing feedback between (2) visualisation and
 68 (3) analysis (Fig. 1). The package is available for direct installation through
 69 R from CRAN (<https://CRAN.R-project.org/package=pavo>), while the devel-
 70 opment version remains available on Github (<https://github.com/rmaia/pavo>).
 71 Comprehensive details and examples of the rich functionality of pavo are avail-
 72 able in help files as well as the package vignettes. Indeed, we strongly encour-
 73 age readers to refer to the vignettes as the primary source for information on
 74 pavo's functionality (accessible through `browseVignettes(pavo)`, and at <http://rafaelmaia.net/pavo/>), since they are updated as necessary with every pack-
 75 age release.
 76

77 **Organisation**

78 Images and spectra can be loaded into pavo in bulk through the use of `getimg` and
 79 `getspec`, respectively. Both are capable of handling multiple data formats, such
 80 as jpeg, bmp and png in the case of images, and over a dozen formats of spectral
 81 data, including the diverse and complex proprietary formats of the various spec-
 82 trometer vendors. Once loaded, the data are stored as objects of an appropriate
 83 custom S3 class, for use in further functions. Spectral data are of class `rspec`, and
 84 inherit methods from `data.frame`, while images are of class `getimg`, and are mul-
 85 tidimensional objects (typically 3D, for an RGB image) that inherits methods from
 86 `array`. If more than one image is imported in a single call to `getimg`, then each
 87 image is stored as an element of a list. This class system allows for — among
 88 other things — the reliable use of generic functions such as `plot` and `summary`,
 89 which can be called any time to inspect and visualise data.

90 Several functions then facilitate the initial processing of colour data. It is of-
 91 ten desirable to process spectra to remove unwanted noise, modify the spectral

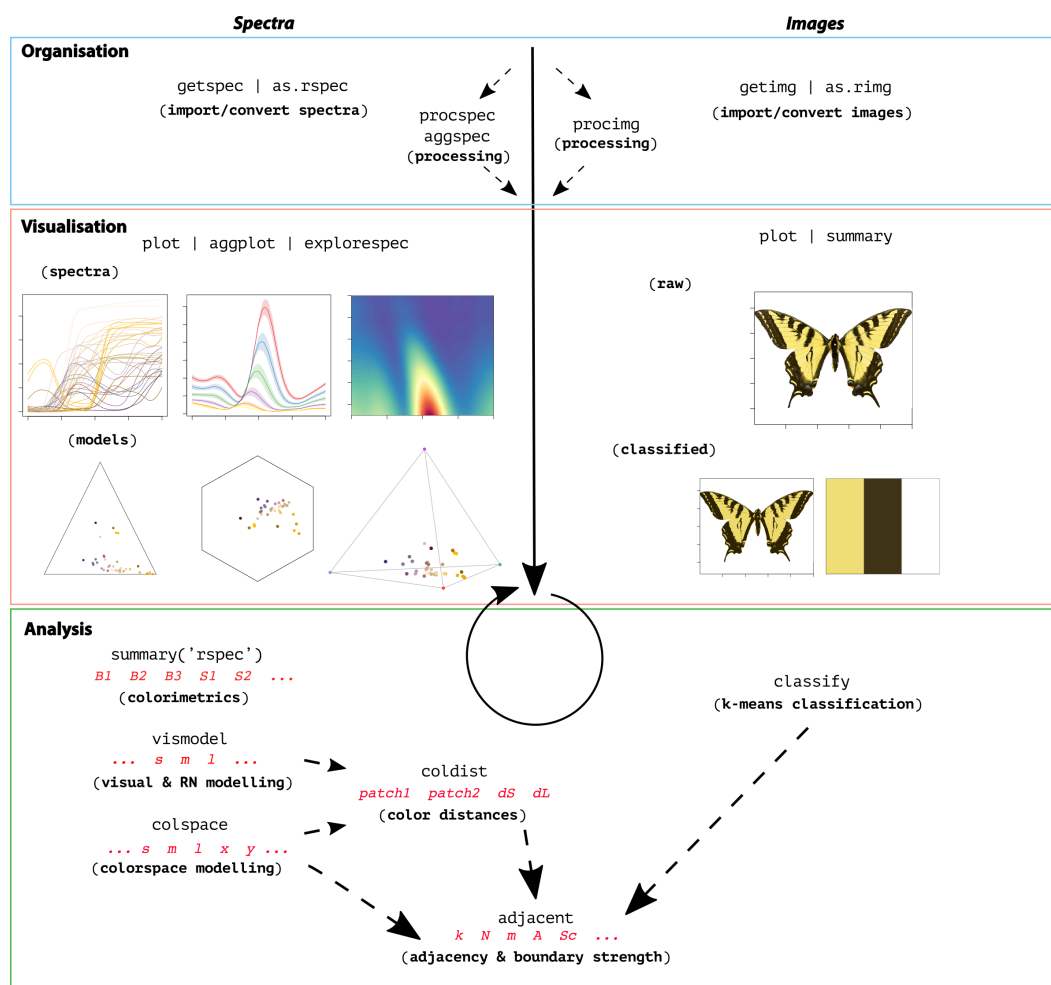


Figure 1: A general overview of the colour-pattern analysis workflow in *pavo*, as of version 2.0, displaying some key functions at each stage.

range, and/or interpolate the standard wavelength intervals, all of which may be achieved through `procspec`. For images, `procimg` offers similar functionality such as the ability to interactively specify the real-world scale of images (in preferred units of measurement), rotate and resize images, or define the boundary between a focal object and the visual background. The scope of image processing in *pavo* 2.0 is relatively limited by design, as much of what might be used during standard image handling are either needs best considered and met by researchers during image capture and data-checking, or are readily achieved within R using existing packages such as *imager* (Barthelme, 2018) and *magick* (Ooms, 2018). Indeed, *pavo* 2.0 includes two convenience functions, `ring2cimg` and `cimg2ring`, to convert be-

102 tween image-classes used by `pavo` and `imager`, allowing ready access to extensive
103 image-processing capabilities.

104 **Visualisation**

105 The repeated visualisation of spectral and spatial data is an essential step during
106 all stages of analysis, and `pavo 2.0` offers numerous tools and publication-ready
107 graphics fit for purpose. Once the package is loaded, the `plot` function recognises
108 objects of class `rspec` and `ring`, as well as `colspace` (the product of visual mod-
109 elling, detailed below), and becomes the conduit to most visualisations. For raw
110 spectral data, for example, `plot` will produce a clean plot of the spectra versus
111 wavelengths (Fig. 1, centre-left). Following visual modelling, di-, tri-, and tetra-
112 chromatic models can instead be visualised, as well as data from more specialised
113 models, such as the colour hexagon (Chittka, 1992), CIEXYZ or LAB spaces (Smith
114 & Guild, 1931; Westland *et al.*, 2012), categorical space (Troje, 1993), segment anal-
115 ysis (Endler, 1990), the colour-opponent coding space (Backhaus, 1991), or the
116 'receptor-noise' space (de Ibarra *et al.*, 2001; Pike, 2012). Images can also be plot-
117 ted, with the result depending on whether and how they have been processed.
118 When given an unprocessed `ring` object, `plot` will produce a simple raster-based
119 plot of the image (Fig. 1, right). Following the results of `classify`, in which image
120 pixels are k-means classified into discrete colour-classes (or if a colour-classified
121 image is loaded directly), the plot will use the mean RGB values of each colour-
122 class to plot the now-classified image (Fig. 2).

123 **Analysis**

124 Since the perception of colour is a subjective experience, significant progress has
125 been made in representing its reception using ecologically relevant 'visual models'
126 (Kelber *et al.*, 2003; Kemp *et al.*, 2015; Renoult *et al.*, 2015), which `pavo 2.0` includes
127 in an extended repertoire. The first step in such analyses is a call to `vismodel`,

128 which models photoreceptor stimulation (quantum-catches, or photon-flux) based
 129 on information about the viewer's visual sensitivity and viewing environments.
 130 While users are free to use their own spectra, pavo includes a suite of built-in
 131 receptor sensitivities, illuminant and transmission data (be it environmental or
 132 ocular), and viewing backgrounds, for convenience.

133 Once quantum catches are estimated the results can be used in a number of mod-
 134 els, depending on the question and analytical objective at hand (Kemp *et al.*, 2015;
 135 Renoult *et al.*, 2015). General colourspaces are available through a call to `colspace`
 136 which, if provided no further arguments, will model the data in a generalist di-
 137 tri- or tetrachromatic space informed by the dimensionality of the visual system.
 138 More specialised colourspaces — which may be informed by specific information
 139 about the visual perception of particular species — are also available via `colspace`.
 140 The CIEXYZ, CIELAB, and CIELch models (designed and intended exclusively
 141 for humans) are available, and `colspace` will check that the appropriate inputs,
 142 such as the human colour-matching function, have been used to model receptor
 143 stimulation, as required (Smith & Guild, 1931; Westland *et al.*, 2012). The colour-
 144 opponent-coding (Backhaus, 1991) and colour-hexagon (Chittka, 1992) models of
 145 bee vision are implemented, as is the categorical model of fly colour-vision de-
 146 tailed by Troje (1993). Plots for every space are accessible through a call to `plot`
 147 which, thanks to the underlying class system, will draw on the appropriate vi-
 148 sualisation for the model at hand — be it a hexagon, a dichromatic segment, a
 149 Maxwell triangle, or a three-dimensional tetrahedron.

150 The receptor-noise limited model of early-stage (retinal) colour processing has
 151 proven exceptionally popular (Vorobyev *et al.*, 2001; Vorobyev & Osorio, 1998), and
 152 has been tested to varying degrees in diverse taxa (Barry *et al.*, 2015; Fleishman
 153 *et al.*, 2016; Kelber *et al.*, 2003; Olsson *et al.*, 2015; White & Kemp, 2016). Following
 154 the estimation of receptor stimulation in `vismodel`, the model incorporates infor-
 155 mation on relative receptor densities and noise through the function `coldist`, and
 156 estimates either quantum- or neural-noise weighted colour distances. Version 2.0

157 of pavo introduces several extensions of this approach, such as the bootstrapped
 158 colour distance of `bootcoldist`, which provides an estimate of the noise-weighted
 159 distances (δS 's and/or δL 's) between the centroids of colour samples in multivari-
 160 ate space, with an appropriate measure of error (Maia & White, 2018). Stimuli can
 161 also now be expressed and plotted as coordinates in 'perceptual' (i.e. receptor-
 162 noise corrected) space by calling `jnd2xyz` on the distances calculated in `coldist`
 163 (de Ibarra *et al.*, 2001; Pike, 2012). Notably, these functions accept n-dimensional
 164 data, allowing for the modelling of extreme (Chen *et al.*, 2016; Cronin & Marshall,
 165 1989) or hypothetical high-dimensional visual systems. Of course `coldist` also
 166 accepts the results of alternative models — such as the hexagon or CIELab — and
 167 will return colour distances in units appropriate for each space.

168 Exciting recent advances now allow for the analysis of colour pattern geometry
 169 — that is, the *spatial* structure of colour patches — in conjunction with the compar-
 170 atively well-developed approaches to the *spectral* analysis of colour outlined above
 171 (Endler, 2012; Endler *et al.*, 2018; Pike, 2018; Troscianko *et al.*, 2017). The most
 172 significant extension of pavo as of 2.0 is the introduction of an image-based work-
 173 flow to allow for the combined analysis of the spectral and spatial structure of
 174 colour patterns, currently centred on the adjacency analysis (Endler, 2012), its ex-
 175 tension, the boundary strength analysis (Endler *et al.*, 2018), and related measures
 176 of overall pattern contrast (Endler & Mielke, 2005). Briefly, this process entails
 177 classifying the pixels of images into a number of discrete colour classes, before
 178 sampling the now-classified image with an evenly spaced grid. The column-wise
 179 and row-wise colour-class transitions between adjacent points are then tallied, and
 180 from this a suite of summary statistics on pattern structure — from simple colour
 181 proportions, through to colour diversity and pattern complexity — are estimated
 182 (e.g. Endler *et al.*, 2014; Rojas *et al.*, 2014; Rojas & Endler, 2013). If the colour 'dis-
 183 tance' between adjacent colour classes is known, such as might be estimated using
 184 receptor-noise modelling above, then this can also be incorporated to derive sev-
 185 eral measures of the salience of patch boundaries, which are important for colour

186 pattern perception (discussed in [Endler *et al.*, 2018](#)). In pavo 2.0, these steps are
 187 carried out through calls to `classify`, which uses k-means clustering to automati-
 188 cally or interactively classify all image pixels into discrete colour-classes, followed
 189 by `adjacent`, which performs the adjacency analysis and, if appropriate colour
 190 distances are also specified, the boundary strength analysis.

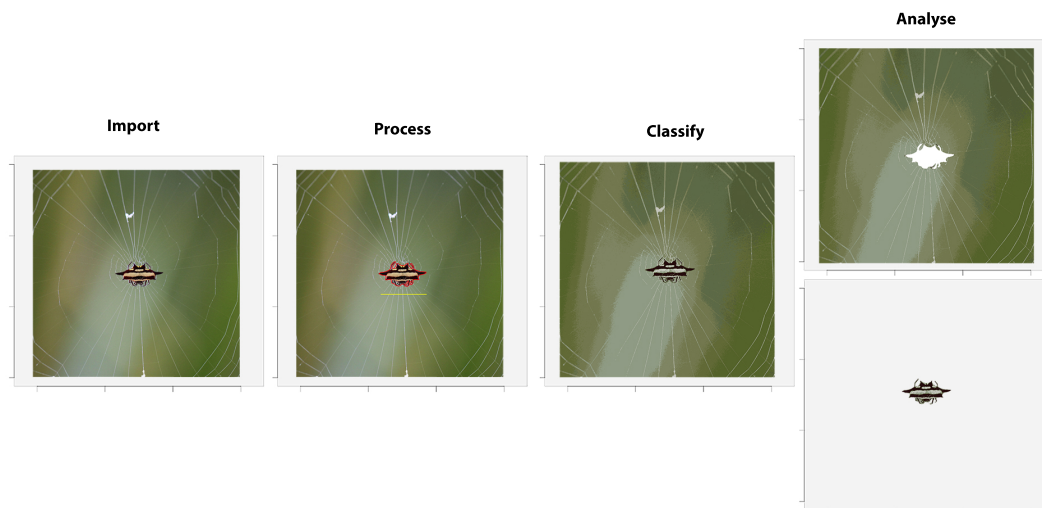


Figure 2: A sample workflow for image handling and analysis in pavo, as of version 2.0. Images are first imported and optionally processed by, for example, setting scales (yellow line) or defining objects and backgrounds (red outline). They may then be colour-classified before being passed to analytical functions, currently centered on the adjacency and boundary-strength analyses. If backgrounds and focal objects are defined then they can be analysed separately, concurrently, or either one can be excluded entirely.

191 As alluded to earlier, our goal is to provide a flexible and relatively simple
 192 analytical framework for the analysis of a colour pattern's spatial structure us-
 193 ing images, without the need for specialised photographic equipment or and/or
 194 extensive calibration and processing (demonstrated in the colour-plate based ex-
 195 ample below). We thus make an analytical and conceptual distinction between
 196 the spectral data afforded by spectrometry, and the spatial data afforded by im-
 197 ages, with the two able to be conveniently combined during latter analyses (Fig.
 198 1). This also minimises the unnecessary duplication of efforts of more general-
 199 purpose tools such as `imager` ([Barthelme, 2018](#)) and `magick` ([Ooms, 2018](#)), and the

200 excellent image analysis toolbox for imageJ (Troscianko & Stevens, 2015), which
201 offer rich functionality for image processing and (in the latter case) analysis.

202 **Worked example: mimicry in *Heliconius* spp.**

203 Butterflies of the genus *Heliconius* are widely involved in mimicry, and have proven
204 an exemplary system for studies of colour pattern development, ecology, and evo-
205 lution (Jiggins, 2016). Here we demonstrate some of pavo 2.0's capabilities by
206 briefly examining the the visual basis of mimicry in this system, with the objective
207 of quantifying the spectral and spatial (dis)similarity between putative models and
208 mimics. For our spatial analyses, we follow Endler (2012) and use colour plate XII
209 from Eltringham (1916), which is arranged into what he described as model and
210 mimic pairs (Fig. 3). For our spectral analyses we collated six reflectance spec-
211 tra from each of the the 'red', 'yellow', and 'black' patches of the forewings of two
212 species — *H. egeria* and *H. melpomene* (Fig. 3, top left pair) — from personal sources
213 and the literature (Bybee *et al.*, 2011; Wilts *et al.*, 2017). For reasons of simplicity
214 and data availability we restrict our visual modelling to these two species, though
215 the below spectral analyses would ideally be repeated for all model/mimic pairs.

216 ***Spectral analysis***

217 We first focus on the spectral data, since some of the results of this work will
218 be drawn on for the latter pattern analyses. We begin by loading the reflectance
219 spectra, which are saved in a single tab-delimited text file available at the package
220 repository along with the image plates (<https://github.com/rmaia/pavo>), before
221 LOESS-smoothing them to remove any minor electrical noise and zeroing spurious
222 negative values.

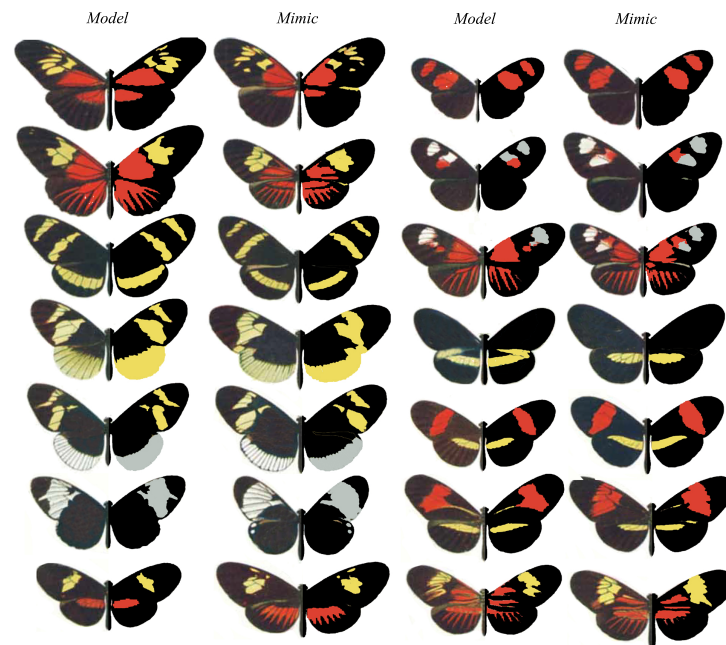


Figure 3: A modification of Eltringham's (1916) colour plate of *Heliconius* butterflies, *sensu* Endler (2012), arranged into putative models and mimics. The left side of each individual is as per the original, while the right half display pattern elements that have been classified into discrete classes through k-means clustering, using the `classify` function.

```
# Load spectra

> heli_specs <- getspec('../data', ext = 'txt')

# Smooth spectra and zero negative values

> heli_specs <- procspec(heli_specs,
>                        opt = 'smooth',
>                        fixneg = 'zero')
```

223 A call to `plot(heli_specs, col = spec2rgb(heli_specs))` displays the now-
 224 clean spectra, with each line coloured according to how it might appear to a hu-
 225 man viewer (Fig. 4, top left).

226 Since our interest is in quantifying the fidelity of visual mimicry, we must
 227 consider the perspective of ecologically relevant viewers (the primary selective
 228 agents) which, in the case of aposematic *Heliconius*, are avian predators (Benson,

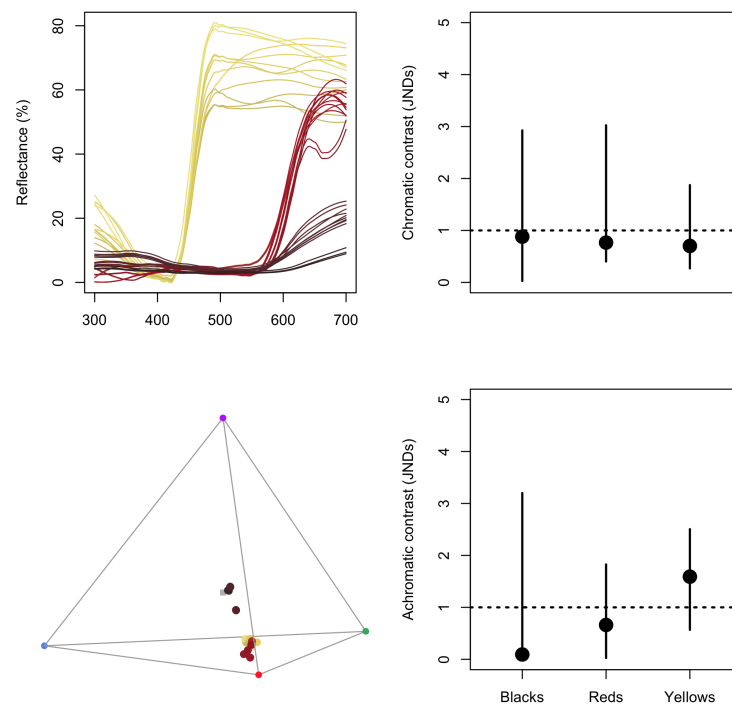


Figure 4: Reflectance spectra from black, red, and yellow patches of *H. egeria* and *H. melpomene*, along with their positions in a tetrahedral model of avian vision (left side). The bootstrapped, noise-corrected chromatic and achromatic patch distances between species (right) predicts that the individual colours of this model/mimic pair are likely indistinguishable to avian predators.

1972; Chai, 1986). We thus use the receptor-noise limited model (Vorobyev *et al.*,
2001; Vorobyev & Osorio, 1998) to predict whether the black, red, and yellow
colour patches of a representative model and mimic are distinguishable to avian
predators. This first entails estimating the photoreceptor quantum catches of a
representative viewer, so we use a built-in average UV-sensitive avian visual phe-
notype for estimating chromatic distances, and the double-cone sensitivity of the
blue tit for luminance distances.

```
> heli_model <- vismodel(heli_specs,  
>                         visual = 'avg.uv',  
>                         achromatic = 'bt.dc',  
>                         relative = FALSE)
```

At this point we may wish to get a quick sense of the relative distribution

of stimuli by converting them to locations in an avian tetrahedral colourspace and plotting the results with `plot(colspace(heli_model))` (Fig. 4). With receptor stimulation estimated, we now calculate noise-corrected chromatic and achromatic distances between patches. The `coldist` function can be used to return the pairwise distances between every spectrum, which might then be averaged to derive a mean distance between species for every patch. This neglects the multivariate structure of such data, however, when the objective is to estimate the separation of groups in colourspace (Maia & White, 2018). We therefore prefer a bootstrapped measure of colour distance using `bootcoldist`, which provides a robust measure of the separation of our focal samples (i.e the red, white, and black patches of model versus mimic), along with a 95% confidence interval, which can be inspected to see if it exceeds the theoretical discrimination threshold of one JND. We specify a relative receptor density of 1:2:2:4 (ultraviolet:short:medium:long wavelength receptors; Maier & Bowmaker (1993)), a signal-to-noise ratio yielding a Weber fraction of 0.1 for both chromatic and achromatic receptors, and assume that noise is proportional to the Weber fraction and independent of the magnitude of receptor stimulation (reviewed in Kelber *et al.* (2003); Olsson *et al.* (2017)).

```
# Calculate the bootstrapped, noise-corrected colour distance
# between groups, using sample names to specify grouping ID's.
> heli_dist <- bootcoldist(heli_model,
>                           by = sub('\\.*', '', rownames(heli_model)),
>                           n = c(1, 2, 2, 4),
>                           weber = 0.1,
>                           weber.achro = 0.1)
```

Inspection of the key comparisons of interest (Fig. 4, right) reveals that the 95% CI of all chromatic and achromatic comparisons includes the theoretical threshold of one JND. This predicts that the individual colour pattern elements of putative model and mimic *H. egeria* and *H. melpomene* are indistinguishable, or difficult to

discriminate, to avian viewers — the assumed intended recipient of the aposematic signals. As noted above, the analysis of this representative pair can be readily scaled to encompass all species given the necessary data, and we can now use this information to inform our study of the spatial structure of these signals.

Pattern analysis

We first load the focal images, which comprise the individual samples from plate XII of [Eltringham \(1916\)](#), saved as jpegs (Fig. 3). We then plot one or all of the images to check they are as expected.

```
# Load all images. Here the 28 jpegs are stored in a folder called
# 'butterflies' located within the current working directory.
> heli_images <- getimg("butterflies")
28 files found; importing images.

# Plot the first image in the list only.
> plot(heli_images[[1]])

# Plot all images, which will progress through
# the sequence automatically.
> plot(heli_images)
```

We then classify the pixels of all images into discrete colour or luminance categories, here using k-means clustering, to create a colour-classified image matrix. The function `classify` will carry this out, though there are numerous specific ways in which it may be achieved, including automatically or ‘interactively’, with the option of a reference image as template. Since our images are heterogeneous, it is simplest to use the interactive version of `classify`, which will cycle through each image and ask the user to manually identify a sample from every discrete colour or luminance class present, which are then used as cluster centres.

```
# Interactively colour-classify all images using k-means clustering.
> heli_class <- classify(heli_images, interactive = TRUE)

# Cycle through plots of the colour-classified images, alongside their
# identified colour palettes.
> summary(heli_class, plot = TRUE)
```

274 Finally, we use an adjacency analysis to estimate a suite of metrics describ-
 275 ing the structure and complexity of the colour pattern geometry of model and
 276 mimic *Heliconius*, and by including the visually-modelled colour distances esti-
 277 mated above, the output will include several measures of the salience of colour
 278 patch edges as part of the boundary strength analysis (Endler, 2012; Endler *et al.*,
 279 2018). We will exclude the white background since it is not relevant, simply by
 280 specifying the colour-category ID belonging to the homogeneous underlay. If the
 281 image was more complex, such as an animal in its natural habitat, we would in-
 282 stead interactively identify and separate the focal animal and background using
 283 procimg (e.g. Fig. 2, second panel).

```
# Construct and inspect a data.frame of pairwise colour and luminance
# distances between all colour classes, constructed from the earlier
# receptor-noise modelled estimates. Note that we do not bother
# including colour-class ID 1, since that is the white background
# which is to be excluded from the analysis (see below).
# (Alternatively we could include it, and it would simply be ignored).
> distances <- data.frame(c1 = c(2, 2, 3),
                          c2 = c(3, 4, 4),
                          dS = c(10.6, 5.1, 4.4),
                          dL = c(1.1, 2.5, 3.2))

> distances
  c1 c2  dS  dL
```

```

2   3   10.50   7.41
2   4   11.76   23.40
3   4   13.29   15.99

# Calculate adjacency and boundary-strength statistics. We specify a
# scale of 50 mm, and note that the 'white' background, which has a class
# ID of 1 in this case, is to be excluded from the analysis.
# We also include the colour distance between all patches, as estimated above.
> heli_adj <- adjacent(heli_class,
>                      xscale = 50,
>                      bkgID = 1,
>                      exclude = 'background',
>                      coldists = distances)

# Inspect a subset of the resulting data.frame. Variable meanings
# are detailed in the function documentation (see ?adjacent),
# or Endler (2012), Endler et al. (2018), and Endler & Mielke (2005).
> head(heli_adj)[, 1:7]
      k  N      n_off  p_2   p_3   p_4   q_2_2  ...
mimic_01 3 345522  6547  0.801  0.130  0.067  0.796
mimic_02 2 1018370 4091  0.835  0.164  NA      0.834
mimic_03 3 265278  6155  0.685  0.198  0.116  0.677
...

```

284 We can now inspect the pattern descriptors of particular interest, and explore
285 the similarity of models and mimics with respect to their broader colour pattern
286 geometry. As seen in Fig. 5, the relative proportions of focal colours (top row),
287 measures of pattern diversity and complexity (centre row), and the salience of
288 patch boundaries (bottom row) are highly correlated between species pairs. This,
289 in conjunction with the above modelling, suggests that the overall colour pat-

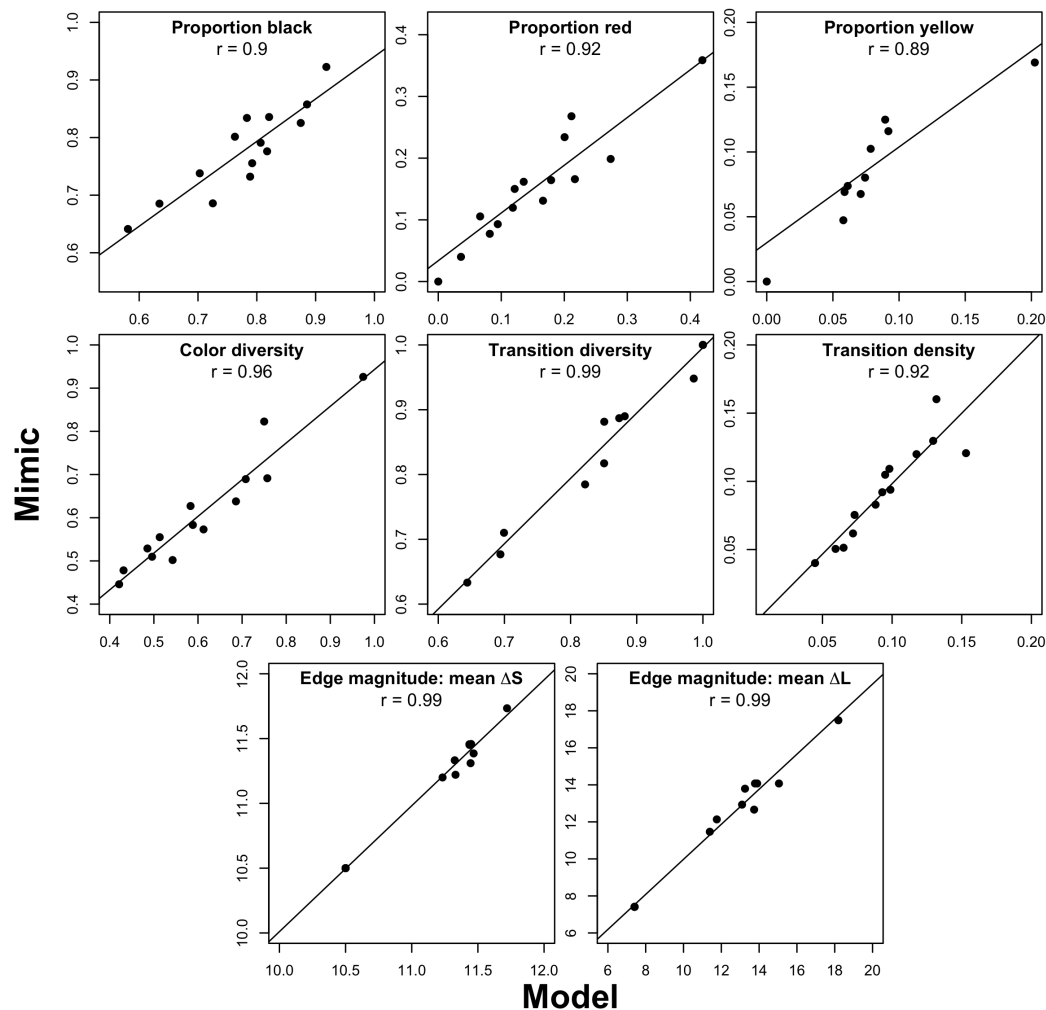


Figure 5: Select results of the colour pattern analysis of model and mimic *Heliconius* (Fig. 3), using adjacency and boundary strength analyses. Strong correlations are evident in colour proportions (top row), measures of colour diversity and complexity (centre row), and estimates of mean chromatic and achromatic edge salience (bottom row).

290 terns of putative model and mimic *Heliconius* — both spectrally and spatially —
 291 are highly similar, and are thus predicted to be very difficult to discriminate to
 292 the intended avian viewers of their aposematic signals, as consistent with theory
 293 (Müller, 1879). More interesting questions remain, of course, including the degree
 294 to which mimics need resemble models to deceive viewers, and the relative impor-
 295 tance of different colour pattern elements (e.g. Fig. 5) in mediating the subjective
 296 resemblance of species pairs, for which pavo is well suited to help answer.

297 **Conclusions**

298 The integrative study of biological colouration has borne rich fruit, though its po-
 299 tential to illuminate the structure and function of much of the natural world is not
 300 nearly realised (Endler & Mappes, 2017). As we have sought to demonstrate, pavo
 301 2.0 (and beyond) provides a flexible framework to assist researchers studying
 302 the physiology, ecology, and evolution of colour patterns and visual perception.
 303 We appreciate bug reports and suggestions, via email or the Github issue tracker
 304 <https://github.com/rmaia/pavo/issues>.

305 **Citation of methods**

306 Many of the methods applied in pavo are described in detail in their original
 307 publications — as listed in the documentation for the relevant functions — to
 308 which users should refer and cite as appropriate, along with pavo itself, via this
 309 publication (as of v2.0).

310 **Acknowledgements**

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 312 image-based methods. TEW thanks Elizabeth Mulvenna and Cormac White for
 313 their support. The authors have no conflicts of interest to declare.

314 **Authors statement**

315 TEW, RM, and HG authored the software and manuscript, JAE developed and
 316 assisted in the implementation of methods, and critically revised the manuscript.

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