

1 APPLICATION

2 Running head: PAVO 2

3 pavo 2: 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`, 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` introduces image-analysis capabili-  
30 ties, providing a cohesive workflow for the comprehensive analysis of colour  
31 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` 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, a major revision and update of the R package pavo  
56 (Maia *et al.*, 2013a). Since its initial release, the package has provided a cohesive  
57 framework for the processing and analysis of spectral data, yet the interceding  
58 years have seen the advent of novel analytical methods and the refinement of  
59 existing ones. As detailed below, pavo 2 has been extensively expanded to incor-  
60 porate a suite of new tools, with the most significant advance being the inclusion  
61 of geometry-based analyses. This allows for the quantification of spectral and spa-  
62 tial properties of colour patterns within a single workflow, thereby minimising the  
63 computational and cognitive overhead associated with their otherwise fragmented  
64 analysis.

## 65 **The pavo package, version 2**

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://](http://rafaelmaia.net/pavo/)  
75 [rafaelmaia.net/pavo/](http://rafaelmaia.net/pavo/)), since they are updated as necessary with every pack-  
76 age release.

## 77 **Organisation**

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

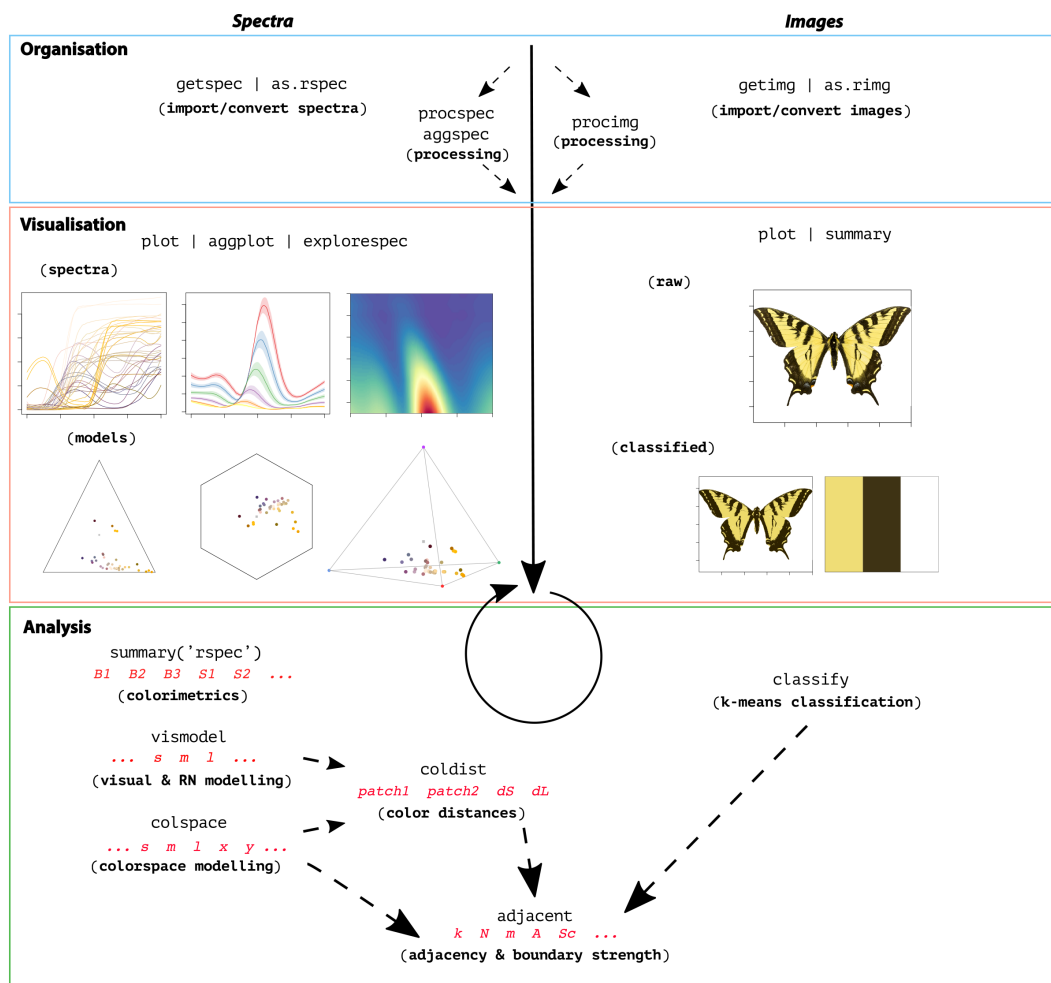


Figure 1: A general overview of the colour-pattern analysis workflow in *pavo*, as of version 2, displaying some key functions at each stage.

92 range, and/or interpolate the standard wavelength intervals, all of which may be  
 93 achieved through `procspec()`. For images, `procing()` offers similar functionality  
 94 such as the ability to interactively specify the real-world scale of images (in pre-  
 95 ferred units of measurement), rotate and resize images, or define the boundary  
 96 between a focal object and the visual background. The scope of image processing  
 97 in *pavo 2* is relatively limited by design, as much of what might be used during  
 98 standard image handling are either needs best considered and met by researchers  
 99 during image capture and data-checking, or are readily achieved within R using  
 100 existing packages such as *imager* (Barthelme, 2018) and *magick* (Ooms, 2018). In-  
 101 deed, *pavo 2* includes convenience functions to convert between image-classes

102 used by `pavo`, `imager`, and `magick`, allowing ready access to extensive image-  
103 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` offers numerous tools and publication-ready  
107 graphics fit for purpose. Once the package is loaded, the `plot()` function recog-  
108 nises objects of class `rspec` and `ring`, as well as `colspace` (the product of visual  
109 modelling, detailed below), and becomes the conduit to most visualisations. For  
110 raw spectral data, for example, `plot()` will produce a clean plot of the spectra  
111 versus wavelengths (Fig. 1, centre-left). Following visual modelling, di-, tri-, and  
112 tetra-chromatic models can instead be visualised, as well as data from more spe-  
113 cialised models, such as the colour hexagon (Chittka, 1992), CIEXYZ or LAB spaces  
114 (Smith & Guild, 1931; Westland *et al.*, 2012), categorical space (Troje, 1993), segment  
115 analysis (Ender, 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 plotted,  
117 with the result depending on whether and how they have been processed. When  
118 given an unprocessed `ring` object, `plot()` will produce a simple raster-based plot  
119 of the image (Fig. 1, right). Following the results of `classify()` (discussed be-  
120 low), in which image pixels are k-means classified into discrete colour-classes (or  
121 if a colour-classified image is loaded directly), the plot will use the mean RGB  
122 values of each colour-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` 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.*,  
135 2015; *Renoult et al.*, 2015). General colourspaces are available through a call to  
136 `colspace()` which, if provided no further arguments, will model the data in a  
137 generalist di- tri- or tetrachromatic space informed by the dimensionality of the  
138 visual system. More specialised colourspaces — which may be informed by spe-  
139 cific information about the visual perception of particular species — are also avail-  
140 able via `colspace()`. The CIEXYZ, CIELAB, and CIELch models (designed and  
141 intended exclusively for humans) are available, and `colspace()` will check that the  
142 appropriate inputs, such as the human colour-matching function, have been used  
143 to model receptor stimulation, as required (*Smith & Guild*, 1931; *Westland et al.*,  
144 2012). The colour-opponent-coding (*Backhaus*, 1991) and colour-hexagon (*Chit-  
145 tka*, 1992) models of bee vision are implemented, as is the categorical model of fly  
146 colour-vision detailed by *Troje* (1993). Plots for every space are accessible through  
147 a call to `plot()` which, thanks to the underlying class system, will draw on the  
148 appropriate visualisation for the model at hand — be it a hexagon, a dichromatic  
149 segment, a 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),  
152 and has been tested to varying degrees in diverse taxa (*Barry et al.*, 2015; *Fleish-  
153 man et al.*, 2016; *Kelber et al.*, 2003; *Olsson et al.*, 2015; *White & Kemp*, 2016).  
154 Following the estimation of receptor stimulation in `vismodel()`, the model incor-  
155 porates information on relative receptor densities and noise through the function  
156 `coldist()`, and estimates either quantum- or neural-noise weighted colour dis-

157 tances. Version 2 of `pavo` introduces several extensions of this approach, such as  
158 the bootstrapped colour distance of `bootcoldist()`, which provides an estimate  
159 of the noise-weighted distances ( $\delta S$ 's and/or  $\delta L$ 's) between the centroids of colour  
160 samples in multivariate space, with an appropriate measure of error (detailed in  
161 [Maia & White, 2018](#)). Stimuli can also now be expressed and plotted as coordi-  
162 nates in 'perceptual' (i.e. receptor-noise corrected) space by calling `jnd2xyz()` on  
163 the distances calculated in `coldist()` ([de Ibarra et al., 2001](#); [Pike, 2012](#)). Notably,  
164 these functions now accept n-dimensional data (derived independently, but see  
165 [Clark et al., 2017](#); [Gawryszewski, 2018](#), for valuable discussion). This allows for the  
166 modelling of extreme ([Chen et al., 2016](#); [Cronin & Marshall, 1989](#), though given the  
167 lack of support for traditional opponency in these systems, the RN model may be  
168 of limited use here) or entirely hypothetical visual systems. Of course `coldist()`  
169 also accepts the results of alternative models — such as the hexagon or CIELab —  
170 and will return colour distances in units appropriate for each space.

171 Exciting recent advances now allow for the analysis of colour pattern geom-  
172 etry — that is, the *spatial* structure of colour patches — in conjunction with the  
173 comparatively well-developed approaches to the *spectral* analysis of colour out-  
174 lined above ([Endler, 2012](#); [Endler et al., 2018](#); [Pike, 2018](#); [Troscianko et al., 2017](#)).  
175 The most significant extension of `pavo` as of version 2 is the introduction of an  
176 image-based workflow to allow for the combined analysis of the spectral and spa-  
177 tial structure of colour patterns, currently centred on measures of overall pattern  
178 contrast ([Endler & Mielke, 2005](#)), the adjacency analysis ([Endler, 2012](#)), and its  
179 extension, the boundary strength analysis ([Endler et al., 2018](#)). In `pavo 2`, the var-  
180 ious steps for such analyses are carried out through calls to `classify()`, which  
181 uses k-means clustering to automatically or interactively classify image pixels into  
182 discrete colour-classes, and/or `adjacent()`, which performs the adjacency analy-  
183 sis and, if appropriate colour distances are also specified, the boundary strength  
184 analysis (discussed in [Endler et al., 2018](#)).

185 Briefly, these analyses entail classifying evenly-spaced points within a visual



186 scene into discrete colour classes using spectrometric measurements and/or pho-  
187 tography. The column-wise and row-wise colour-class transitions between adja-  
188 cent points are then tallied, and from this a suite of summary statistics on pattern  
189 structure — from simple colour proportions, through to colour diversity and pat-  
190 tern complexity — are estimated (e.g. [Endler \*et al.\*, 2014](#); [Rojas \*et al.\*, 2014](#); [Rojas  
& Endler, 2013](#); [White, 2017](#)). The precise procedure that might be followed by  
192 researchers may vary considerably depending on the goal and tools at hand, and  
193 `pavo 2` is designed to accommodate such flexibility. In relatively simple cases (as  
194 in the below example), users may import and calibrate images via `getimg()` and  
195 `procimg()`, k-means classify the entire image using `classify()`, and combine it  
196 with spectrometric measurements and visual modelling of the few discrete colour-  
197 classes in a call to `adjacent()`. In more complex cases, such as animals in their  
198 natural habitats, users may instead wish to collect spectrometric measurements  
199 along a grid-sample of the visual scene, visually model and statistically cluster the  
200 results (e.g. using `vismodel()`), then feed the resulting colour-classified grid into  
201 `adjacent()` directly (as per ‘method 1’: [Endler, 2012](#)), without the use of images  
202 or the `classify()` function at all.

203 As alluded to earlier, our goal is to provide a flexible and relatively simple an-  
204 analytical framework for the analysis of a colour pattern’s spatial structure using im-  
205 ages, without the requirement for specialised photographic equipment or and/or  
206 extensive calibration and processing (demonstrated in the colour-plate based ex-  
207 ample below). We thus make an analytical and conceptual distinction between  
208 the spectral data afforded by spectrometry, and the spatial data afforded by im-  
209 ages, with the two able to be conveniently combined during latter analyses (Fig.  
210 1). This also minimises the unnecessary duplication of efforts of more general-  
211 purpose tools such as `imager` ([Barthelme, 2018](#)) and `magick` ([Ooms, 2018](#)), and the  
212 excellent image analysis toolbox for `imageJ` ([Troscianko & Stevens, 2015](#)), which  
213 offer rich functionality for image processing and (in the latter case) analysis. We  
214 emphasise, however, that the convenience of the toolkit provided by `pavo 2` belies

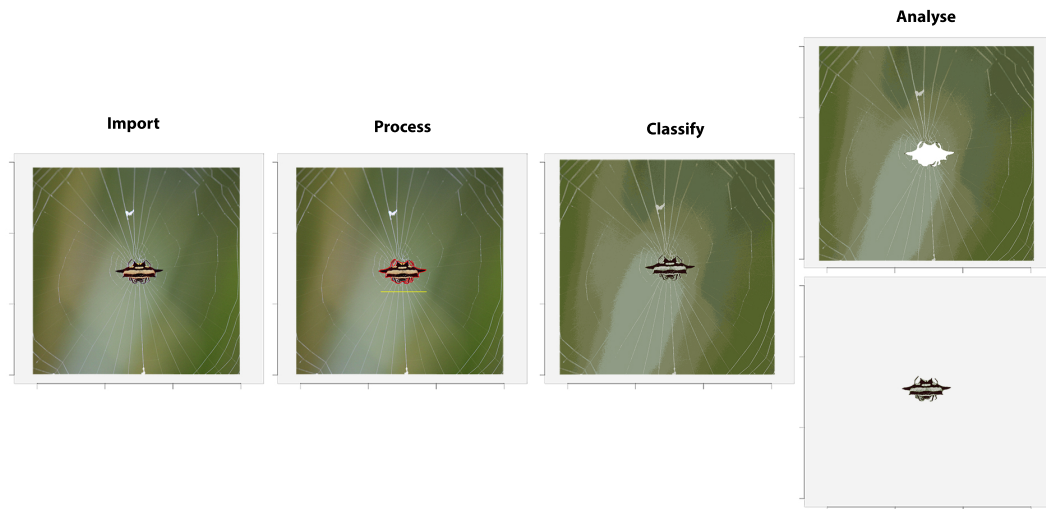


Figure 2: A sample workflow for image handling and analysis in pavo, as of version 2. Images are first imported and optionally processed by, for example, setting scales (yellow line) or defining objects and backgrounds (red outline). They may then be colour-classified before being passed to analytical 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.

215 the complexity of the choices demanded of researchers, and that every parameter  
216 and option requires close consideration and justification. It is rare, for example,  
217 that image analyses should be used without any input from visually-modelled  
218 spectrometric data, since naive clustering performed on uncalibrated images will  
219 typically offer a poor representation of a visual scene as relevant to non-human an-  
220 imals. For example, even in simple cases, as below, the number of discrete patches  
221 present (i.e. the argument `kcols` in `cluster()`) is best estimated using spectro-  
222 metric data in an ecologically relevant model, rather than relying exclusively on  
223 human-subjective estimates of colour segregation. One possible approach is inte-  
224 grated into the below example, and Endler (2012) details others, such as estimating  
225 `kcols` as the number of receptor-noise ellipsoids required to encompass the entire  
226 sample of spectra.

## 227 **Worked example: mimicry in *Heliconius* spp.**

228 Butterflies of the genus *Heliconius* are widely involved in mimicry, and have proven  
229 an exemplary system for studies of colour pattern development, ecology, and evo-  
230 lution (Jiggins, 2016). Here we demonstrate some of pavo 2's capabilities by briefly  
231 examining the the visual basis of mimicry in this system, with the objective of  
232 quantifying the spectral and spatial (dis)similarity between putative models and  
233 mimics. For our spatial analyses, we follow Endler (2012) and use colour plate XII  
234 from Eltringham (1916), which is arranged into what he described as model and  
235 mimic pairs (Fig. 3). For our spectral analyses we collated six reflectance spectra  
236 from each of the assumed-discrete 'red', 'yellow', and 'black' patches (confirmed  
237 by spectral measurement, below) of the forewings of two species — *H. egeria* and  
238 *H. melpomene* (Fig. 3, top left pair) — from personal sources and the literature  
239 (Bybee *et al.*, 2011; Wilts *et al.*, 2017). For reasons of simplicity and data availability  
240 we restrict our visual modelling to these two species, though the below spectral  
241 analyses would ideally be repeated for all model/mimic pairs.

### 242 *Spectral analysis*

243 We first focus on the spectral data, both to confirm the assumption that there  
244 are discrete colour patches and because some of the results of this work will be  
245 drawn on for the latter pattern analyses. We begin by loading the reflectance  
246 spectra, which are saved in a single tab-delimited text file along with the image  
247 plates (available at the package repository; <https://github.com/rmaia/pavo>, or  
248 via figshare; <https://dx.doi.org/10.6084/m9.figshare.7445840.v1>), before LOESS-  
249 smoothing them to remove any minor electrical noise and zeroing spurious nega-  
250 tive values.

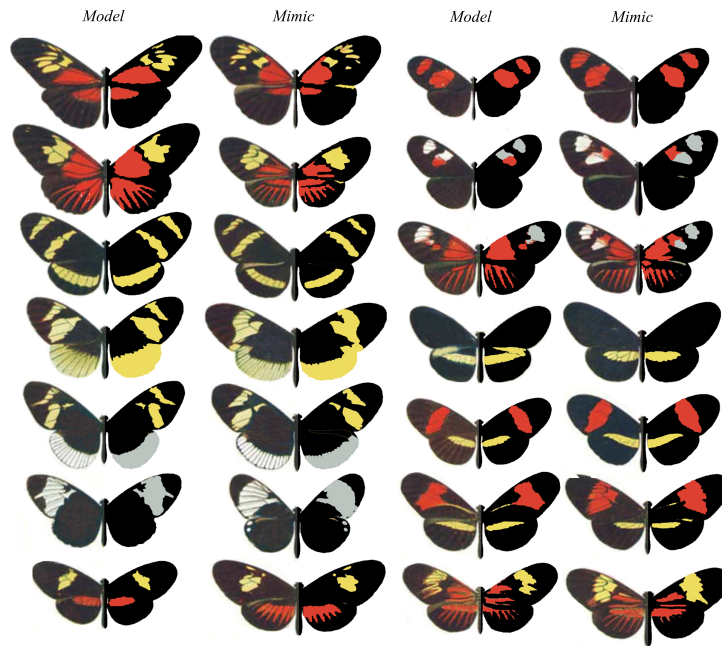


Figure 3: A modification of Eltringham's (1916) colour plate of *Heliconius* butterflies, *sensu* Endler (2012), arranged into putative models and mimics. The left side of each individual is as per the original, while the right half display pattern elements that have been classified into discrete classes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

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

254 Our interest is in quantifying the fidelity of visual mimicry, so we must con-  
255 sider the perspective of ecologically relevant viewers (the primary selective agents)  
256 which, in the case of aposematic *Heliconius*, are avian predators (Benson, 1972;

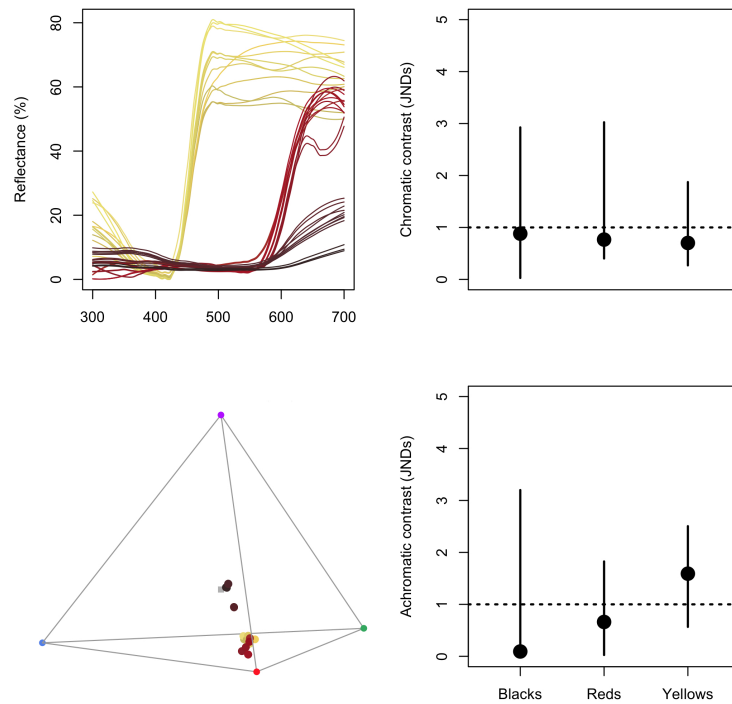


Figure 4: Reflectance spectra from black, red, and yellow patches of *H. egeria* and *H. melpomene*, along with their positions in a tetrahedral model of avian vision (left side). The bootstrapped, noise-corrected chromatic and achromatic patch distances between species (right) predicts that the individual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

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

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

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

265 of stimuli by converting them to locations in an avian tetrahedral colourspace  
266 and plotting the results with `plot(colspace(heli_model))` (Fig. 4). With receptor  
267 stimulation estimated, we now calculate noise-corrected chromatic and achromatic  
268 distances between patches. The `colldist()` function can be used to return the pair-  
269 wise distances between every spectrum, which might then be averaged to derive  
270 a mean distance between species for every patch. This neglects the multivariate  
271 structure of such data, however, when the objective is to estimate the separation of  
272 groups in colourspace (Maia & White, 2018). We therefore prefer a bootstrapped  
273 measure of colour distance using `bootcolldist()`, which provides a robust mea-  
274 sure of the separation of our focal samples (i.e the red, white, and black patches  
275 of model versus mimic), along with a 95% confidence interval, which can be in-  
276 spected to see if it exceeds the theoretical discrimination threshold of one JND. We  
277 specify a relative receptor density of 1:2:2:4 (ultraviolet:short:medium:long wave-  
278 length receptors; Maier & Bowmaker (1993)), a signal-to-noise ratio yielding a  
279 Weber fraction of 0.1 for both chromatic and achromatic receptors, and assume  
280 that noise is proportional to the Weber fraction and independent of the magnitude  
281 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 <- bootcolldist(heli_model,  
>                               by = sub('\\\\.\\.*', '', rownames(heli_model)),  
>                               n = c(1, 2, 2, 4),  
>                               weber = 0.1,  
>                               weber.achro = 0.1)
```

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

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

### 290 *Pattern analysis*

291 We first load the focal images, which comprise the individual samples from plate  
292 XII of Eltringham (1916), saved as jpegs (Fig. 3). We then plot one or all of the  
293 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)
```

294 We then classify the pixels of all images into discrete colour or luminance cat-  
295 egories, here using k-means clustering, to create a colour-classified image matrix.  
296 The function `classify()` will carry this out, though there are numerous specific  
297 ways in which it may be achieved, including automatically or 'interactively', with  
298 the option of a reference image as template. Since our images are heterogeneous, it  
299 is simplest to use the interactive version of `classify()`, which will cycle through  
300 each image and ask the user to manually identify a sample from every discrete  
301 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)
```

302 Finally, we use an adjacency analysis to estimate a suite of metrics describ-  
303 ing the structure and complexity of the colour pattern geometry of model and  
304 mimic *Heliconius*, and by including the visually-modelled colour distances esti-  
305 mated above, the output will include several measures of the salience of colour  
306 patch edges as part of the boundary strength analysis (Endler, 2012; Endler *et al.*,  
307 2018). We will exclude the white background since it is not relevant, simply by  
308 specifying the colour-category ID belonging to the homogeneous underlay. If the  
309 image was more complex, such as an animal in its natural habitat, we might in-  
310 stead interactively identify and separate the focal animal and background using  
311 `procing()` (e.g. Fig. 2, second panel). Alternatively, we might forego the use of  
312 images altogether, and instead grid-sample and cluster the spectra across the vi-  
313 sual scene and use these in directly in the call to `adjacent()` (*sensu* 'method 1' in  
314 Endler 2012, mentioned above).

```
# Construct and inspect a data.frame of pairwise colour and luminance  
# distances between all colour classes, built 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 the 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
...
```

315 We can now inspect the pattern descriptors of particular interest, and explore  
316 the similarity of models and mimics with respect to their broader colour pattern  
317 geometry. As seen in Fig. 5, the relative proportions of focal colours (top row),

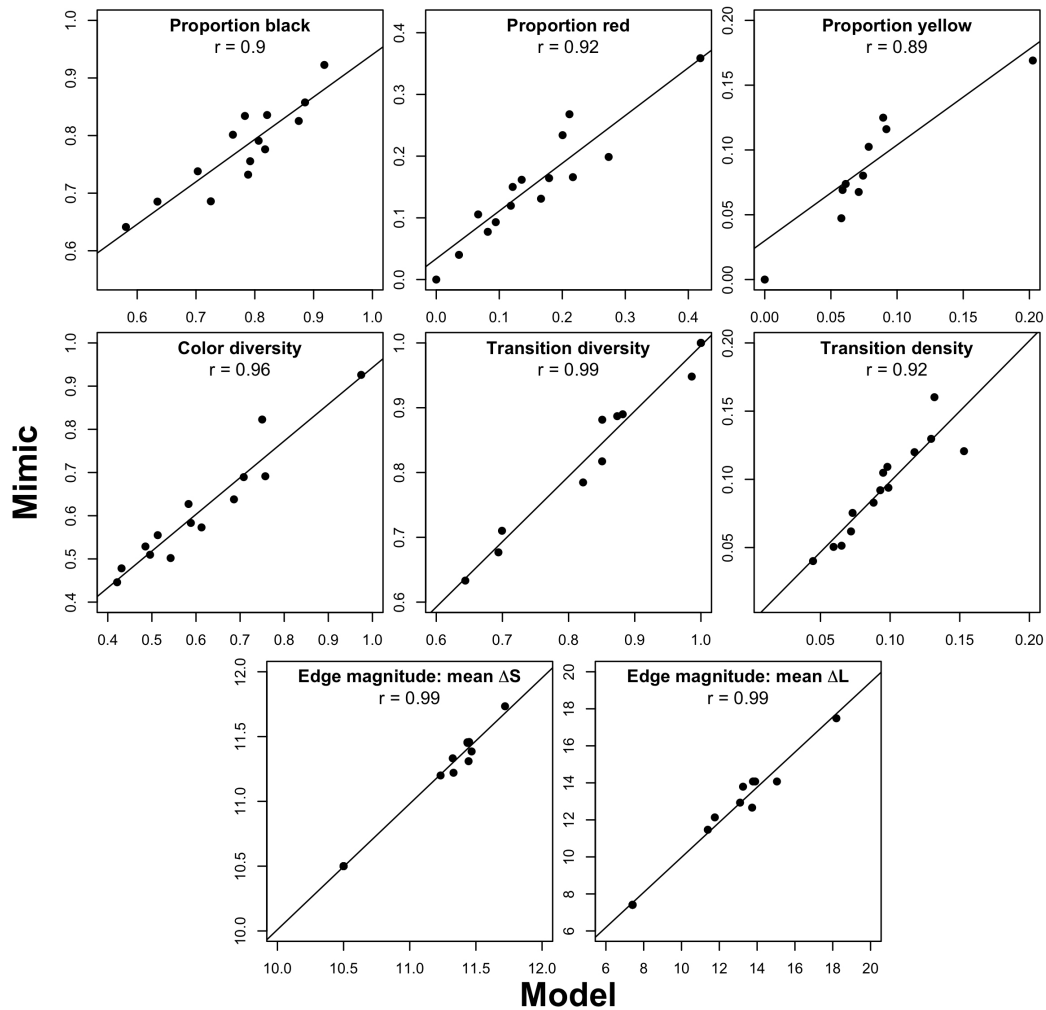


Figure 5: Select results of the colour pattern analysis of model and mimic *Heliconius* (Fig. 3), using adjacency and boundary strength analyses. Strong correlations are evident in colour proportions (top row), measures of colour diversity and complexity (centre row), and estimates of mean chromatic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

318 measures of pattern diversity and complexity (centre row), and the salience of  
319 patch boundaries (bottom row) are highly correlated between species pairs. This,  
320 in conjunction with the above modelling, suggests that the overall colour pat-  
321 terns of putative model and mimic *Heliconius* — both spectrally and spatially —  
322 are highly similar, and are thus predicted to be very difficult to discriminate to  
323 the intended avian viewers of their aposematic signals, as consistent with theory  
324 (Müller, 1879). More interesting questions remain, of course, including the degree

325 to which mimics need resemble models to deceive viewers, and the relative impor-  
326 tance of different colour pattern elements (e.g. Fig. 5) in mediating the subjective  
327 resemblance of species pairs, for which pavo 2 is well suited to help answer.

## 328 **Conclusions**

329 The integrative study of biological colouration has borne rich fruit, though its  
330 potential to illuminate the structure and function of much of the natural world is  
331 not nearly realised (Endler & Mappes, 2017). As we have sought to demonstrate,  
332 pavo 2 (and beyond) provides a flexible framework to assist researchers studying  
333 the physiology, ecology, and evolution of colour patterns and visual perception.  
334 We appreciate bug reports and suggestions, via email or the Github issue tracker  
335 <https://github.com/rmaia/pavo/issues>.

## 336 **Citation of methods**

337 Many of the methods applied in pavo 2 are described in detail in their original  
338 publications — as listed in the documentation for the relevant functions — to  
339 which users should refer and cite as appropriate, along with pavo itself, via this  
340 publication.

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347 **Authors statement**

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

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