

APPLICATION

pavo: an R package for the analysis, visualization and organization of spectral data

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Summary

1. Recent technical and methodological advances have led to a dramatic increase in the use of spectrometry to quantify reflectance properties of biological materials, as well as models to determine how these colours are perceived by animals, providing important insights into ecological and evolutionary aspects of animal visual communication.

2. Despite this growing interest, a unified cross-platform framework for analysing and visualizing spectral data has not been available. We introduce *pavo*, an R package that facilitates the organization, visualization and analysis of spectral data in a cohesive framework. *pavo* is highly flexible, allowing users to (a) organize and manipulate data from a variety of sources, (b) visualize data using R's state-of-the-art graphics capabilities and (c) analyse data using spectral curve shape properties and visual system modelling for a broad range of taxa.

3. In this paper, we present a summary of the functions implemented in *pavo* and how they integrate in a workflow to explore and analyse spectral data. We also present an exact solution for the calculation of colour volume overlap in colour space, thus expanding previously published methodologies.

4. As an example of *pavo*'s capabilities, we compare the colour patterns of three African glossy starling species, two of which have diverged very recently. We demonstrate how both colour vision models and direct spectral measurement analysis can be used to describe colour attributes and differences between these species. Different approaches to visual models and several plotting capabilities exemplify the package's versatility and streamlined workflow.

5. *pavo* provides a cohesive environment for handling spectral data and addressing complex sensory ecology questions, while integrating with R's modular core for a broader and comprehensive analytical framework, automated management of spectral data and reproducible workflows for colour analysis.

Key-words: animal communication, colour, colour space, receptor noise, reflectance, sensory ecology, spectrometry, visual model

Introduction

The role of colouration and colour vision in animal communication has been a fundamental question in evolutionary biology for many decades (Darwin 1859, 1896; Poulton 1890; Bennett & Théry 2007). Studies on visual communication have shed light on various aspects of natural (Chittka & Menzel 1992) and sexual selection (Hill 2002), and how these interact (Kemp *et al.* 2009). It is also an ideal system for truly integrative biological research, spanning from the optical processes generating colour (Shawkey *et al.* 2009), hormonal and genetic mechanisms regulating phenotype (Muller & Eens 2009), physiological processes involved in perceiving the signal (Hart 2001), and its adaptive and evolutionary patterns (Badyaev & Hill 2003; Darst *et al.* 2006).

However, 'colour' refers to a sensory experience, not an objective quantity, and the realization that animals can vary quite considerably in their visual system and how they process this information prompted two important methodological advances. First, it highlighted the need for an objective quantification of the energy reflected at different wavelengths, as a first approximation of a 'receiver-independent' measure of an organism or object's colour (Endler 1993; Eaton & Lanyon 2003; Bennett & Théry 2007). Over the last 20 years, the rising popularity of portable spectrometers has made objective quantification of the spectral properties of animal and plant integuments commonplace (Endler 1990; Eaton & Lanyon 2003; Andersson & Prager 2006). Second, advances in the understanding of perception and processing of colour have allowed analysis of reflectance data using visual models that estimate how animals see and differentiate these colours (Goldsmith 1990; Tovee 1995; Vorobyev & Osorio 1998; Vorobyev *et al.* 1998).

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Because of these advances, a cohesive framework for working with and analysing colour from reflectance data is needed. Output file types from spectrometer manufacturers are not standardized, and though existing software programs have provided helpful implementations of several methodologies (Gomez 2006; Stoddard & Prum 2008; Montgomerie 2006), they are often limited in the number or types of methodologies implemented, the types of data they can import and process, or the platforms in which they are available. Moreover, many are proprietary and/or closed, hindering customization (for a review of available software, see Montgomerie 2006). Furthermore, once the relevant colour data are extracted, they often require additional conversion and export into statistical software for analyses, which precludes protocol standardization across laboratories, batch processing, and automation of workflows.

Here, we introduce *pavo* – a package for R (R Development Core Team 2013) that addresses these problems by providing a flexible, yet cohesive, environment in which researchers can organize, analyse and visualize colour data generated by spectrometry. R is open source and multi-platform and is rapidly becoming the working language for scientific programming and data analysis, particularly in ecology and evolution (e.g. Paradis *et al.* 2004; Bolker 2008). *pavo* incorporates R’s flexibility using object classes that can seamlessly interpret each

other, providing functions that can be used to import, explore, process, and analyse spectral colour data under a variety of user-defined models. We propose that combining these procedures under a coherent framework not only streamlines workflow, but also allows data to be explored and manipulated in ways that can be used to visualize patterns, obtain information, and develop and test hypotheses (Fig. 1).

The pavo package

The stable release of *pavo* is available from CRAN (<http://CRAN.R-project.org/package=pavo>) for direct installation from R, and the development version is available from github (<https://github.com/rmaia/pavo>). *pavo* was developed with three main workflow stages in mind (Fig. 1): organization of spectral data by inputting raw files and processing their spectral content; visualization of the output, including exploratory capacities to identify further required manipulations and previously unconsidered patterns; and analysis of data from the spectral shape of reflectance curves or by incorporating receiver psychophysiology in visual models. As noted by Bennett & Théry (2007) and others (Andersson & Prager 2006; Montgomerie 2006), though spectral data have become commonplace in studies of animal colouration, it is easy to obtain poor-quality or inaccurate data. Therefore, a workflow for spectral

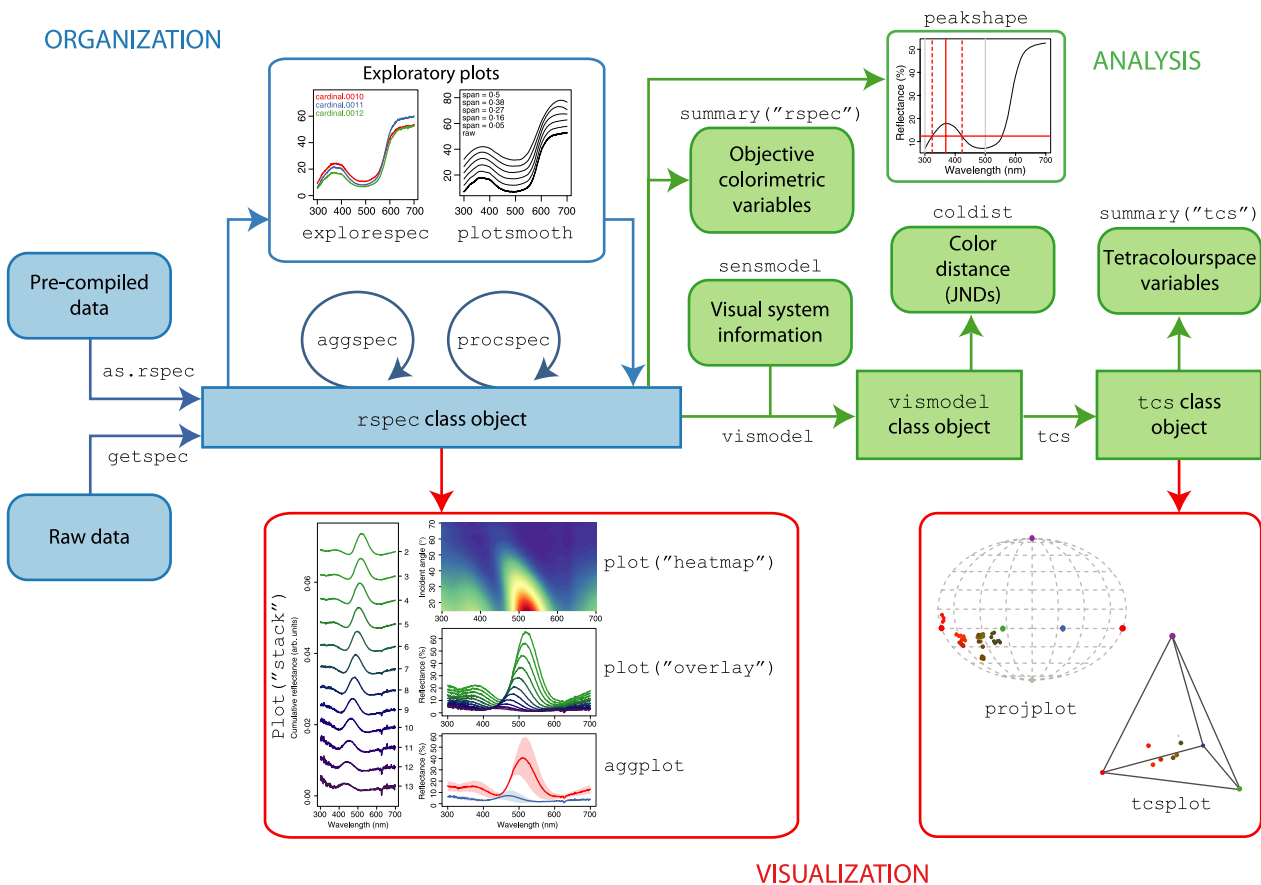


Fig. 1. Example *pavo* workflow, highlighting its main functions and plotting capabilities.

colour data analysis has to go beyond a ‘plug and chug’ implementation, requiring thorough exploratory investigation. With this in mind, *pavo* takes advantage of R’s object-oriented programming environment to implement modular functions that allow each of these steps to be explored and to work cohesively. Below we outline *pavo*’s organization, visualization and analysis capabilities, followed by a detailed worked example using African glossy starlings to demonstrate some of its functionalities.

ORGANIZATION

Spectral data are stored in *pavo* and recognized for its functions by use of a new object class, ‘*rspec*’, which inherits methods from *data.frame*. Objects of class *rspec* are characterized by having individual reflectance spectra as columns of the data frame, with a first column containing the associated wavelength values. Raw spectral data can be imported using the function *getspec*, which currently supports data from a variety of software (including Ocean Optics OOIBase and SpectraSuite files, Avantes AvaSpec and CRAIC). In addition, spectral data that have been previously compiled (into a spreadsheet, for example) can be imported into R and converted to *rspec* objects using the *as.rspec* function.

The use of dedicated R object classes allows generic functions such as *plot* and *summary* to identify the object as a particular type of data frame and interpret it accordingly (see below). The class ‘*vismodel*’ is used to interpret spectral data that have been processed through one of the visual models implemented and also stores information on how it was generated (e.g. the visual phenotype, background and illuminant used, and any transformations applied; see below). Additionally, the ‘*tcs*’ class refers to tetrahedral colour space models (Endler & Mielke 2005; Stoddard & Prum 2008), and the *summary* function can be used to extract summary variables, such as the colour volume or hue span (Stoddard & Prum 2008) for groups and subsets of points.

It is common when collecting spectral data to take multiple measurements from the same sample, averaging these to avoid sampling error (Quesada & Senar 2006). *pavo* provides the *aggspec* function for this purpose, as well as the *procspec* function for noise removal via smoothing, and transformations to standardize and clean spectral data.

VISUALIZATION

With *pavo* installed and loaded, the *plot* function recognizes *rspec* objects and plots them accordingly – interpreting the first column as wavelengths (usually using it as the *x* axis) and the remaining columns as reflectance values (*y* axis) for individual spectra. Several plotting options for multiple spectra are implemented (Fig. 1). In addition, the *aggplot* function provides plotting capabilities for among-spectra summary statistics plotting (Fig. 1), with a similar syntax to *aggspec*. *pavo* also offers exploratory plotting capabilities that can be combined with data processing and formatting,

such as *explorespec* (for visualizing groups of spectra) and *smoothplot* (for choosing smoothing parameters; Fig. 1).

Finally, *pavo* offers plotting capabilities for the avian tetrahedral colour space model (Stoddard & Prum 2008; Endler & Mielke 2005) through the *tcsplot* and *projplot* functions (Fig. 1, see below).

ANALYSIS

The *summary* function can be applied to *rspec* objects to extract several objective (‘receiver-independent’) reflectance shape variables, relevant to specific or universal mechanisms of colour signalling (e.g. pigments, structures) or colour perception (spectral intensity, location and purity). Description and discussion of these variables can be found in Andersson & Prager (2006) and Montgomerie (2006), as well as in the package vignette. Additionally, the function *peakshape* provides descriptors of spectral peaks, such as the wavelength of maximum reflectance and the full width at half-maximum, and can be fine-tuned to extract information from specific areas of the curve. This implementation can be useful when the spectral curve has multiple peaks or a complex shape (e.g. the UV peak of carotenoid curves, Fig. 1).

pavo also allows the easy production of models that incorporate the visual system of the receiver through the *vismodel* function. Models can be calculated incorporating the visual phenotype (cone absorbance), background colour, and ambient illuminant (Vorobyev & Osorio 1998). Several avian receptor phenotypes (Hart 2001; Endler & Mielke 2005) are implemented as options, but user-defined receptor data from any taxon can be used as model input. Further, the *sensmodel* function implements the calculation of cone absorbance curves based on peak sensitivity information (available from the literature, for example Hart 2001) and can also include oil droplet and ocular transmission information in the calculations (Govardovskii *et al.* 2000; Hart & Vorobyev 2005).

Visual models can be calculated in terms of absolute photon catches, in which case the receptor noise model can be used to infer contrast between colours (implemented in the function *coldist* Vorobyev & Osorio 1998), or in relative cone stimulation, in which case the model reduces to a colour space model represented in $n-1$ dimensions (where n is the number of different receptors involved in colour vision; Goldsmith 1990; Endler & Mielke 2005; Stoddard & Prum 2008). Absolute or relative cone stimulation can be selected by the logical argument *relative* from the *vismodel* function. In the case of the avian tetrahedral colour space, several additional variables can be calculated based on spherical coordinates which represent the hue angles and saturation (the distance from the achromatic centre; see Stoddard & Prum 2008) by calling the *tcs* function. This function generates an object of class *tcs*; a *summary* call from a *tcs* object will return summary statistics described in Stoddard & Prum (2008) for sets of points (see below).

pavo also builds upon previously described visual model methods. For example, Stoddard & Stevens (2011) presented the useful technique of calculating the overlap between the

volumes defined by two sets of points in colour space. They used this metric to quantify mimicry (Stoddard & Stevens 2011; Stoddard 2012), such that a greater volume overlap would indicate greater overall colour similarity. Given the complexity of calculating the intersection of three-dimensional convex hulls, Stoddard & Stevens (2011) used a Monte Carlo approach to estimate the degree of volume overlap. *pavo*, instead, provides the exact solution for the calculation of the intersection of colour volumes using a method originally implemented to calculate the overlap between multidimensional niches (Villéger *et al.* 2011) through the computational geometry capabilities available from the *rcdd* package (Geyer *et al.* 2012) (For performance and precision comparison, see Supporting Information).

Worked example: colour divergence in glossy starlings (Sturnidae)

Glossy starlings from the African clade have bright and diverse iridescent colours likely used in courtship displays and social competition (Rubenstein & Lovette 2009). Here, we demonstrate some of *pavo*'s capabilities through a worked example to compare the colours of a monophyletic clade of three glossy starling species, two of which (*Lamprolornis chloropterus* and *L. elisabeth*) have recently diverged and whose status as full species is debated (Gill & Donsker 2012; Craig *et al.* 1998; Lovette & Rubenstein 2007). The data used for this example have been deposited online and is available from the Dryad Database (Maia *et al.* 2013). Further information and a 'how-to' of *pavo*'s main functions, including the formulae used for the calculations, can be found in detail in the package vignette – available online from CRAN or directly from R using the command `vignette('pavo')`.

STEP 1: ORGANIZATION AND PROCESSING

The data consist of reflectance spectra in Avantes '.txt' output format, which in this example are located in the folder '/Desktop/glossystarlings' (by default, the current working directory and the '.txt' extension are used). The files are named to indicate the species, bird ID, plumage patch and measurement ID (for example, 'LAAC.14423.belly.001.txt' would be the first measurement taken from the belly of individual 14423, a *Lamprolornis acuticaudus* specimen). This is important because *pavo* uses the file name (minus the file extension) to label the columns of the *rspec* object with the imported reflectance spectra. We recommend that users carefully consider and adhere to a rigorous file naming scheme to make subsetting and data manipulation easier (see Andersson & Prager 2006 for a useful discussion and suggestions in this regard). Additionally, we suggest that sample identity labels should have the same number of characters, which simplifies character string manipulation and subsetting based on partial matching.

We measured reflectance spectra from 11 plumage patches (see Fig. 2 for list) of four males from museum specimens of each of the three species. Three measurements from different

locations within each patch were collected. We used the *getspec* function to load these 396 raw spectral data files, then used the *aggspec* function to average the spectra within patches (as determined by the *by* argument) and the *procspec* function to remove electrical noise arising from the spectrometer (using local polynomial regression fitting, or *loess*) with the following annotated lines of code:

```
#get raw data
>specs <- getspec(when = '~ /Desktop/
glossystarlings', ext = 'txt', lim = c(300, 700))
396 files found; importing spectra
=====
#average by groups of 3 spectral curves
>specs <- aggspec(specs, by = 3, FUN = "mean")
#remove electrical noise using
#Gaussian smoothing
>specs <- procspec(specs, opt = "smooth")
processing options applied:
  smoothing spectra with a span of 0.25
```

STEP 2: VISUALIZATION

Next, we plotted spectra contained in the resulting *rspec* object. We used *aggplot* to visualize the mean reflectance curves for each body part from each species, as can be seen in the example below for the 'belly' body patch (Fig. 2b):

```
#subset wavelength column and the
#12 spectra from the belly patch
>specs.belly <- subset(specs, "Belly")
#extract first 4 characters from column names
#(species labels)
>spp <- substr(names(specs.belly), 1, 4)
#average and plot spectral data by species
>aggplot(specs.belly, by = spp)
```

STEP 3: ANALYSIS

Colour distances

To explore how these colours may be perceived by birds, we first used the *vismodel* function, which takes into account avian visual sensitivities (sensory phenotype), to calculate the quantum catches for each photoreceptor. *pavo* allows for user-defined cone sensitivities, but also incorporates available data from several taxa, including the European starling (*Sturnus vulgaris*, *visual* = "star", Hart *et al.* 1998), which we used for our example. We used the *relative* = FALSE option from the *vismodel* function to obtain raw photon catch values for the four avian photoreceptor classes (*usml*), suitable for calculating chromatic distances ΔS , Vorobyev *et al.*, 1998).

vismodel can return either the calculated quantum catches (Q_i , the default) or values transformed according to Fechner's law (f_i ; the signal being proportional to the logarithm of the quantum catch), as determined by the argument *qcatch* (not used in the code below, hence the default, Q_i , is returned). It can also apply the von Kries transformation (normalizing by

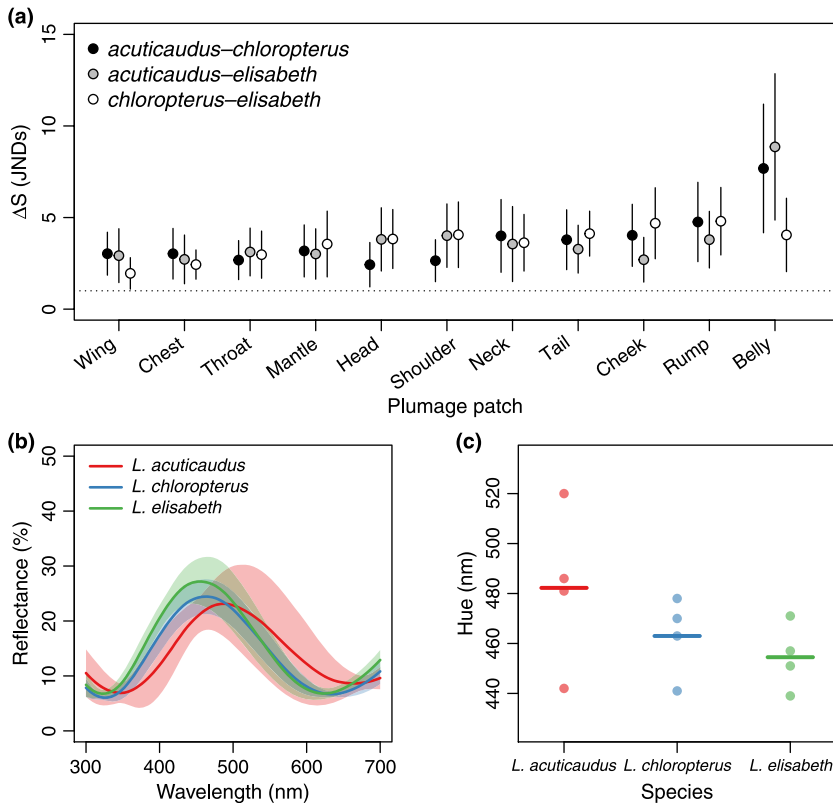


Fig. 2. (a) Plot showing colour distances (in units of just noticeable differences, JNDs) by patch (y-axis) for three pairs of African starling species (Sturnidae). The dotted horizontal line indicates JND = 1, above which the pair of colour patches is considered to be distinguishable by birds. Points and error bars indicate mean \pm standard error chromatic distances between different pairs of species: *Lamprotornis chloropterus* and *L. elisabeth* (open circles); *L. acuticaudus* and *L. elisabeth* (grey circles); and *L. acuticaudus* and *L. chloropterus* (black circles). (b) Plot of mean smoothed spectra for the belly body patch. Line colours indicate species (green: *L. acuticaudus*, red: *L. chloropterus*, blue: *L. elisabeth*), and shaded areas indicate the standard deviation of the spectral data. (c) Hue (wavelength of maximum reflectance) values for each specimen measured (points) and mean per species (bars) for the belly patch ($N = 4$ individuals each; colours as in b).

the stimulus' background to account for receptor adaptation) through the `vonkries` argument (which defaults to `FALSE`). These settings are stored as arguments in the `vismodel` object, are referenced by functions downstream in the workflow (e.g. `coldist`, below), and can be called using the summary function:

```
>vm.star <- vismodel(specs, visual="star",
relative = FALSE)
>summary(vm.star)
visual model options:
* Quantal catch: Qi
* Visual system: star bt.dc
* Illuminant: ideal, scale = 1 (von Kries color
correction not applied)
* Background: ideal
* Relative: FALSE
```

Second, we used the `coldist` function to calculate colour distances with receptor noise based on the relative photoreceptor densities (Vorobyev & Osorio 1998). We used relative cone abundances (arguments `n1`, `n2`, `n3` and `n4`) for the European starling (Hart *et al.* 1998), and the `v` argument was set to 0.1 to give a $\{l\}$ -cone Weber fraction (calculated as $v/\sqrt{n_i}$) of approximately 0.05 (Vorobyev & Osorio 1998; Vorobyev *et al.* 1998). The results of `coldist` give colour distances (chromatic, ΔS , shown in Fig. 2; and achromatic, ΔL) between all possible combinations of plumage patches in a `vismodel` object and can be further subsetted (using the `subset` argument) to focus on comparisons of interest (e.g. colour difference between homologous patches of two species, or between colour patches and a given background).

This argument employs regular expressions functionality and thus allows for partial string matching of row names to the rule specified in the argument. In this case, if we want comparisons between a single type of patch, the `subset` argument will have two values to match both `patch1` and `patch2` columns:

```
>deltas <- coldist(vm.star, n1=1, n2=1.38,
n3=3.34, n4=3.46, v=0.1)
#subset only comparisons between wing spectra
>deltas.wing <- coldist(vm.star, n1=1, n2=1.38,
n3=3.34, n4=3.46, v=0.1, subset=c("Wing", "Wing"))
>head(deltas.wing, 3)
```

	patch1	patch2	dS	dL
1	LAAC.260046.Wing	LAAC.264607.Wing	2.661180	8.714508
2	LAAC.260046.Wing	LAAC.347959.Wing	2.790763	3.033686
3	LAAC.260046.Wing	LAAC.347960.Wing	5.158210	5.629931

We can see from Fig. 2a that the recently divergent *L. chloropterus* and *L. elisabeth* have accumulated similar levels of colour disparity as they have to their sister species, *L. acuticaudus*. Considering a value of 1 as a threshold for Just Noticeable Differences (JNDs, Fig. 2a dashed line), nearly all plumage patch comparisons yield discernible colours, both within the *L. chloropterus* – *L. elisabeth* subclade as well as compared with the *L. acuticaudus* outgroup.

Spectral analysis

Another strength of `pavo` is its ability to compare different colour metrics (Butler *et al.* 2011), such as those extracted directly from the spectral curves (Fig. 2b) and thus do not consider

receiver visual system phenotype (see Andersson & Prager (2006) for discussion). As many as 23 objective colourimetric variables are calculated by the summary of an `rspec` object (for a complete description and summary of formulae, see the package vignette and Montgomerie 2006), but the `subset` argument can be used to filter these variables. If `subset = TRUE`, the variables described in Andersson & Prager (2006) are returned, but a string of variable names can also be used as a filter. For our example, we extracted and plotted an objective metric of hue (wavelength of peak reflectance, Fig. 2c) for the most contrasting body patch ('belly', Fig. 2a) in the visual model analysis:

```
# extract colour variables
> colr <- summary(specs.belly, subset=TRUE)
> head(colr, 3)
              B2          S8      H1
LAAC.260046.Belly  18.92453  1.518254  520
LAAC.264607.Belly  12.31255  1.257920  481
LAAC.347959.Belly  11.20595  1.233001  486
# extract hue (H1)
> hue <- colr$H1
# extract species names
#(first 4 letters from row names)
> spp <- substr(rownames(colr), 1, 4)
# make box plots of hue (H1) by species
> boxplot(hue~spp)
```

Avian tetrahedral colourspace

To analyse these spectra in avian tetrahedral colourspace, we used `vismodel` again, but now specifying `relative=TRUE` to indicate that $\{usm\}$ values should be scaled to sum of unity for each observation. We then used the `voloverlap` function to calculate the volumes occupied by each species' plumage patches, as well as their overlap. The `plot=TRUE` option provides a useful graphical representation of the overlap in colourspace (Fig. 3).

```
> vm.star.rel <- vismodel(specs, visual="star",
relative=TRUE)
> tcs.star <- tcs(vm.star.rel)
#subset points from the two species
> tcs.lael <- subset(tcs.star, "LAEL")
> tcs.lacl <- subset(tcs.star, "LACL")
> voloverlap(tcs.lael, tcs.lacl, plot=TRUE)
```

Conclusions and future directions

`pavo` implements colour analyses for reflectance data based on both spectral properties and visual system receptor stimulation, as well as a modular framework that allows the assumptions and parameters of such models to be tuned as necessary. As such, it is particularly suited to compare and validate the use of different colourimetrics (Butler *et al.* 2011), determine the influence of various assumptions in visual models, or help choose parameter values that would introduce the least bias in visual models (Hanley 2013). `pavo` can also be used to investigate the information content of colour signals using

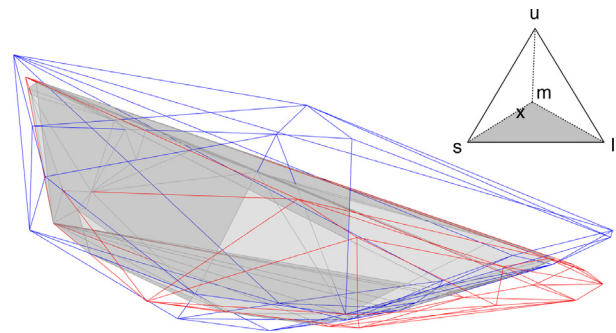


Fig. 3. Colourspace occupied by the recently diverged *Lamprolornis chloropterus* (red) and *L. elisabeth* (blue). The colour volume overlap between the two species is shown in grey. For reference, the avian tetrahedral colourspace (inset) shows the $\{usm\}$ colour vertices, with the location of the volumes in the colourspace indicated by an 'x'.

receiver-specific visual systems (Pike *et al.* 2011) and answer questions pertaining to signal production mechanisms (e.g. comparing the relative contribution of pigments and structural colours in various integuments; D'Alba *et al.* 2012). Currently, the visual modelling functions implemented in `pavo` already allow for any number of photoreceptors to be considered, but colour distances can so far only be calculated for di-, tri- and tetrachromats. Implementing extensions of the Vorobyev & Osorio (1998) model will allow `pavo` to calculate colour distances considering the visual systems of organisms such as butterflies (e.g. six photoreceptors in Pierids and *Papilio* butterflies; Morehouse & Rutowski 2010, Arikawa 2003) and mantis shrimps (Order Stomapoda; 16 photoreceptors; Cronin *et al.* 1993).

Furthermore, as an R package, `pavo` provides a direct integration with the growing number of statistical procedures that the language incorporates, facilitating a streamlined workflow from initial data input to final analysis. It therefore allows the inclusion of colour analysis from spectral data in open and reproducible workflows, which can be directly automated and adjusted for consistency. Finally, `pavo` will continue to incorporate reference data for illuminants, backgrounds, photoreceptors, and other components of visual systems (e.g. ocular media; Siebeck & Marshall 2001) as they become available, providing a repository of spectral and colour data for physiological models across a broad range of taxa.

Citation of methods implemented in pavo

Most of the methods implemented in `pavo` have been thoroughly described in their original publications, to which users should refer for details and interpretation. For reflectance shape variables ('objective colourimetrics') and their particular relation to signal production and perception, see Andersson & Prager (2006) and Montgomerie (2006). Visual models based on photon catches and receptor noise are detailed in the study by Vorobyev & Osorio (1998) and Vorobyev *et al.* (1998), and photoreceptor sensitivity curve estimation in the study by Govardovskii *et al.* (2000) and Hart & Vorobyev (2005). For tetra-

hedral colour space model implementations and variable calculations, see Endler & Mielke (2005) and Stoddard & Prum (2008), and for colour volume overlap, see Stoddard & Stevens (2011) and Stoddard (2012). Users of the functions that apply these methods should cite the original sources as appropriate, along with `pavo`.

Data accessibility

Data for the examples have been deposited in the Dryad repository: <http://dx.doi.org/10.5061/dryad.298b1>

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Exact determination of volume overlap.

Figure S1. Results of Monte Carlo simulations of volume overlaps for various 3-dimensional shapes (a: cube, b: regular tetrahedron, c: cuboid) compared to the exact result.

Figure S2. Results of Monte Carlo simulations of proportional volume overlaps for arbitrary polyhedra with varying overlap volumes (a: 40%, b: 16%, c: 3%) compared to the exact calculations.