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APPLICATION

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

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Summary

1. Animal colouration has been a powerful model for the study of a diverse range of topics in biology. Recent technical, methodological and empirical advances have led to a dramatic increase in the use of spectrophotometry to quantify reflectance properties of biological material and of models to determine how these colours are perceived by the animals themselves , providing important insights to ecological and evolutionary aspects of animal visual communication.

2. Despite this growing interest, a unified framework for analyzing 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: it allows the user to (a) organize and manipulate data from a variety of sources, (b) visualize data with easy-to-use tools, and (c) analyze data using visual system modeling for a broad range of taxa.

3. In this paper, we present a summary of the functions implemented in `pavo`, suggest a workflow to explore spectral data, and model colours using user-defined visual systems. We also present an exact solution for the calculation of colour volume overlap in colourspace, 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. Using spectral data and avian visual models, we show that colour disparity between the recently diverged species is as great as that between them and the more distantly related species. The flexibility and streamlined workflow of pavo is demonstrated through use of different visual models and several plotting capabilities.

5. `pavo` provides a unique environment capable of addressing complex sensory ecology questions that previously required the use of multiple, sometimes restricted, software and programs.

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Introduction

The role of colouration and colour vision in animal communication has been a fundamental question in evolutionary biology for centuries (Darwin, 1859, 1896; Poulton, 1890; Bennett & Théry, 2007). Visual communication has provided a unique opportunity to investigate 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 systems and how they process this information prompted two important methodological advances. First, it highlighted the need for an objective quantification of the colour reflected by surfaces, 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 falling cost and rising popularity of portable spectrophotometers have 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).

Because of these advances, a cohesive framework for working with and analyzing colour from reflectance data is needed. Output file types from spectrophotometer manufacturers are not standardized, and software programsfor analyses are limited either in the methods they implement, the types of data they import and process, or the platforms in which they are available. Moreover, many are proprietary and/or closed, making customization impossible (for a review of available software, see Montgomerie, 2006). Colour data often require additional conversion and export into statistical software for analyses, restricting protocol standardization across labs, and batch processing automation of workflows. Combined with published descriptions that are often poorly detailed or inconsistent, the current procedures and implementations severely hinder the reproducibility of research and the reimplementation of methods.

We introduce `pavo` – a package for R (R Core Team, 2012) that addresses the aforementioned problems by providing a flexible, yet cohesive, environment in which researchers can organize, analyze and visualize colour data generated by spectrophotometry. R is also 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 by using object classes that can seamlessly interpret each other, providing functions that can be used to intuitively import, explore, process and analyze 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 new ways that can be used to visualize patterns, obtain information and develop and test hypotheses (Figure 1).

Figure 1 about here

The `pavo` package

The stable release of `pavo` is available from CRAN (`http://cran.r-project.org/`) for direct installation from R, and the development version is available from github (`https://github.com/rmaia/pavo`). `pavo` was developed with

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three main workflow stages in mind (Figure 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 (Figure 1). 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 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, as outlined below.

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 wavelength identification data. Raw spectral data can be imported using the function `getspec`, which currently supports data from a variety of spectrophotometer and microspectrophotometer models (Ocean Optics OOIBase and SpectraSuite files, Avantes AvaSpec, and CRAIC). In addition, previously compiled data frames 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 like `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 has 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 colourspace models (Endler & Mielke, 2005; Stoddard & Prum, 2008), and the `summary` function can be used to extract summary variables, like 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 values) for individual spectra. Several plotting options for multiple spectra are implemented (Figure 1). In addition, the `aggplot` function provides plotting capabilities for among-spectra summary statistics plotting (Figure 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 smoothening parameters; Figure 1).

Finally, `pavo` offers plotting capabilities for the avian tetrachromatic colourspace model (Stoddard & Prum, 2008; Endler & Mielke, 2005) through the `tcsplot` and `projplot` functions (Figure 1, see below).

ANALYSIS

The `summary` function can be applied to `rspec` objects to produce several tristimulus (hue, saturation and brightness) colour variables extracted from the spectral shape of the reflectance curve. This function calculates variables previously described in the literature (summarized in Montgomerie, 2006) for the spectra contained in the `rspec` input object (description and formulae for the variables can be found in the help file, by typing `?summary.rspec`, and 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. describing the UV peak of carotenoid curves, Figure 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, e.g. 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 `colordist` Vorobyev & Osorio, 1998) or in relative cone stimulation, in which case the model reduces to a colourspace 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 colourspace, several additional variables can be calculated based on spherical coordinates which represent the hue based on the angles and saturation based on the distance from the achromatic center (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) present the useful technique of calculating the overlap between the volumes defined by two sets of points in colourspace. 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 Supplemental Information)

Example: colourspace divergence in glossy starlings (Sturnidae)

Here we exemplify some of `pavo`'s capabilities and workflow by comparing the colour patterns of a monophyletic clade of three glossy starling species. Glossy starlings from the African clade have bright and diverse iridescent colours likely used in sexual selection and social competition (Rubenstein & Lovette, 2009). The lesser blue-eared starling (*Lamprotornis chloropterus*) and the southern blue-eared starling (*L. elisabeth*) form a recently diverged clade that some (including the International Ornithological Committee; Gill & Donsker, 2012) have considered full species, whereas others have classified them as subspecies of *L. chloropterus* (Craig *et al.*, 1998; Lovette & Rubenstein, 2007). We compared the extent to which these two species have diverged in their colours and compared each to their most recently diverged sister species, the sharp-tailed starling (*L. acuticaudus*).

STEP 1: ORGANIZATION AND PROCESSING

The data consist of reflectance spectra (in Avantes ".ttt" output format) taken from museum specimens of the three species. We measured three reflectance spectra from 11 plumage patches (see Figure 2 for list) of four males per species. We loaded, averaged and removed the electrical noise arising from the spectrometer (using local polynomial regression fitting, or loess) from these 396 raw spectral data files using the following annotated lines of code in `pavo`:

```
> specs <- getspec(where = '/Desktop/glossystarlings', ext = 'ttt', lim = c(300, 700)) #get raw data
> specs <- aggspec(specs, by = 3, FUN = mean) #average by groups of 3 spectral curves
> specs <- procspec(specs, opt='smooth') #remove electrical noise
```

STEP 2: VISUALIZATION

Next, we plotted spectra contained in this `rspec` object. We used `aggplot` to visualize the mean reflectance curves for each body part from each species. Below we show the code for one of these plots, as well as the results for the "belly" and "wing" body patches: (Figure 2):

```
> specs.belly <- specs[,1:13]  # subset wavelength column and the 12 spectra from the belly patch
> spp <- substr(names(specs.belly),1,4)[-1]  # 4 first characters from column names (species labels)
> aggplot(specs.belly, by = spp)  # average spectral data by species
```

STEP 3: ANALYSIS

To understand how these colours are perceived by birds, we used the `vismodel` function to compare colours taking into account avian visual sensitivities (sensory phenotype). `pavo` incorporates available data on the retinal sensitivities of numerous taxa, including the European starling (*Sturnus vulgaris*, `visual='star'`), which we incorporated here (Hart *et al.*, 1998). We used both the `relative=FALSE` and `relative=TRUE` options of the `vismodel` function to measure colour attributes using the receptor noise and colourspace models, respectively.

Colour distances

First, `relative=FALSE` was used to calculate raw photon catch values for the four avian photoreceptor classes ($\{usml\}$), suitable for calculating chromatic distances (ΔS, Vorobyev *et al.*, 1998). We used relative cone abundances (arguments `n1, n2, n3, and n4`) for the European starling (Hart *et al.*, 1998), which can be easily passed as arguments to the `coldist` function. The `v` argument was set to 0.1 to give a $\{"l"\}$ cone Weber fraction of approximately 0.05 (Vorobyev & Osorio, 1998; Vorobyev *et al.*, 1998). The results of `coldist` give colour distances (chromatic , ΔS, Figure 2; and achromatic, ΔL, not shown) between all possible combinations of plumage patches in the `tcs` object, and can be further subset to only analyse comparisons of interest (e.g., colour difference between homologous patches of two species). For example:

```
> vm.star1 <- vismodel(specs, visual = 'star', relative = FALSE)
> delta.S <- coldist(vm.star1, qcatch = 'fi', n1 = 1, n2 = 1.38, n3 = 3.34, n4 = 3.46, v = 0.1)
> # subset only comparisons between wing spectra
> head(delta.S[intersect(grep('wing', delta.S$patch1), grep('wing', delta.S$patch2)),])
```

	patch1	patch2	tetra.dS	dL
1031	LAEL.668721.MAL.wing	LAEL.668723.MAL.wing	4.1937305	5.427964
1042	LAEL.668721.MAL.wing	LAEL.668724.MAL.wing	0.6092773	2.042986
1053	LAEL.668721.MAL.wing	LAEL.668727.MAL.wing	1.3653852	1.085405
1064	LAEL.668721.MAL.wing	LACL.162946.MAL.wing	2.5464770	6.832917
1075	LAEL.668721.MAL.wing	LACL.668619.MAL.wing	0.6138824	2.187837
1086	LAEL.668721.MAL.wing	LACL.668623.MAL.wing	1.7393595	2.252061

The calculation of both chromatic and achromatic distances of these 8646 pairs of colors took about 0.9 seconds in a MacBook Pro running R version 2.15.2. We can see from Figure 2 that the recently divergent *L. chloropterus* and *L. elisabeth* have accumulated similar levels of color disparity among them as they have to their sister species, *L. acuticaudus*. Considering a value of 1 as a threshold for Just Noticeable Differences (JNDs, Figure 2A dashed lines) nearly all plumage patch comparisons yield colors that are discernible to the starling visual system, both within the

L. chloropterus-L. elisabeth subclade as well as compared to the *L. acuticaudus* outgroup. Given the high satuturation of iridescent colors, small differences in the spectral shape can yield noticeably different colors. This becomes clear when comparing the results for the most and least contrasting body patches ("belly" and "wing", respectively) to their spectral curves (see Figure 2B,C). Even groups of spectra that considerably overlap in their average reflectance, as in the case of the "wing" patch (Figure 2B), the pairwise comparisons of individual spectra yield values above the threshold of JND=1 (Figure 2A). Even within-species comparsions yield discernible colors for the "wing" patch, as evidenced by the output from `coldist` (above).

Figure 2 about here

Avian tetrahedral colourspace

Finally, to produce a tetrahedral colourspace plot, we used `relative=TRUE` in the `vismodel` input to indicate that $\{usml\}$ values should be scaled to sum of unity for each patch. These results indicate that the expansion of occupied colourspace for both subspecies is largely due to divergence in the colour of the "belly" patch (see circles labeled 'bel' at edges of red and blue polyhedrons in Figure 3a). Interestingly, the "cheek" plumage patch of *L. elisabeth* is more similar to *L. acutacaudus* than *L. chloropterus*, to which it is more closely related (circles labeled 'ch' in Figure 3A).

We also used the `voloverlap` function to calculate the volumes occupied by each species' plumage patches, as well as their overlaps. As an example, the code below shows the result for the overlap between the two recently diverged species (see Figure 3B):

```
> vm.star2 <- vismodel(specs, visual = 'star', relative = TRUE)
> tcs.star <- tcs(vm.star2)
> #subset points from the two species
> tcs.lael <- tcs[grep('LAEL',row.names(tcs.star))]
> tcs.lacl <- tcs[grep('LACL',row.names(tcs.star))]
> voloverlap(tcs.lael, tcs.lacl, plot = TRUE)

          vol1        vol2   overlapvol vsmallest      vboth
1 1.916209e-05 4.05405e-05 4.673727e-06 0.2439049 0.08493229
```

Figure 3 about here

The `plot=TRUE` option provides a useful graphical representation of the overlap in colourspace (Figure 3b). Again, the calculated volume in `pavo` represents the exact solution to the geometric problem (i.e. not an approximation obtained through Monte Carlo simulation, Stoddard & Stevens, 2011).

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 summary tristimulus variables and spectral shape analysis of reflectance curves, see Montgomerie (2006). For visual models based on photon catches and receptor noise, see Vorobyev & Osorio (1998) and Vorobyev *et al.* (1998). For visual phenotype sensitivity curve estimation, see Govardovskii *et al.* (2000) and Hart & Vorobyev (2005). For tetrahedral colourspace models, 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 must cite the original sources along with `pavo`.

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References

Andersson, S. & Prager, M. (2006) Quantifying colors. *Bird Coloration Vol. I* (eds. K.J. Mcgraw & G.E. Hill). Harvard Univ. Press Cambridge, MA, pp. 41–89.

Badyaev, A.V. & Hill, G.E. (2003) Avian sexual dichromatism in relation to phylogeny and ecology. *Annual Review Of Ecology Evolution And Systematics*, **34**, 27–49.

Bennett, A.T.D. & Théry, M. (2007) Avian color vision and coloration: multidisciplinary evolutionary biology. *The American Naturalist*, **169**(S1), S1–S6.

Bolker, B. (2008) *Ecological Models and Data in R*. Princeton University Press, ISBN 9781400840908.

Chittka, L. & Menzel, R. (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **171**, 171–181, URL `http://dx.doi.org/10.1007/BF00188925`. 10.1007/BF00188925.

Craig, A., Feare, C., Croucher, B. & Shields, C. (1998) *Starlings and Mynas*. Helm Identification Guides, A&C Black, ISBN 9781408135228.

Darst, C.R., Cummings, M.E. & Cannatella, D.C. (2006) A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proceedings Of The National Academy Of Sciences Of The United States Of America*, **103**(15), 5852–5857.

Darwin, C. (1859) *On the Origin of the Species by Means of Natural Selection: Or, The Preservation of Favoured Races in the Struggle for Life*. John Murray.

Darwin, C. (1896) *The Descent of Man, and Selection in Relation to Sex*. The Works of Charles Darwin, V. 9, AMS Press, ISBN 9780404084097.

Eaton, M.D. & Lanyon, S. (2003) The ubiquity of avian ultraviolet plumage reflectance. *Proceedings Of The Royal Society B - Biological Sciences*, **270**(1525), 1721–1726.

Endler, J.A. (1990) On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal Of The Linnean Society*, **41**(4), 315–352.

Endler, J.A. (1993) The color of light in forests and its implications. *Ecological Monographs*, **63**(1), 1–27.

Endler, J.A. & Mielke, P. (2005) Comparing entire colour patterns as birds see them. *Biological Journal Of The Linnean Society*, **86**(4), 405–431.

Geyer, C.J., Meeden, G.D. & incorporates code from cddlib written by Komei Fukuda (2012) *rcdd: rcdd (Computational Geometry)*. URL `http://CRAN.R-project.org/package=rcdd`. R package version 1.1-7.

Gill, F. & Donsker, D. (2012) Ioc world bird names (version 3.2). `http://www.worldbirdnames.org`. [Online; accessed 12-December-2012].

Goldsmith, T.H. (1990) Optimization, constraint, and history in the evolution of eyes. *Quarterly Review Of Biology*, **65**(3), 281–322.

Govardovskii, V.I., Fyhrquist, N., Reuter, T., Kuzmin, D.G. & Donner, K. (2000) In search of the visual pigment template. *Visual Neuroscience*, **17**(4), 509–528.

Hart, N.S. (2001) The visual ecology of avian photoreceptors. *Progress In Retinal And Eye Research*, **20**(5), 675–703.

Hart, N.S., Partridge, J.C. & Cuthill, I.C. (1998) Visual pigments, oil droplets and cone photoreceptor distribution in the European starling (Sturnus vulgaris). *Journal of Experimental Biology*, **201**, 1433–1446.

Hart, N. & Vorobyev, M. (2005) Modelling oil droplet absorption spectra and spectral sensitivities of bird cone photoreceptors. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **191**(4), 381–392.

Hill, G.E. (2002) *A red bird in a brown bag: the function and evolution of colorful plumage in the house finch*. Oxford University Press, USA.

Kemp, D.J., Reznick, D.N., Grether, G.F. & Endler, J.A. (2009) Predicting the direction of ornament evolution in Trinidadian guppies (Poecilia reticulata). *Proceedings Of The Royal Society B - Biological Sciences*, **276**(1677), 4335–4343.

Lovette, I.J. & Rubenstein, D.R. (2007) A comprehensive molecular phylogeny of the starlings (Aves: Sturnidae) and mockingbirds (Aves: Mimidae): congruent mtDNA and nuclear trees for a cosmopolitan avian radiation. *Molecular Phylogenetics and Evolution*, **44**(3), 1031–1056.

Montgomerie, R. (2006) Analyzing colors. *Bird Coloration Vol. I* (eds. K.J. Mcgraw & G.E. Hill). Harvard Univ. Press Cambridge, MA, pp. 90–147.

Muller, W. & Eens, M. (2009) Elevated yolk androgen levels and the expression of multiple sexually selected male characters. *Hormones and Behavior*, **55**(1), 175–181.

Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.

Poulton, E. (1890) *The colours of animals: their meaning and use, especially considered in the case of insects*. International scientific series, D. Appleton and Company.

Quesada, J. & Senar, J.C. (2006) Comparing plumage colour measurements obtained directly from live birds and from collected feathers: the case of the great tit Parus major. *Journal Of Avian Biology*, **37**(6), 609–616.

R Core Team (2012) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria, URL `http://www.R-project.org/`. ISBN 3-900051-07-0.

Rubenstein, D.R. & Lovette, I.J. (2009) Reproductive skew and selection on female ornamentation in social species. *Nature*, **462**(7274), 786–789.

Shawkey, M.D., Morehouse, N.I. & Vukusic, P. (2009) A protean palette: colour materials and mixing in birds and butterflies. *Journal of the Royal Society, Interface / the Royal Society*, **6**, S221–S231.

Stoddard, M.C. (2012) Mimicry and masquerade from the avian visual perspective. *Current Zoology*, **58**(4), 630–648.

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

Stoddard, M.C. & Stevens, M. (2011) Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution*, **65**(7), 2004–2013.

Tovee, M.J. (1995) Ultra-violet photoreceptors in the animal kingdom: their distribution and function. *Trends In Ecology & Evolution*, **10**(11), 455–460.

Villéger, S., Novack-Gottshall, P.M. & Mouillot, D. (2011) The multidimensionality of the niche reveals functional diversity changes in benthic marine biotas across geological time. *Ecology Letters*, **14**(6), 561–568.

Vorobyev, M. & Osorio, D. (1998) Receptor noise as a determinant of colour thresholds. *Proceedings Of The Royal Society Of London Series B-Biological Sciences*, **265**(1394), 351–358.

Vorobyev, M., Osorio, D., Bennett, A.T.D., Marshall, N. & Cuthill, I. (1998) Tetrachromacy, oil droplets and bird plumage colours. *Journal Of Comparative Physiology A-Neuroethology Sensory Neural And Behavioral Physiology*, **183**(5), 621–633.

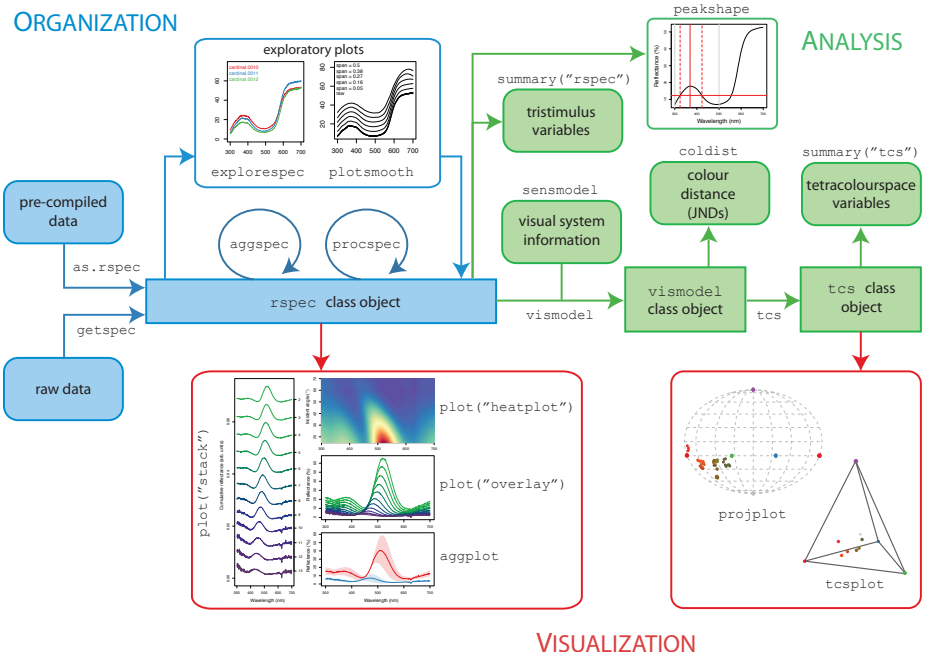

Fig. 1. Example pavo workflow, highlighting its main functions and plotting capabilities

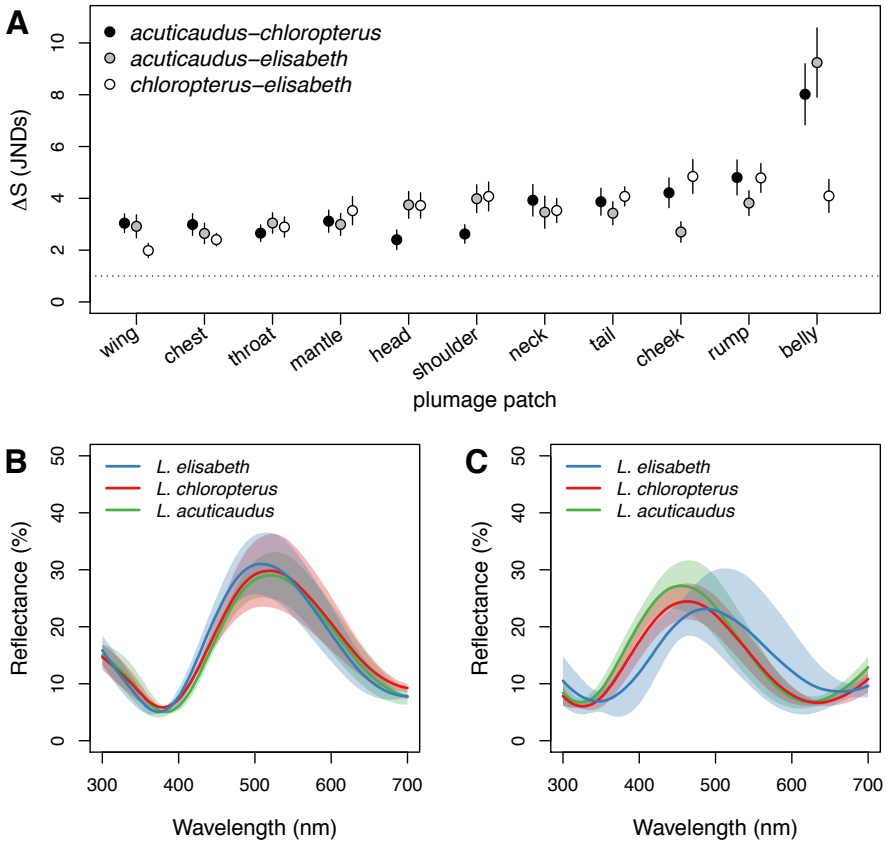

Fig. 2. **A**: Dotplot showing color distances (in units of just noticeable differences, JNDs) by patch (y-axis) for three pairs of African starling species (Sturnidae). Dotted vertical lines indicate JND=1, above which the pair of colour patches is considered to be distinguishable by birds. Points indicate 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). Error bars show +/- one standard error. **B,C**: Plots of mean smoothed spectra for different body patches (B: wing, C: belly). Line colors indicate species (green: *L. acuticaudus*, red: *L. chloropterus*, blue: *L. elisabeth*) and shaded areas indicate the standard deviation of the spectral data.

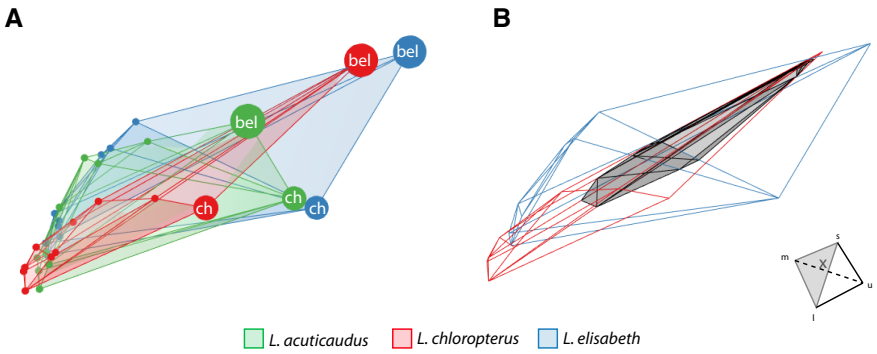

Fig. 3. **A**: Tetrahedral colorspace plot showing minimum convex polygons (convex hulls) containing reflectance spectra for plumage patches from *Lamprotornis acuticaudus* (green), *L. chloropterus* (blue) and *L. elisabeth* (red). Labeled points show representative plumage patches belly (bel) and cheek (ch). **B**: Colourspace occupied by *L. chloropterus* (blue) and *L. elisabeth* (red). Convex hulls volume overlap (similar colourspace occupied) is shown in grey. For reference, tetrahedron in lower right corner shows {*usml*} colour vertices and location of points in colourspace as indicated by an 'x'.