Reproducible research in the study of biological coloration

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Keywords: methods, photography, pigment, spectrometry, structural colour, visual ecology, visual modelling

Highlights:

- We review the reproducibility of contemporary studies of biological coloration.
- Methodological details are often missing, and data are seldom publicly available.
- We present guidelines for methods reporting, and for improving reproducibility.

Introduction

The study of colour in nature has generated insights into fundamental evolutionary and ecological processes, and research into colour traits is a rapidly growing field (Kelber & Osorio 2010). The ongoing interest in biological coloration has in part been driven by the increased availability of key technologies, including spectrometry and photography, and concurrent advances in methods for analysing colour data, such as visual models (e.g., Kelber et al. 2003; Endler & Mielke 2005; Stevens et al. 2007). While these developments are positive for the field, the increasingly complex analyses being run on ever-greater amounts of data heighten the need for comprehensive methods reporting and diligent data management (Alsheikh-Ali et al. 2011; Nekrutenko & Taylor 2012).

Replication and transparency lie at the heart of the scientific enterprise. Beyond simply allowing independent verification of results, reproducible research ensures greater comparability between studies and provides a foundation for testing new ideas and methods (Piwowar et al. 2007; Van Noorden 2011; Whitlock 2011). A study may be considered truly reproducible when it satisfies three broad criteria: (i) methods are reported completely, (ii) data are publicly available and archived, and (iii) the chain of modification of raw data is documented and preserved. While completely reproducible research (e.g., FitzJohn et al. 2014) is a laudable goal, the considerable demands it imposes on researchers means that it will often, in practice, be unattainable. Nevertheless, even partial reproducibility through the relatively simple practices of complete methods reporting and public data archiving is of tremendous value.

Our aim was to explore the state of reproducibility in the study of biological coloration, and to outline simple ways in which it may be improved. We first outline common methods for studying biological coloration and present guidelines for comprehensive methods reporting. We then explore how well some of these important criteria are reported in the literature. We also quantify the availability of publicly archived data and code and suggest some useful tools for increasing the reproducibility of colour trait research more broadly.

Measuring colour

Generations of biologists have endeavoured to explain the mechanisms and functions of animal and plant colouration (Poulton 1890; Wallace 1891; Thayer & Thayer 1909; Endler & Mielke 2005), and uncovering best practices in measuring colour has been a great challenge. The direct measurement of reflectance and/or transmittance through spectrometry revolutionized the study of biological coloration (Dyck 1966), and has been widely adopted as the standard (Andersson et al. 2006). Digital photography is also increasingly being used to quantify colour (Stevens et al. 2007), as high-resolution cameras are inexpensive and allow for the

simultaneous, rapid sampling of multiple colour patches (McKay 2013).

Expansion in the availability of objective methods for the measurement of colour has been matched by advances in theory and analysis. In particular, the development of visual models has enabled researchers to move beyond quantitative comparisons of reflectance spectra and adopt potentially more biologically relevant perspectives when defining and testing hypotheses (Chittka 1992; Vorobyev & Osorio 1998; Endler & Mielke 2005). Visual models typically attempt to describe the reception and early-stage processing of chromatic and achromatic information as a function of an object's reflectance, the ambient illumination, and a receiver's sensory system (Vorobyev & Osorio 1998; Endler & Mielke 2005). Although relatively easy to implement, visual models are built on multiple assumptions about the way in which stimuli are processed that can dramatically shape the results of a given analysis (e.g., Lind & Kelber 2009; Pike 2012).

Guidelines for methods reporting

Given that methodological variation may shape results in significant and unpredictable ways (Tables 1 and 2, and references therein), the comprehensive reporting of methods is a simple and crucial step in ensuring research is reproducible. Accordingly, we developed a list of information about the capture (Table 1) and analysis (Table 2) of colour data that should ideally be reported. With regards to the measurement of colour, we focus on the two most frequently used methods; photography and spectrometry. Analytical techniques are diverse, mathematically complex, and are being developed rapidly (Kelber & Osorio 2010; Théry & Gomez 2010). Such progress means that the need for a deep understanding of common methods can quickly outstrip the working knowledge of the average researcher. As a consequence, the subtle complexity of many analytical techniques can be overlooked by empiricists, leading to critical methodological information not being reported. Our guidelines for reporting the details of colour analyses (Table 2) thus cover two broad, common methods: colourimetric (or 'spectral') analyses, and visual modelling.

While we wish to emphasize that these details are essential to ensuring full reproducibility of data capture and analysis, we recognize that space restrictions in the main text of manuscripts may preclude the incorporation of all these details. In such cases, we suggest that details be included in meta-data or in a supplementary file so that the necessary information is available to researchers. It is also the case that there is variability in the degree to which each parameter may affect results, and so we have aimed to provide a brief, qualitative outline of the potential effects that variation in each parameter may have on the data (Table 1 and 2). These tables are not intended as a guide to the selection of methods, however, for which we refer readers to excellent recent reviews as well as the original publications (Kelber et al. 2003; Montgomerie et al. 2006; Stevens et al.

2007; Kemp et al. 2015, and references in Tables 1 and 2).

Assessing reporting and reproducibility

To determine the current state of reproducibility in the field we assessed a sample of the literature against a set of our criteria (Table 1 and 2), which we expected should be commonly reported based on our background reading. We searched papers from 2013 in 22 leading journals: *American Journal of Botany*, *The American Naturalist*, *Animal Behaviour*, *Behavioral Ecology*, *Behavioral Ecology and Sociobiology*, *Biological Journal of the Linnean Society*, *Biology Letters*, *Current Zoology*, *Ecology*, *Ecology and Evolution*, *Ecology Letters*, *Ethology*, *Evolution*, *Functional Ecology*, *Journal of Ecology*, *The Journal of Evolutionary Biology*, *The Journal of Experimental Biology*, *Naturwissenschaften*, *New Phytologist*, *Oikos*, *PLoS ONE*, *Proceedings of the Royal Society B: Biological Sciences*. On each of the journals' homepages, we used the Boolean phrase 'colour*' or 'color*' or 'spectra*'to search the title and/or abstract. Journals were haphazardly divided up between the authors to review the 216 papers returned from our search. On first pass, we excluded review papers, methodological papers, papers quantifying spatial (i.e., pattern) rather than chromatic properties of a colour patch, and studies taking micro-spectrometric measurements of retinal absorbance. The final set of 60 papers included only those that used either a spectrometer or camera to quantify coloration. In order to reduce the risk of observer bias in our assessment, each paper included in the final set was read and reassessed by two further authors. Any discrepancies between assessment scores were discussed by the three authors that had read the article and resolved prior to analysis. We also recorded whether data (in either a 'raw' or 'processed' form) and/or any code were publicly available. The data along with our analysis script has been stored as a github repository. We have kept the papers used in our dataset anonymous as our aim was to explore the general question of reproducibility in the field.

Methods reporting in colour studies

Our literature survey suggests there is surprising inconsistency and incompleteness in commonly reported methodological details (Fig. 1). Most studies (n = 51) used a spectrometer to measure colour, yet integration times (20%), and probe-sample geometry (49%) and distance (20%) were often not reported. Among studies that used photography (n = 18), 67% detailed the number of pixels averaged, though camera models were more frequently reported (89%). Light sources were detailed in 76% and 65% of spectrometer- and camera-based studies, respectively. With regard to data analysis, of the 35 studies that calculated colourimeteric variables, 77% specifically defined their measure of brightness, hue and/or chroma. The receptor-noise limited model

(Vorobyev et al. 1998) was commonly used among studies with visual modelling (11 of 22), though there was considerable variation in the detailing of the type of receptor noise used (45% reported), the type of quantum catch used (59%), or whether photoreceptor adaptation (43%) was modelled. In contrast, details of the species' visual system being modelled (95%), the background used (82%), and the modelled illuminant (74%) were more commonly reported.

While some of the figures reported above seem troubling, it is important to note that 38% of papers made reference to previous work for details on some or all methods. The referenced works may have comprehensively covered some of these criteria, but were often incomplete as well, or referenced yet another paper. To avoid "decay" of methodological detail reporting over successive papers, we suggest reporting all details along with the current manuscript. That aside, the remaining 62% of studies did not reference previous work, and were missing potentially important methodological details.

Public availability of data and code

Of the 60 studies analysed, 1.7% publicly provided the raw underlying data, 31.7% provided data in a pre-processed form, and 66.7% of studies did not provide any publicly accessible data. The paucity of data being made available post-publication in studies of biological coloration is in line with other fields (Wolkovich et al. 2012; Drew et al. 2013; Vines et al. 2013), including the broader field of animal behaviour (Caetano & Aisenberg 2014). There is evidence, however, that this trend is shifting as funding agencies and publishers increasingly mandate the release of data (Whitlock et al. 2010).

The clearest benefit of open access to data is that it allows researchers to build upon previous work for the testing and refinement of new ideas and methods. As discussed above, it is also essential that complete methodological details be provided, either in the manuscript or associated meta-data, to ensure data can be used to their full potential. This is particularly relevant in the study of colour, where new analysis methods are frequently developed (e.g., Endler 2012; Allen & Higham 2013; Stoddard et al. 2014). More generally, the provision of open data is likely to foster collaborations as researchers draw upon existing work (Piwowar et al. 2007; Whitlock 2011), and considerably reduce the difficulty of meta-analyses and large scale comparative work (e.g., Maia et al. 2013a; Burd et al. 2014). We thus encourage researchers in our field to publicly archive raw data (e.g., individual reflectance spectra), to maximise its utility. The use of modest data embargoes and appropriate licenses can help to ensure that original authors are able to make the best use of data and are subsequently credited, though we acknowledge that tensions over data-sharing exist (discussed in Roche et al. 2014).

None of the included studies linked to any form of code. This is not surprising given the popularity of graphical-user-interface (GUI) based statistical and colour-analytical software (e.g., AVICOL; Gomez 2006). When such software is freely available, and is combined with the comprehensive reporting of methods, its use represents a valuable approach to conducting reproducible research. We also note that there are advantages in adopting a programming workflow, in spite of the initial time investment required. Programming languages such as Python (Van Rossum & Drake Jr 1995) and R (R Core Team 2014), for example, are free, open-source, and are host to communities of developers that continuously build and implement analytical tools. Indeed, tools specifically for the analysis of colour data already exist (e.g., the package "pavo" for R; Maia et al. 2013b). While no program can compensate for having a clear understanding of the analysis at hand, programming languages are flexible by nature. This allows individual researchers to rapidly explore new methods as they are published, and methods developers to implement their analyses to increase the reach and impact of their work. A further advantage of programmatic analyses is that analysis scripts, when properly curated, represent a complete documented history of a study's methods. The chain of modification of raw data may thus be preserved, both for researchers revisiting their own work in the future, and for other scientists looking to build directly on the results of previous studies.

Conclusions

Our review of the recent literature highlights some impediments to reproducibility in the study of biological coloration. The simplest step towards reproducible research is through the comprehensive reporting of methods, yet key information is often unreported (Fig. 1). Here we provide a list of methodological information that we suggest be specified in studies that focus on the spectrometric- or camera-based analysis of colour (Tables 1 and 2). In addition to the suggested information, we also recommend that raw spectra be presented where possible, as these figures allow for the rapid assessment of the nature and quality of the data independent of downstream processing. We also emphasise the importance of explicitly outlining the biological justification underlying all choices for data capture and analysis (Kemp et al. 2015). Finally, the public storage of data along with detailed meta-data plays a pivotal role in reproducibility, yet public data storage in studies of colour traits is not yet the norm. We therefore encourage researchers to consider the substantial benefits of publicly archiving their data (Piwowar et al. 2007; Whitlock 2011). Overall, we hope that our guidelines will encourage researchers to think about the reproducibility of their work and the advantages of increased transparency, which will continue the advance of an exciting era in colour research.

Acknowledgements

We thank Rafael Maia, the Morehouse lab, and three anonymous reviewers for valuable discussions and suggestions. TEW and RLD were supported by Australian Postgraduate Awards, and DWAN was supported by an NSERC PGSD and MQRES scholarship.

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