



DR RHIANNON LIA DALRYMPLE (Orcid ID : 0000-0002-2660-1424)

Article type : Regular Manuscript

Macroecological patterns in flower colour are shaped by both biotic and abiotic factors.

Rhiannon L. Dalrymple¹, Darrell J. Kemp², Habacuc Flores-Moreno^{1,3}, Shawn W. Laffan⁴, Thomas E. White⁵, Frank A. Hemmings⁶ and Angela T. Moles¹.

1 – Evolution & Ecology research Centre, School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW 2052, Australia

2 – Department of Biological Sciences, Faculty of Science and Engineering, Macquarie University, North Ryde, Sydney, NSW 2109, Australia

3 – Department of Ecology, Evolution, and Behavior, University of Minnesota, St Paul, MN, USA 55108

4 – School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW 2052, Australia

5 — School of Life and Environmental Sciences, The University of Sydney, Camperdown, Sydney, NSW, 2109, Australia

6 – John T. Waterhouse Herbarium, School of Biological, Earth and Environmental Sciences, UNSW Sydney, Sydney, NSW 2052, Australia

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/NPH.16737](https://doi.org/10.1111/NPH.16737)

This article is protected by copyright. All rights reserved

Author for correspondence: Rhiannon Dalrymple

Phone: +61412828914

Email: Rhiannon.dalrymple@gmail.com

Received: 11 February 2020

Accepted: 24 May 2020

ORCiD:

Dalrymple, R.L. - orcid.org/0000-0002-2660-1424

Kemp, D.J. - orcid.org/0000-0002-5845-5513

Flores-Moreno, H. - orcid.org/0000-0002-7083-0005

Laffan, S.W. - orcid.org/0000-0002-5996-0570

White, T.E - orcid.org/0000-0002-3976-1734

Hemmings, F.A. - orcid.org/0000-0002-0355-0070

Moles, A.T. - orcid.org/0000-0003-2041-7762

Summary

- There is a wealth of research on the way interactions with pollinators shape flower traits. However, we have much more to learn about influences of the abiotic environment on flower colour.
- We combine quantitative flower colour data for 339 species from a broad spatial range covering tropical, temperate, arid, montane and coastal environments from 9.25° S to 43.75° S with 11 environmental variables to test hypotheses about how macro-ecological patterns in flower colouration relate to biotic and abiotic conditions.
- Both biotic community and abiotic conditions are important in explaining variation of flower colour traits on a broad scale. The diversity of pollinating insects and the plant community have the highest predictive power for flower colouration, followed by mean annual

precipitation and solar radiation. On average, flower colours are more chromatic where there are fewer pollinators, solar radiation is high, precipitation and net primary production is low, and growing seasons are short, providing support for the hypothesis that higher chromatic contrast of flower colours may be related to stressful conditions.

- To fully understand the ecology and evolution of flower colour, we should incorporate the broad selective context that plants experience into research, rather than focussing primarily on effects of plant-pollinator interactions.

Keywords

Flower, colour, pollinator, diversity, resource availability, productivity, solar radiation, precipitation

Introduction

The mutualism between angiosperms and their pollinators is arguably the most influential biological interaction in evolutionary history (Kiester *et al.*, 1984; Ollerton, 1996; Lunau, 2004; Ollerton *et al.*, 2011). Conventional wisdom (*sensu* Rausher 2008) is that pollinators exert the primary selective pressure on flower colour, with pollinator mediated selection driving different flower colours and ‘pollination syndromes’ which are suites of traits adapted to pollinator attraction (Chittka & Menzel, 1992; Menzel & Shmida, 1993; Proctor *et al.*, 1996; Fenster *et al.*, 2004; Rausher, 2008; Shrestha *et al.*, 2019). Other aspects of the biotic environment, such as the colour of other plant community members, are also known to influence flower colour (Gumbert *et al.*, 1999; McEwen & Vamosi, 2010). However, while abiotic dimensions of the environment can also impose selection on flowers as certain conditions may favour species with certain colouration traits over others (Chalker-Scott, 1999; Galen, 1999; Strauss & Whittall, 2006; Arista *et al.*, 2013), the effects of the abiotic environment in influencing flower colour have received far less attention than have the effects of biotic interactions and overall, we know little about the relative importance of abiotic and biotic selection pressures in shaping the colour of flowers (Strauss & Whittall, 2006). To come to a more comprehensive understanding of the ecology of flower colour, we must synthesize ideas about the evolutionary

pressures and ecological contexts which shape it. This paper makes a first attempt at doing so across broad spatial and taxonomic scales, by addressing a number of hypotheses about the evolution of flower colour and determining if flower colour is more affected by aspects of the biotic environment than it is by abiotic conditions.

Biotic environment and community diversity

We began by exploring the relative importance of the diversity of the angiosperm community, and the diversity of the bird and insect guilds that play important roles in pollination in shaping flower colour. We studied the diversity of insects and birds at guild level because plants and pollinators tend to interact in broad, variable networks rather than as sets of partner pairs (Waser *et al.*, 1996; Williams & Adam, 2010; Popic *et al.*, 2013).

Flower colour presents a primary signal for pollinator attraction (Stebbins, 1974; Waser & Price, 1985; Melendez-Ackerman & Campbell, 1998; Bradshaw & Schemske, 2003; Fenster *et al.*, 2004), and research has repeatedly demonstrated pollinator-mediated directional selection on flower colour (Waser & Price, 1981; Hopkins & Rausher, 2012; Ortiz *et al.*, 2015). Flower colour traits are often adapted to attract particular guilds of pollinators, selected to suit their visual systems, hue discrimination optima and innate colour preferences (Chittka & Menzel, 1992; Bradshaw & Schemske, 2003; Cronk & Ojeda, 2008; Dyer *et al.*, 2012; Shrestha *et al.*, 2013a; Bergamo *et al.*, 2016; Shrestha *et al.*, 2019). For instance, red, bird-visited flowers often display another colour of a complementary hue (sometimes referred to as ‘parrot colours’), supposedly to attract the attention of avian visitors (Proctor *et al.*, 1996; Cronk & Ojeda, 2008). Based on this, we expect that plant species in communities with high bird diversity would, on average, display a greater number of different spectra on their flowers and that there would be a greater disparity between the hues on their flowers than do species living in communities with low bird diversity. In insect-pollinated flowers, nectar guides that point at or form a ‘bulls-eye’ to guide a pollinator more efficiently towards the nectar rewards/pollen centre of a flower, are often in colours that contrast strongly to the main petal hue or in UV colours (Penny, 1983; Waser & Price, 1985; Leonard & Papaj, 2011; Papiorek *et al.*, 2016). We therefore test the hypothesis that plant species in environments with a higher diversity of pollinating

insects will tend to have greater average contrast between their flower colours than do species in regions with a lower diversity of pollinating insects.

The “pollination market” hypothesis predicts that a range of distinct and discriminable flower colours should exist in a diverse habitat (Feinsinger, 1987; Friedman & Shmida, 1995; Gumbert *et al.*, 1999; Bergamo *et al.*, 2018). This is because plant species may benefit from flower constancy, in which pollinators tend to selectively visit flowers of only one species (Chittka *et al.*, 1999). There is evidence that flower colour can play a role in reproductive character displacement and reinforcing selection to reduce hybridization of sympatric species by promoting flower constancy (Levin & Kerster, 1967; Hopkins & Rausher, 2012; Grossenbacher & Stanton, 2014). It has been shown that diverse plant communities (especially those containing rarer species) may display a range of colours that are more distinct and divergent from each other (Gumbert *et al.*, 1999; McEwen & Vamosi, 2010; Bergamo *et al.*, 2018), and selection may favour increased flower pigmentation (and thus greater colour chromaticity, spectral purity or intensity) in sympatric congeners to promote insect pollinator constancy (Hopkins & Rausher, 2012; Kemp *et al.*, 2015; van der Kooi *et al.*, 2019a). Thus, we predict that in more species-rich plant communities, there will be higher average chromatic contrast (relative to an achromatic point) of floral colours, and that on average species will tend to have a greater number of different spectra on their flowers compared to those that live in less species-rich plant communities, as having more than one colour per species greatly increases the range of identifiable and discriminable colouration patterns possible.

Abiotic environment

Many of the pigments responsible for flower colours may also have a role in resistance to abiotic stressors, including high temperatures, low precipitation, high ultraviolet radiation and low-productivity environments (Chalker-Scott, 1999; Schemske & Bierzychudek, 2001; Mori *et al.*, 2005; Arista *et al.*, 2013; Koski & Ashman, 2015).

Anthocyanin pigments (a subgroup of flavonoids) are largely responsible for many flower colours, producing nearly all pink, red, orange, purple, blue and blue-black hues (Davies, 2008), with greater amounts of anthocyanins resulting in higher colour chroma (Hopkins & Rausher, 2012). The biosynthesis of these pigments is affected by day-length and the ratio of red to near-infrared light (which varies with solar angle / latitude)(Taylor, 1965; reviewed by Jaakola & Hohtola, 2010). The number of sunlight hours has been shown to be important predictor of the frequency of flower colour morphs within some species (Arista *et al.*, 2013). Anthocyanins also have a role in protecting fruits and flowers from ultraviolet (UV) radiation (Ben-Tal & King, 1997; Chalker-Scott, 1999; Mori *et al.*, 2005; Koski & Ashman, 2015). As an increase in anthocyanin can be induced by longer day lengths and higher UV radiation, we predict that on average flowers from regions with greater solar radiation will have more chromatically contrasting (i.e. more heavily saturated) flower colours than do those from regions with lower solar radiation.

Precipitation can affect plant colours directly. Negative correlations have been demonstrated between water availability and the amount of flavonoids (including anthocyanin) that plants produce (Chalker-Scott, 1999; Tattini *et al.*, 2004; Strauss & Whittall, 2006). Low precipitation and/or drought stress has been shown to favour colour saturated flower morphs over unpigmented or less colour saturated flowers (Schemske & Bierzychudek, 2001; Warren & Mackenzie, 2001), and has been shown to predict the frequency of colour morphs in *Lysimachia arvensis* across its range (Arista *et al.*, 2013). Precipitation may also indirectly effect plant colours through altering soil conditions, by leaching nutrients or altering pH in soil (Austin & Vitousek, 1998). As anthocyanin production is affected by both nutrient levels and pH (Asen, 1976; Chalker-Scott, 1999 and references therein), we may expect high precipitation to reduce the production of highly saturated flower colours. Therefore, we predict that broad gradients of precipitation will correlate with patterns of flower colour across-species, with water-stress favouring higher average flower colour chromaticity than in regions with higher precipitation.

Flower colour may have important effects on flower temperature (van der Kooi *et al.*, 2019b), with the spectral composition and intensity of light that is reflected or absorbed impacting internal

temperature (e.g. Jewell *et al.*, 1994; McKee & Richards, 2008). The development of flavonoids can be affected by temperature (Ben-Tal & King, 1997; Jaakola & Hohtola, 2010), which may indicate that anthocyanins have a function in ameliorating heat stress (Coberly & Rausher, 2003). It has been shown that temperature can differentially affect the fitness of morphs of low- and high-colour saturated flowers (Coberly & Rausher, 2003). If anthocyanins provide resistance from heat stress, higher temperatures are predicted to select for greater average chromatic contrast of flower colours than that found at lower temperatures.

The resources-cost hypothesis predicts that if less conspicuous flowers require less investment of essential resources than more conspicuous flowers, then a reduction in investment in related floral traits will be advantageous in resource-poor conditions (Galen, 1999). Thus, plant species in areas with abundant resources may evolve and maintain more colourful flowers than do plants in regions with resource scarcity. This hypothesis has been upheld in animals, with birds and butterflies in regions with higher net primary productivity (NPP) and longer growing season showing a greater number of different spectra and more saturated colours (Dalrymple *et al.*, 2018). We predict that species in areas with greater NPP and longer growing seasons will have a greater average number of different spectra and greater chromatic contrast in flowers than those species in areas with lower NPP and shorter growing seasons.

The theory of sensory drive predicts that biological colours used in signalling will be influenced by light environment as this can alter the conspicuousness of colour signals (Endler, 1992). The intensity and colour of ambient light can be shaped by physical structure of a habitat (Endler, 1992; Endler, 1993). While ambient light environment has been a major focus of research for animal colouration (Marchetti, 1993; Macedonia, 2001; McNaught & Owens, 2002; Gomez & Théry, 2004; Dalrymple *et al.*, 2018), there has been far less interest in the impacts of light environment on floral signal design (but see Altshuler, 2003; Binkenstein & Schaefer, 2015).

We considered three aspects of habitat which can affect the light environment: plant height, leaf area index and cloud cover. Plant canopy height is a key feature of habitat structure and complexity which

can alter ambient light environments (Endler, 1993). Taller habitats can have greater structural complexity such as shrub layers and branches, obscuring long-distance visual communication which impacts predictions for colour signal design, as low contrast between colour patches may enhance shape recognition and reduce shape disruption (Zahavi & Zahavi, 1997; Heindl & Winkler, 2003). Leaf area index (LAI, the area of leaves per m² of ground area) is a good indicator of shadiness in a habitat as greater leaf mass means greater absorption and reflection of light by leaves (Falster & Westoby, 2003). The light beneath a forest canopy is generally less intense with a green hue, which amplifies the green colour of leaves (Endler, 1993). In areas with high LAI, we predict that flowers may display more saturated colours as these may be more colour-constant in this type of light environment. We also expect species to display fewer colours that are brighter (i.e. have higher total reflectance, perceived as more luminant) for salience in these darker habitats and to encourage the recognition of flowers by their shape in the more spatially complex conditions (Endler, 1993; Zahavi & Zahavi, 1997; but see Binkenstein & Schaefer, 2015). This is because insects have poor visual-spatial resolution and not likely to use colour perception in the initial detection of flowers (Chittka & Raine, 2006), but rather to use shape and achromatic cues to identify their preferred foraging flowers (Lehrer *et al.*, 1995; Howard *et al.*, 2019). The colour and intensity of light in an environment is also affected by absorption and scattering by clouds (Endler, 1993; Altshuler, 2003; McKenzie *et al.*, 2003; Australian Government Bureau of Meteorology (BOM), 2014). Due to the homogenizing effect of cloud cover on light environments, selection for certain colour combinations within a given light environment may be relaxed (Endler 1993). Thus, we predict that regions with higher cloud cover will be home to species with greater average differences between hues of flowers than those species in regions with lower cloud cover.

Synthesis: the relative importance of biotic vs abiotic factors in shaping macro-ecological gradients in flower colour

We have identified three predictions about how diversity of certain guilds in the community should be correlated with flower colour traits, and seven predictions for how abiotic conditions should influence flower colours (Table 1). In addition to testing these hypotheses, we quantified the relative importance

of abiotic and biotic conditions in influencing flower colour traits at a broad spatial and taxonomic scale. Given that discussion of flower colour almost invariably relates to its function in attracting pollinators, we predicted that biotic conditions would be more influential overall. Finally, we assess the extent to which abiotic and biotic variables are able to explain previously observed latitudinal gradients in flower colours (Dalrymple *et al.*, 2015). We believe that taking a pluralistic approach to the exploration of flower colour at a broad scale is an important step towards better understanding flower evolution and ecology, which will be crucial in the development of novel hypotheses in the field.

Methods

Flower colour sampling

We built a database of reflectance spectra of flowers of 339 angiosperm species or subspecies (from 74 families). Flowers were sampled across four different habitat types (rainforest, grassland, woodland and heathland) at 17 sites between Cairns, Queensland and southern Tasmania, Australia during the spring of 2012. Five 100 m transects were sampled at each grassland, woodland and heathland site. Transects in rainforest sites were approximately 200 m due to the low density of flowers. Transects aimed to capture as much of the floristic diversity at the site as feasible, and wherever possible we sampled a flower from three individuals from all flowering angiosperm species in each site. We excluded endangered species, non-native species, species known to possess strong ontogenetic colour variation, and species with flowers so small they could not be accurately measured. A full species list is provided in Notes S1, and a complete record of sampling can be found in Appendix S5 of Dalrymple *et al.* (2015).

We quantified five flower colour traits: the (1) *chromatic contrast* of all colour patches, the (2) *hue disparity* and (3) *maximum contrast* between colours displayed, the (4) *total reflectance* of all colour patches, and (5) *number of different spectra* displayed by the species. The measurement of colours with reflectance spectrometry is described in Notes S2.

As we have 339 study species, some of whom span the entire 30° latitude range our study area and a broad array of habitats and communities, we have opted to use visual system-independent colour indices and not make any assumptions or data transformations related to any one specific viewer. This is because different species are likely to be viewed by and interact with a broad range of ecologically-relevant viewers at any one time. Thus, we undertook a visual system-independent appraisal based on the ‘segment analysis’ approach of Endler (1990), integrating the area under reflectance curves in four equal spectral segments (300-400 nm; 400-500 nm; 500-600 nm; 600-700nm), producing relative segment reflectance data (more details can be found in (Dalrymple *et al.*, 2015)). This handling weights each segment equally, and thus neither relies upon the visual sensitivity of any particular species, nor makes any assumptions about colour perception. We did, however, adjust the segment reflectances using the Michaelis-Menton transformation (Endler & Mielke, 2005) to account for approximately log-normal nature of opponency-based processing that is fundamental to colour perception in vertebrates and invertebrates alike. The relative segment reflectances values were used to calculate X, Y and Z coordinates for location in a tetrahedral colour space, using the equations of Endler and Mielke (2005).

The *chromatic contrast* of each colour patch was calculated as the Euclidean distance between the achromatic centre of this tetrahedron and the colour patch’s projected location (Endler & Mielke, 2005); note that this is contrast to an achromatic point (the achromatic centre of our tetrahedral colour space) and not between any two spectra. Spectra that have high chromatic contrast would be perceived as a more saturated colour than spectra that have lower chromatic contrast. From the relative position of the species’ colours in the tetrahedron, we derived *maximum contrast* between any two colours on a species’ flowers (calculated as maximum Euclidean distance between the colours displayed by the species), and *average disparity between hues* on a species’ flowers (the angular difference between points in colour space, which represents a measure of differences in hue independent of chromaticity (ranging from 0 for identical hues and 1.0 for ‘complementary’ hues; following Endler & Mielke,

2005). Note that our calculations of maximum contrast and average hue disparity quantify differences between multiple colours displayed in the same flower, but do not provide any comparison of one species' flower colour traits to those of other neighbouring species. The *total reflectance* of each colour patch was calculated as the sum of the area under the reflectance curve relative to the white standard (following Dalrymple *et al.*, 2015a; see Notes S1). Spectra that have higher total reflectance would be perceived as brighter or more luminant than spectra that have lower total reflectance. The *number of different spectra* on a flower is a count of distinct colours displayed on the patches of a flower. Determining patches of distinct colour was assessed using spectrometer (and are thereby not hindered by being of human-visual colour assessment), patches were of large enough size to be measured well within patch boundaries, and were not forming a colour intergrade between two other colour patches (as per Dalrymple *et al.*, 2015).

Diversity

Spatial range data for all species of plants in the Flora of Australia Online (<http://www.environment.gov.au/biodiversity/abrs/online-resources/flora/main/>) were derived from the Atlas of Living Australia (<http://www.ala.org.au>). Spatial range data for animals were also derived from Atlas of Living Australia, by exporting all records of higher taxonomic groups. We have included birds (Aves) and a subset of insect orders — Lepidoptera, Coleoptera, Hymenoptera, Diptera, and Thysanoptera. While coarse, we have included these broad groups for three reasons: 1) they are the taxonomic designation of orders which contain the most pollinators of our study range (Proctor *et al.*, 1996; Williams & Adam, 2010); 2) Pollinator data are most often merely assumed from taxonomy or morphology (Waser *et al.*, 1996; Ollerton *et al.*, 2009; Williams & Adam, 2010), or from flower visitation despite the fact that visitation does not always result in pollination (King *et al.*, 2013; Popic *et al.*, 2013). As such, key pollinators of many Australian plant species have not been established, and a detailed pollination network matrix is not possible at this time; 3) many flower visitors can affect plant fitness (not only those that provide pollination services – *e.g.* pollen robbers),

and flower colour may therefore be impacted by interactions with multiple perceivers simultaneously (Renoult *et al.*, 2014).

Range data were filtered to only spatially valid records which were identified to species level, excluding non-vegetative areas and non-native vegetation. The diversity of each group was calculated by mapping species across their recorded ranges, and taking a count of plant, bird or insect species present in 0.5° grid cells in Biodiverse (Laffan *et al.*, 2010), using longitude and latitude of the data-points. We excluded grid cells for which less than five species were recorded to reduce the noise associated with taking averages from small groups of numbers.

Habitat variables

Datasets concerning abiotic aspects of the environment are described in Notes S1. They are the same as those employed in Dalrymple *et al.* (2018): solar radiation, mean annual rainfall, mean annual temperature, leaf area index (LAI), plant canopy height, growing season length, net primary productivity (NPP) and cloud cover. All environmental variables were mapped into 0.5° grid cells in Biodiverse (Laffan *et al.*, 2010), using the longitude and latitude of the data-points.

Statistical analysis

Data for rainfall, LAI, plant height, insect and plant diversity were \log_{10} transformed before analysis for normality.

All statistical analyses were performed in R 3.6.0 (R Core Team, 2014) using the manymodel framework in the mvabund package 4.0.1 (Wang *et al.*, 2012). This enabled us to improve model outputs through the permutation of residuals to relax the normality assumptions of linear models (Wang *et al.*, 2010; Winkler *et al.*, 2014) as colour data are typically non-normal (Endler & Mielke, 2005; Maia & White, 2018). This technique has the additional advantage of being robust to spatial auto-correlation. Variance explained by the models is calculated using Hooper's R^2 statistic; this is also used to compare models as AICc comparisons cannot be applied to models with multiple response variables.

We calculated variance explained for the individual predictors within a model (alike to partial R²) as (eta² statistic /number of response variables)/R² of the model.

Analyses were performed on 0.5° latitude grid-cell level data, which are the averages of the response and predictor variable data from each spatial cell. Sample sizes vary between models as data are not available for all predictor variables for all grid-cells. All models presented incorporate main effects only, with no interactions. Many ecosystem processes co-vary. However the sheer number of possible combinations makes posterior testing and interpretation of significant interactions complicated, and increases the potential for model over-fitting (Burnham & Anderson, 2002).

We first tested the ten predictions from the literature (Table 1) using simple linear models where the independent variable (x) was an environmental or community variable at a grid cell, and dependant variable (y) was the average value of a flower colour trait for all species of angiosperms present in that grid cell, using the significance and slope of relationships to assess the prediction.

Next, we examined the relative importance of all variables in driving all flower colour traits simultaneously on a broad scale. We did this using a multivariate multiple regression analysis which included all five flower colour traits as a single multi-response variable, and all abiotic and biotic variables as explanatory variables (see Figure 1; we refer to this as the ‘full model’).

We then sought to establish if the macroecological patterns in flower colour were more influenced by abiotic or biotic conditions. We built two multivariate multiple regression models both with all five flower colour traits as a single multi-response variable. The explanatory variables for one of these models were all variables related to the abiotic environment, and the other had the three biotic community variables (see Figure 1). Given that there are fewer variables in the biotic category, we also compared the variance explained by the biotic model to a model which contained only the three abiotic variables with the highest partial R² values in the full model (See table S2 in Notes S3).

We then sought to determine the amount of variation in each colour trait could be explained by models that incorporate both abiotic and biotic components of environment. We used the R^2 of single predictor linear models (each colour trait vs each abiotic and biotic predictor variable separately) to build a model for each trait using the abiotic variable and the biotic variable which explained the most variance in the colour trait, and then added two more variables (from either category) which explained the next two highest amounts of variation in the colour trait. We refer to these as the “selected models” for the traits. The variance explained in these selected models was then compared to models that also included a term for latitude to see if there may be an unaccounted factor shaping broad patterns in flower colour traits which co-varies with latitude at this scale (i.e. Martin *et al.*, 2010), using ANOVA analyses to establish if adding latitude significantly improved explanatory power (Crawley, 2013). We have also compared these models using AICc values, with greater penalty for model complexity to reduce the probability of model overfitting (Burnham & Anderson, 2002).

Results

None of the predictions about how diversity would relate to flower colouration were supported

Contrary to our first prediction, plants in communities with high bird species richness tend to signal with patches that are more similar in hue (e.g. orange and red, rather than complimentary hues such as orange and purple; ($P \leq 0.001$, $R^2 = 0.03$; Table 2; Figure 2b)). There was also no significant relationship between the number of different spectra on each flower and bird community diversity (Figure 2a, $P = 0.055$)

Contrary to our second prediction, insect community diversity was not positively correlated with average contrast between flower colour patches ($P = 0.09$).

Contrary to our third prediction, greater plant species richness is associated with less chromatically contrasting colours ($P \leq 0.001$, $R^2 = 0.036$). Flowers in communities with 400 or more plant species are on average 5.4 % less chromatically contrasting than those in communities with 100 plant species

or less. There was no significant effect of plant diversity on number of different spectra ($P = 0.81$; Table 2; Figure 2c and d).

Stressful abiotic environments are correlated with more chromatic flowers

In line with our fourth and fifth predictions, there was a positive correlation between chromatic contrast and solar radiation ($P = 0.003$, $R^2 = 0.02$), and a negative correlation between chromatic contrast and precipitation ($P \leq 0.001$, $R^2 = 0.08$). That is, in regions where plants experience stress due to high solar radiation or low water availability, flowers are significantly more chromatic.

However, effect sizes are relatively small. Regions with 20 MJ/m^2 solar exposure or higher (much of the area above the tropic of Capricorn) have flowers that are 2.5 % more chromatic than those in areas that experience 15 MJ/m^2 or lower (much of Tasmania). Regions where mean annual precipitation is $\leq 500\text{ mm}$ (generally inland, west of 145°E) are home to species whose flowers are 3.8 % more chromatic on average than those in areas which get $\geq 1500\text{ mm}$ (Table 2, Figure 3a and b).

Our sixth prediction was that there would be a positive correlation between temperature and chromatic contrast, however, this was unsupported ($P = 0.145$; Figure 3c).

Counter to our seventh prediction, regions with higher NPP or longer growing seasons have resident species with flowers that are significantly less chromatic (NPP: $P \leq 0.001$, $R^2 = 0.08$; growing season length: $P \leq 0.001$, $R^2 = 0.02$; Figure 3d and e). For instance, species in regions with a growing season of 11 months or more have flowers that are on average 3.9 % less chromatic than those in regions in which the growing season is 9 months or less. There was no significant effect of either NPP or growing season length on the number of spectra per species (NPP: $P = 0.52$; growing season length: $P = 0.32$; Table 2, Figure 3f and g).

Counter to prediction eight, increased cloud cover was associated with the display of more similar hues ($P \leq 0.001$, $R^2 = 0.09$; Table 2; Figure 3f).

Supporting our ninth prediction, there was a negative correlation between plant height and maximum colour contrast ($P \leq 0.001$, $R^2 = 0.04$). In habitats that are 25 m tall or more (such as rainforests), flowers have colours that are on average 15.3 % less contrasting than in habitats that are 10 m tall or less (such as savanna grasslands; Table 2; Figure 3g).

In line with our tenth prediction, leaf area index was positively correlated with total reflectance ($P = 0.004$, $R^2 = 0.02$; Figure 3i). On average, species in regions with an LAI of 1.5 or less (typically grassland or shrubland) display colours that reflect 3.6 % less light overall than flowers of those species in regions with LAI of 5 or more (such as forests (Asner *et al.*, 2003)). However, contrary to this prediction, there was a negative correlation between LAI and chromatic contrast ($P = 0.004$, $R^2 = 0.02$; Figure 3j), with the same increase in LAI resulting in a 5.5 % increase in average flower colour chromaticity. There was no significant relationship between LAI and the number of flower colours (Table 2).

Abiotic and biotic environment are both important in explaining broad patterns in flower colouration

Modelling the effect of all abiotic and biotic environment variables on all flower colour traits showed that insect community diversity was the single most influential variable shaping flower colouration, explaining 5.3 % of the total variation across species for the five flower colour traits. Plant community diversity, mean annual precipitation and solar radiation were also significant in shaping the macro-ecological patterns in flower colours, explaining 4.4 %, 4.3 % and 0.8 % of the total variation, respectively. A total of 14.6 % of the variation in flower colouration across the range was explained by this model (Table S2 in Notes S3).

A model including only variables related to the abiotic environment explained 10.8 % of the total variation in our five flower colour traits across the broad study range. If we restrict that analysis to only include the three abiotic variables with the highest R^2 (mean annual precipitation, cloud cover and plant height; see Table S2 in Notes S3; see methods), abiotic environment still explains 9.5 % of

the variation overall. By contrast, a model with the three variables related to the biotic community only explained 4.7 % of the total variation in flower colouration.

In the models built for each trait using the single linear predictors with the highest R^2 (based on Table 2), plant community diversity was included for four of five colour traits (Table 3). Plant height was included in selected models for three of five traits. The best model for number of different spectra per species included temperature, solar radiation, and bird community diversity. Interestingly, insect community diversity, which explained the greatest amount of variation in the full model for all colour traits, was not selected in any individual colour trait model. The model for average hue disparity between flower colours had the highest explanatory power, and included plant height, plant community diversity, cloud cover and LAI, and explained 20.6 % of the total variation in this trait.

The addition of the term for latitude did not significantly improve the selected models for any of the flower colour traits, and only increased variance explained by models between 0.05 % and 0.49 %. AICc values indicate that models without terms for latitude were more parsimonious for the data in all traits (Table 3).

Discussion

Both abiotic and biotic factors are important in explaining macro-ecological patterns in flower colours. The importance of the abiotic environment might not be surprising given the wealth of knowledge about environmental correlations in global patterns of other plant traits (Cunningham *et al.*, 1999; Wright & Cannon, 2001; Wright *et al.*, 2005; Moles *et al.*, 2009; Moles *et al.*, 2014). However, the flower colour literature has focused disproportionately on the effects of the pollinating and flowering communities (see Notes S4). Our results support the notion that if we wish to derive a more holistic understanding of flower colour ecology and evolution, we need to incorporate more information about species' abiotic environment.

The diversity of pollinating insects was the best single predictor of flower colouration at a broad scale, which reflects the role of insects as the most important pollinators globally (Stebbins, 1974; Faegri & van der Pijl, 1979; Proctor *et al.*, 1996; Williams & Adam, 2010). While there is long history of research into how the biotic community influences floral traits (Hervey, 1899; Stebbins, 1974; Waser *et al.*, 1996; Gumbert *et al.*, 1999; Ollerton *et al.*, 2009; Dyer *et al.*, 2012; Grossenbacher & Stanton, 2014), none of the specific directional predictions derived from the literature about how biotic community diversity would correlate with flower traits were upheld across species at a broad spatial scale. In fact, most of our results did not support *a priori* predictions, with multiple correlations going in the opposite direction from that predicted.

In attracting pollinators, plants are interacting with their broader flowering community, suggesting that the angiosperm community at large will play an important role in shaping flower colour traits (Gumbert *et al.*, 1999; McEwen & Vamosi, 2010). While we demonstrate the importance of the plant community in shaping the colours of resident species, an important prediction about the relationship between plant diversity and flower colour was not supported. Instead of the increase in colour signal salience predicted by the idea of character displacement, increased plant community diversity correlated with species who had multiple flower colour patches displaying more similar hues that are less contrasting and less chromatic. This may indicate that in highly diverse plant communities, angiosperms tend to converge on a colour strategy, which could reflect a strategy where plants of different species facilitate each other's pollinator visitation by displaying similar signals (Hegland *et al.*, 2009; Hegland & Totland, 2012). Our results therefore support the idea that such a facilitative strategy may be a common phenomenon in tropical forests (Feinsinger, 1987). Rainforest residents may benefit from a facilitative strategy of pollinator attraction as rainforest flowers vary in abundance through space and time (Williams & Adam, 2010), and these habitats are generally highly species diverse (see Figure S1 in Notes S3) and contain a higher proportion of rarer species (Hegland *et al.*, 2009; Bergamo *et al.*, 2020). Most biotically-pollinated angiosperms employ a generalist pollination strategy (Ollerton, 1996; Waser *et al.*, 1996; Popic *et al.*, 2013), and colour convergence, rather than character displacement or divergence, may reflect generalisation in plant-pollinator interactions in species rich plant communities (Kantsa *et al.*, 2017). Both field and laboratory based research has

shown that convergence of flower communities on a more limited set of flower colours than is possible may also reflect adaptation to broad preferences and visual abilities of key pollinator guilds, which can form strong community assembly filters and selective pressures shaping flower colouration globally (Chittka & Menzel, 1992; Gumbert *et al.*, 1999; Chittka & Raine, 2006; Arnold *et al.*, 2009; Shrestha *et al.*, 2013a; Shrestha *et al.*, 2013b; Bukovac *et al.*, 2017). This supports our viewer independent approach to colour traits at this scale; while our metrics are independent of any one visual system and assume only a general ‘viewer’ - sharing only the fundamentals of visual models – we are returning findings that broadly converge on those from studies that incorporate colour perception and acuities of particular viewers (e.g. Gumbert *et al.*, 1999; Chittka & Raine, 2006; Shrestha *et al.*, 2013b).

Our data are consistent with the hypothesis that flower colouration can be a mechanism for protection from abiotic stressors (Chalker-Scott, 1999; Schemske & Bierzychudek, 2001; Warren & Mackenzie, 2001; Mori *et al.*, 2005; Strauss & Whittall, 2006). Despite chromatic contrast of colours being strongly correlated with solar radiation, precipitation, NPP and growing season length, we saw no effect of mean annual temperature. This is consistent with findings of no association between colour and temperature in 30 Australian plant species (Shrestha *et al.*, 2018), and a review that highlights there is no clear effect of flower colour polymorphisms on the difference between ambient and internal flower temperature at the within species level (van der Kooi *et al.*, 2019b). Perhaps resistance of heat stress through flower colour is not a successful way of protecting such fragile organs, and other floral characteristics such as shape, orientation, opening and closing behaviour and floral pubescence may be more important in this regard (van der Kooi *et al.*, 2019b). Maintaining flowers through heat waves may not pay off, as extreme heat could lead to negative effects on meiosis, pollen germination, or pollen tube growth defects (Larkindale *et al.*, 2005), and abscission of flowers can be initiated or accelerated by extreme temperatures (Addicott & Lynch, 1955). At the other end of the scale, it has been shown that cold temperatures can affect the biosynthesis of pigments in numerous species (reviewed by Jaakola & Hohtola 2010), including the genus *Anigozanthos* which display greatest colour saturation (perceived chroma) when temperatures are low during development (Ben-Tal & King 1997). This may also offset any signal of heat selecting on chromaticity of flower colours.

The relative importance of the abiotic and biotic dimensions of environment in shaping broad-scale patterns in flower colour are broadly similar those found for the colours of birds and butterflies shown by Dalrymple *et al.* (2018). However, we have been able to explain far less of broad-scale variation in flower colour than was possible for animals with a similar set of predictors (14.6 % vs 48.9%). Indeed, the effect sizes we have shown and variance that we have been able to explain in flower colour at this broad scale is generally low, although this is common for such taxonomically and spatially broad studies of functional traits in plants (e.g. see table 1 of Moles *et al.*, 2014). Our results do indicate, however, that the biotic and abiotic variables considered here do cumulatively account for the known patterns in flower colour across latitudes (Dalrymple *et al.*, 2015), as addition of latitude did not improve models for any flower colour trait. Comparison of the patterns shown here to those of Dalrymple *et al.* (2018) reveals that animal and plant colours often respond very differently to abiotic and biotic gradients, which likely relates to the different roles of colouration in plants and animals. For instance, flower signal design is often driven by the need to be visually conspicuous to pollinators (Chittka & Menzel, 1992; Lunau, 2004; Shrestha *et al.*, 2013a; Shrestha *et al.*, 2013b; Bukovac *et al.*, 2017), while animal colours may reflect the balance between sexual selection, which can drive evolution of more gaudy displays (Endler, 1983; Hill, 1990; Iwasa & Pomiankowski, 1994) and the need for crypsis or camouflage in the face of predation (Endler, 1983; Caro, 2005).

Animals tend to have more saturated colours in regions with high NPP and/or long growing seasons, (Dalrymple *et al.*, 2018), but flowers display lower chromatic contrast in those conditions (Figure 1). This suggests that while more productive environments provide better resource conditions for the expression of vibrant animal colouration, plants (which can synthesise all of their colour pigments *de novo*) may invest more in anthocyanins in less productive regions due to their protective roles. For instance, high chromatic contrast in regions with high solar radiation may potentially reflect increases in UV absorbing pigments in the flowers of resident species. Further, flowers with different hues experience differential florivory pressure (McCall *et al.*, 2013), probably because anthocyanins (which share biosynthetic pathways of other flavonoid compounds known to deter herbivory) may act as defensive agents (reviewed by Fineblum & Rausher, 1997). Applying the resource availability

hypothesis (Coley *et al.*, 1985), we would expect that if flowers with higher anthocyanin levels (and thereby greater colour chromaticity and perceived saturation) experience less florivory, this may be a strategy to reduce loss of expensive reproductive organs where resources are scarcer. In this way, flower pigmentation may be considered as part of a resource economics spectrum (Reich, 2014). The application of resource economics to floral traits may be a worthwhile future research direction.

The great taxonomic and spatial breadth of the current work required us to derive biologically meaningful colour-metrics, while making few assumptions about the specific viewer(s) and microsites of floral signals. This has implications for the extrapolation of our results to predictions for particular viewers, as our colour metrics will not correlate perfectly with those derived from other colour models because of the assumptions and data transformations applied for different colour processing and phenomenon such as colour constancy (Renoult *et al.*, 2014; Kemp *et al.*, 2015). While our findings are likely to reflect 'real' effects across the relevant suite of viewers, that viewers may vary in terms of specific perceptual/behavioural implications because of finer-scale psychophysical differences should be considered in extensions of this work. The scale of our approach also required us to take average species values of colour metrics and apply them to the entirety of the species range. We have then derived average values of traits displayed in the community of a 0.5° grid cell. Thus, we have not been able to include any variation in a species' colour across their geographic range, nor any variation in the ambient conditions in which a species' flowers would be encountered such as the intensity and colour of ambient light in a flowers' microsite or the background against which the signal is displayed. We have also not explored the variation in species values which were present in each grid cell community. Further, interactions between our abiotic and biotic variables may be significant and meaningful biologically, but have not been included in our models (for reasons outlined in methods). As such, the importance of some of the explored factors may hinge on other variables considered, and a more refined understanding of the impact of a particular dimension of the environment in driving flower colouration may be sought in the exploration of interactions. Finally, flower colouration can result not only from different pigment types, but also the amount and localisation of those pigments and other structures that reflect light(van der Kooi *et al.*, 2016). An exploration of the physiological characteristics underlying the reflectance

spectra of flowers and how these may vary across biological and abiotic gradients may be a worthy future direction for research into pathways of adaptation in flower colouration. Addressing some of these limitation in future research may allow us to embark on more nuanced explorations of the patterns in variation in our data, and ask new questions about patterns in and drivers of variation in colouration.

Summary

The best predictors of broad-scale patterns of flower colouration are the diversity of the pollinating insects and the plant community, and the amount of mean annual precipitation and solar radiation. We have long understood the importance of pollinator mediated selection on flower colours, but the abiotic environment is also significantly correlated with flower colouration at the macro-ecological levels and can explain a similar amount of the overall variation in flower traits. Our results are supportive of recent work that attempts to disentangle the whole context of flower colour selection – covering both abiotic and biotic conditions - rather than focussing solely on pollinator-mediated selection (i.e. Arista *et al.*, 2013; Ortiz *et al.*, 2015). This is fundamental for the design of studies that seek to compare colouration of floral communities across regional, latitudinal or global scales, as differences in the abiotic environments that species and communities experience are just as important to consider as the network of plants and pollinators.

Acknowledgements

Thanks go to Will Edwards and Peter Vesk for sampling support, and to Evan Webster and Jatin Kala for data wrangling and computational support. R.L.D. was funded by an Australian Postgraduate Award, UNSW Research Excellence Award, an E&ERC start up grant, and the Wiley Blackwell fundamental ecology award, A.T.M. was supported by a QEII fellowship from the Australian Research Council (DP0984222), and D.J.K. by the Australian Research Council (FT170100417). Herbarium specimens are held at John T Waterhouse Herbarium (UNSW), with some duplicates at the Queensland Herbarium (BRI) and the National Herbarium of Victoria (MEL). Adrian Dyer, Casper van der Kooi and an anonymous reviewer provided helpful comments during the review process.

Author contributions

RLD, ATM and DJK planned and designed the research; RLD, HF-M, TEW, SWL and FAH collected the data; RLD, HF-M and SWL analysed the data; RLD wrote the manuscript with input from all coauthors.

References

- Addicott FT, Lynch RS. 1955.** Physiology of Abscission. *Annual Review of Plant Physiology* **6**(1): 211-238.
- Altshuler DL. 2003.** Flower Color, Hummingbird Pollination, and Habitat Irradiance in Four Neotropical Forests. *Biotropica* **35**(3): 344-355.
- Arista M, Talavera M, Berjano R, Ortiz PL. 2013.** Abiotic factors may explain the geographical distribution of flower colour morphs and the maintenance of colour polymorphism in the scarlet pimpernel. *Journal of Ecology* **101**(6): 1613-1622.
- Arnold SE, Savolainen V, Chittka L. 2009.** Flower colours along an alpine altitude gradient, seen through the eyes of fly and bee pollinators. *Arthropod-Plant Interactions* **3**(1): 27-43.
- Asen S. 1976.** Known factors responsible for infinite flower color variations. *Acta Horticulturae* **63**: 217-223.
- Asner GP, Scurlock JMO, A. Hicke J. 2003.** Global synthesis of leaf area index observations: implications for ecological and remote sensing studies. *Global Ecology and Biogeography* **12**(3): 191-205.
- Austin AT, Vitousek PM. 1998.** Nutrient dynamics on a precipitation gradient in Hawai'i. *Oecologia* **113**(4): 519-529.
- Australian Government Bureau of Meterology (BOM) 2014.** Daily Solar Radiation Model Description.
<http://www.bom.gov.au/climate/austmaps/about-solar-maps.shtml> Accessed 05/04/2014.
- Ben-Tal Y, King RW. 1997.** Environmental factors involved in colouration of flowers of Kangaroo Paw. *Scientia Horticulturae* **72**(1): 35-48.
- Bergamo PJ, Rech AR, Brito VL, Sazima M. 2016.** Flower colour and visitation rates of Costus arabicus support the 'bee avoidance' hypothesis for red-reflecting hummingbird-pollinated flowers. *Functional Ecology* **30**(5): 710-720.
- Bergamo PJ, Susin Streher N, Traveset A, Wolowski M, Sazima M. 2020.** Pollination outcomes reveal negative density-dependence coupled with interspecific facilitation among plants. *Ecology Letters* **23**(1): 129-139.
- Bergamo PJ, Telles FJ, Arnold SE, de Brito VLG. 2018.** Flower colour within communities shifts from overdispersed to clustered along an alpine altitudinal gradient. *Oecologia* **188**(1): 223-235.
- Binkenstein J, Schaefer HM. 2015.** Flower colours in temperate forest and grassland habitats: a comparative study. *Arthropod-Plant Interactions* **9**(3): 289-299.
- Bradshaw HD, Schemske DW. 2003.** Allele substitution at a flower colour locus produces a pollinator shift in monkeyflowers. *Nature* **426**(13): 176-178.

Bukovac Z, Dorin A, Finke V, Shrestha M, Garcia J, Avarguès-Weber A, Burd M, Schramme J, Dyer A. 2017.

Assessing the ecological significance of bee visual detection and colour discrimination on the evolution of flower colours. *Evolutionary Ecology* **31**(2): 153-172.

Burnham KP, Anderson DR. 2002. *Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach*. New York: Springer Science+Business Media.

Caro T. 2005. The Adaptive Significance of Coloration in Mammals. *Bioscience* **55**(2): 125-125.

Chalker-Scott L. 1999. Environmental Significance of Anthocyanins in Plant Stress Responses. *Photochemistry and Photobiology* **70**(1): 1-9.

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.

Chittka L, Raine NE. 2006. Recognition of flowers by pollinators. *Current Opinion in Plant Biology* **9**(4): 428-435.

Chittka L, Thomson JD, Waser NM. 1999. Flower Constancy, Insect Psychology, and Plant Evolution. *Naturwissenschaften* **86**(8): 361-377.

Coberly LC, Rausher MD. 2003. Analysis of a chalcone synthase mutant in *Ipomoea purpurea* reveals a novel function for flavonoids: amelioration of heat stress. *Molecular Ecology* **12**(5): 1113-1124.

Coley PD, Bryant JP, Chapin FS. 1985. Resource availability and plant antiherbivore defense. *science* **230**(4728): 895-899.

Crawley MJ. 2013. *The R Book*. Chichester, UK.: John Wiley & Sons, Ltd.

Cronk Q, Ojeda I. 2008. Bird-pollinated flowers in an evolutionary and molecular context. *Journal of Experimental Botany* **59**(4): 715-727.

Cunningham SA, Summerhayes B, Westoby M. 1999. Evolutionary Divergences in Leaf Structure and Chemistry, Comparing Rainfall and Soil Nutrient Gradients. *Ecological Monographs* **69**(4): 569-588.

Dalrymple RL, Flores-Moreno H, Kemp DJ, White TE, Laffan SW, Hemmings FA, Hitchcock TD, Moles AT. 2018. Abiotic and biotic predictors of macroecological patterns in bird and butterfly coloration. *Ecological Monographs* **88**(2): 204-224.

Dalrymple RL, Kemp DJ, Flores-Moreno H, Laffan SW, White TE, Hemmings FA, Tindall ML, Moles AT. 2015. Birds, butterflies and flowers in the tropics are not more colourful than those at higher latitudes. *Global Ecology and Biogeography* **24**(12): 1424-1432.

Davies KM 2008. Modifying Anthocyanin Production in Flowers. In: Gould K, Davies K, Winefeld C eds.

Anthcyanins: Biosynthesis, Functions and Applications. NY, USA: Springer Science+Business Media, 49-80.

Dyer AG, Boyd-Gerny S, McLoughlin S, Rosa MGP, Simonov V, Wong BBM. 2012. Parallel evolution of angiosperm colour signals: common evolutionary pressures linked to hymenopteran vision.

Proceedings of the Royal Society B: Biological Sciences **279**(1742): 3606-3615.

Endler JA. 1983. Natural and sexual selection on color patterns in poeciliid fishes. *Environmental Biology of Fishes* **9**(2): 173-190.

Endler JA. 1990. On the measurement and classification of colour in studies of animal colour patterns. *Biological Journal of the Linnean Society* **41**: 315-352.

Endler JA. 1992. Signals, Signal Conditions, and the Direction of Evolution. *The American Naturalist* **139**: S125-S153.

Endler JA. 1993. The Color of Light in Forests and Its Implications. *Ecological Monographs* **63**(1): 1-27.

Endler JA, Mielke PW. 2005. Comparing entire colour patterns as birds see them. *Biological Journal of the Linnean Society* **86**: 405-431.

Faegri K, van der Pijl L. 1979. *The principles of pollination ecology*. Oxford: Pergamon Press.

Falster DS, Westoby M. 2003. Plant height and evolutionary games. *Trends in Ecology & Evolution* **18**(7): 337-343.

Feinsinger P. 1987. Effects of Plant Species on Each Other's Pollination: Is Community Structure Influenced? *Trends in Ecology & Evolution* **2**(5): 123-126.

Fenster CB, Armbruster WS, Wilson P, Dudash MR, Thomson JD. 2004. Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution, and Systematics* **35**: 375-403.

Fineblum WL, Rausher MD. 1997. Do Floral Pigmentation Genes Also Influence Resistance to Enemies? The W Locus in *Ipomoea Purpurea*. *Ecology* **78**(6): 1646-1654.

Friedman JW, Shmida A. 1995. Pollination, gathering nectar and the distribution of flower species. *Journal of Theoretical Biology* **175**(2): 127-138.

Galen C. 1999. Why Do Flowers Vary?: The functional ecology of variation in flower size and form within natural plant populations. *Bioscience* **49**(8): 631-640.

Gomez D, Théry M. 2004. Influence of ambient light on the evolution of colour signals: comparative analysis of a Neotropical rainforest bird community. *Ecology Letters* **7**(4): 279-284.

- Grossenbacher DL, Stanton ML. 2014.** Pollinator-mediated competition influences selection for flower-color displacement in sympatric monkeyflowers. *American Journal of Botany* **101**(11): 1915-1924.
- Gumbert A, Kunze J, Chittka L. 1999.** Floral colour diversity in plant communities, bee colour space and a null model. *Proceedings of the Royal Society B: Biological Sciences* **266**(1429): 1711-1716.
- Hegland SJ, Grytnes JA, Totland Ø. 2009.** The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research* **24**(4): 929-936.
- Hegland SJ, Totland Ø. 2012.** Interactions for pollinator visitation and their consequences for reproduction in a plant community. *Acta Oecologica* **43**: 95-103.
- Heindl M, Winkler H. 2003.** Interacting effects of ambient light and plumage color patterns in displaying Wire-tailed Manakins (Aves, Pipridae). *Behavioral Ecology and Sociobiology* **53**(3): 153-162.
- Hervey EW. 1899.** *Observations on the colours of flowers*. New Bedford: E. Anthony & Sons, Incorp. Printers.
- Hill GE. 1990.** Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Animal Behaviour* **40**: 563-572.
- Hopkins R, Rausher MD. 2012.** Pollinator-Mediated Selection on Flower Color Allele Drives Reinforcement. *science* **335**(6072): 1090-1092.
- Howard SR, Shrestha M, Schramme J, Garcia JE, Avarguès-Weber A, Greentree AD, Dyer AG. 2019.** Honeybees prefer novel insect-pollinated flower shapes over bird-pollinated flower shapes. *Current zoology* **65**(4): 457-465.
- Iwasa Y, Pomiankowski A. 1994.** The Evolution of Mate Preferences for Multiple Sexual Ornaments. *Evolution* **48**(3): 853-867.
- Jaakola L, Hohtola A. 2010.** Effect of latitude on flavonoid biosynthesis in plants. *Plant, Cell & Environment* **33**(8): 1239-1247.
- Jewell J, McKee J, Richards AJ. 1994.** The keel colour polymorphism in *Lotus corniculatus* L.: differences in internal flower temperatures. *New Phytologist* **128**(2): 363-368.
- Kantsa A, Raguso RA, Dyer AG, Sgardelis SP, Olesen JM, Petanidou T. 2017.** Community-wide integration of floral colour and scent in a Mediterranean scrubland. *Nature Ecology & Evolution* **1**(10): 1502-1510.
- Kemp DJ, Herberstein ME, Fleishman LJ, Endler JA, Bennett AT, Dyer AG, Hart NS, Marshall J, Whiting MJ. 2015.** An integrative framework for the appraisal of coloration in nature. *The American Naturalist* **185**(6): 705-724.

Kiester AR, Lande R, Schemske DW. 1984. Models of Coevolution and Speciation in Plants and Their Pollinators. *The American Naturalist* **124**(2): 220-243.

King C, Ballantyne G, Willmer P. 2013. Why flower visitation is a poor proxy for pollination: measuring single-visit pollen deposition, with implications for pollination networks and conservation. *Methods in Ecology and Evolution* **4**(9): 811-818.

Koski MH, Ashman T-L. 2015. Floral pigmentation patterns provide an example of Gloger's rule in plants. *Nature Plants* **1**(14007): 14007.

Laffan SW, Lubarsky E, Rosauer DF. 2010. Biodiverse, a tool for the spatial analysis of biological and related diversity. *Ecography* **33**(4): 643-647.

Larkindale J, Mishkind M, Vierling E 2005. Plant responses to high temperature. In: Jenks MA, Hasegawa PM eds. *Plant Abiotic Stress*. Oxford: Blackwell Publishing, 144.

Lehrer M, Horridge GA, Zhang SW, Gadagkar R. 1995. Shape Vision in Bees: Innate Preference for Flower-Like Patterns. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **347**(1320): 123-137.

Leonard AS, Papaj DR. 2011. 'X' marks the spot: The possible benefits of nectar guides to bees and plants. *Functional Ecology* **25**(6): 1293-1301.

Levin DA, Kerster HW. 1967. Natural Selection for Reproductive Isolation in *Phlox*. *Evolution* **21**(4): 679-687.

Lunau K. 2004. Adaptive radiation and coevolution — pollination biology case studies. *Organisms Diversity & Evolution* **4**(3): 207-224.

Macedonia JM. 2001. Habitat light, colour variation, and ultraviolet reflectance in the Grand Cayman anole, *Anolis conspersus*. *Biological Journal of the Linnean Society* **73**(3): 299-320.

Maia R, White TE. 2018. Comparing colors using visual models. *Behavioral Ecology* **29**(3): 649-659.

Marchetti K. 1993. Dark habitats and bright birds illustrate the role of the environment in species divergence. *Nature* **362**: 149-152.

Martin PR, Montgomerie R, Lougheed SC. 2010. Rapid sympatry explains greater color pattern divergence in high latitude birds. *Evolution* **64**(2): 336-347.

McCall A, Murphy S, Venner C, Brown M. 2013. Florivores prefer white versus pink petal color morphs in wild radish, *Raphanus sativus*. *Oecologia* **172**(1): 189-195.

McEwen JR, Vamosi JC. 2010. Floral colour versus phylogeny in structuring subalpine flowering communities. *Proceedings of the Royal Society B: Biological Sciences* **277**(1696): 2957-2965.

- McKee J, Richards AJ. 2008.** Effect of flower structure and flower colour on intrafloral warming and pollen germination and pollen-tube growth in winter flowering *Crocus* L. (Iridaceae). *Botanical Journal of the Linnean Society* **128**(4): 369-384.
- McKenzie RL, Björn LO, Bais A, Ilyasd M. 2003.** Changes in biologically active ultraviolet radiation reaching the Earth's surface. *Photochemical & Photobiological Sciences* **2**: 5-15.
- McNaught MK, Owens IPF. 2002.** Interspecific variation in plumage colour among birds: species recognition or light environment? *Journal of Evolutionary Biology* **15**: 505-514.
- Melendez-Ackerman E, Campbell DR. 1998.** Adaptive Significance of Flower Color and Inter-Trait Correlations in an *Ipomopsis* Hybrid Zone. *Evolution* **52**(5): 1293-1303.
- Menzel R, Shmida A. 1993.** The ecology of flower colours and the natural colour vision of insect pollinators: the Israeli flora as a study case. *Biological Reviews of the Cambridge Philosophical Society* **68**: 81-120.
- Moles AT, Perkins SE, Laffan SW, Flores-Moreno H, Awasthy M, Tindall ML, Sack L, Pitman A, Kattge J, Aarssen LW, et al. 2014.** Which is a better predictor of plant traits: temperature or precipitation? *Journal of Vegetation Science* **25**(5): 1167-1180.
- Moles AT, Warton DI, Warman L, Swenson NG, Laffan SW, Zanne AE, Pitman A, Hemmings Fa, Leishman MR. 2009.** Global patterns in plant height. *Journal of Ecology* **97**(5): 923-932.
- Mori M, Yoshida K, Ishigaki Y, Matsunaga T, Nikaido O, Kameda K, Kondo T. 2005.** UV-B protective effect of a polyacylated anthocyanin, HBA, in flower petals of the blue morning glory, *Ipomoea tricolor* cv. Heavenly Blue. *Bioorganic & Medicinal Chemistry* **13**(6): 2015-2020.
- Ollerton J. 1996.** Reconciling Ecological Processes with Phylogenetic Patterns: The Apparent Paradox of Plant-Pollinator Systems. *Journal of Ecology* **84**(5): 767-769.
- Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter Cl, Rotenberry J. 2009.** A global test of the pollination syndrome hypothesis. *Annals of botany* **103**(9): 1471-1480.
- Ollerton J, Winfree R, Tarrant S. 2011.** How many flowering plants are pollinated by animals? *Oikos* **120**(3): 321-326.
- Ortiz PL, Berjano R, Talavera M, Rodríguez-Zayas L, Arista M. 2015.** Flower colour polymorphism in *Lysimachia arvensis*: How is the red morph maintained in Mediterranean environments? *Perspectives in Plant Ecology, Evolution and Systematics* **17**(2): 142-150.

Papiorek S, Junker RR, Alves-dos-Santos I, Melo GA, Amaral-Neto LP, Sazima M, Wolowski M, Freitas L,

Lunau K. 2016. Bees, birds and yellow flowers: pollinator-dependent convergent evolution of UV patterns. *Plant Biology* **18**(1): 46-55.

Penny JHJ. 1983. Nectar guide colour contrast: a possible relationship with pollination strategy. *New Phytologist* **95**(4): 707-721.

Popic TJ, Wardle GM, Davila YC. 2013. Flower-visitor networks only partially predict the function of pollen transport by bees. *Austral Ecology* **38**: 76-86.

Proctor M, Yeo P, Lack A. 1996. *The natural history of pollination*. Portland, OR: Timber Press.

R Core Team 2014. R: A language and environment for statistical computing. Vienna, Austria, ISBN 3-900051-07-0, <http://www.R-project.org/> R Foundation for Statistical Computing.

Rausher Mark D. 2008. Evolutionary Transitions in Floral Color. *International Journal of Plant Sciences* **169**(1): 7-21.

Reich PB. 2014. The world-wide ‘fast–slow’ plant economics spectrum: a traits manifesto. *102*(2): 275-301.

Renoult JP, Valido A, Jordano P, Schaefer HM. 2014. Adaptation of flower and fruit colours to multiple, distinct mutualists. *New Phytologist* **201**(2): 678-686.

Schemske DW, Bierzychudek P. 2001. Perspective: Evolution of Flower Color in the Desert Annual *Linanthus parryae*: Wright Revisited. *Evolution* **55**(7): 1269-1282.

Shrestha M, Burd M, Garcia J, Dorin A, Dyer A. 2019. Colour evolution within orchids depends on whether the pollinator is a bee or a fly. *Plant Biology* **21**(4): 745-752.

Shrestha M, Dyer AG, Boyd-Gerny S, Wong BBM, Burd M. 2013a. Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision. *The New Phytologist* **198**(1): 301-310.

Shrestha M, Dyer AG, Burd M. 2013b. Evaluating the spectral discrimination capabilities of different pollinators and their effect on the evolution of flower colors. *Communicative & integrative biology* **6**(3): 301-310.

Shrestha M, Garcia JE, Bukovac Z, Dorin A, Dyer AG. 2018. Pollination in a new climate: assessing the potential influence of flower temperature variation on insect pollinator behaviour. *PLoS one* **13**(8): e0200549.

Stebbins GL 1974. Adaptations for Cross-Pollination. *Flowering Plants: evolution above the species level*. Cambridge, MA: The Belknap Press of Harvard University Press.

- Strauss SY, Whittall JB 2006.** Non-pollinator agents of selection on floral traits. In: Harder LD, Barrett SCH eds. *Ecology and evolution of flowers*. New York: Oxford University Press, 120-138.
- Tattini M, Galardi C, Pinelli P, Massai R, Remorini D, Agati G. 2004.** Differential accumulation of flavonoids and hydroxycinnamates in leaves of *Ligustrum vulgare* under excess light and drought stress. *New Phytologist* **163**(3): 547-561.
- Taylor AO. 1965.** Some Effects of Photoperiod on the Biosynthesis of Phenylpropane Derivatives in Xanthium. *Plant Physiology* **40**(2): 273-280.
- van der Kooi CJ, Dyer AG, Kevan PG, Lunau K. 2019a.** Functional significance of the optical properties of flowers for visual signalling. *Annals of botany* **123**(2): 263-276.
- van der Kooi CJ, Elzenga JTM, Staal M, Stavenga DG. 2016.** How to colour a flower: on the optical principles of flower coloration. *Proceedings of the Royal Society B: Biological Sciences* **283**(1830): 20160429.
- van der Kooi CJ, Kevan PG, Koski MH. 2019b.** The thermal ecology of flowers. *Annals of botany* **124**(3): 343-353.
- Wang Y, Meng L-L, Yang Y-P, Duan Y-W. 2010.** Change in floral orientation in *Anisodus luridus* (Solanaceae) protects pollen grains and facilitates development of fertilized ovules. *American Journal of Botany* **97**(10): 1618-1624.
- Wang Y, Naumann U, Wright ST, Warton DI. 2012.** mvabund— an R package for model-based analysis of multivariate abundance data. *Methods in Ecology and Evolution* **3**(3): 471-474.
- Warren J, Mackenzie S. 2001.** Why Are All Colour Combinations Not Equally Represented as Flower-Colour Polymorphisms? *New Phytologist* **151**(1): 237-241.
- Waser N, Price M. 1985.** The effect of nectar guides on pollinator preference: experimental studies with a montane herb. *Oecologia* **67**(1): 121-126.
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996.** Generalisation in pollination systems and why it matters. *Ecology* **77**(4): 1043-1060.
- Waser NM, Price MV. 1981.** Pollinator Choice and Stabilizing Selection for Flower Color in *Delphinium nelsonii*. *Evolution* **35**(2): 376-390.
- Williams G, Adam P. 2010.** *The flowering of Australia's rainforests: a plant and pollination miscellany*. Collingwood, VIC: CSIRO Publishing.
- Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. 2014.** Permutation inference for the general linear model. *NeuroImage* **92**: 381-397.

- Accepted Article**
- Wright IJ, Cannon K.** 2001. Relationships between Leaf Lifespan and Structural Defences in a Low-Nutrient, Sclerophyll Flora. *Functional Ecology* **15**(3): 351-359.
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets Ü, Oleksyn J, et al.** 2005. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* **14**(5): 411-421.
- Zahavi A, Zahavi A.** 1997. *The Handicap Principle: a Missing Piece of Darwin's Puzzle*. New York: Oxford University Press.

Supporting information:

Notes S1 List of study species.

Notes S2 Additional methods.

Notes S3 Additional results.

Notes S4 Details of ISI web of knowledge search.

Tables

Table 1: Ten directional predictions for how biotic and abiotic environmental variables would correlate with flower colour traits were derived from the literature for testing on a broad taxonomic and geographic scale.

	Category	Prediction
1	Biotic	The diversity of bird species will positively correlate with the average number of different spectra on a species' flowers and the disparity between the hues displayed.
2	Biotic	The diversity of insects in a community is expected to positive correlate to the average contrast between the colours displayed on a species' flowers
3	Biotic	Plant species richness is expected to positively correlate with the average chromatic contrast of flower colours and the number of flower colours displayed on a species
4	Abiotic	There will be a positive correlation between solar radiation and chromatic contrast of flower colour
5	Abiotic	Temperature is expected to positively correlate with the chromatic contrast of flower colours
6	Abiotic	Precipitation is expected to be negatively correlated with chromatic contrast of flower colours
7	Abiotic	NPP and growing season length are expected to positively correlate species who display a greater number of different spectra and display higher chromatic contrast in their flower colours.

8	Abiotic	Cloud cover is expected to be positively correlated with average hue disparity
9	Abiotic	Plant canopy height is expected to negatively correlate with average maximum contrast between flower colours
10	Abiotic	leaf area index is expected to negatively correlate with number of flower colours, and to positively correlate with chromatic contrast and total reflectance

Table 2: linear regressions between flower colour traits and variables which reflect the abiotic and biotic environment.

	Number of different spectra	Chromatic contrast	Average hue disparity	Maximum contrast	Total reflectance
Abiotic environment					
Solar radiation	P = 0.008 r = -0.118 n = 540	P = 0.003 r = 0.130 n = 540	P ≤ 0.001 r = 0.237 n = 520	P = 0.755 r = -0.014 n = 520	P = 0.068 r = -0.077 n = 540
Net primary productivity	P = 0.52 r = -0.028 n = 543	P ≤ 0.001 r = -0.279 n = 543	P ≤ 0.001 r = -0.232 n = 522	P ≤ 0.001 r = -0.259 n = 522	P ≤ 0.001 r = 0.200 n = 543
Growing season length	P = 0.322 r = -0.045 n = 558	P ≤ 0.001 r = -0.138 n = 558	P = 0.016 r = -0.110 n = 537	P = 0.021 r = -0.100 n = 537	P ≤ 0.001 r = 0.182 n = 558
Mean annual rainfall	P = 0.534 r = -0.026 n = 558	P ≤ 0.001 r = -0.283 n = 558	P = 0.008 r = -0.122 n = 537	P ≤ 0.001 r = -0.326 n = 537	P = 0.004 r = 0.134 n = 558
Mean annual temperature	P = 0.004 r = -0.122 n = 558	P = 0.145 r = 0.063 n = 558	P ≤ 0.001 r = 0.214 n = 537	P = 0.079 r = -0.077 n = 537	P = 0.111 r = -0.071 n = 558
Plant height	P = 0.225 r = 0.045 n = 534	P ≤ 0.001 r = -0.285 n = 534	P ≤ 0.001 r = -0.303 n = 513	P ≤ 0.001 r = -0.202 n = 513	P ≤ 0.001 r = 0.141 n = 534
Leaf Area Index	P = 0.195 r = 0.055 n = 544	P ≤ 0.001 r = -0.257 n = 544	P ≤ 0.001 r = -0.255 n = 534	P ≤ 0.001 r = -0.210 n = 534	P = 0.004 r = 0.130 n = 544
Cloud cover	P = 0.425 r = 0.055 n = 206	P = 0.003 r = -0.245 n = 206	P ≤ 0.001 r = -0.302 n = 203	P = 0.047 r = -0.138 n = 203	P = 0.873 r = -0.010 n = 206

Biotic environment					
Bird community diversity	P = 0.055 r = -0.077 n = 558	P = 0.22 r = 0.055 n = 558	P ≤ 0.001 r = -0.158 n = 537	P = 0.054 r = -0.084 n = 537	P = 0.75 r = 0.010 n = 558
Plant community diversity	P = 0.808 r = 0.010 n = 558	P ≤ 0.001 r = -0.189 n = 558	P ≤ 0.001 r = -0.198 n = 537	P ≤ 0.001 r = -0.279 n = 537	P = 0.475 r = 0.030 n = 558
Pollinating insect community diversity	P = 0.862 r = -0.008 n=528	P ≤ 0.001 r = -0.159 n = 528	P = 0.433 r = -0.033 n = 510	P = 0.088 r = -0.079 n = 510	P = 0.77 r = -0.015 n = 528

While the data confirmed the hypothesis that abiotically stressful conditions would be associated with higher flower colour chromaticity, many of other the predictions regarding how the light environment and the biotic community would be correlated with flower colour were rejected. P values, *r* and number of observations (n) are provided for all tests. *r* is the Pearson's correlation coefficients (squared to give the R²), the direction of which indicates the slope of the relationship.

Table 3: Selected models for flower colour traits. None of the selected models for flower colour traits were significantly improved by the addition of a term for latitude.

Flower colour trait	Explanatory variables	R²	AICc	R² after adding a term for latitude	AICc after adding a term for latitude	P value (<0.05 if latitude term improves model)
Chromatic contrast	Plant height + plant community diversity + mean annual rainfall + net primary production	0.103	-3069.109	0.105	-3068.232	0.283
Average hue disparity	Plant height + plant community diversity + cloud cover + leaf area index	0.206	-206.628	0.207	-204.746	0.607
Number of different spectra	Temperature + bird community diversity + solar radiation + cloud cover	0.040	-240.021	0.044	-238.639	0.377
Maximum colour span	Mean annual rainfall + plant community diversity + net primary production + leaf area index	0.120	-337.733	0.120	-335.986	0.577
Total reflectance	Net primary production + plant community diversity + growing season length	0.074	8930.149	0.078	8929.397	0.082

	+ plant height					
--	----------------	--	--	--	--	--

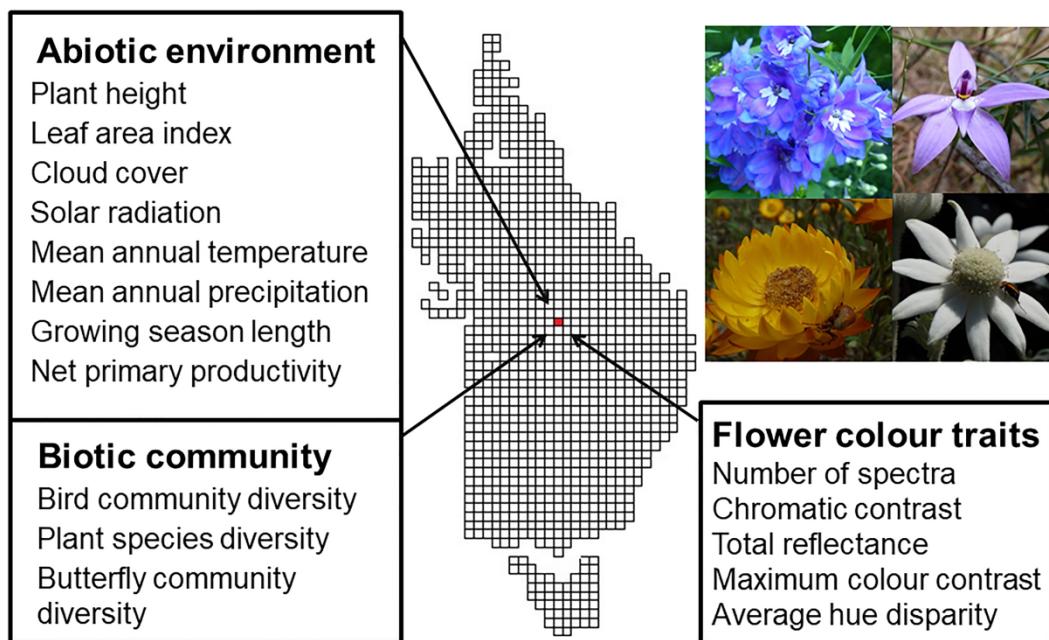
Variance explained by the selected models (which incorporated the four explanatory variables listed) was compared to the variance explained by models which incorporated latitude using ANOVA model comparison. P values of ANOVA analyses are provided, in which values ≤ 0.05 indicate that models were significantly different. AICc values for model with and without a term for latitude are also provided; lower AICc values indicate a better fit for the data, and are indicated in bold. P values are for ANOVA comparison of models with and without a term for latitude, with a P value < 0.05 indicating that latitude significantly improved the model. Models without a term for latitude were always more parsimonious than those that include latitude. In the explanatory variables column NPP = net primary production and LAI = leaf area index.

Figure legends

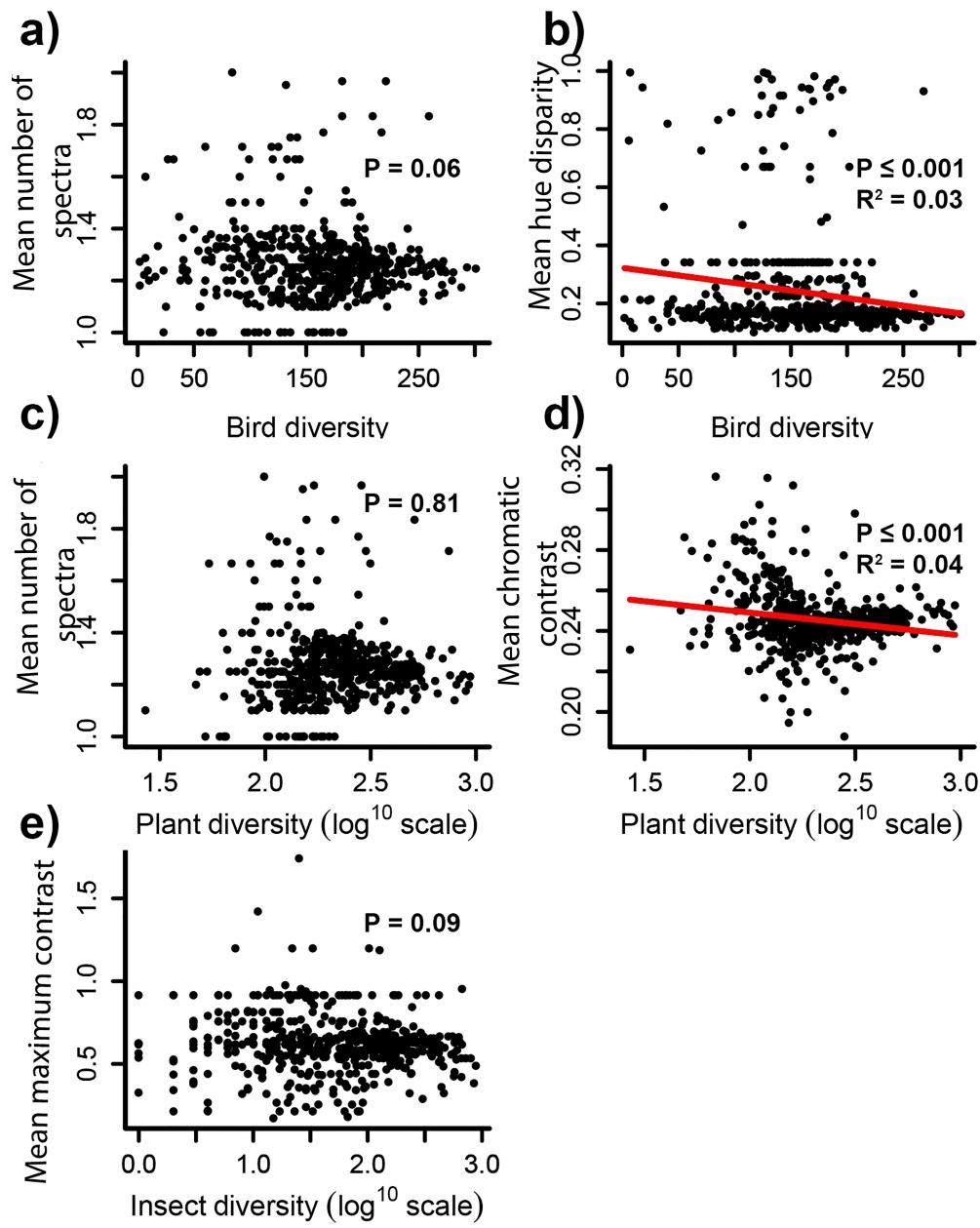
Figure 1: For each 0.5° latitude grid-cell we determined the community diversity of birds, butterflies and plants, and the mean value of eight environmental variables. These form our biotic and abiotic explanatory variables in analyses. Using reflectance spectra, we have calculated five colour traits for 339 angiosperm species' flowers, and then, using spatial range data, we have calculated the mean of the trait values of resident species in each spatial grid cell. These form our response variables in analyses.

Figure 2: None of the specific predictions about how the diversity of birds, plants or pollinating insects would relate to the average flower trait values of the community were upheld on a broad scale. Relationships between the number of different spectra and bird community species richness (a) and plant community species richness (c), and between the maximum colour contrast and the species richness of the insect community (e), are all non-significant. Significant relationships between bird community species richness and average hue disparity (b) and chromatic contrast and plant community species richness (d) are denoted with regression lines, however, both of these relationships are negative in direction which is opposite to that predicted.

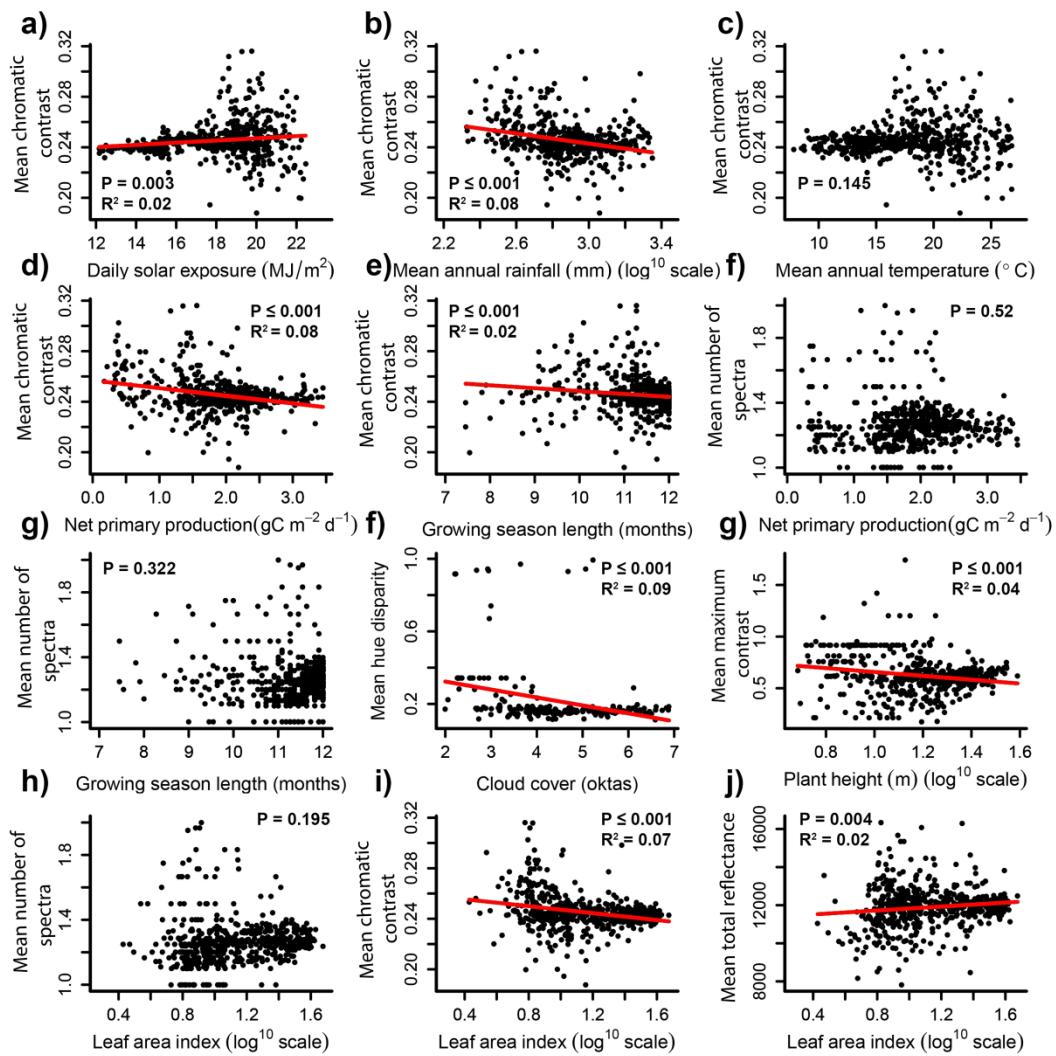
Figure 3: Only a few of the predictions about how flower colour traits and the abiotic conditions of the environment would correlate were upheld by our data. Significant relationships are denoted with regression lines. Flower colours are more chromatically contrasting (displaying higher chromaticity) in conditions of higher solar radiation (a), lower mean annual rainfall (b), lower net primary production (d), and shorter growing season length (e). Flowers species display colours with greater disparity between their hues in less cloudy conditions (f). Flowers also have brighter, more chromatic and more contrasting colours in tall, shady habitats (g, i and j). There are no significant relationships between chromatic contrast and temperature (c), nor between mean number of spectra and net primary production (NPP) (f), growing season length (g) or leaf area index (h).



nph_16737_f1.tif



nph_16737_f2.tif



nph_16737_f3.tif