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Research

Shades of red: bird-pollinated flowers target the specific colour discrimination abilities of avian vision

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Summary

- Colour signals are a major cue in putative pollination syndromes. There is evidence that the reflectance spectra of many flowers target the distinctive visual discrimination abilities of hymenopteran insects, but far less is known about bird-pollinated flowers. Birds are hypothesized to exert different selective pressures on floral colour compared with hymenopterans because of differences in their visual systems.
- We measured the floral reflectance spectra of 206 Australian angiosperm species whose floral visitors are known from direct observation rather than inferred from floral characteristics. We quantified the match between these spectra and the hue discrimination abilities of hymenopteran and avian vision, and analysed these metrics in a phylogenetically informed comparison of flowers in different pollination groups.
- We show that bird-visited flowers and insect-visited flowers differ significantly from each other in the chromatic cues they provide, and that the differences are concentrated near wavelengths of optimal colour discrimination by whichever class of pollinator visits the flowers.
- Our results indicate that angiosperms have evolved the spectral signals most likely to reinforce their pollinators' floral constancy (the tendency of individual pollinators to visit flowers of the same species) in communities of similarly coloured floral competitors.

Introduction

Animal pollinators play an essential role in the reproduction of many flowering plants. Although pollinator behaviour is influenced by a variety of floral traits, including morphology, nectar availability, size and odour (Sutherland & Vickery, 1993; Kunze & Gumbert, 2001; Schiestl & Schüter, 2009), colour plays a major role in the attraction and decision making of pollinators (Schemske & Bradshaw, 1999; Streisfeld & Kohn, 2007; Morawetz & Spaethe, 2012). In particular, colour is a central element in putative pollination syndromes that relate floral traits to the perceptions and preferences of different classes of pollinators (Fenster *et al.*, 2004).

The colour component of pollination syndromes has usually been presented in very general terms. For example, to a human observer, bee-pollinated flowers are often violet or blue while bird-pollinated flowers are often orange or red (Raven, 1972; Sutherland & Vickery, 1993). More recent analyses have been based on the reflectance spectra of flowers, and have found a correspondence between the colour discrimination abilities of hymenopteran vision and the floral reflectance characteristics in multispecies samples from Israel (Chittka & Menzel, 1992) and Australia (Dyer *et al.*, 2012). These studies did not, however, classify species by their floral visitors, and so did not explicitly address colour differences between insect-pollinated and bird-pollinated flowers; they also lacked phylogenetically informed statistical analyses. Here we analyse a larger sample of the Australian flora in an explicit phylogenetic framework to compare floral reflectance features among flowers visited by insects or birds, or both, and relate these differences to the specific colour discrimination abilities of hymenopteran and avian visual systems.

Pollination by insects, particularly hymenoptera, is very common, with about two-thirds of angiosperms species relying on insect visitation for their persistence (Tepedino, 1979). However, evolutionary shifts from insect to bird pollination are frequent and phylogenetically widespread among angiosperms (Kay *et al.*, 2005; Cronk & Ojeda, 2008; Rausher, 2008; Smith *et al.*, 2008; Thomson & Wilson, 2008) and are often accompanied by large shifts in floral colour signals owing to alterations of anthocyanin synthetic pathways (Rausher, 2008; Thomson & Wilson, 2008; Des Marais & Rausher, 2010). Reflection of light in the (human) orange and red part of the spectrum by bird-pollinated flowers has usually been interpreted as a means to reduce their conspicuousness to bees (Raven, 1972; Rodríguez-Gironés & Santamaría, 2004; Lunau *et al.*, 2011). Indeed, colour discrimination by bees at wavelengths > 530 nm

is very poor, although they can distinguish achromatic intensity (brightness) differences at longer wavelengths (Chittka & Waser, 1997). If inconspicuousness to bees was the only selective factor acting on floral colour of bird-pollinated flowers, any chromatic signal in the long-wavelength region of visible radiation might suffice. However, there are reasons to expect that pollinator-driven selection will lead to the evolution of more specific floral colour traits among bird-pollinated flowers.

Coexisting angiosperm species that share a guild of pollinators may often be in competition for pollinator services (Rathcke, 1983). For example, greater regional diversity of angiosperms is associated with higher levels of pollen limitation of seed set (Vamosi *et al.*, 2006), a pattern consistent with competition over pollinator attraction. In a competitive environment, traits that promote floral constancy – the tendency of pollinators to visit flowers of the same species – will be favoured, because heterospecific pollen transfers waste pollen, stigmatic space and floral rewards (Waser, 1986; Chittka *et al.*, 1999). Floral colour signals that are readily discriminated from the signals of competing species promote floral constancy (Chittka *et al.*, 1999; Dyer *et al.*, 2012). These considerations suggest that if the ability of pollinators to distinguish colours was uniform across their visible spectrum, floral spectral signals might be relatively uniformly distributed to maximize differences among potential competitors. However, the chromatic acuity of a visual system is not uniform, as colour information is interpreted by neural processes from the differential stimulation of photoreceptors with different spectral sensitivities (Dyer *et al.*, 2011). The wavelength separation and breadth of these sensitivities produce asymmetries in colour discrimination. Thus, we may expect certain flower colours to be more successful because they are more readily distinguished from their background and from heterospecific flowers that share the same pollinators (Gumbert *et al.*, 1999).

Maximal colour discrimination occurs at wavelengths where the sensitivities of two photoreceptor types overlap and change rapidly in opposite directions (Peitsch *et al.*, 1992; Kelber *et al.*, 2003; Dyer *et al.*, 2011). Both behavioural data and electrophysiological recording and modelling suggest that the trichromatic vision of hymenopteran insects, based on UV-, blue- and green-sensitive receptors, allows the finest colour discriminations at wavelengths near 400 nm and 500 nm (Helversen, 1972; Peitsch *et al.*, 1992; Chittka, 1996a). Avian visual systems are typically tetrachromatic and fall into two broad groups: violet sensitive (VS) and ultraviolet sensitive (UVS; Hart & Hunt, 2007; Ödeen & Håstad, 2010). Violet-sensitive vision is phylogenetically ancestral, while UVS vision appears to be associated principally with the radiation of the Passerida, although it has arisen independently in other bird lineages (Ödeen & Håsted, 2003, 2010). Maximum hue discrimination in the pigeon, *Columba livia*, a species with VS vision, occurs near 460, 540, and 600 nm (Emmerton & Delius, 1980). Although the pigeon is not a pollen vector, its visual capabilities are considered a model for VS vision (Hart & Hunt, 2007; Dyer *et al.*, 2012). The hummingbird *Archilochus alexandri*, a VS species that is an important pollinator, shows a similar pattern of discrimination ability at medium and long wavelengths, although it can discriminate wavelengths near 420 nm much better than the pigeon (Goldsmith *et al.*, 1981). Physiological measurement and modelling of vision in the budgerigar, *Melopsittacus undulates*, which has a UVS visual system, suggest that its hue discrimination optima occur near 416, 489 and 557 nm (Goldsmith & Butler, 2003). The paucity of quality behavioural data on avian hue discrimination and the potential for interspecific variation within a visual system are currently impediments to a better understanding of the role of avian pollinators in floral colour evolution. Our aim here, however, is a broad comparison between insect- and bird-pollinated flowers. We use the colour discrimination abilities of the pigeon and the budgerigar as standards for VS and UVS vision, although we recognize that avian pollinators as a group may have a greater complexity of vision among individual species (Hart & Hunt, 2007).

We compared the colour information provided by a large sample of flowers of Australian plants visited by various pollinators. Australia's native hymenopteran fauna includes important pollinators, especially stingless bees, and hymenopteran vision likely has had an important influence on floral colour evolution among Australian angiosperms (Dyer *et al.*, 2012). Birds are also important pollinators in Australia, especially for some of the most species-rich families such as Myrtaceae and Proteaceae (Ford *et al.*, 1979; Barker *et al.*, 2007). If competition among bird-pollinated flowers favours the evolution of readily discriminated colour signals, the signature of pollinator-driven adaptation should include not only floral colour differences between insect- and bird-pollinated flowers, but also fine tuning of colour signals within a syndrome to those wavelengths of radiation that the preferred floral visitor can most easily distinguish. We tested this hypothesis using quantitative metrics of the match between floral colour cues and the visual capacities of hymenopteran insects and of birds.

Materials and Methods

Sampling sites and species

We sampled 206 native Australian angiosperm species from 119 genera in 40 families (APG III, 2009) occurring in two natural communities and in a botanical garden. The two communities were at Boomers Reserve (37°37′39″ S, 145°15′21″ E) and Baluk Willam Flora Reserve (37°55′32″ S, 145°20′45″ E) to the north and east, respectively, of Melbourne in central Victoria, Australia. These sites are each *c.* 75 ha of *Eucalyptus* woodland with well-developed shrub and herb layers. We set up 43 permanent 0.01 ha circular quadrats at Boomers Reserve and 46 quadrats at Baluk Willam Flora Reserve. All quadrats were visited fortnightly from March 2010 to August 2011 and all herbaceous species in flower were identified and sampled. The third site was the Maranoa Gardens in the Melbourne urban area (37°48′37″S, 145°05′24″E), which maintains a collection of species representative of plant communities that occur around Australia. The Gardens were visited once per month from May 2009 to January 2010 and again in June 2012. We randomly selected herbaceous and woody species that were in flower. Sampled species were identified with the aid of several

local floras (Ross, 2000; Jeans & Backhouse, 2006; Richardson *et al.*, 2006; Corrick & Fuhrer, 2008).

Each species was assigned to one of three pollination categories on the basis of published information on floral visitors or occasionally on our own field observations: flowers visited only by insects (147 spp), by both insects and birds (22 spp) or only by birds (38 sp). A list of species and their APG III families, the floral visitation category to which each species belongs and references to literature supporting the category assignments are presented in the Supporting Information, Table S1.

Colour measurement

A colour photograph of a representative flower of each species was taken as an identification record. We also photographed flowers along with a calibrated UV grey scale using a digital UV camera (Fuji Finepix Pro S3 (Fujifilm Corp., Tokyo, Japan) UV-IR modified CCD for UV imaging and fitted with a 105 mm f4.5 quartz UV-Nikkor lens and optically polished Baadar U-filter with 325–369 nm half bandwidth). This allowed us to identify any areas of UV reflectance so that they could be included in the spectrophotometric measurement. Reflectance spectra for wavelengths from 300 to 700 nm were measured on at least two flowers of each species using an Ocean Optics spectrophotometer (Dunedin, FL, USA) with a PX-2 pulsed xenon light source. A UV-reflecting white standard (freshly pressed pellet of dry $BaSO_4$) was used to calibrate the spectrophotometer. Spectra from multiple flowers were averaged within each species. For flowers with multiple colours (including colours with a UV component), we obtained reflectance spectra from the predominant colour based on area. For species with colour polymorphisms, we selected the most common morph in our sites, which generally meant omitting white floral morphs. All spectra from our study will be made available through the Floral Reflectance Database (www.reflectance.co.uk).

Similar floral colours can best be distinguished by a visual system when their reflectance changes rapidly near wavelengths where photoreceptor sensitivities overlap. We identified rapid changes in colour signal by locating inflection points in the reflectance profile of a flower. To locate inflections, we first smoothed a spectrum using a Gaussian kernel method with a bandwidth of 20 nm. (This procedure eliminated spurious inflections associated with small-scale noise in the reflectance measurements.) We then numerically calculated the first and second derivatives of the smoothed spectra at 1 nm intervals using the differentiation functions of MATHCAD 8 (Mathsoft, Inc., Cambridge, MA, USA). By definition, inflections occur at wavelengths where the second derivative equals zero and the corresponding first derivative is at a local maximum or minimum. We imposed the further requirement that inflections must be associated with a total change in reflectance of 20% or more, to correspond to a requirement used by Chittka & Menzel (1992) and Dyer *et al.* (2012). Reflectance spectra and inflection points of a representative bee-pollinated flower and a bird-pollinated flower are shown in Fig. 1. A complete list of species and their inflection points is presented in Table S2.

Our analysis of spectral signals involves comparing the wavelengths at which inflection points occur with experimentally determined hue-discrimination functions for different animal pollinators. This procedure enables direct comparisons between pollinator visual capabilities and colour signals that are independent of the assumptions required for specific colour perception models (Chittka & Menzel, 1992; Dyer *et al.*, 2012). This direct comparison addresses the most fundamental level of visual processing because, while manipulation of photoreceptor signals can occur through different neural processing (Dyer *et al.*, 2011a,b) and colour learning abilities (Reser *et al.*, 2012) in different taxa, the spectral breadth and position of the photoreceptors will most directly influence the characteristics of a visual system (Goldsmith, 1990). In addition, previous work has established that experimentally determined spectral discrimination functions are an appropriate measure for interpreting animal discrimination of broadband reflection spectra as occur in natural flower colours (Chittka & Waser, 1997). This is especially important for comparing hymenoptera with trichromatic vision and birds with tetrachromatic visual systems, where no direct colorimetric comparison is possible because of underlying physiological differences in both receptor and post-receptor processing. The value of such direct comparisons of animal visual capabilities is that the underlying principles of colour vision based on wavelength discrimination (Helversen, 1972; Emmerton & Delius, 1980) hold true for a number of alternative colour models, even if the colour models have very different underlying assumptions about how photoreceptor signals are processed and how colour opponent mechanisms weight such signals (Goldsmith, 1990; Chittka, 1996b; Vorobyev & Brandt, 1997).

Thus, the wavelength discrimination methodology that we use reveals the most important components of a flower colour signal that a vision system could use to discriminate between two perceptually similar flower colours. By making available the reflectance spectra from our study on the Floral Reflectance Database, it would be possible in the future to test how specific colour models (Endler, 1990; Goldsmith, 1990; Vorobyev & Brandt, 1997) may enhance colour discrimination for our sample species. However, a comparative evaluation of different colour models that currently exist (Endler, 1990; Goldsmith, 1990; Vorobyev & Brandt, 1997), and continue to be developed to understand animal colour vision, is beyond the scope of the present study.

Data analysis

The dependent variables in our analyses were metrics of the match between a flower's inflection points and the hue-discrimination optima of the hymenopteran, avian VS and avian UVS visual systems. The first metric was the mean absolute deviation (MAD) of inflection points from the wavelengths of visual optima. To calculate the MAD, each inflection was compared with its closest visual optimum. Thus, smaller values of MAD imply a closer fit between inflections and a particular visual system. For example, *Wahlenbergia gloriosa* (Fig. 1), an insect-visited species, has inflections at 413, 504 and 653 nm. Relative to the hymenopteran optima at 400 nm

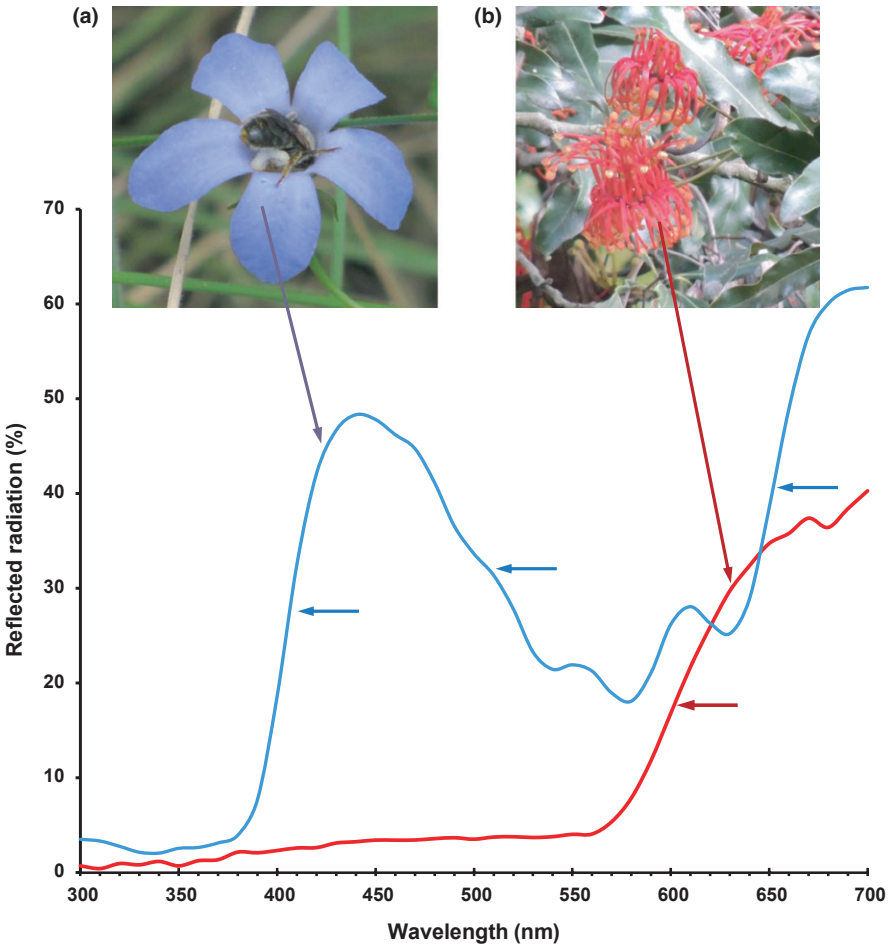

Fig. 1 Reflectance spectra and inflection points (indicated by arrows) of a representative insect-pollinated flower (*Wahlenbergia gloriosa*: Campanulaceae) (a) and bird-pollinated flower (*Stenocarpus sinuatus*: Proteaceae) (b).

and 500 nm, the mean average deviation for *W. gloriosa* is $MAD_{Hym} = ({}^{|}413 - 400^{|} + {}^{|}504 - 500^{|} + {}^{|}653 - 500^{|})/3 = 56.7$. Relative to the optima at 460, 540 and 600 nm of avian VS vision, $MAD_{VS} = 45.3$ and relative to the avian UVS optima at 416, 489, and 557 nm, $MAD_{UVS} = 38$.

The MAD quantifies the match between an ensemble of inflection points and a visual system, but it may be that one inflection holds more significance than others for a pollinator's colour discrimination. Therefore, we defined a second metric of fit between flower colour and visual system: the minimum absolute deviation (minAD) of any single inflection in a reflectance spectrum from a specific discrimination optimum (that is, from 400 nm and 500 nm for hymenopteran vision, from 460, 540 and 600 nm for avian VS vision, and from 416, 489 and 557 nm for avian UVS vision. Thus, *W. gloriosa* has the following minAD values: $minAD_{400} = 13$, $minAD_{500} = 4$, $minAD_{460} = 44$, $minAD_{540} = 36$, $minAD_{600} = 53$, $minAD_{416} = 3$, $minAD_{489} = 15$ and $minAD_{557} = 53$.

The MAD and minAD metrics for all species are given in Table S2. Comparing these metrics among plants in the three floral visitation categories is an analysis of variance problem. Owing to phylogenetic structure within our multispecies sample, we used the phylogenetic ANOVA technique of Garland *et al.* (1993) as implemented in the R package 'phytools' (Revell, 2012). Phylogenetic ANOVA uses a conventional *F*-ratio of within-group to among-group variances calculated for the empirical data, but then compares this ratio with a null distribution of *F*-ratios derived from simulated evolution of the characters along the branches of the phylogenetic tree, without regard to the group membership of the species at the tips (Tables S5, S6). The simulation in phytools assumes a Brownian motion model of trait evolution. *Post-hoc* tests for pairwise group comparisons were also conducted in phytools based on distributions of pairwise *t*-statistics derived from the simulations, with a correction for multiple tests. We used 10 000 simulations for each test and sequential Bonferroni correction for the *post-hoc* comparisons. Additional detail on the analyses is available in the Methods S1.

To construct a phylogenetic tree for our species, we obtained a backbone tree for the families and genera in our sample using phylomatic (www.phylodiversity.net/phylomatic), which uses an APG III (2009) family-level topology in its reference megatree (except we resolved Dilleniaceae as sister to (Caryophyllales + Asterids) following Soltis *et al.*, 2011). We grafted subfamilial and infrageneric topology for the species in our sample on to this backbone based on information available in current systematics literature (Table S3). Where the available literature lacked sufficient resolution, we left unresolved polytomies. We dated internal nodes of the tree using the maximum likelihood ages of

Wikström *et al.* (2001), supplemented with estimated dates from family- or genus-specific analyses (Table S4). In total, 31 of 36 nodes basal to family-level crown nodes were dated, along with 29 of the remaining distal nodes. Undated internal nodes were evenly spaced between the nearest dated basal and distal nodes. Branch lengths in terminal clades were set according to Pagel's (1992) method and the clade scaled to the most recent dated node from which it descended. The resulting tree is shown in Fig. S1, and is available as a Nexus file from the Dryad database (www.datadryad.org).

Results

Although flowers in all three pollination classes had inflection points throughout the 300–700 nm range of wavelength, the inflections were aggregated differently among groups (Fig. 2). Insect-visited flowers showed pronounced clustering of inflections near 400 nm and 500 nm. About one-quarter of all inflections in this group occurred in the range 380–420 nm, and anther quarter occurred in the range 480–520 nm (Fig. 2a). Flowers visited exclusively by birds had fewer short-wavelength inflections and lacked a strong peak near 400 nm: only *c.* 10% of inflections occurred between 380 nm and 420 nm. However, they had a cluster of inflection points surrounding 600 nm representing about one-quarter of all inflections in that group (Fig. 2c). Flowers that receive both insect and bird visitors showed a strong spike of inflections near 500 nm containing 17% of all inflections in the group (Fig. 2b).

The MAD values show that colour cues of insect- and of bird-pollinated flowers match the hue discrimination abilities of their own floral visitors better than that of nonvisitors (Table 1). MAD_{Hym} was significantly smaller for insect-pollinated flowers than for flowers visited exclusively by birds, which had a MAD_{Hym} value 50% larger ($P = 0.0062$, Table 1). By contrast, bird-pollinated flowers had the lowest value of MAD_{VS} while insect-pollinated flowers had a value 40% higher – a significant difference ($P = 0.0032$, Table 1). Flowers with both types of visitors had intermediate values of both MAD_{Hym} and MAD_{VS} that did not differ significantly from the other two groups (Table 1). MAD_{UVS} did not differ significantly among pollination groups ($P = 0.38$, Table 1).

The minAD metrics select a single inflection for each species that is closest to a specified hue discrimination optimum of a given pollinator type. These metrics were widely spread within all three pollination groups: that is, each group contained some species with inflections near a given optimum and some with inflections far from a given optimum (Fig. 3). Nonetheless, there are significant differences among the pollination groups for several of the minAD metrics (Table 2).

The mean values of the $minAD_{400}$ and $minAD_{600}$ metrics had the strongest contrast between insect-pollinated and bird-pollinated flowers, with approximately a twofold difference in each case (Table 2). The floral group with the best fit (lowest mean value of the metric) matched a hue-discrimination optimum of their own pollinator (insect-pollinated for $minAD_{400}$ and bird-pollinated for $minAD_{600}$). *Post-hoc* pairwise comparisons showed

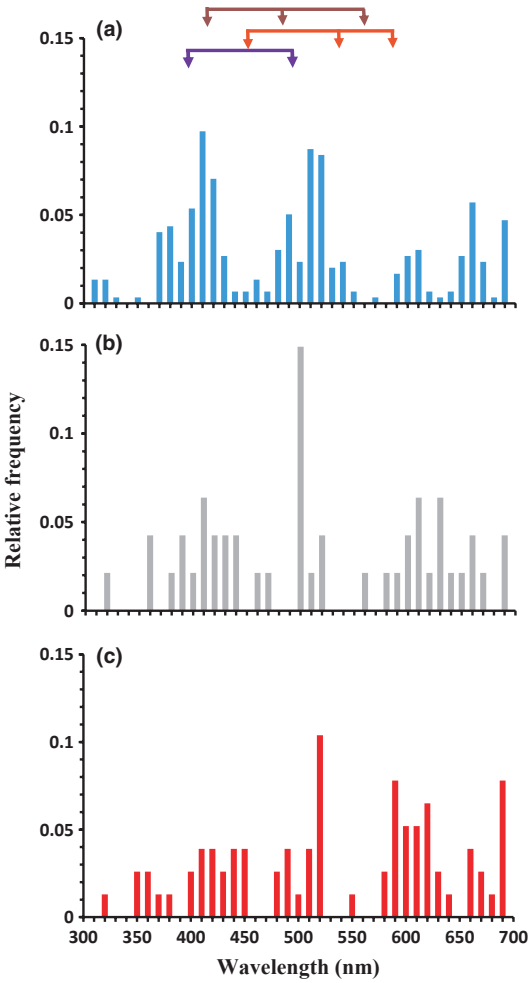

Fig. 2 Relative frequency distribution of inflection points of Australian flowers in three pollination categories. (a) Insect-pollinated flowers, $n = 148$ species; (b) insect- and-bird-pollinated flowers, $n = 22$ species; (c) bird-pollinated flowers, $n = 38$ species. Bars with arrows at the top of the figure indicate the hue-discrimination optima of the three visual systems under consideration: violet, hymenopteran optima at 400 nm and 500 nm; red, avian violet sensitive (VS) optima at 460, 540, and 600 nm; dark red, avian ultraviolet sensitive (UVS) optima at 416, 489, and 557 nm.

that insect-pollinated and bird-pollinated flowers differed significantly from each other in each case, while flowers visited by both types of pollinators had intermediate values of minAD that did not differ significantly from either the insect-only or bird-only pollination groups (Table 2).

Significant differences between insect- and bird-pollinated flowers also occurred for $minAD_{416}$ and $minAD_{557}$ (Table 2). These differences were less pronounced than for $minAD_{400}$ and $minAD_{600}$, and are more difficult to interpret. Insect-pollinated flowers had the lowest mean $minAD_{416}$ value, even though 416 nm is an optimum of avian UVS vision, probably because this wavelength is also close to the hymenopteran optimum at 400 nm. Similarly, no floral group had an abundance of inflections near 557 nm and the low mean value of $minAD_{557}$ for bird-pollinated flowers (Table 2) likely results from the abundance of inflections near 600 nm among the flowers in this group (Fig. 2).

Table 1 Average values of mean absolute deviation (MAD) metrics for plant species within each floral-visitor group, and results of phylogenetic ANOVA testing differences among group means

	Visitor group				
Metric	Insects	Insects + Birds	Birds	*F*	Phylogenetic *P*
MAD_{Hym}	47.6^{a}	63.8^{ab}	72.1^{b}	6.24	0.0062
MAD_{VS}	45.2^{a}	39.2^{ab}	32.7^{b}	6.69	0.0032
MAD_{UVS}	35.8	39.8	43.0	1.24	0.3779

Smaller MAD values indicate closer average fit of floral inflection points to the hue-discrimination optima of hymenopteran, avian violet sensitive (VS), or avian ultraviolet sensitive (UVS) vision (see the Materials and Methods section for further explanation). Phylogenetic *P* values were determined from null distributions of *F* derived from 1000 simulations of Brownian motion evolution of the corresponding metric. Groups sharing a common superscript letter on the group means were not significantly different from each other at the 0.05 level in *post-hoc* tests.

We can gain some further insight from distributions of minAD (Fig. 3). Insect-pollinated flowers had a large proportion of $minAD_{400}$ values < 40 (i.e. inflection points within 40 nm of the hymenopteran discrimination optimum at 400 nm) and few species whose nearest inflection is no closer than 180 nm (i.e. at 580 nm or greater). By contrast, about one-third of flowers with bird visitors and one-quarter of those with bird and insect visitors had $minAD_{400}$ values > 180 (Fig. 3a). The pattern for $minAD_{416}$ is similar to that of $minAD_{400}$ (Fig. 3b). Thus, distinguishing between floral chromatic cues aimed at bees or at birds with UVS vision will be challenging given the proximity of their discrimination optima at short wavelengths. The match between bird-pollinated flowers and the avian VS optimum at 600 nm is more definitive. Nearly two-thirds of flowers in this group have an inflection point within 40 nm of 600 nm ($minAD_{600} \leq 40$), while this is true of < 20% of insect-pollinated flowers in our sample (Fig. 3d). All three pollination groups have somewhat similar distributions of $minAD_{557}$ (Fig. 3c), although the mean values of $minAD_{557}$ still differed significantly among the groups (Table 2).

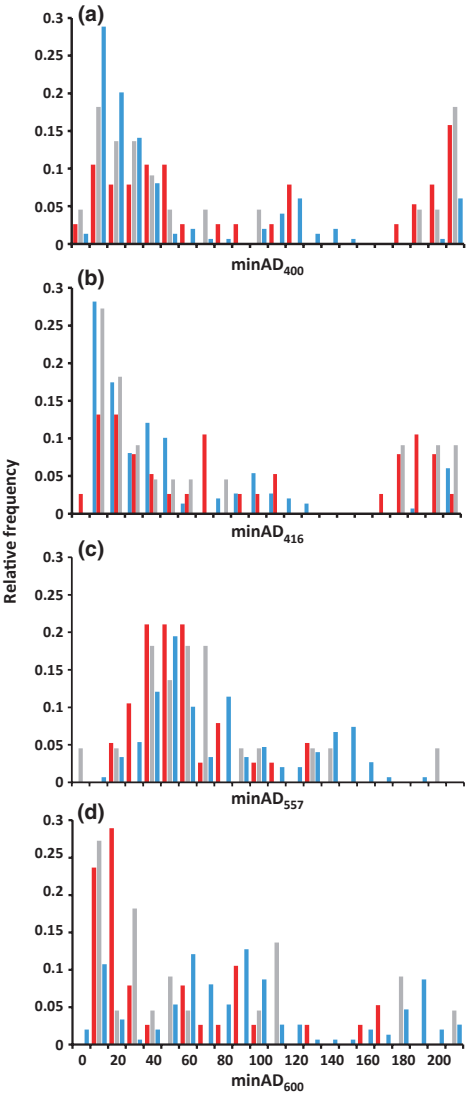

Fig. 3 Relative frequency distribution of minAD (minimum absolute deviation) values of Australian flowers in three pollination categories (bird visitors, red; bird and insect visitors, grey; insect visitors, blue). For each species, minAD measures the minimum absolute deviation (in nm) of an inflection points from a hue-discrimination optimum at (a) 400 nm (hymenopteran vision), (b) 416 nm (avian ultraviolet sensitive (UVS) vision), (c) 557 nm (avian UVS vision), and (d) 600 nm (avian violet sensitive (VS) vision).

Discussion

Floral colour signals differed significantly between bird- and insect-pollinated flowers in a sample of the Australian flora, and the flowers within each pollination category had spectral reflectance characteristics that that are clustered at wavelengths that would maximize their distinctiveness to the visual systems of their respective pollinators. In particular, insect-pollinated flowers frequently have inflections in their reflectance spectra near 400 nm or 500 nm, a result that is in agreement with previous studies (Chittka & Menzel, 1992; Dyer *et al.*, 2012). These inflections would make the flowers optimally distinguishable by the highly conserved colour processing in hymenopteran trichormats (von Helversen, 1972; Chittka, 1996a). By contrast, bird-pollinated flowers frequently have an inflection near 600 nm, which is close to a hue discrimination optimum for birds with the VS visual system. These patterns do have exceptions. In particular, a substantial portion of bird-pollinated flowers in our sample had inflections at short wavelengths, near the hymenopteran discrimination optimum at 400 nm (Fig. 3a) but also near the avian UVS optimum near 416 nm (Fig. 3b). Given that the budgerigar, with VS vision, may also have good colour discrimination near 420 nm (Goldsmith *et al.*, 1981), floral reflectance spectra with short wavelength inflections may be readily discriminated by birds. Thus, our results suggest that bird-pollinated species may use both long- and short-wavelength cues to attract and inform their floral visitors (Fig. 2c).

Surprisingly, many inflections from insect-pollinated flowers occur at long wavelengths of 600 nm or greater (Fig. 2a). Plant tissues in general, floral or otherwise, consistently have high reflectance at infrared wavelengths (Chittka *et al.*, 1994), often

Table 2 Average values of minimum absolute deviation (minAD) metrics within each floral-visitor group and results of phylogenetic ANOVA testing differences among group means

	Visitor group				
	Insects	Insects + Birds	Birds	*F*	Phylogenetic *P*
Hymenopteran vision					
$minAD_{400}$	49.7[a]	76.9[ab]	94.2[b]	6.19	0.0059
$minAD_{500}$	43.8	55.3	54.7	1.28	0.3749
Avian VS vision					
$minAD_{460}$	54.0	70.0	73.7	3.30	0.0669
$minAD_{540}$	62.8	63.0	51.9	1.13	0.4184
$minAD_{600}$	88.1[a]	60.4[ab]	43.6[b]	9.87	0.0003
Avian UVS vision					
$minAD_{416}$	48.1[a]	70.9[ab]	85.0[b]	5.05	0.0167
$minAD_{489}$	44.6	64.4	60.4	2.32	0.1518
$minAD_{557}$	74.5[a]	64.4[ab]	51.8[b]	5.04	0.0120

minAD values indicate the proximity of any one inflection point in a species' floral reflectance profile to specific wavelengths of best hue-discrimination optima in the hymenopteran, avian violet sensitive (VS) or avian ultraviolet sensitive (UVS) vision (see the Materials and Methods section for further explanation). Phylogenetic *P* values were determined from null distributions of *F* derived from 1000 simulations of Brownian motion evolution of the corresponding metric. Groups sharing a common superscript letter on the group means were not significantly different from each other at the 0.05 level in *post-hoc* tests.

with a strong rise in the far red spectrum (680–690 nm; Buschmann & Nagel, 1991). Thus, the long-wavelength inflections that occurred in insect-pollinated flowers could be an artefact of the general reflectance properties of plant tissue (Chittka & Menzel, 1992; Dyer *et al.*, 2012). The much greater proportion of inflections at wavelengths from 580 nm to 620 nm in flowers with bird visitors (Fig. 2b,c) implies that there is a functional basis to the reflectance traits at these wavelengths in these species. By contrast, the long-wavelength inflections in insect-pollinated flowers are less clustered than in bird-pollinated flowers.

There was a paucity of inflections at middle wavelengths among bird-pollinated flowers, despite the high colour acuity at these wavelengths in both the VS and UVS systems. This pattern may result from the effect of background vegetation. Leaves and vegetation generally have a peak in reflectance at *c.* 530–550 nm (Gates *et al.*, 1965; Chittka *et al.*, 1994; Asner, 1998). Vegetation may therefore present an overwhelming distraction that interferes with effective signalling by flowers to pollinators in this region of the spectrum, as flowers needs to be distinguishable from the background to be detected (Spaethe *et al.*, 2001; Dyer *et al.*, 2008). Chittka & Menzel (1992) proposed that for colour signals to stand out from the background and from synchronously blooming competitors, flowers must use combinations of pigments that generate sharp steps in their reflectance spectra, preferably at the boundaries between receptors. While the evidence from the current study suggests that many flowers have evolved colour signals at spectral positions of maximal pollinator colour discrimination (Fig. 2), which would enhance their capacity to identify flowers, our data do not support the hypothesis that these signals evolved to maximize detection against a foliage background. If signal detection against a background with peak reflectance *c.* 530–550 nm were the major factor driving flower signal evolution, then the inflections in our data set should have been maximally distant from this region of the spectrum. However, bird-pollinated flowers (Fig. 2c) have inflection points clustered near 600 nm and 500 nm but relatively fewer *c.* 450 nm and 650 nm. Our data thus suggest that the need for flowers to be reliably identified by pollinators is the major driver of flower colour evolution. This conclusion is also supported by behavioural evidence that there is an achromatic visual pathway in both bees (Giurfa *et al.*, 1996) and birds (Lind & Kelber, 2011.) for detecting fine detail and stimuli at a small visual angle, while chromatic vision is mainly tuned for stimuli viewed at a relatively large visual angle. Thus pollinator colour vision appears to be mainly used when a pollinator approaches a flower that had been detected at a greater distance via the achromatic channel.

The potential role of floral colour in shifts from insect to bird pollination has previously been demonstrated experimentally in two sister species of *Mimulus*, bee-pollinated *M. lewisii* and hummingbird-pollinated *M. cardinalis*. In a natural environment planted with F_2 hybrids segregating for floral colour quantitative trait loci, anthocyanin and carotenoid concentrations in petals dramatically affected the probability of visitation by either bees or hummingbirds (Schemske & Bradshaw, 1999). A similar result was obtained with nearly isogenic lines of the two species containing the heterospecific allele of a single gene affecting carotenoid synthesis (Bradshaw & Schemske, 2003). *Mimulus lewisii* flowers appear purple to human observers and are normally visited almost exclusively by bees, but in the experimental lineage expressing the *M. cardinalis* gene, the flower appeared yellow-orange and experienced very large increases in hummingbird visitation rates. Similarly, *M. cardinalis* flowers expressing the *M. lewisii* gene appeared dark pink and experienced bee visitation rates many times greater than wild-type *M. cardinalis* flowers (Bradshaw & Schemske, 2003). Selection acting on floral colour genes may arise following geographic dispersal or ecological changes that alter the relative proportion of floral visits by birds and bees, turning a formerly mutualistic relationship with bees into a parasitic one in which less efficient pollen transfer by self-grooming bees reduces the availability of pollen to the more efficient and newly abundant bird visitors (Thomson & Wilson, 2008).

The suggestion that floral colours are adaptations to the visual systems of their pollinators is often made, but supporting evidence usually involves pronounced colour differences between species with different pollinator guilds (Schemske & Bradshaw, 1999; Bradshaw & Schemske, 2003; Rausher, 2008; Thomson & Wilson, 2008). Our results imply that species that undergo bee-to-bird transitions in pollination evolve not merely 'non-bee' floral colours, but colours that emphasize a narrow region of the electromagnetic spectrum providing the best opportunity to be distinguished by birds from competing bird-pollinated flowers (Fig. 2). We suggest that when shifts to bird pollination occur, an initial phase of selection, possibly acting on genes of large effect that shift floral reflectance to longer wavelengths (Schemske & Bradshaw, 1999), will be followed by a secondary phase of selection in which signals to birds become more precise.

The VS visual system is phylogenetically ancestral in birds, while the UVS system is a more recent shift found principally in the Passerida (Hart & Hunt, 2007; Ödeen & Håstad, 2010). The Australian honeyeaters (Meliphagidae) are a lineage of early diverging oscines that have VS vision (Ödeen & Håstad, 2010). They are the most important family of pollinating birds in Australia. For example, over 80% of > 3000 bird visits to flowers observed by Paton & Ford (1977) in the Mount Lofty Ranges and Murray Valley of South Australia were made by honeyeaters, and 23 of the 38 bird-pollinated species in our sample are known to be visited by honeyeaters. The coincident Eocene radiation of meliphagids (Barker *et al.*, 2004) and of bird-pollinated Embothriinae (Proteaceae) in Australia (Barker *et al.*, 2007) also points to the important role these birds have played in angiosperm evolution. The match between the reflectance characteristics of bird-pollinated flowers in our sample and the VS vision of meliphagids is consistent with strong selection for floral colour driven by these birds.

New World hummingbirds (Trochilidae) have a VS visual system, as do the Australian honeyeaters (Ödeen & Håstad, 2010). If our interpretation of chromatic evolution in Australian flowers is correct, then we would expect New World plant species adapted to hummingbird pollination to show a similar aggregation of reflectance inflections near 600 nm. Sunbirds (Nectariniidae) are a large and important family of pollinating birds in Africa and Asia but they have the UVS visual system (Ödeen & Håstad, 2010). We predict that the bird-pollinated flowers of these continents will be characterized by a shift in the wavelengths of inflection points toward the UVS optimum of 557 nm, although colour cues at middle wavelengths near this optimum may not be sufficiently distinct against a foliage background (as discussed above) to allow frequent evolution of reflectance inflection points near the 557 nm discrimination optimum.

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Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Phylogenetic tree of the sample species.

Table S1 Species used in the analysis and their floral visitor group

Table S2 (a) Reflectance profile inflection points and MAD (mean absolute deviation) values of species in the sample; (b) minAD (minimum absolute deviation) values of species in the sample

Table S3 Studies used to provide subfamilial topology for certain well-represented taxa in our data set

Table S4 Ages of nodes indicated in the phylogenetic tree in Fig. S1

Table S5 Maximum-likelihood estimates of Pagel's (1999)λ, and significance of tests against null hypotheses of $\lambda = 0$ and $\lambda = 1$

Table S6 Results of phylogenetic ANOVA testing differences among pollination groups after transformation of the phylogenetic tree

Methods S1 Detail about maximum-likelihood estimation and phylogenetic ANOVA test.

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