Colour polymorphic lures target different visual channels in prey

Thomas E. White[1,2], Darrell J. Kemp[1]

[1]Department of Biological Science,

Macquarie University, North Ryde, 2109, Australia

[2] Corresponding author.

Phone: +61 2 9850 6279

E-mail: thomas.white@mq.edu.au

Keywords: deception, communication, sensory trap, signal, spider

Word count (excluding abstract): 4997

Number of figures: 4

Number of tables: 0

Data archival location: Figshare (doi: 10.6084/m9.figshare.1517656)

Supplementary information: supplementary table, methods, results

Abstract

Selection for signal efficacy in variable environments may favour colour polymorphism, but little is known about this possibility outside of sexual systems. Here we used the colour polymorphic orb-web spider *Gasteracantha fornicata*, whose yellow- or white-banded dorsal signal attracts dipteran prey, to test the hypothesis that morphs may be tuned to optimize either chromatic or achromatic conspicuousness in their visually noisy forest environments. We used data from extensive observations of naturally occurring spiders and precise assessments of visual environments to model signal conspicuousness according to dipteran vision. Modelling supported a distinct bias in the chromatic (yellow morph) or achromatic (white morph) contrast presented by spiders at the times when they caught prey, as opposed to all other times at which they may be viewed. Hence, yellow spiders were most successful when their signal produced maximum colour contrast against viewing backgrounds, whereas white spiders were most successful when they presented relatively greatest luminance contrast. Further modelling across a hypothetical range of lure variation confirmed that yellow-versus-white signals should respectively enhance chromatic-versus-achromatic conspicuousness to flies, in *G. fornicata*'s visual environments. These findings suggest that colour polymorphism may be adaptively maintained by selection for conspicuousness within different visual channels in receivers.

Introduction

The study of colour polymorphism has revealed much about the evolutionary processes that maintain variation, and the role of such variation in predator-prey dynamics, sexual selection, and speciation (Roulin 2004; Bond 2007; Wellenreuther et al. 2014; White & Kemp 2016). Less is known

about the drivers of visual signal polymorphism outside the contexts of sexual signalling and crypsis (Bond 2007; Wellenreuther et al. 2014). A possibility that transcends these particular contexts is that variation in light environments and/or receiver sensory biology may favour polymorphism by offering a greater spread of signalling 'niches' (Lythgoe 1979). This hypothesis, formalized in the theory of sensory drive (Endler 1992), is supported by a growing body of evidence (albeit largely from sexual-signalling systems) that shows selection for efficacy drives signal diversity (Fuller 2002; Gomez and Théry 2004; Stuart-Fox et al. 2007; Chunco et al. 2007; Rojas et al. 2014).

The way in which selection shapes the design of signals will vary, depending on the functional context in which they are used. This difference is especially pronounced between the contexts of intraspecific communication, as in sexual systems, and deceptive signaling, such as aggressive mimicry or prey luring (e.g. Hauber 2002; O'Hanlon et al. 2014; Barry et al. 2015). In sexual systems the interests of signalers and receivers are broadly, albeit inexactly, aligned. Receivers (i.e. potential mates or rivals) are thus expected to respond to signals differentially, which often drives selection for the transmission of information about individual compatibility (such as species or sex identification) and/or 'quality' (e.g. Ryan et al. 2005; Barry et al. 2015; Umbers et al. 2013) on the part of signalers. Deceptive signaling systems, in contrast, are antagonistic. The most effective signals are those that are readily received and misclassified by receivers (e.g. as a potential food source, or simply an object of possible interest), thereby eliciting a maladaptive response (Searcy and Nowicki 2005). Receivers are strongly selected to avoid such signals, and so the information encoded in deceptive signals is typically restricted to the 'identity' of the signaler (which may be highly non-specific; White and Kemp 2015). Since the potential confounds of complex information exchange are minimized in deceptive systems (though not absent; see discussion), the evolutionary costs of signaling are weighted towards efficacy (signal transmission), rather than strategy (signal content). Deceptive systems are therefore particularly well suited for testing efficacy-focused hypotheses for the maintenance of polymorphism (Lythgoe 1979; Endler 1992). To that end, sit-and-wait predators such as orb-web spiders often attract prey with conspicuous and polymorphic visual

lures (e.g. Muma 1971; Fan et al. 2009; Rao et al. 2015), but almost nothing is known about the adaptive maintenance of polymorphism in this context.

A basic task for most visual systems is to extract colour and luminance (i.e., subjective 'brightness') information from the environment. Such information is typically separated at the earliest stages of neural processing (Strausfeld and Lee 1991; Chittka 1996; Osorio et al. 1999; Anderson and Laughlin 2000; Paulk et al. 2008; Borst 2009); that is, apportioned into chromatic (colour) versus achromatic (luminance) channels (though this separation may not be absolute; Wardill et al. 2012). Tightly controlled laboratory-based behavioural and psychophysical studies have isolated how the information offered by each channel can be prioritised to guide different visual tasks (Giurfa et al. 1997; Osorio and Vorobyev 2005; Kelber 2005; Zhou et al. 2012). However, natural environments vary greatly in the overall level and spectral quality of illumination depending on weather and/or vegetative structure (Endler 1993b), as well as visual backgrounds that encompass a diversity of viewing perspectives. This implies potential for signal designs that achieve conspicuousness via maximising contrast in either colour or luminance channels (e.g. Cummings 2007). The potential is highest in heterogeneous environments such as forests, where visual conditions can change greatly across fine spatial and temporal scales (Endler 1993b). In such environments, one largely unexplored possibility is that selection for signal efficacy may favour polymorphic signalling solutions wherein different morphs are maintained because they maximize colour or luminance contrast, respectively.

Gasteracantha fornicata is a diurnal orb-web spider found in tropical and sub-tropical forests that uses conspicuous banded colouration as a lure for attracting primarily dipteran prey (Muma 1971; Hauber 2002). Subsequent work has determined that the colour scheme of this species is stably polymorphic, exhibiting human-perceived 'white' and 'yellow' bands against a black outline (Kemp et al. 2013). The coloured bands of both morphs reflect strongly and equivalently across wavelengths longer than 530 nm, and absorb strongly below 400 nm, yet differ by ca. 50 nm in their transition from low to high reflectance (i.e. their 'hue', Fig. 1a; and Kemp et al. 2013). Because orb-spiders are

sit-and-wait predators, the environments in which their signals are viewed and their intended receivers (i.e. prey) are uniquely well defined, and individual fitness may be readily estimated via prey capture. This group is thus well suited to empirical tests of hypotheses about the demands of signal efficacy — which encompasses signal design, viewing conditions, and viewer perception (Lythgoe 1979; Endler 1993a) — and the evolution of polymorphism.

Here we set out to test the hypothesis that colour polymorphism in visually noisy environments may be maintained by the use of two distinct strategies for achieving visual conspicuousness; colour and luminance. Specifically, we predicted that the white-banded morphs may benefit by stimulating their prey's achromatic channel, given that reflectance of light across a broader spectral range will generate relatively stronger positive achromatic contrast against darker visual backgrounds (as well as maximizing luminance contrast between the dark and light bands of the spider itself; Fig. 1a). Conversely, we predicted that the yellow morph would maximize the stimulation of their prey's chromatic channel, given that many flies possess a well-documented preference for yellow stimuli (as per the comprehensive recent review of Lunau 2014).

Methods

We tested the hypothesis that yellow and white spider morphs target chromatic and achromatic visual pathways in prey, respectively, using two complementary approaches. First, we made extensive focal observations on wild *G. fornicata* of both morphs, recording and identifying captures, and precisely characterizing their visual environments at times of prey capture and at frequent intervals across the entire day. We then coupled data on illumination and backgrounds with known parameters of dipteran vision to model the envelopes of chromatic and achromatic contrast presented by spiders. This is analogous to estimating a fly-specific 'visual signal niche' for our focal spider sample, as defined by variation in their natural choice of microhabitat as well as changes in

visual properties at these microhabitats throughout the day and due to weather conditions. We aimed to test whether the visual contrast of spiders at times when they actually caught prey was non-randomly distributed within this envelope; that is, whether and how the modelled visual contrast of each morph related to a key measure of fitness; capture success. Specifically, our hypotheses predict that yellow morphs should experience greater capture success at the times when they are presenting relatively greater chromatic contrast (i.e. a particularly 'colourful' signal, from the perspective of their dipteran prey; Fig. 2a), whereas white morphs should be most successful at the times when they present relatively greater achromatic contrast (i.e. a particularly 'luminant' signal; Fig. 2b).

Second, we used our microhabitat light data and a dipteran visual model to explore the consequence of colour variation extending beyond that displayed by the extant *G. fornicata* morphs. We limited colour variation in this exercise such that the sigmoidal spectral shape common to both morphs (Fig. 1a) was maintained, yet varied in its transition point (or 'hue'). This explored a range across human-perceived red, orange, yellow and white (then extending into the ultraviolet); a dimension of spectral tuning readily achieved in animals via the variation of pigment suites (Watt 1969; Grether 2005). We aimed specifically to predict the colour phenotype(s) that would maximise either chromatic or achromatic conspicuousness to dipteran viewers, given the overall set of visual conditions *G. fornicata* were seen to inhabit.

Focal individual and environmental assessments

We haphazardly selected individual spiders for observation in Cairns (September 2013 & March 2014), and Townsville (September 2014), in Queensland, Australia. Colour morphs co-occur at both locations, and are intermixed at fine spatial scales (Kemp et al. 2013). Each spider (n = 65 white, 69 yellow) was observed continuously from 0900 to 1600 hours. All capture events over this period were recorded, with prey items immediately inspected at close range for identification. Prompt inspection is critical because the spiders completely wrap their captures in silk within several

minutes. All prey larger than ca. 5 mm were identified to at least arthropod class (as insects, where possible, to order). Every hour, we collected two measures of viewing environments at the precise location of each individual spider: illuminating irradiance (with the collector held parallel to each spider's dorsum), and background irradiance (with the collector held parallel to each spider's ventrum). Spectra were recorded with a JAZ EL-200 portable spectrometer fitted with a cosine-corrected, spectralon diffused irradiance module (OceanOptics Ltd., Dunedin, USA).

We measured spider reflectance (n = 80 yellow, 44 white spiders, distinct from those above) using a JAZ EL-200 portable spectrometer (boxcar width = 10, integration time = 100 ms, scans to average = 10), with a PX-2 pulsed xenon light source (White et al. 2015). The 500 um light source and collector probes (fit with Ocean Optics 74-UV lenses) were set at 90° and 45° relative to the sample plane, respectively. Reflectance was measured from an approximately 5 mm area on each side of the medial line of the spider's largest dorsal band, and subsequently averaged (as per Kemp et al. 2013). The spectrometer was calibrated against a 99% diffuse "spectralon" reflectance standard (Labsphere, New Hampshire) between each individual. Spectra were captured with OceanOptics SpectraSuite software (ver. 1.6.0_11), and subsequently binned at 1 nm wavelength intervals before minor LOESS smoothing (α = 0.15). All post-capture spectral processing and visual modelling (detailed below, and in the supplementary methods) was done using R (ver. 3.2.0; R Core Team 2014), primarily with the development version of the package 'pavo' (Maia et al. 2013).

Visual modelling; signal bias and extant lure efficacy

We used visual modelling to address the hypothesis that yellow and white spider morphs respectively target colour versus luminance channels in dipteran receivers. Chromatic contrast was estimated using a simple tetrahedral stimulation space, based on the photoreceptor sensitivities of *Drosophila melanogaster* (opsin λ_{max}; R7p = 345 nm, R7y = 375 nm, R8p = 437 nm, R8y = 508 nm; Salcedo et al. 1999), because dipterans in the families Tephritidae and Drosophilidae account for the vast majority of captures by *G. fornicata* (Hauber 2002; Kemp et al. 2013; also see supplementary

table S1). Such a model estimates the colour information available to dipteran viewers at the earliest stage of photoreception (supposing the involvement of all four photoreceptor types in drosophilid colour vision, as supported by physiological evidence; Morante and Desplan 2008) and makes minimal assumptions about subsequent neural processing, such as specific opponency mechanisms or colour categorisation (following Brembs and Ibarra 2006; Kelly and Gaskett 2014; Renoult et al. 2014; see full details in the supplementary methods). An alternate model of dipteran vision proposes that all colours are perceptually grouped into one of four colour categories (based on behavioural data from the blowfly *Lucilia* sp.; Troje 1993), but we do not include this assumption as subsequent behavioural studies have demonstrated the apparent ability of several fly species, including *D. melanogaster*, to discriminate colours that would fall within the same putative category (e.g. Sutherland et al. 1999; Brembs and Ibarra 2006; Yamaguchi et al. 2010). Given the tentative nature of this model however, and recent evidence challenging traditional views of how photoreceptor subtypes contribute to colour and luminance-based tasks in *Drosophila* (Yamaguchi et al. 2008, 2010; Wardill et al. 2012), we repeated the process outlined below with a more general model of animal vision based on a non-specific, modified segment analysis (Dalrymple et al. 2015; Endler 1990; and detailed in Fig. S1). This assumes even less about the visual perception of particular prey groups, and therefore relies more heavily on our empirical data on the signals of naturally occurring spiders, their visual backgrounds, and ambient lighting.

We estimated receptor quantum catches as the integrated product of mean spider reflectance (Fig. 1a), illuminating irradiance, and each given photoreceptor's absorbance, from 300 to 700 nm in 1 nm increments (equation 4 in Endler and Mielke 2005). Quantum catches values were then log transformed in accordance with the Weber-Fechner law (Vorobyev et al. 2001; Endler and Mielke 2005) and converted to points in a tetrahedral space (as per equations 19 - 20 in Endler and Mielke 2005). Chromatic contrast was estimated as the Euclidean distance between a spider and its background. To estimate achromatic contrast, we first calculated luminance as the product of *D. melanogaster*'s R1-6 photoreceptor sensitivity (with primary and secondary peaks at 355 nm and

478 nm; Salcedo et al. 1999) and a given spider or background spectrum. Achromatic contrast was then estimated as the difference between spider and background luminance, divided by the sum of spider and background luminance (Fleishman and Persons 2001; Fleishman et al. 2009; and supplementary methods). Positive values thus indicate that spiders are 'brighter' than their backgrounds, whereas negative values indicate that spiders are relatively darker, to a dipteran viewer (as estimated according to the *Drosophila* visual system). Note that neither chromatic or achromatic contrast values are scaled in relation to discrimination thresholds (just noticeable differences; 'JNDs'), because we are exploring questions about highly conspicuous signals at supra-threshold distances, and there is little empirical support that JNDs pose the appropriate unit for perception in such cases (Kemp et al. 2015).

To test the prediction that yellow morphs should experience greater capture success when presenting a relatively stronger chromatic signal, whereas white morphs should experience greater capture success when presenting a relatively stronger achromatic signal (Fig. 2a, b), we first modelled the appearance of each spider according to its specific combination of background and ambient illumination as measured at every hour. This created a pool of 1072 data points (134 spiders with 8 hourly light measures each per day) that estimated the conspicuousness of spiders to their dipteran prey throughout each diurnal period, and encompasses a reasonable spread of gross seasonal and habitat variation. Every prey capture could be attributed to an individual spider at a specific time of day, so we extracted the subset of points corresponding to fly capture events (n = 77 and 67 captures, for white and yellow morphs, respectively). We restricted our subset to large (>5 mm) captures, because these are thought to constitute the primary fitness-affecting targets of orb-web spiders (Venner and Casas 2005). We then used a randomization procedure to test whether the mean chromatic and achromatic contrast presented by each spider morph at the specific times of prey capture differed from the contrast values expected if captures occurred at random (i.e., to statistically test if the results presented in Fig. 2c, d, conform to the predictions illustrated in Fig. 2a, b). We derived distributions of mean fly-subjective contrast estimates by taking 5000 randomized

sub-samples of the total pool of contrasts, with the size of each sub-sample equal to the observed number of fly captures (n = 77 and 67 for white and yellow spiders, respectively; supplementary table 1). These distributions describe the probability of observing a given mean spider-background contrast value under the null hypothesis that fly capture events occur at random with respect to the spiders' visual appearance (Fig. 3). We then derived p-values by which to test the observed data against the generated distributions by calculating the proportion of contrast values in the null distribution that were equally or more extreme than the observed mean contrast value, and multiplying it by two (for a two-tailed test; Adams and Anthony 1996). As a measure of effect size, we report Cohen's *d* — the distance between the mean of the observed contrast values and the mean of the associated null distribution, in units of pooled standard deviation — for each of the four tests.

We also tested for differences in the 'brightness' and saturation (or 'chromaticity') of ambient light at the times of prey captures, that were independent of any particular visual system. Here, we calculated brightness as the mean intensity (μmol m^{-2} s^{-1} nm^{-1}) across the range of 300 - 700 nm. We estimated saturation by calculating the relative intensity of four 'segments' in a given spectrum (300 - 700 nm, at 100 nm intervals; as per equations 4 and 6 in Endler 1990), converting it to a point in a tetrahedral space (as per equations 19 - 20 in Endler and Mielke 2005), and calculating the Euclidean distance between this point and the tetrahedron's achromatic origin. We used one-way ANOVAs to statistically test the effects of between-morph background brightness, background saturation, illuminating brightness, and illuminating saturation. Intensity data were $\log_e(1+x)$ transformed to normality prior to analysis. Finally, we used chi-square tests to explore between-morph differences in both total versus dipteran capture success.

Visual modelling; hypothetical lure efficacy

We used visual modelling to explore the related question of which lure colours maximise chromatic and achromatic conspicuousness to Diptera in the environments inhabited by *G. fornicata*. We

calculated the mean reflectance of 124 *G. fornicata* (as described above) and used it to generate 398 'hypothetical' spider reflectance curves with distinct hues (defined as the low-to-high inflection point, as per Kemp et al. 2013) spaced at 1 nm intervals (Fig. 1b). Each hypothetical spider was modelled using all of the 1072 illuminant/background spectra that we recorded during observations, using the same visual modelling procedure outlined above. The end product is a function that describes the fly-subjective chromatic or achromatic contrast of all possible lure colours (taking the sigmoid-shaped reflectance of *G. fornicata* as constant), across the precise set of conditions in which spiders could have been potentially viewed (Fig 4a, b).

To assess the performance of *G. fornicata* in the context of both models, each function was first normalised such that the area under the curve equalled one. This was necessary to ensure that each individual *G. fornicata* had equal 'scoring potential' in the context of each model. We then calculated the hue of 124 *G. fornicata* (n = 44 white, 80 yellow, as above), and extracted each spider's corresponding conspicuousness 'score' in both models. Morph differences in within-model scores were assessed with a Wilcoxon two-sample test. We refrained from directly comparing scores between models because not enough is known about how the dipteran perceptual system weighs chromatic versus achromatic information under varied ecological settings (Osorio and Vorobyev 2005).

Data availability

All raw data are available via Figshare (doi: 10.6084/m9.figshare.1517656).

Results

At the times of prey capture, yellow spider morphs presented a disproportionate chromatic (d = 0.422, P = 0.007), but not achromatic (d = 0.018, P = 0.908), contrast against their backgrounds, compared to all other times of the day (Fig. 2c, Fig. 3a, c). That is, yellow spiders presented

significantly greater chromatic contrast with their backgrounds at the time of fly capture events than would be expected if captures occurred at random with respect to a spider's subjective appearance. Conversely, white spider morphs presented a disproportionate achromatic (d = 0.515, P < 0.001), but not chromatic (d = 0.029, P = 0.833), contrast during the subset of occasions when they were seen to capture prey (Fig. 2d, Fig. 3b, d).

These results were qualitatively unchanged, though the effects were less pronounced, when considering a more general model of receiver vision (supplementary Fig. S1). Yellow spiders presented inordinate chromatic (d = 0.384, P = 0.012), but not achromatic (d = 0.102, P = 0.328) background contrast at the times of prey capture. White spiders, conversely, generated disproportionate achromatic (d = 0.374, P = 0.021), but not chromatic (d = 0.121, P = 0.261), contrast.

Subsequent visual modelling considering a range of hypothetical lure phenotypes suggested that the signal of extant yellow morphs is broadly optimised to maximise chromatic contrast under the range of conditions in which spiders are potentially viewed (Fig. 4a, c; Wilcoxon two-sample, W = 3520, P = < 0.001). The signal of white morphs generates greater achromatic contrast across environments relative to yellow morphs (Fig. 4c; Wilcoxon two-sample, W = 0, P = < 0.001). However, unlike the theoretical maximal chromatic contrast presented by yellow morphs, the achromatic contrast of white spiders is not optimal in an 'absolute' sense (i.e., greater achromatic contrast could be achieved under natural conditions if their reflectance extended into the UV; Fig. 4b). Prey composition was indistinguishable between morphs (supplementary table 1), and confirmed that dipterans present the vast majority (> 90%) of captures. Of these, tephritids featured prominently (> 65%), with muscids (ca. 15%) and drosophilids (ca. 10%) comprising most of the remainder. Hymenoptera, particularly native stingless bees *Tetragonula* sp., were the most abundant non-dipteran prey item (ca. 9%), while various Hemiptera, Coleoptera, and Orthoptera were also

infrequently caught. There were no differences between morphs in the rate of prey capture, either in terms of Diptera (χ^2 = 3.384, df = 1, P = 0.404) or all prey pooled (χ^2 = 14.067, df = 7, P = 0.707).

There was a subtle difference in the intensity of background light at the times when each morph caught prey ($F_{1,142}$ = 18.76, P = < 0.001, ω^2 = 0.110), with white spiders presented against brighter backgrounds than yellow spiders at those times (supplementary Fig. S2). There was otherwise no morph difference in the intensity of illuminating light ($F_{1,142}$ = 0.01, P = 0.921), or the saturation of illuminating ($F_{1,142}$ = 2.3, P = 0.132) or background ($F_{1,142}$ = 0.19, P = 0.921) light at times of prey capture.

Discussion

The theory of sensory drive has provided great insight into signal diversification, including polymorphism, in sexual signalling systems (e.g. Fuller 2002; Chunco et al. 2007; Gray et al. 2008). Here we show how sensory drive principles might be profitably applied to deceptive predator-prey signalling. Our data indicate a distinct bias in the fly-subjective chromatic and achromatic appearance of *G. fornicata* morphs at the times of prey capture as compared to all viewing times. Yellow spiders presented unusually high chromatic contrast against their viewing backgrounds when successful, whereas white spiders presented an unusually high achromatic contrast at such times (Fig. 2c, d; Fig. 3). Although these results rely, in part, on a tentative model of drosophilid vision, the correspondence between dipteran (Fig. 3) and non-specific (supplementary Fig. S1) sensory analyses shows that our findings are robust against gross variation in assumed viewer spectral sensitivities and inputs (and, hence, also implies that these visual effects may be similarly generated in a suite of potential viewers, albeit to varying degrees). Further visual modelling across a hypothetical range of stimulus variation was consistent with the notion that extant lure colours accentuate colour and luminance contrast in the environments that *G. fornicata* inhabit (Fig. 4). We found no evidence of

differences in capture success or prey composition between morphs (supplementary table 1). Finally, at times when prey was caught, white morphs often signalled against brighter backgrounds than yellow spiders (supplementary Fig. S2). Although our data are strictly observational in nature, they support specific predictions for how variation in light environments and receiver visual ecology, that is, the contextual use of colour and luminance information (discussed below), may underlie colour-lure polymorphism.

Our findings support the novel hypothesis that a colour polymorphism may be adaptively maintained by selection for conspicuousness within different visual channels. This contrasts with most colour polymorphic systems, in which phenotypic variation is maintained by temporal and/or spatial variation in selection primarily for the chromatic properties of a signal. In sexual systems, for example, selection for chromatic conspicuousness in variable environments is known to drive both intra-specific (Fuller 2002; Gray et al. 2008; Hancox et al. 2013) and inter-specific signal diversity (Leal and Fleishman 2002; Gomez and Théry 2004; Stuart-Fox et al. 2007). A notable exception occurs among sister species of dichromatic surfperch, in which diverse ambient light conditions are thought to have driven selection for either chromatic or achromatic visual biases in females (Cummings 2004, 2007). This variation in signaling environments is reflected in the design of male sexual signals, with males of chromatically biased species expressing signals with greater colour contrast, and males from achromatically biased populations expressing greater luminance contrast (Cummings 2007). The scarcity of polymorphic signals tuned to discrete visual channels in sexual systems may, in part, be a result of constraints introduced by common strategy-based demands. There is likely to be physiological limits, for example, on how information about mate quality may possibly be communicated (McGraw and Hill 2000; Kemp and Rutowski 2007; White et al. 2015). In contexts where strategy-based costs are reduced (i.e. where simple conspicuousness is paramount), such as lures, these constraints may be relaxed or at least shifted, allowing for the use of otherwise elusive signal designs.

These findings are also consistent with the notion that selection for signal efficacy may broadly shape diversity in colour-lures. The prevalence of 'yellow' and 'white' colours in this class of signal (e.g. Heiling et al. 2005; Chiao et al. 2009; Llandres et al. 2011; Kemp et al. 2013), for example, may reflect common solutions to the challenge of maximising conspicuousness in similarly noisy environments and/or to receivers with common visual biases. That lures are often polymorphic (e.g. Muma 1971; Fan et al. 2009; Rao et al. 2015) may also result from selection in environments that vary in common ways. Along these lines, shifts in the attractiveness of yellow-versus-white coloration of spider webs (*Nephila clavipes*; Craig et al. 1996) and bodies (*G. fornicata*; Kemp et al. 2013) have been linked to small-scale changes in ambient lighting driven by local weather conditions. That is, variation in the incidence of cloudy (hence relatively dull and spectrally flat), versus sunny (hence bright and saturated) skies. Our finding for a difference in the brightness of visual backgrounds at the times when each morph actually caught prey is consistent with such a threshold-type scenario, since our measures of signaling environments necessarily capture some variation in gross ambient lighting. The between-morph difference in environments is subtle however (supplementary Fig. S2), which cautions against strong interpretations of its possible significance in the absence of more direct tests. Another possibility is that lure polymorphism may impede learned or innate resistance in prey, which is analogous to how polymorphism in cryptic species is known to defeat the search images of predators (e.g. Bond and Kamil 2002; Spottiswoode and Stevens 2012). A key signature of this mechanism in cryptic species is frequency dependent fitness (Bond 2007). This is not supported by present data in *G. fornicata,* however, as evidenced here by the equivalent overall capture success of morphs across a broad spatial scale (supplementary table S1; though these data are essentially a point sample, and so are unlikely to capture temporal variation in fitness), and also seen in previous dedicated empirical tests of this hypothesis (Kemp et al. 2013).

Extensive laboratory-based work continues to inform the complex physiological basis of colour and luminance processing (Strausfeld and Lee 1991; Anderson and Laughlin 2000; Morante and Desplan

2008; Paulk et al. 2008; Schnaitmann et al. 2013), but understanding how visual information is used and may influence signal evolution in the wild poses a far greater challenge. In semi-natural settings, receivers may switch between prioritising chromatic and achromatic information depending on which is more informative (Kelber 2005; Schaefer et al. 2006), and/or based on past experience (Kelber 2005). This implies predictable consequences for the design of effective visual signals. In fruit-foraging crows, for example, the alternating use of colour and luminance information may explain the occurrence of red accessory signals in fruit displays (e.g. Burns and Dalen 2002), which Schaefer et al. (2006) suggest enhances overall (i.e. combined colour and luminance) conspicuousness. Similarly, our results support the contextual use of colour and luminance information by receivers as generating 'opportunities' for deceptive signalling (Fig. 3). Given enough information about signalling environments and receiver visual ecology, it may prove possible to predict where such opportunities lie in terms of signal design (as we attempt here, e.g. Fig. 4).

In *Gasteracantha fornicata*, it is notable that modelling predicts optimal achromatic contrast should be achieved by a true UV-white morph (Fig. 4b); a phenotype expressed in the closely related *Gasteracantha cancriformis* (Muma 1971; Gawryszewski and Motta 2012). Although it has not been examined in this genus, UV reflectance across diverse spider taxa is known to be a result of incoherent scattering by guanine crystals (reviewed in Oxford and Gillespie 1998). Since guanine is a readily available excretory product (Anderson 1966), the absence of a UV-white signal in *G. fornicata* is unlikely to be the result of physiological limitations. It instead may reflect biotic constraints, such as a possible trade-off between the benefit of increased achromatic conspicuousness (Fig. 4b), and the cost of decreased chromatic conspicuousness (the chromatic-contrast 'valley'; Fig. 4a) under the conditions in which relevant receivers, including predators, view them. Alternately, limitations may be imposed by strategy-based costs which, while often minimised, are never absent in deceptive signaling systems (Searcy and Nowicki 2005; White & Kemp 2015). Some dipterans, for example, have an apparent innate aversion to UV-positive stimuli (Burg et al. 1984; Ishikawa et al. 1985; Judd et al. 1988). This may shape the set of effective signal designs available to *G. fornicata,* for which

flies are the putative 'intended' receiver (supplementary table S1). Other potential prey items, such as honeybees, however, often prefer UV-positive stimuli (Giurfa et al. 1995). This implies greater potential for the evolution of true UV-white lures in environments where the marginal fitness benefit of actively attracting bees outweighs the cost of deterring flies. Again, a deeper understanding of the relevant receivers, and how they weigh and respond to colour versus luminance information will prove essential to realistic tests of such possibilities. Due to their empirical tractability and colour polymorphic nature, orb-web spiders such as *Gasteracantha* present an excellent avenue for future progress.

Acknowledgements

We thank Leo Fleishman, Marie Herberstein, Martin Stevens, Eric Warrant, Daniel Osorio, Rafael Rodriguez, and anonymous reviewers for valuable discussions and suggestions. TEW thanks Elizabeth Mulvenna for, like, everything. TEW was supported by an Australian Postgraduate Award and the Australasian Evolution Society. DJK was supported by the Australian Research Council (grant DP140104107).

References

Adams, D. C., and C. D. Anthony. 1996. Using randomization techniques to analyse behavioural data. Anim, Behav. 51:733–738.

Anderson, J. F. 1966. The excreta of spiders. Comp. Bio. Phys. 17:973-982.

Anderson, J. C., and S. B. Laughlin. 2000. Photoreceptor performance and the co-ordination of achromatic and chromatic inputs in the fly visual system. Vis. Res. 40:13–31.

Barry, K. L., T. E. White, D. N. Rathnayake, S. A. Fabricant, and M. E. Herberstein. 2015. Sexual signals for the colour-blind: Cryptic female mantids signal quality through brightness. Fun. Ecol. 29:531–539.

Bond, A. B. 2007. The evolution of color polymorphism: Crypticity, searching images, and apostatic selection. Ann. Rev. Ecol. Evol. Syst. 38:489–514.

Bond, A. B., and A. C. Kamil. 2002. Visual predators select for crypticity and polymorphism in virtual prey. Nature 415:609–613.

Borst, A. 2009. Drosophila's view on insect vision. Curr. Biol. 19:R36–R47.

Brembs, B., and N. H. de Ibarra. 2006. Different parameters support generalization and discrimination learning in *Drosophila* at the flight simulator. Learn. Mem. 13:629–637.

Burg, J. G., and R. C. Axtell. 1984. Monitoring house fly, *Musca domestica* (Diptera: Muscidae), populations in caged-layer poultry houses using a baited jug-trap. Env. ent. 13:1083-1090.

Burns, K., and J. Dalen. 2002. Foliage color contrasts and adaptive fruit color variation in a bird-dispersed plant community. Oikos 96:463–469.

Chiao, C.-C., W.-Y. Wu, S.-H. Chen, and E.-C. Yang. 2009. Visualization of the spatial and spectral signals of orb-weaving spiders, *Nephila pilipes*, through the eyes of a honeybee. J. Exp. Biol. 212:2269–2278.

Chittka, L. 1996. Optimal sets of color receptors and color opponent systems for coding of natural objects in insect vision. J. Theor. Biol. 181:179–196.

Chunco, A. J., J. S. McKinnon, and M. R. Servedio. 2007. Microhabitat variation and sexual selection can maintain male color polymorphisms. Evolution 61:2504–2515.

Craig, C. L., R. S. Weber, and G. D. Bernard. 1996. Evolution of predator-prey systems: Spider foraging plasticity in response to the visual ecology of prey. Am. Nat. 147:205–229.

Cummings, M. E. 2004. Modelling divergence in luminance and chromatic detection performance across measured divergence in surfperch (embiotocidae) habitats. Vis. Res. 44:1127–1145.

Cummings, M. E. 2007. Sensory trade-offs predict signal divergence in surfperch. Evolution 61:530–545.

Dalrymple, R., D. Kemp, H. Flores-Moreno, S. Laffan, T. White, F. Hemmings, M. Tindall, and A. Moles. 2015. Birds, butterflies and flowers in the tropics are not more colourful than those in higher latitudes. Global Ecol. Biogeo. 848–860.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biol. J. Linn. Soc. 41:315–352.

Endler, J. A. 1992. Signals, signal conditions, and the direction of evolution. Am. Nat. 139:S125–S153.

Endler, J. A. 1993a. Some general comments on the evolution and design of animal communication systems. Phil. Trans. Roy. Soc. B 340:215–225.

Endler, J. A. 1993b. The color of light in forests and its implications. Ecol. Mono. 63:1–27.

Endler, J. A., and P. W. Mielke. 2005. Comparing entire colour patterns as birds see them. Biol. J. Linn. Soc. 86:405–431.

Fan, C. M., E. C. Yang, and I. M. Tso. 2009. Hunting efficiency and predation risk shapes the color-associated foraging traits of a predator. Behav. Ecol. 20:808–816.

Fleishman, L. J., M. Leal, and M. H. Persons. 2009. Habitat light and dewlap color diversity in four species of puerto rican anoline lizards. J. Comp. Phys. A 195:1043–1060.

Fleishman, L., and M. Persons. 2001. The influence of stimulus and background colour on signal visibility in the lizard anolis cristatellus. J. Exp. Biol. 204:1559–1575.

Fuller, R. C. 2002. Lighting environment predicts the relative abundance of male colour morphs in bluefin killifish (*Lucania goodei*) populations. *Proc. R. Soc. B* 269:1457–1465.

Gawryszewski, F., and P. Motta. 2012. Colouration of the orb-web spider *Gasteracantha cancriformis* does not increase its foraging success. Ethol. Ecol. Evo. 24:23–38.

Giurfa, M., J. Nunez, L. Chittka, and R. Menzel. 1995. Colour preferences of flower-naive honeybees. J. Comp. Phys. A 177:247-259.

Giurfa, M., M. Vorobyev, R. Brandt, B. Posner, and R. Menzel. 1997. Discrimination of coloured stimuli by honeybees: Alternative use of achromatic and chromatic signals. J. Comp. Phys. A 180:235–243.

Gomez, D., and M. Théry. 2004. Influence of ambient light on the evolution of colour signals: Comparative analysis of a neotropical rainforest bird community. Ecol. Lett. 7:279–284.

Gray, S. M., L. M. Dill, F. Y. Tantu, E. R. Loew, F. Herder, and J. S. McKinnon. 2008. Environment-contingent sexual selection in a colour polymorphic fish. Proc. R. Soc. B 275:1785–1791.

Grether, G. F. 2005. Environmental change, phenotypic plasticity, and genetic compensation. Am. Nat. 166:E115–E123.

Hancox, D., R. S. Wilson, and C. R. White. 2013. Visual habitat geometry predicts relative morph abundance in the colour-polymorphic ornate rainbowfish. Proc. R. Soc. B 280.

Hauber, M. E. 2002. Conspicuous colouration attracts prey to a stationary predator. Ecol. Ento. 27:686–691.

Heiling, A. M., L. Chittka, K. Cheng, and M. E. Herberstein. 2005. Colouration in crab spiders: Substrate choice and prey attraction. J. Exp. Biol. 208:1785–1792.

Ishikawa, Y., S. Tanaka, Y. Matsumoto, K. Yamashita, M. Yoshida, and E. Shirai. 1985. Color preference of the onion fly, *Hylemya antiqua Meigen* (Diptera: Anthomyiidae) with reference to ultraviolet reflection. App. Ento. Zool., 20:20-26.

Judd, G. J. R., J. H. Borden, and A. D. Wynne. 1988. Visual behaviour of the onion fly, *Delia antiqua*: antagonistic interaction of ultraviolet and visible wavelength reflectance. Ent. exp. app. 49:221-234.

Kelber, A. 2005. Alternative use of chromatic and achromatic cues in a hawkmoth. Proc. R. Soc. B 272:2143–2147.

Kelly, M., and A. Gaskett. 2014. UV reflectance but no evidence for colour mimicry in a putative brood-deceptive orchid *Corybas cheesemanii*. Curr. Zool. 60:104-113.

Kemp, D. J., and R. L. Rutowski. 2007. Condition dependence, quantitative genetics, and the potential signal content of iridescent ultraviolet butterfly coloration. Evolution 61:168–183.

Kemp, D. J., M. E. Herberstein, L. J. Fleishman, J. A. Endler, A. T. D. Bennett, A. G. Dyer, N. S. Hart, J. Marshall, and M. J. Whiting. 2015. An integrative framework for the appraisal of coloration in nature. Am. Nat. 185:705–724.

Kemp, D. J., C. Holmes, B. C. Congdon, and W. Edwards. 2013. Color polymorphism in spiny spiders (*Gasteracantha fornicata*): Testing the adaptive significance of a geographically clinal lure. Ethology 1126–1137.

Leal, M., and L. J. Fleishman. 2002. Evidence for habitat partitioning based on adaptation to environmental light in a pair of sympatric lizard species. Proc. R. Soc. B 269:351–359.

Llandres, A. L., F. M. Gawryszewski, A. M. Heiling, and M. E. Herberstein. 2011. The effect of colour variation in predators on the behaviour of pollinators: Australian crab spiders and native bees. Ecol. Ento. 36:72–81.

Lunau, K. 2014. Visual ecology of flies with particular reference to colour vision and colour preferences. J. Comp. Phys. A 1–16.

Lythgoe, J. N. 1979. Ecology of vision. Clarendon Press; Oxford University Press.

Maia, R., C. M. Eliason, P.-P. Bitton, S. M. Doucet, and M. D. Shawkey. 2013. Pavo: An r package for the analysis, visualization and organization of spectral data. Methods Ecol. Evo. 906–913.

McGraw, K. J., and G. E. Hill. 2000. Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc. R. Soc. B 267:1525–1531.

Morante, J., and C. Desplan. 2008. The color-vision circuit in the medulla of *Drosophila*. Curr. Biol. 18:553–565.

Muma, M. H. 1971. Biological and behavioral notes on *Gasteracantha cancriformis* (arachnida: Araneidae). Flor. Ento. 345–351.

O'Hanlon, J. C., G. I. Holwell, and M. E. Herberstein. 2014. Pollinator deception in the orchid mantis. Am. Nat. 183:126-132.

Osorio, D., and M. Vorobyev. 2005. Photoreceptor sectral sensitivities in terrestrial animals: Adaptations for luminance and colour vision. Proc. R. Soc. B 272:1745–1752.

Osorio, D., A. Miklósi, and Z. Gonda. 1999. Visual ecology and perception of coloration patterns by domestic chicks. Evol. Ecol. 13:673–689.

Oxford, G. S., and R. G. Gillespie 1998. Evolution and ecology of spider coloration. Ann. Rev. Ento. 43:619-643.

Paulk, A. C., J. Phillips-Portillo, A. M. Dacks, J.-M. Fellous, and W. Gronenberg. 2008. The processing of color, motion, and stimulus timing are anatomically segregated in the bumblebee brain. J. Neuro 28:6319–6332.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rao, D., E. Castañeda-Barbosa, N. Nuñez-Beverido, and F. Díaz-Fleischer. 2015. Foraging benefits in a colour polymorphic neotropical orb web spider. Ethology 121:187–195.

Renoult, J. P., A. Valido, P. Jordano, and H. M. Schaefer. 2014. Adaptation of flower and fruit colours to multiple, distinct mutualists. New Phytol. 201:678–686.

Rojas, B., P. Rautiala, and J. Mappes. 2014. Differential detectability of polymorphic warning signals under varying light environments. Behav. Proc. 109, Part B:164–172.

Roulin, A. 2004. The evolution, maintenance and adaptive function of genetic colour polymorphism in birds. Biol. Rev. 79:815–848.

Ryan, M. J., M. E. Cummings, and D. Futuyma. 2005. Animal signals and the overlooked costs of efficacy. Evolution 59:1160–1161. BioOne.

Salcedo, E., A. Huber, S. Henrich, L. V. Chadwell, W.-H. Chou, R. Paulsen, and S. G. Britt. 1999. Blue- and green-absorbing visual pigments of drosophila: Ectopic expression and physiological characterization of the r8 photoreceptor cell-specific rh5 and rh6 rhodopsins. J. Neuro. 19:10716–10726.

Searcy, W. A., and S. Nowicki. 2005. The evolution of animal communication: reliability and deception in signaling systems. Princeton University Press, New Jersey.

Schaefer, H. M., D. J. Levey, V. Schaefer, and M. L. Avery. 2006. The role of chromatic and achromatic signals for fruit detection by birds. Behav. Ecol. 17:784–789.

Schnaitmann, C., C. Garbers, T. Wachtler, and H. Tanimoto. 2013. Color discrimination with broadband photoreceptors. Curr. Biol. 23:2375–2382. Elsevier.

Spottiswoode, C. N., and M. Stevens. 2012. Host-parasite arms races and rapid changes in bird egg appearance. Am. Nat. 179:633–648.

Strausfeld, N. J., and J.-K. Lee. 1991. Neuronal basis for parallel visual processing in the fly. Vis. Neuro. 7:13–33.

Stuart-Fox, D., A. Moussalli, and M. J. Whiting. 2007. Natural selection on social signals: Signal efficacy and the evolution of chameleon display coloration. Am. Nat. 170:916–930.

Sutherland, J. P., M. S. Sullivan, and G. M. Poppy. 1999. The influence of floral character on the foraging behaviour of the hoverfly, *Episyrphus balteatus*. Ento. Exp. App. 93:157–164.

Troje, N. 1993. Spectral categories in the learning behaviour of blowflies. Zeit. Naturfor. C 48:96–96.

Umbers, K. D. L., N. J. Tatarnic, G. I. Holwell, and M. E. Herberstein. 2013. Bright turquoise as an intraspecific signal in the chameleon grasshopper (*Kosciuscola tristis*). Behav. Ecol. Sociobiol. 67:439–447.

Venner, S., and J. Casas. 2005. Spider webs designed for rare but life-saving catches. Proc. R. Soc. B 272:1587–1592.

Vorobyev, M., R. Brandt, D. Peitsch, S. B. Laughlin, and R. Menzel. 2001. Colour thresholds and receptor noise: Behaviour and physiology compared. Vis. Res. 41:639–653.

Wardill, T. J., O. List, X. Li, S. Dongre, M. McCulloch, C.-Y. Ting, C. J. O'Kane, S. Tang, C.-H. Lee, R. C. Hardie, and others. 2012. Multiple spectral inputs improve motion discrimination in the *Drosophila* visual system. Science 336:925–931.

Watt, W. B. 1969. Adaptive significance of pigment polymorphisms in colias butterflies, iI. thermoregulation and photoperiodically controlled melanin variation in *Colias eurytheme*. PNAS 63:767–774.

Wellenreuther, M., E. I. Svensson, and B. Hansson. 2014. Sexual selection and genetic colour polymorphisms in animals. Mol. Ecol. 5398–5414.

White, T. E., and D. J. Kemp. 2015. Technicolour deceit: A sensory basis for the study of colour-based lures. Anim. Behav. 105:231–243.

White, T. E., and D. J. Kemp. 2016. Quick guide: colour polymorphism. Curr. Biol.: In press

White, T. E., J. Zeil, and D. J. Kemp. 2015. Signal design and courtship presentation coincide for highly biased delivery of an iridescent butterfly mating signal. Evolution. 69:14-25.

White, T. E., R. L. Dalrymple, D. W. A. Noble, J. C. O'Hanlon, D. B. Zurek, and K. D. L. Umbers. 2015. Reproducible research in the study of biological coloration. Anim. Behav. 106:51–57.

Yamaguchi, S., C. Desplan, and M. Heisenberg. 2010. Contribution of photoreceptor subtypes to spectral wavelength preference in *Drosophila*. PNAS 107:5634–5639.

Yamaguchi, S., R. Wolf, C. Desplan, and M. Heisenberg. 2008. Motion vision is independent of color in *Drosophila*. PNAS 105:4910–4915.

Zhou, Y., X. Ji, H. Gong, Z. Gong, and L. Liu. 2012. Edge detection depends on achromatic channel in *Drosophila melanogaster*. J. Exp. Biol. 215:3478–3487.

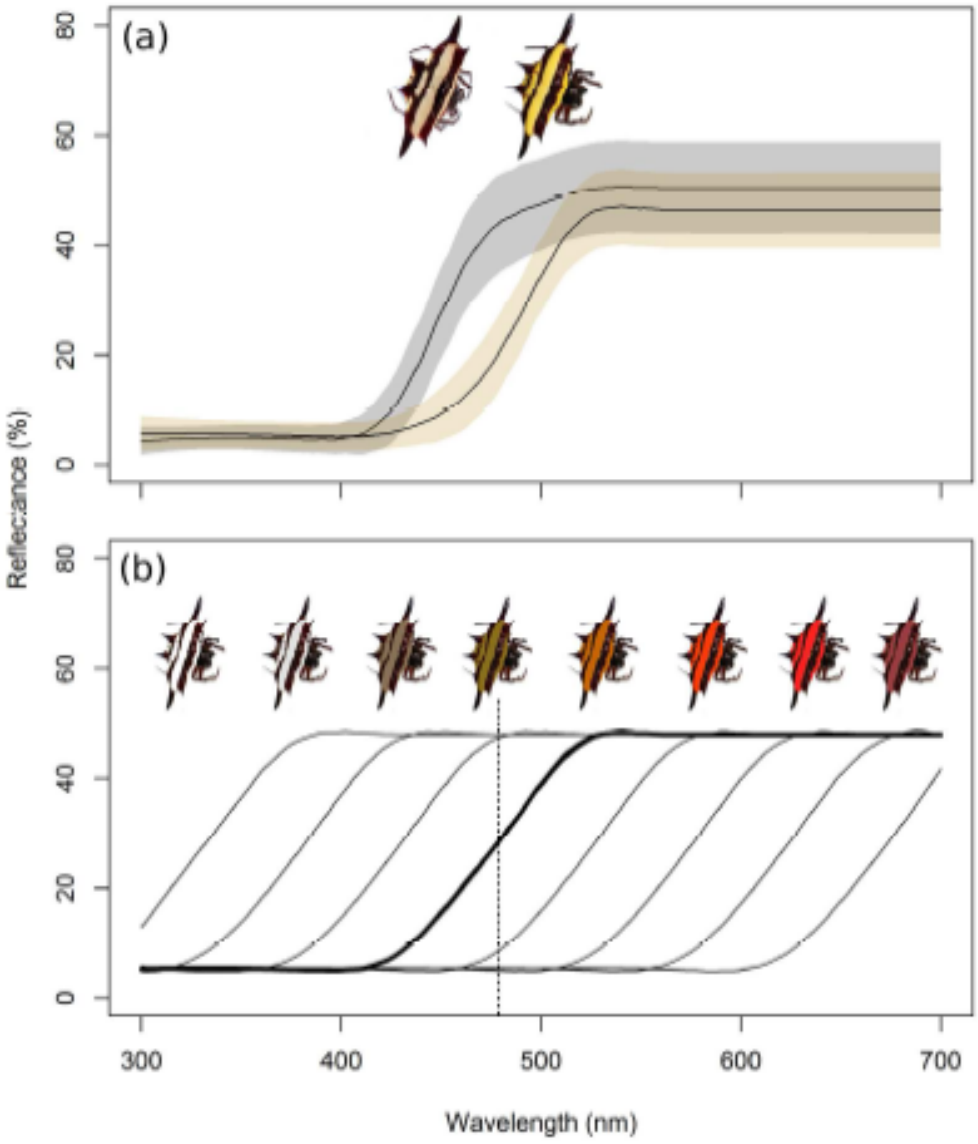

Figure 1: (a) Aggregated reflectance spectra (mean ± sd) of female *G. fornicata* (n = 80 'yellow', 44 'white', inset). (b) Examples of the hypothetical reflectance spectra used to address the question of which lure colours maximise fly-subjective contrast in the environments of *G. fornicata*. The hues (low-to-high inflection points) of spectra are here spaced at 50 nm intervals, though for visual modelling the spacing was 1 nm. The template spectrum from which all other spectra were generated (mean of 80 yellow and 44 white *G. fornicata*) is highlighted in bold, and its inflection point indicated by the dashed line. Manipulated spider images are included only as an illustrative approximation of how they may appear to a human observer. 169x198mm (300 x 300 DPI)

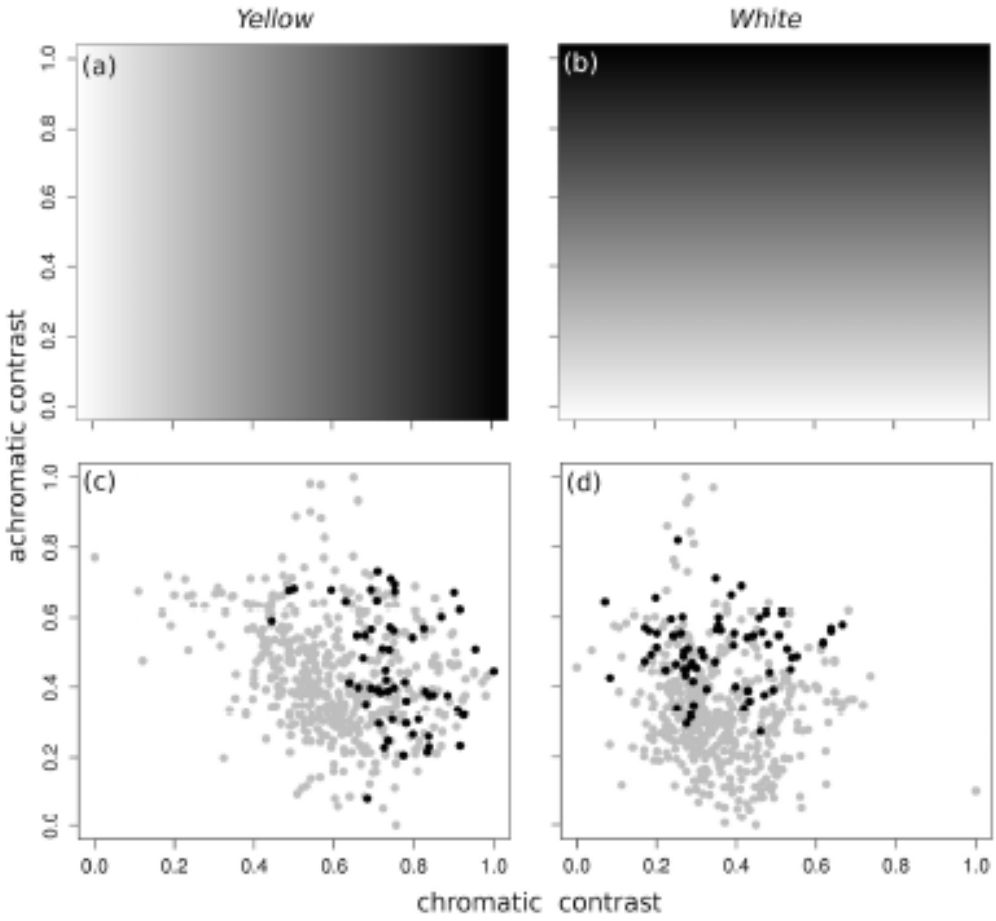

Figure 2: (a, b) A heuristic illustration of our predictions regarding the frequency of prey capture events (gradient fill, with darker area indicating greater capture success) of spider morphs (indicated by column headings), as a function of fly-subjective chromatic and achromatic contrast (normalised, unitless). If, as hypothesised, yellow and white morphs are targeting different visual channels, we may expect (a) yellow individuals to experience greater capture success at the viewing times when they are generating relatively greater chromatic contrast (i.e. presenting a disproportionately 'colourful' signal), as opposed to all other possible viewing times. Conversely, we may expect (b) white individuals to capture more prey at the viewing times when they are generating relatively greater achromatic contrast (i.e. presenting a disproportionately 'luminant' signal). (c, d) Observed data indicate the visual appearance of individuals of each morph at all potential viewing times (grey points), and the subset of times at which prey were captured (black points). Data are presented as plots of normalised fly-subjective chromatic versus achromatic background contrast (unitless, see main text and supplement for details). Note that we cannot say whether chromatic and achromatic contrasts are similarly weighted by dipteran prey.
180x161mm (300 x 300 DPI)

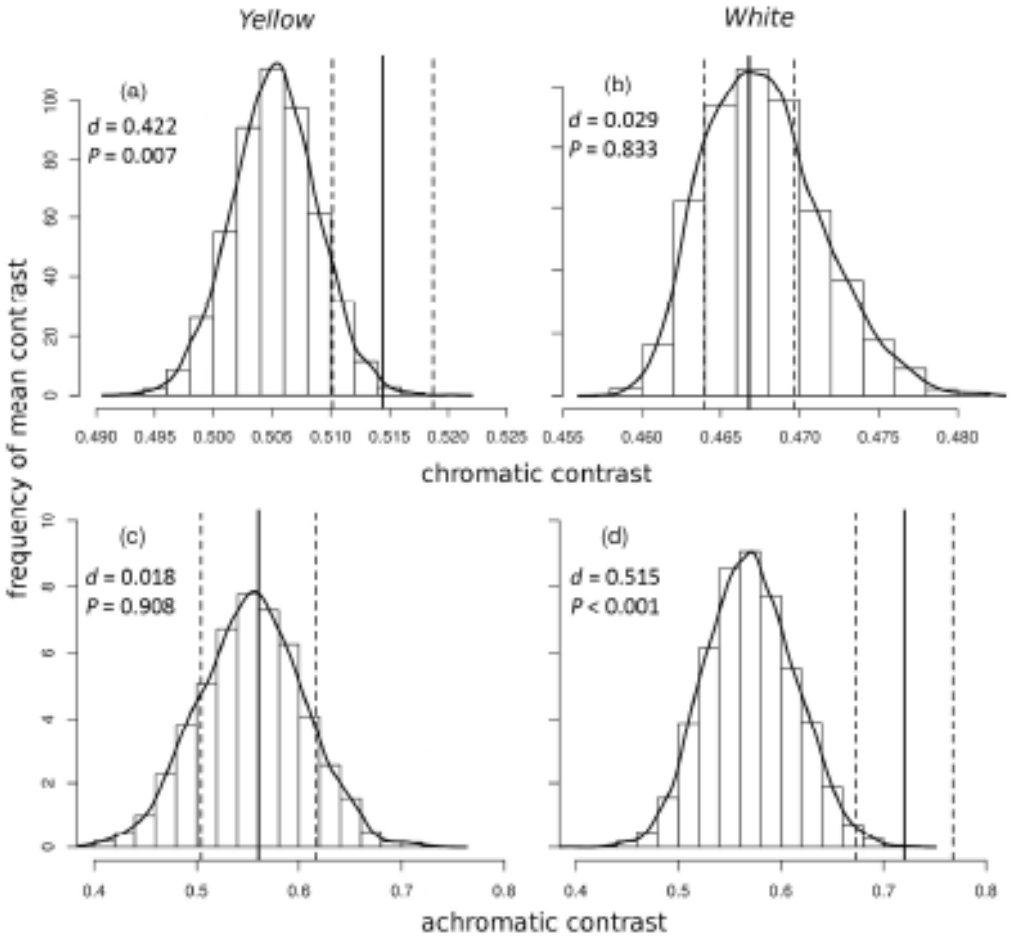

Figure 3: Null distributions of mean fly-subjective chromatic and achromatic contrasts (unitless) of (a, c) yellow, and (b, d) white *G. fornicata* against their backgrounds. Column headings indicate morphs. These are empirically generated distributions that describe the probability of observing a given mean spider-background contrast value, under the null hypothesis that fly captures occur at random with respect to the spiders' visual appearance. The distribution is generated from 5000 randomized sub-samples of a total pool of 1072 potential contrast values, which were calculated by visually modelling every individual spider under its specific combination of background and ambient illumination as recorded at every hour (134 spiders with 8 hourly light measures per day; see main text and supplementary methods). The size of each sub-sample corresponded to the number of large (>5 mm) fly captures (n = 77 and 67 for white and yellow spiders, respectively; supplementary table 1). Vertical lines indicate the mean ± se of the observed data, that is, the mean ± se of the observed chromatic and achromatic spider-background contrast values at the times of fly capture events.
180x167mm (300 x 300 DPI)

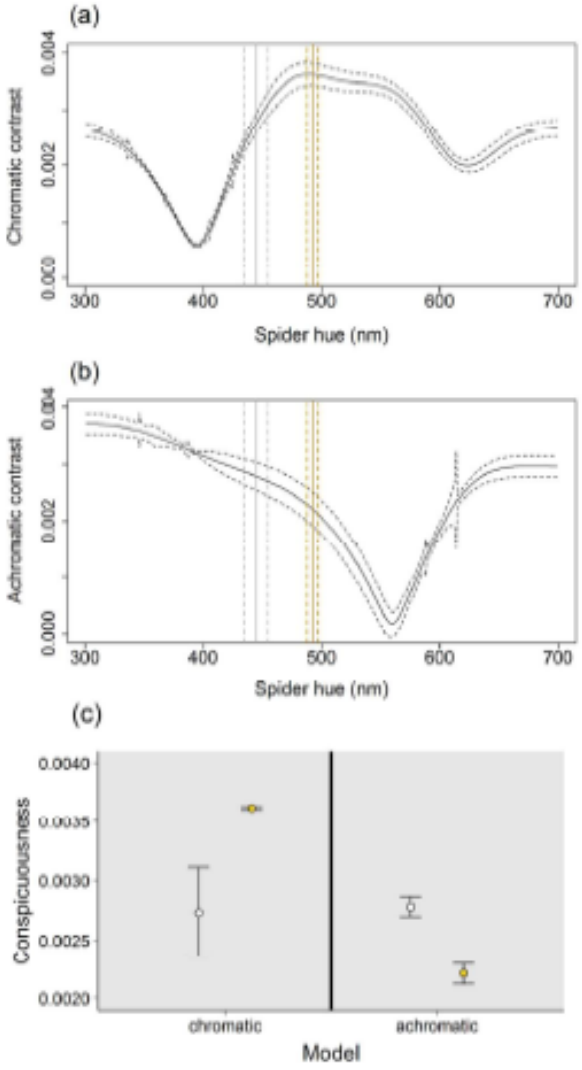

Figure 4: The fly-subjective (a) chromatic and (b) achromatic contrast (unitless) of all possible lure colours (taking the sigmoid-shaped reflectance of *G. fornicata* as constant) in the visual environments of *G. fornicata*. Functions describe the mean ± sd normalised spider-background contrast of 398 hypothetical spiders with hues (low-to-high inflection point of reflectance curves) spaced at 1 nm intervals, modelled using the 1072 recorded background/illuminant spectra (as represented by standard deviations) collected during spider observations. Grey and yellow vertical lines indicate the mean ± sd observed hue of 44 white, and 80 yellow *G. fornicata*, respectively. (c) The mean ± sd conspicuousness \'score\' of observed *G. fornicata* morphs (white and yellow points, respectively) in the context of each model (shown in a, b). Larger values indicate greater contrast against backgrounds. Note that the scales on the ordinate of (a) and (b) differ from those on the abscissa of Fig. 3, because the values here were normalised so that the area under the curve equals one. This ensured that individual G. fornicata had equal \'scoring potential\' in the context of each model, which was necessary to quantify the relative performance of each morph, as in (c).
91x170mm (300 x 300 DPI)

Electronic supplementary material (ESM):

White TE, Kemp DJ (2016) Colour polymorphic lures target different visual channels in prey

Supplementary methods

Visual modelling

The quantum catch for a given photoreceptor i in the tetrahedral model of drosophilid vision was calculated as:

$$Q_i = \int_\lambda R_i(\lambda)S(\lambda)I(\lambda)d\lambda \tag{1}$$

where λ denotes wavelength, and R_i, S, and I represent spectra for photoreceptor sensitivity, the stimulus, and illuminant respectively (Endler and Mielke 2005). Quantum catches were then log transformed in accordance with the Weber-Fechner law (Vorobyev et al. 2001; Endler and Mielke 2005), before being normalised to sum to one. Co-ordinates in the tetrahedral stimulation space (with height = 1; Endler and Mielke 2005) were then calculated as:

$$x = \frac{1 - 2s - m - u}{2}\sqrt{\frac{3}{2}} \tag{2}$$

$$y = \frac{-1 + 3m + u}{2\sqrt{2}} \tag{3}$$

$$z = u - \frac{1}{4} \tag{4}$$

where the subscripts u, s, m, and l - when dealing with fly vision - refer to quantum catches by R7p, R7y, R8p, R8y photoreceptors respectively (Salcedo et al. 1999). Chromatic contrast was then calculated as the Euclidean distance (unitless) between points:

$$\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2 + (z_1 - z_2)^2} \tag{5}$$

Achromatic contrast was calculated as:

$$\frac{Q_s - Q_b}{Q_s + Q_b} \tag{6}$$

where Q_s and Q_b refer to the quantum catches of *Drosophila melanogaster*'s R1-6 photoreceptor when viewing the spiders and backgrounds (i.e. 'luminance', calculated as per equation 1), respectively (Salcedo et al. 1999; Fleishman and Persons 2001).

Supplementary results

Table S1: The identity and abundance of large (>5 mm) prey captured by yellow and white *G.fornicata* throughout the observational period. Ordered by overall (across-morph) abundance.

prey order	prey family	captured by white spiders	captured by yellow spiders
Diptera	Tephritidae	37	34
Diptera	Muscidae	16	9
Hymenoptera	Apidae	6	12
Diptera	Drosophilidae	9	8
Diptera	Calliphoridae	4	6
Coleoptera	Scarabaeidae	4	4
Coleoptera	unknown	4	4
Diptera	Tipulidae	5	2
Hemiptera	unknown	3	1
Diptera	unknown	2	3
Hemiptera	Aphididae	2	3
Diptera	Dolichopodidae	1	3
Unknown	unknown	3	0
Hemiptera	Pyrrhocoridae	1	1
Diptera	Culicidae	1	1
Diptera	Stratiomyidae	1	1
Hemiptera	Fulgoroidea	0	2
Orthoptera	unknown	2	0
Araenidae	unknown	1	0
Diptera	Tabanidae	1	0
Hemiptera	Cicadidae	0	1

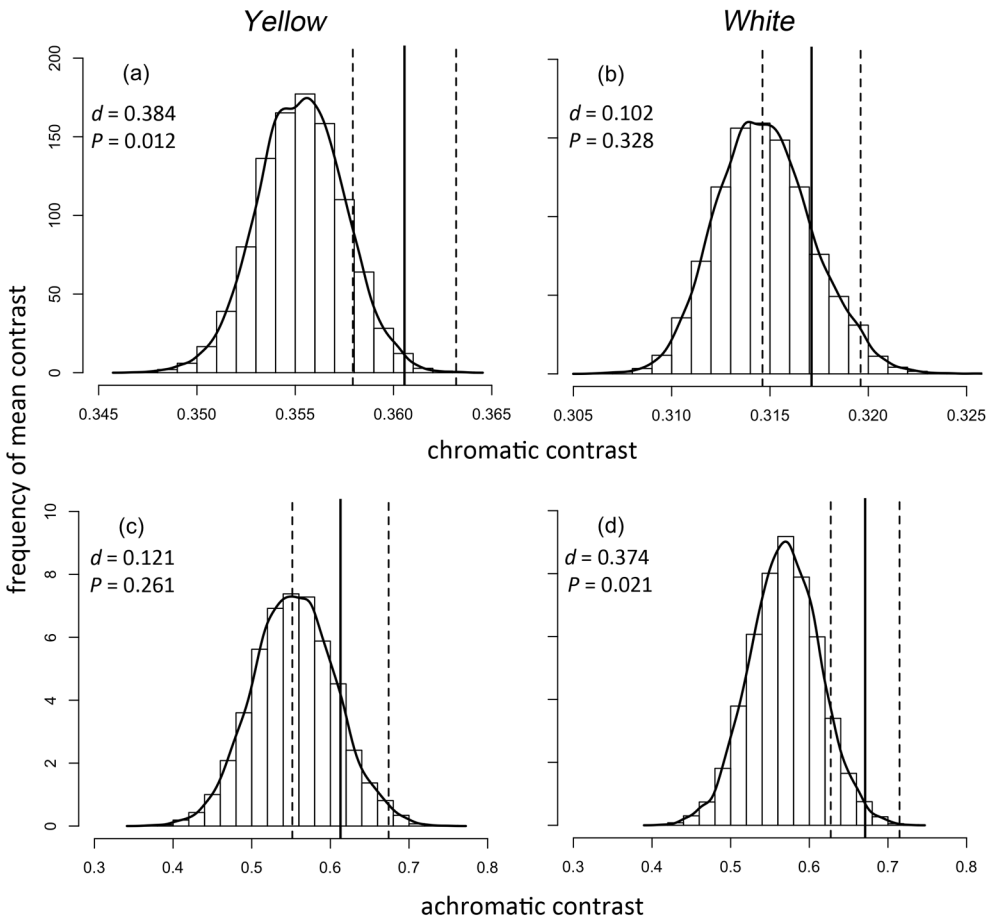

Figure S1: Null distributions of mean fly-subjective chromatic and achromatic contrasts (unitless) of (a, c) yellow, and (b, d) white *G. fornicata* against their backgrounds (as in Fig. 3, main text), repeated using a modified segment-based analysis (Endler 1990; Dalrymple et al. 2015). Column headings indicate morphs. We estimated chromatic contrast by calculating the relative intensity of four 'segments' in spider and background spectra (300 - 700 nm, at 100 nm intervals; as per equations 4 and 6 in Endler 1990), converting each spectrum to a point in a tetrahedral space (as per equations 19 - 20 in Endler and Mielke 2005, or equations 1-5 above), and calculating the Euclidean distance between spiders and their relevant backgrounds. Achromatic contrast was calculated as per equations 6 (above), but with an 'achromatic photoreceptor' sensitivity set to one across the 300 - 700 nm range. As in Fig. 3 (main text), these distributions are empirically generated, and describe the probability of observing a given mean spider-background contrast value, under the null hypothesis that fly captures occur at random with respect to the spiders' visual appearance. The distribution is generated from 5000 randomized sub-samples of a total pool of 1072 potential contrast values, which were calculated by visually modelling every individual spider under its specific combination of background and ambient illumination as recorded at every hour (134 spiders with 8 hourly light measures per day). The size

of each sub-sample corresponded to the number of large (>5 mm) fly captures (n = 77 and 67 for white and yellow spiders, respectively). Vertical lines indicate the mean ± se of the observed data, that is, the mean ± se of the observed chromatic and achromatic spider-background contrast values at the times of fly capture events. We derived p-values (inset, along with Cohen's d) by which to test the observed data against the generated distributions by calculating the proportion of contrast values in the null distribution that were equally or more extreme than the observed mean contrast value, and multiplying it by two (for a two-tailed test; Adams and Anthony 1996).

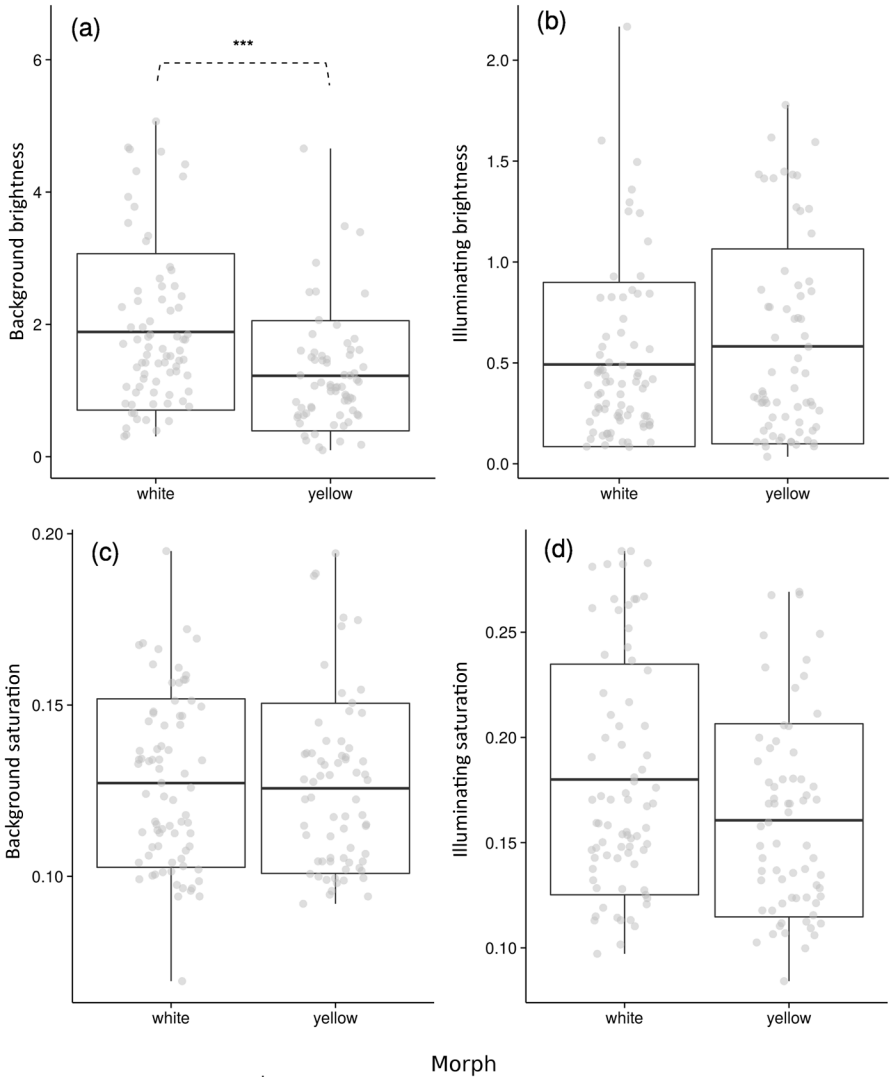

Figure S2: The 'brightness' (μmol m^{-2} s^{-1} nm^{-1}) and saturation (unitless) of illuminating and background light around spiders at the times of prey capture. Boxplots show mean $\pm$ sd, maximum and minimum (a) background brightness, (b) illuminating brightness, (c) background saturation, and (d) illuminating saturation. Grey circles show raw data (n = 77 and 67 large dipteran captures for white and yellow spiders, respectively), and asterisks' indicate significance at $P < 0.001$.

References

Adams, D. C., and C. D. Anthony. 1996. Using randomization techniques to analyse behavioural data. Animal Behaviour 51:733–738. Elsevier.

Dalrymple, R., D. Kemp, H. Flores-Moreno, S. Laffan, T. White, F. Hemmings, M. Tindall, and A. Moles. 2015. Birds, butterflies and flowers in the tropics are not more colourful than those in higher latitudes. Global Ecology and Biogeography 848–860.

Endler, J. A. 1990. On the measurement and classification of colour in studies of animal colour patterns. Biological Journal of the Linnean Society 41:315–352. Wiley Online Library.

Endler, J. A., and P. W. Mielke. 2005. Comparing entire colour patterns as birds see them. Biological Journal of the Linnean Society 86:405–431.

Fleishman, L., and M. Persons. 2001. The influence of stimulus and background colour on signal visibility in the lizard anolis cristatellus. Journal of Experimental Biology 204:1559–1575. The Company of Biologists Ltd.

Salcedo, E., A. Huber, S. Henrich, L. V. Chadwell, W.-H. Chou, R. Paulsen, and S. G. Britt. 1999. Blue-and green-absorbing visual pigments of drosophila: Ectopic expression and physiological characterization of the r8 photoreceptor cell-specific rh5 and rh6 rhodopsins. The Journal of Neuroscience 19:10716–10726. Soc Neuroscience.

Vorobyev, M., R. Brandt, D. Peitsch, S. B. Laughlin, and R. Menzel. 2001. Colour thresholds and receptor noise: Behaviour and physiology compared. Vision Research 41:639–653.