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Colour Discrimination Functions of the Humming Bird Hawkmoth (Macroglosum Stellatarum) Modelled From Behavioural Choices

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Abstract

The hummingbird hawkmoth (*Macroglossum stellatarum)* is a diurnal, visually oriented insect that has become an important animal model for comparative studies. Hawkmoths use colour information to find and identify profitable flowers, and they are major pollinators in various regions of the world. Recent research on other flower visiting animals including bees, hoverflies and birds shows that their respective colour choices for target-distractor stimuli of varying similarity can be reliably modelled using sigmoidal-type continuous functions, as postulated by von Helversen 50 years ago. We formally test if colour choices by *M. stellatarum* can also be reliably explained by these sigmoidal functions that are important for modelling plant-pollinator interactions in other animals. We fit multiple formulations of sigmoidal functions selecting those which best fit the observed data. We show that colour vision in hawkmoths varies across various regions of colour space. Our results are consistent with theories that colour choices are principally mediated by higher level neural processes in the animal brain, since it is at this level physiological signal modulation varies with stimulus modulation in a probabilistic fashion. These functions thus enable important insights into how to model how animals interact with colour stimuli like flowers in complex environments.

Introduction

Humans typically sense the colour visual environment with three spectrally different photoreceptor classes, whose maximum sensitivity is respectively located at about 420 (blue-sensitive), 534 (green) and 564 (red) nm (Bowmaker and Dartnall 1980). Each receptor class pools all available sensed photons as described by the principle of univariance, and subsequent signals are projected to multiple higher level processing areas in the brain which enable the generation of colour perception via opponent neural mechanisms (Rushton 1972; Hurvich 1981; Lee 2005).

Flowers are amongst the most colourful objects in the natural environment, and from the earliest days of visual ecology seminal researchers appreciated that flowers typically evolved signals to suit the visual capabilities of the most important pollinators, which have very different colour visual capabilities compared to humans (von Frisch 1914; Kühn 1927; Daumer 1958; Dyer et al. 2021). Nevertheless, studies on human colour vision set the main foundation for animal colour vision studies (Kelber et al. 2003b; Kemp et al. 2015).

Our trichromatic visual system allows us to differentiate between saliently different colours like ripe fruit from green foliage (Mollon 1989). However, as colours become increasingly similar, discrimination accuracy drops until reaching a limit after which it is no longer possible to discern between the two stimuli, even if these differ in their spectral properties (MacAdam 1985). This relationship between colour (dis)similarity and accuracy of discrimination will take the characteristic shape of a letter “S” and often referred to as being sigmoidal. The sigmoidal shape of the colour discrimination function arises from the presence of both lower and upper asymptotes where discrimination accuracy remains constant in spite of changes in colour difference. The upper limit of a colour discrimination function is imposed by the

impossibility of significantly increasing accuracy in colour discrimination after approaching approximately 100% of correct choices in spite of an increment in colour difference. On the other hand, an observer will continuously choose at random, i.e. an accuracy around 50%, when discriminating between colour stimuli that are so similar to each other as to be below a perceptual threshold. Only when the difference between stimuli is large enough to become apparent to the observer the function will start to rise, indicating the end of the lower plateau region, until reaching the upper asymptote of the function (Garcia et al. 2017).

Von Helversen (1972a), inspired by the experimental results of colour discrimination by the trichromatic European honeybee (*Apis mellifera*) (von Frisch 1914; Daumer 1956; von Helversen 1972b), proposed that the relationship between colour dissimilarity and discrimination accuracy by an animal observer should also be described by a sigmoidal curve. However, he did not provide a mathematical formulation for a function which could match his theoretical model, nor explicitly examine how the theory was supported by empirical data for different bee species or other animals.

The first empirical evidence of sigmoidal functions in hymenopteran insects was reported by Dyer and Chittka (2004a) for bumblebee colour choices, who noted that such a probabilistic way of processing colour information was consistent with evidence showing that the discharge rates of single neurons in macaque monkeys (*Macaca fuscata*) correlates with the relative position of colour similarity of stimuli on a colour map for primate vision (Komatsu and Ideura 1993). This fits with other evidence that whilst there are some differences in colour processing mechanisms between primates and insects, overall there are highly convergent visual strategies for chromatic discrimination (Pichaud et al. 1999, Hempel de Ibarra et al. 2014). Other research on bumblebees (Dyer and Chittka 2004c) and honeybees (Giurfa 2004; Avarguès-Weber et al. 2010; Dyer and Garcia 2014) shows that conditioning experience has on effect on the ability of an hymenopteran observer to discriminate colours and lead to changes in the brain and long-term memory (Sommerlandt et al. 2016), suggesting that colour discrimination is influenced by neural tuning processes in these animals.

Hawkmoths include various diurnal and nocturnal visually driven moth species which feed on flower nectar and are considered to be major pollinators in various ecosystems across their distribution ranges. For example, hawkmoths are responsible for pollinating 10% of all tree species in tropical dry forests in addition to various species of shrubs, herbs and epyphites (Haber and Frankie 1989), and are the main pollinators of orchid species in Madagascar (Nilsson et al. 1992) and North America (Fox et al. 2013). In Costa Rica, long tongued hawkmoths are reported as being generalist flower visitors, feeding on many different species of flowers, including those adapted to other animal pollinators such as bats and hummingbirds, which display a variety of different colours (Haber and Frankie 1989).

The hummingbird hawkmoth (*Macroglossum stellatarum)* is a diurnal, visually oriented insect which uses colour information to find and identify profitable flowers (Kelber et al. 2003a), and can override innate preferences following training to discriminate between distinct colour stimuli (Kelber 1996). *M. stellatarum* demonstrates a capacity to be a food generalist and forage on a variety of different spectrally

different colour stimuli to obtained preferred sugar nutrition (Kelber 2003). *M. stellatarum* has functional trichromatic vision (Kelber and Henique 1999) which facilitates a capacity to discriminate between spectral colours at a level which is finer than honeybees (Telles et al. 2016). It is also known that hawkmoths show evidence of improved capacity to discriminate between colours following differential conditioning to stimuli (Kelber 2010). However, in spite of the current knowledge on the colour vision of *M. stellatarum*, it remains untested if discrimination for (dis)similar colour differences in this species can be reliably described by a continuous, sigmoidal function as has recently been demonstrated for other major pollinators including insects like bees and flies (Garcia et al. 2017, 2018; Hannah et al. 2019; Garcia et al. 2022), and violet sensitive birds (Garcia et al. 2021).

Here we specifically test a hypothesis that sigmoidal functions may describe colour choices in hawkmoths by using previously recorded behavioural data on colour discrimination of (dis)similar colour differences by *M. stellatarum* (Telles et al. 2016). If this hypothesis proves true, our model allows predicting accuracy of colour discrimination without resorting to assumptions of currently unknown physiological parameters of *M. stellatarum* colour vision. We discuss the outcomes of our modelling with respect to underlying mechanistic accounts of whether biologically relevant colour discrimination in these animals is principally mediated due to receptor level, and/or higher level neural processes.

Materials And Methods

Colour modelling

We modelled colour discrimination functions for the hummingbird hawkmoth (*Macroglosum stellatarum*) corresponding to the six different reference quasi-monochromatic stimuli reported by (Telles et al. 2016). To control for potential differences in discrimination accuracy across the spectrum, we fitted functions to the respective colour differences corresponding to stimuli of either longer- or shorter-wavelength to each training reference stimulus..

Functions were fitted following the protocol detailed in Garcia et al. (2017). Firstly, the spectral profile of the stimuli used for the behavioural experiment by Telles et al. (2016) were expressed as a loci in the Maxwell colour space (Fig. 1, panel a) implementing standard colorimetric calculations (Renoult et al. 2017). The selection of the colour space was based on the fact that this model is suitable for trichromatic vision and makes no assumptions of the currently unknown potential opponent mechanisms in the vision of *M. stellatarum* (Kelber et al. 2003a).

We modelled each monochromatic using a Gaussian function of form:

$$f(x) = \exp - \left(\frac{(x - b)^2}{2c^2} \right)$$

1

where *b* denotes the wavelength corresponding to the peak transmission of the stimuli and *c* = 5.24. The constant *c* ensures that the half bandwidth of each stimulus is equal to about 15 nm, as reported by Telles et al. (2016). Eq. 1 was evaluated within 300 to 700 nm at 5 nm intervals (Fig. 1 panels b and c).

We used the exponential functions proposed by Stavenga (1993) as template to model the spectral sensitivity functions for the three photoreceptors present in the visual system of *M. stellatarum*. Peak absorption of each photoreceptwas located at 349, 440 and 521nm (Telles et al. 2014), with a secondary peak of absorption, β-band, located at 320 nm.

For our calculations we employed the illumination profile available for the behavioural experiments consisting on a combination of white light emitting diodes and florescent light tubes (Telles et al. 2014, 2016). We assumed a spectrum uniformly reflecting 30% of all incident radiation across the 300 to 700 nm interval to match the experimental conditions reported by Telles et al (2016) as adaptation background for colour modelling. Finally, we modelled chromatic adaptation by *M. stellatarum* using a standard von Kries type scaling function (Garcia et al. 2017). Colour difference between reference and each tested stimuli pair is here expressed as the Euclidean distance between their respective loci in the Maxwell colour space, although the formulation of colour discrimination function as a continuous function can accommodates other colour distance metrics; see for example (Garcia et al. 2017, 2018, 2022). All colour modelling was done using custom written scripts for Matlab release 2017b.

Fitting of the colour discrimination function

The response variable of the colour discrimination functions for *M. stellatarum* consists of the proportion of correct choices corresponding to each colour difference tested. We thus calculated the mean proportion of correct choices for each pair discrimination experiment by dividing the number of correct choices over the total number of choices made by all tested hawkmoths as reported in the supplementary material of Telles et al. (2016).

We fitted two sigmoidal functions (Equations 2 and 3) of different complexity to each dataset, as the shape of these functions has been shown to successfully describe colour discrimination by hymenopterans and dipteran pollinators (Garcia et al. 2017, 2018, 2022).

$$\pi(\Delta C) = \frac{M_o K}{M_o + \left(K - M_o\right)\exp(-r \bullet \Delta C)}$$

2

$$\pi(\Delta C) = \frac{M_o + (K - M_o)}{1 + \exp\left(\frac{xmid - \Delta C}{r}\right)}$$ s(3)

In Equations 2 and 3, the variable π denotes the probability of accurate discrimination of the colour difference (ΔC). We expressed ΔC as the Euclidean distance of two loci in the Maxwell triangle, although

other metrics can also be implemented (Garcia et al. 2017, 2018, 2022), and the constants define the unique shape of the function.

Values for the constants *K, r, Mo, xmid* and *scal* were found by implementing the non-linear least square routine (nls) available in the nlme package (Pinheiro et al. 2013) for R release 4.0.2. If for any given colour discrimination function both Equations fitted the data, a loglikelihood test was performed to identify the model which better described the dataset. If the null hypothesis of equality failed to be rejected, the simpler model was selected in favour of the more complex alternative following standard model selection practices (Crawley 2013).

Once we identified the best model fitting each data set, we used simulations to recover the uncertainty around model parameters as proposed by (Garcia et al. 2017). Briefly, for each function we generated 100,000 realisations of each discrimination experiment by randomly sampling from a binary distribution whose defining parameters are given by results reported by Telles et at (Telles et al. 2016) and fitted the selected equation to each simulated response set. Median and 95% confidence intervals (CI) for each coefficient were then calculated from the distribution of values obtained from the simulation.

Results

We obtained eight different colour discrimination functions out of the twelve data sets analysed (Table 1). Functions were obtained for the long wavelength stimuli tested relative to a reference of 380 nm, and for both the short and long wavelength stimuli of references centred at 400, 440 and 480 nm.

Three and four parameter functions fitted the datasets corresponding to the longer wavelength stimuli tested for references centred at 400 and 440 nm. In both cases the more complex function provided a better fit than the simpler, three parameters alternative ($\chi^2_{400\ nm} = 11.1$, df = 1, P = 0.001, ($\chi^2_{440\ nm} = 5.54$, df = 1, P = 0.018), thus the more complex function (Eq. 3) was selected for these two cases (Table 1). All remaining discrimination functions were fitted by a three parameter logistic model (Eq. 2). Figure 1 depicts the colour discrimination function obtained for those reference wavelengths showing a significant increase in probability of accurate discrimination as a function of colour difference.

Table 1

Reference and tested stimuli used to derive the seven different colour discrimination functions for the hummingbird hawkmoth (*Macroglossum stellatarum*), values for their defining coefficients and colour difference (ΔC) predictions for accuracy thresholds (π) of 60 and 0 %. Colour differences are expressed as units in the Maxwell triangle and obtained by inverting Equations 2 or 3 and solving for ΔC for each accuracy value. * Correct choices for this reference did not significantly increase from chance level so it is not possible to predict colour distances from this function.

Reference wavelength	Tested stimuli	Discrimination function	Parameter values (95% Confidence interval).	$\Delta C_{\pi 0}$ %	$\Delta C_{\pi 0}$ %
380	Shorter n = 4	No fit			
	Longer n = 6	3-log (Eq. 2)	*K* = 1.02 (0.870, 1.33) *r* = 20.9 (3.33, 111) *Mo* = 0.448 (0.083, 0.705)	0.028	0.049
400	Shorter n = 5	3-log (Eq. 2	*K* = 0.934 (0.697, 1.92) *r* = 15.5 (1.65, 304) *Mo* = 0.42 (0.02, 0.758)	0.051	0.084
	Longer n = 6	4-log (Eq. 3)	*K* = 0.323 (-0.456, 0.591) *Mo* = 0.892 (0.667, 2.93) *xmid* = 0.329 (0.213, 0.497) *scal* = 0.003 (0.002, 0.01)	0.329	0.331
440	Shorter n = 6	3-log (Eq. 2)	*K* = 0.928 (0.725, 2.38) *r* = 57 (8.57, 389) *Mo* = 0.387 (0.004, 0.677)	0.026	0.016
	Longer n = 5	4-log (Eq. 3)	*K* = 0.330 (-1.04, 0.634) *Mo* = 1.00 (0.821, 1.21) *xmid* = 0.143 (0.004, 0.190) *scal* = 0.015 (0.001, 0.076)	0.146	0.137
480	Shorter n = 5	3-log (Eq. 2)	*K* = 0.932 (0.723, 2.73) *r* = 16.1 (-53.9, 75.7) *Mo* = 0.079 (0.00, 0.887)	0.216	0.184

Reference wavelength	Tested stimuli	Discrimination function	Parameter values (95% Confidence interval).	$\Delta C_{\pi 0}$ %	$\Delta C_{\pi 0}$ %
	Longer n = 5	3-log (Eq. 2)	*K* = 0.941 (0.771, 1.71) *r* = 18.0 (2.21, 140) *Mo* = 0.5 (0.035, 0.772)	0.052	0.024
520	Shorter n = 6	No fit			
	Longer n = 4	3-log (Eq. 2)	*K* = 0.725 (0.449, 1.68) *r* = -348 (-6810, 462) *Mo* = 0.661 (0.000, 1.45)	NaN*	NaN*
560	Shorter n = 5	No fit			
	Longer n = 4	No fit			

Discussion

In the current manuscript we used mathematical descriptions of the sigmoidal functions originally proposed by von Helversen (1972a) to predict accuracy of colour discrimination by hummingbird hawkmoths based on perceived colour difference to different reference stimuli (Fig. 2). These functions provide a prediction to the question of the effect of colour dissimilarity on colour discrimination by hawkmoths based on behavioural observations. In bees (Dyer and Chittka 2004c; Sommerlandt et al. 2016) several studies show that conditioning can enable improved colour discrimination, and enable long-term memory formation that results in fundamental changes in the brain. These changes likely define the “inner conditions” responsible for the behavioural responses observed, as proposed by von Helversen (1972a). It has also been reported that hawkmoths improve colour discrimination with conditioning (Kelber 2010), although evidence of how this may influence the physiology of the brain remains outstanding. The sigmoidal shaped functions have now been demonstrated to reliably predict colour choices in hawkmoths (Fig. 2), bees (Garcia et al. 2017) and a variety of other animal pollinators (Garcia et al. 2021, 2022), and physiological evidence suggests that the way neurons respond to perceptually (dis)similar colour stimuli (Komatsu and Ideura 1993; Dyer and Chittka 2004c) is a plausible explanation for such observations.

Initial estimates of colour discrimination thresholds for *M. stellatarum* (Telles et al., 2016) were based on the receptor-noise threshold model (Vorobyev and Osorio 1998) which assumes that colour discrimination is limited by purely physiological aspects of vision namely, the fixed signal to noise ratio imposed by the density and number of photoreceptor available in the visual system of the observer (Vorobyev et al. 2001). This assumption, however, does not seem to hold entirely when considering the

data obtained from discrimination experiments of similar colours by this species. More specifically, Telles et al. (2016), noted that whilst the receptor-noise model provides a partial fit to the observed hawkmoth choices, an overall fit was not observed. The colour discrimination functions presented (Fig. 2) account for the entire processing of the colour signals by visual system of the animal without making assumptions of currently unknown physiological aspects of the system as these are modelled directly on the animal's response. Thus with quality psychophysics and modelling, the problem of unknown physiological data can be avoided, and improved predicted power is possible (Table 1).

Modelling of the behavioural data by Telles et al., (2016) revealed evidence of considerable variability in animal choices for using colour information (Fig. 2). Such evidence is consistent with data for bee foragers where other ecological factors impact on how and why flower visitors use colour information to make decisions. For example, both honeybees (Ng et al. 2021) and bumblebees (Chittka et al. 2003, 2009; Dyer and Chittka 2004b) show that bees trade of speed for accuracy, and Evans and Raine (2014) show that such a variability in accuracy may be optimal in a foraging environment with many hive mates engaging in different exploratory behaviour. An important component of sigmoidal functions describing animal colour choices is that these functions also enable capturing such complexity of animal behaviour through the variability around their defining coefficients (Table 1).

Our results also confirm differences in sensitivity to colour differences across the sampled colour space of *M. stellatarum* (Figs. 1 and 2), as was reported by Telles et al. (2016). This aspect of colour perception has been well appreciated in humans (Hurvich 1981), bees (von Helversen 1972b; Dyer and Neumeyer 2005; Garcia et al. 2017), flies (Hannah et al. 2019) and pigeons (Wright 1972; Garcia et al. 2021) suggesting that across different regions of the visible spectrum, not all colour are discriminated in the same way. Such differences are likely to be due to both physiological mechanisms including how photoreceptors overlap and opponent neural processing of photoreceptor input (Rushton 1972; Hurvich 1981; Kelber et al. 2003b; Lee 2005; Kemp et al. 2015).

To model colour discrimination by *M. stellatarum* using the proposed method it is first necessary to identify the more likely colour to be discriminated and choose one of the functions in Fig. 2a-g whose reference wavelength is closest to that of the problem stimuli. Then the respective coefficients in Table 1 are used in either Eq. 1 or 2 to predict accuracy of discrimination of a colour distance input by user. Alternatively Equations 1–2 can be inverted to solve for the colour difference required for attaining a given accuracy level as shown in Table 1.

An important caveat of the model predictions are that these are based on quasi-monochromatic, narrow band stimuli which are not often observed on natural stimuli. This limitation implies that the accuracy of the predicted discrimination around threshold colour differences may be different to those potentially observed for natural stimuli. However we expect that discrimination accuracy for small colour differences between natural stimuli are within the uncertainty bounds of our functions (Fig. 2). Likewise it is also important to point out that none of the tested quasi-monochromatic stimuli used for our modelling correspond to those of lavender flowers often visited by *M. stellatarum* in natural environments and that

any potential effect of this preference in colour discrimination is not accounted for by the proposed models. Finally another important point to consider is that our model assumes a flat, achromatic background which is not representative of most naturally occurring scenarios. Indeed, colour discrimination in heterogenous background, as those typically occurring in nature, tend to be more difficult than when considering homogenous backgrounds and hymenopteran vision (Bukovac et al. 2017). Therefore, under most naturally occurring viewing conditions, accuracy of discrimination by *M. stellatarum* may be lower than the one predicted by our models.

Plant-pollinator interactions are one of the most conspicuous and well-studied fields of visual ecology (Kemp et al. 2019; Shrestha et al. 2019), dating back to seminal formulations of evolutionary theory proposed by Darwin (1876). Whilst it has been established for some time that colour is the main signal used by different pollinators as their respective colour discrimination capabilities match the colours that most frequently evolve (Chittka and Menzel 1992; Dyer et al. 2012), until recently it has remained difficult to interpret how individual pollinators of different species may process (dis)similar colours (Dyer and Chittka 2004a, Shrestha et al., 2019). The methods here proposed provide a valuable new solution to approach questions about complex plant-pollinator interactions involving the model hawkmoth *M. stellatarum*, based on empirical behavioural data reflecting the outcome of colour signal processing by the entire visual system of an observer.

Declarations

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Figures

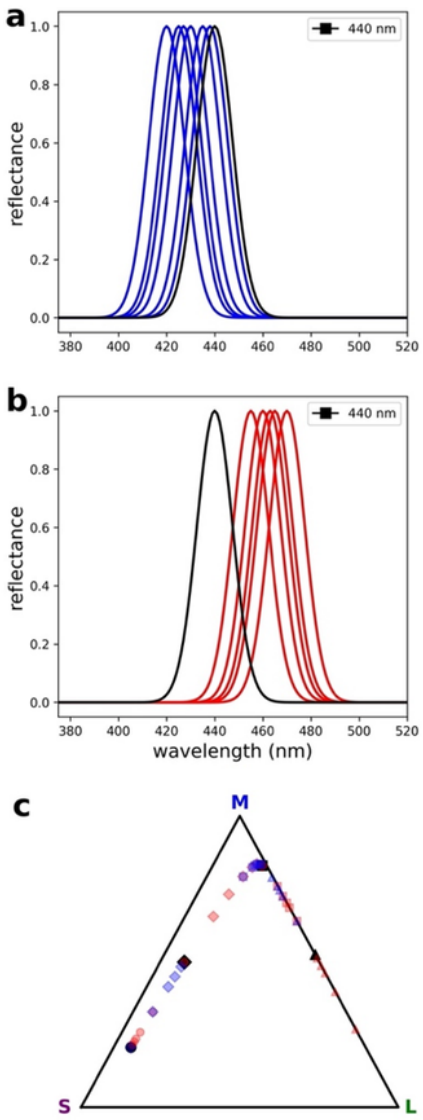


Fig 1 Colour modelling of the quasi-monochromatic stimuli used to derive the colour discrimination function for the hummingbird hawkmoth (*Macroglosum stellatarum*). Panel a depicts the spectral profile of the reference stimulus centred at 440 nm (black line) and the shorter wavelength stimuli (blue lines) tested in the discrimination experiments by Telles et al. (2016). Panel b illustrates the long wavelength (red lines) stimuli tested for the same reference stimulus. Panel c shows the loci corresponding to quasi-monochromatic reference stimuli centred at 380 (●), 400 (◆), 440 (■) and 480 (▲) nm and their respective short (blue markers) and long (red markers) wavelength test stimuli

Figure 1

See image above for figure legend.

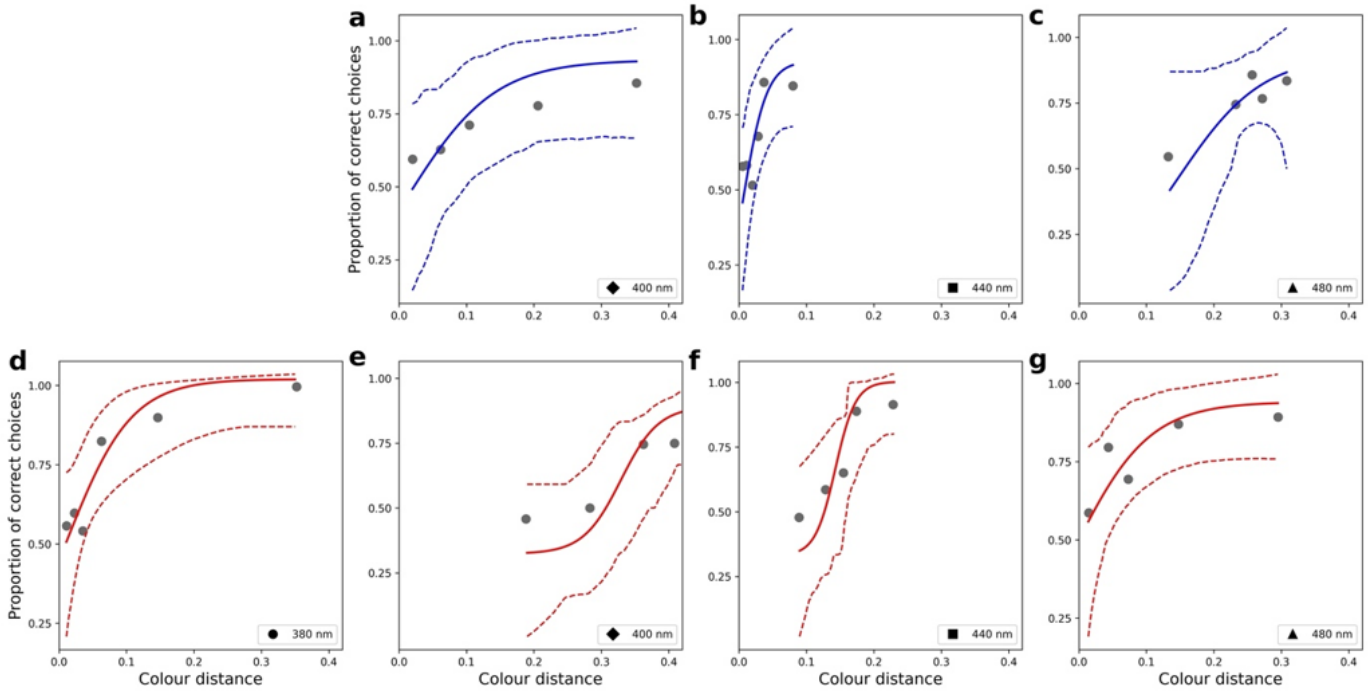


Fig 2 Continuous, colour discrimination functions for four different quasi-monochromatic reference stimuli centred at 380 (●), 400 (◆), 440 (■) and 480 (▲) nm predicting the probability of accurate discrimination of a reference and test stimuli (y-axis) dependent on their colour difference (x-axis), here expressed as Euclidean distance in the Maxwell triangle with a maximum height of 1.00. Panels a, b and c illustrate the colour discrimination functions for wavelengths shorter than reference stimuli centred at 400, 440 and 480 nm; dashed lines indicate the 95 % confidence interval (CI) for each function, whilst panels d, e, f and g depict the colour discrimination function and 95 % CI when considering test stimuli of longer wavelength relative to each reference. Solid markers in all panels represent the results of the pair choice behavioural experiments reported by Telles et al. (2016). Refer to Table 1 in the main text for details of the various stimuli tested for each reference, and values for the parameters defining each function.

Figure 2

See image above for figure legend.