TITLE: Hoverflies are imperfect mimics of wasp colouration

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ABSTRACT

Many Batesian mimics are considered to be inaccurate copies of their models, including a number of hoverfly species which are judged poor mimics of bees and wasps. This inaccuracy is surprising since more similar mimics are expected to deceive predators more frequently and therefore have greater survival. One suggested explanation is that mimics which appear inaccurate to human eyes may be perceived differently by birds, the probable agents of selection. In particular, if patterns contain an ultra-violet (UV) component, this would be visible to birds but overlooked by humans. So far, indirect comparisons have been made using human and bird responses to mimetic stimuli, but direct colour measurements of mimetic hoverflies are lacking. We took spectral readings from a wide range of hoverfly and wasp patterns which show very low reflectance in the UV range, and do not display any human-invisible colour boundaries. We modelled how the recorded spectra would be perceived by both birds and humans. While colour differences between wasps and hoverflies are slightly more distinct according to human visual abilities, bird vision is capable of discriminating the two taxa in almost all cases. We therefore conclude that hoverflies are imperfect mimics even in the “eye of the beholder”.

INTRODUCTION

Colour is widely used by animals as a signal, for example to attract mates (Andersson 1994) or as an anti-predator warning display (Ruxton et al 2004). However, colour is an experience as much as a physical property, and therefore the perceived signal depends on the visual and cognitive abilities of the observer (Endler 1990). For example, male blue tits (*Cyanistes caeruleus*) use an ultra-violet (UV) signal to attract a mate, which is striking to female conspecifics but invisible to humans (Andersson et al 1998). The butterfly *Heliconius numata* displays a colourful wing pattern that conveys different signals to other butterflies and to potential predators (Llaurens et al 2014). Cases like these demonstrate the importance of considering the signal receiver when assessing the colour component of any biological signal, and show that doing so can shed new light on well-studied systems.

Many well-defended prey use bright, conspicuous colour patterns to warn away potential predators (Poulton 1890). Some harmless organisms attempt to hijack this means of communication and deceive those same predators by mimicking the display of a more dangerous "model", in a process known as Batesian mimicry (Bates 1862). Mimetic displays can incorporate a range of different cues, including shape (Jones et al 2013), pattern (Bain et al 2007) and movement (Golding et al 2005), but among these, colour is thought to be particularly salient to predators (Aronsson and Gamberale-Stille 2012; Kazemi et al 2014; Marples et al 1994). Most experimental evidence suggests that Batesian mimics should gain the greatest protection by resembling their models as accurately as possible (Dittrich et al 1993; Lindström et al 1997). However, to human eyes there is great variation in the degree of resemblance between mimics and models in nature, which raises the question of why the less accurate mimics persist in the face of predicted selection towards perfect resemblance (Edmunds 2000; Kikuchi and Pfennig 2013).

One proposed solution to the problem is that a perceived lack of mimetic accuracy as observed by humans might be specific to our particular visual abilities (Cuthill and Bennett 1993). If mimicry is in the “eye of the beholder”, those mimics that we (as humans) consider to be inaccurate might be highly accurate when viewed by an observer with different sensory and cognitive capabilities. A key part of Cuthill and Bennett’s (1993) argument was that in systems with avian predators, the birds’ ability to detect ultra-violet (UV) light (Chen and Goldsmith 1986) might lead them to interpret patterns very differently to humans.

In their “eye of the beholder” hypothesis, Cuthill and Bennett (1993) make particular reference to hoverflies (Diptera: Syrphidae), which, together with their models, are a key study system for understanding the evolution of imperfect mimicry (see e.g. Azmeh et al 1998; Dittrich et al 1993; Holloway et al 2002; Penney et al 2012). The family comprises a large number of species, many of which are abundant and widespread, ranging from non-mimetic to highly accurate mimics of various hymenopteran models, with a wide range of inaccurate mimics in between (Gilbert 2005; Rotheray and Gilbert 2011). Predation from birds is thought to provide the main selective pressure on hoverfly colour patterns (Bain et al 2007; Gilbert 2005; Waldbauer 1988) and therefore it is vital to consider avian perception of the patterns. Suitable methods are well-developed for both collection of spectral data and its subsequent interpretation through the eyes of a particular observer (Endler 1990; Endler and Mielke 2005; Vorobyev and Osorio 1998). These methods have been used to investigate mimetic accuracy in animals such as fish (Cheney and Marshall 2009), butterflies (Llaurens et al 2014) and salamanders (Kraemer and Adams 2014), but to our knowledge, detailed colour analysis is conspicuously lacking for hoverflies and their hymenopteran models.

Most animals, including birds, are thought to perceive the chromatic (hue and saturation) and achromatic (brightness) components of colour separately, and the information in these different channels may be used in different contexts by the signal receiver (Giurfa et al 1997; Osorio et al 1999). Chromatic stimuli are useful for comparison among disparate objects, as the chromatic properties do not change much under different illumination conditions. Achromatic signals are strongly affected by illumination, but are useful for detecting local changes in spectral properties, such as at the border between two colour patches (Osorio et al 1999). From this, we predict two possible ways in which spectral properties could influence mimicry. The absolute values of chromatic stimuli could be important, as birds have been shown to learn and recognise particular colour combinations in potential prey (Aronsson and Gamberale-Stille 2012; Kazemi et al 2014; Svádová et al 2009). For achromatic stimuli, the absolute values are less likely to be relevant, since they are difficult to compare for samples that are separated in space and time (Osorio et al 1999). However, the achromatic contrast between colour patches within a single pattern will be easier to detect, and could form an important signal (Aronsson and Gamberale-Stille 2013).

In this study, we present data on both chromatic and achromatic components of the colours of wasp-mimicking hoverflies and their potential models, confirming that there is no “hidden” UV signal in the patterns of either taxon. Then, we interpret the colours through the eyes of avian predators and estimate the level of mimetic accuracy that is achieved. In doing so, we show that mimicry of wasp colours by hoverflies is, to varying degrees, imperfect.

MATERIALS AND METHODS

Specimens

Insects were collected using a hand net from wild communities in Nottinghamshire, UK and surrounding areas, during July to September 2014. Target insects were any hoverflies or stinging Hymenoptera bearing a two-colour (typically yellow and black) pattern (Fig. 1), but excluding bumblebees and their putative mimics because they are very likely part of a different mimicry ring (Gilbert 2005), and their hairiness makes taking reliable colour measurements difficult. A total of 247 individuals were identified to species level and sexed using relevant keys (Richards 1980; Stubbs and Falk 2002).

Specimens were euthanised by freezing for 10 to 20 minutes on the day of capture and then pinned. Colour measurements (see below) were taken within one hour of death to minimise any colour changes that might occur (colours of some species fade during the days following death: C Taylor, pers. obs.).

Eight different model species were sampled, but only four were found more than twice: *Apis mellifera* (N = 14), *Vespula vulgaris* (N = 10), *V. germanica* (N = 3) and *Vespa crabro* (N = 5). We know from both theory (Getty 1985) and experiments (Lindström et al 1997) that a model's importance in shaping predator behaviour increases with its abundance, and therefore we have excluded rare models (N < 3) from the bulk of the analysis. However, for comparison, we also conducted a repeat analysis using all eight model species.

Spectrophotometry

Reflectance measurements were taken using a 100 μm bifurcating optic fibre probe (Ocean Optics, Dunedin, FL, USA, custom spec) with one fibre connected to a pulsed xenon light source (Ocean Optics PX-2) and the other to a spectrophotometer (Ocean Optics USB 2000+ UV-VIS-ES). The probe was held steady and targeted using a micro-manipulator (Prior, Cambridge, UK). The probe was fixed at an angle of 45° to horizontal, and the patch under measurement was placed as close to horizontal as possible. A custom-made aluminium probe cover cut off at an angle of 45° aided with this alignment, and also helped to maintain a constant distance (approximately 2 mm) between the specimen and the probe (Endler 1990). The light source pulsed at a frequency of 50 Hz and spectral readings were integrated over 10 pulses, or 200 ms. Measurements were recorded for wavelengths over the range 300 to 700 nm at intervals of 0.4 nm. Measurements were taken in relation to a white standard (Ocean Optics WS-1 Diffuse Reflectance Standard) and recalibrated to the standard after approximately every four specimens in order to account for lamp drift. All measurements were taken in a dark room with the xenon lamp being the only source of light.

Pilot testing revealed that readings taken from within 0.2 mm of a colour border were inaccurate (see Appendix) and we therefore targeted the centres of insect colour patches that were at least 0.5 mm in width. In order to check that, in doing so, we did not overlook any colour boundaries invisible to humans, we moved the probe across adjacent areas and monitored any changes to the spectral read-out in real time. We recorded an example of such a process in the form of a transect along the abdomen of an individual of *Helophilus hybridus*, with intervals of 0.2 mm.

For each hoverfly or wasp specimen, we took spectral readings from both ‘black’ (low reflectance, black or dark red/brown to human eyes) and ‘coloured’ (higher reflectance, usually yellow or orange to human eyes) patches of the abdomen where possible. In a few cases, patches of one type were too small to take accurate readings and therefore we only recorded spectra of the predominant patch type in those cases. At least three readings were taken for both of the patch types (where present), ideally taken from different patches on different abdominal tergites, again limited in cases where patches were small or absent on some tergites.

Statistical analysis

Analysis was carried out in R version 3.1.2 (R Core Team 2014) making use of the package ‘pavo’ for spectral processing and visual models (Maia et al 2013). Noise was removed from the spectra using loess smoothing over a span of 0.4. Any smoothed spectra showing negative reflectance values, which can occasionally result from noise or a drift in calibration, were adjusted by adding a constant to the spectrum such that the minimum reflectance value was zero.

Given that the main selective pressure on hoverfly mimicry is considered to come from passerine birds (Dlusski 1984; Gilbert 2005) but that no single species stands out as an obvious candidate, we modelled the colour perception on a generalised ‘UV-type’ retina, with four cone types (U, S, M and L) with peak sensitivity at 372, 456, 544 and 609 nm respectively (Maia et al 2013; Ödeen and Håstad 2003). We used models of photon catch to calculate cone stimulation values for each spectrum, with illumination modelled as ‘D65’ daylight (Maia et al 2013; Vorobyev and Osorio 1998). We then used receptor noise models to calculate the chromatic (ΔS) or achromatic (ΔL) contrast between a given pair of spectra, with units of 'Just Noticeable Differences' or JNDs (Maia et al 2013; Vorobyev and Osorio 1998), and based on a Weber fraction (a measure of signal to noise ratio) of 0.06 (Olsson et al 2015).

In accordance with the way in which birds are thought to perceive spectral information (Osorio et al 1999), we analysed chromatic and achromatic components of the signals separately. To compare the chromatic properties of patterns from a pair of species, we treated black and coloured patch types separately, and calculated ΔS for each. We then made the assumption that, in attempting to discriminate two patterns, a predator will attend to the patch type within the patterns that shows the larger difference. Thus the chromatic distance within a given model-mimic pairing was taken as the larger of the ΔS values for black and coloured patches.

As achromatic signals are typically used to detect within-pattern variation (Osorio et al 1999), we calculated the within-pattern achromatic contrast (ΔL) between the black and coloured patches for each individual insect. We then calculated the absolute difference between model and mimic in values of within-pattern contrast as a measure of achromatic distance.

We repeated the model-mimic comparisons using a different visual model based on human vision, in order to examine any differences from bird perception. Human cone sensitivity data was taken from Stockman and Sharpe (2000) and we assumed a Weber fraction of 0.018 (Wyszecki and Stiles 2000). To compare achromatic perception between the two systems, we regressed human against avian estimates of within-pattern contrast across the insect species sampled, with the intercept fixed at zero. The slope value gives an estimate for the ratio in achromatic sensitivity between birds and human. We carried out similar regressions on chromatic contrast data, with separate regressions for the four different model species and for the two patch types (these eight sets of data could not be pooled as they are not independent of each other).

RESULTS

We examined spectra from 209 individual hoverflies of 33 species, and 38 individual Hymenoptera of eight species, sampling both “black” (very low reflectance) and “coloured” (higher reflectance; usually yellow or orange) patch types within the pattern. At no point did we detect a marked change in spectral properties of any individual that did not correspond to a human-visible boundary (see example with *Helophilus pendulus*, Fig. 2). None of the patterns sampled has a strong UV component in either the coloured or black patches (Fig. 3).

Human “Just Noticeable Difference” (JND) estimates for within-pattern achromatic contrast (ΔL) are related to but considerably higher than the avian equivalents (slope = 4.4, $r^2 = 0.996$, $p < 0.001$). Human and avian JND estimates for chromatic similarity (ΔS) are closer to each other, but human values are usually higher. Slopes for black patches range from 0.88 to 1.41, and for coloured patches from 1.19 to 1.94 (all $r^2 > 0.75$, $p < 0.001$; Fig. 4). Hence, the colour differences that we perceive among model and mimic species are generally rather larger than those evident to avian predators.

All remaining values in the results section are calculated with respect to avian vision. The four main model species (those with $N \geq 3$) are distinguishable from each other in terms of their spectra (Fig. 3). For coloured patches, chromatic contrast ranges from 2 to 12 JNDs

(Table S1). Differences among black patches are smaller, ranging from 0.6 to 4.7, with the largest differences being between *Vespa crabro* and the other three models. The three vespid species (*Vespa crabro, Vespula vulgaris* and *V. germanica*) have similar levels of within-pattern achromatic contrast ($\Delta L = 40$-45 JNDs), whereas contrast for *Apis mellifera* is much lower ($\Delta L = 22$; Table S1).

All of the mimic species sampled are theoretically distinguishable from each of the four main model species in chromatic terms, although some have ΔS values only just larger than one (e.g. *Episyrphus balteatus* differs from *A. mellifera* by $\Delta S = 1.3$; Table 1, Figs. S2 and S3). The species sampled are split roughly half and half between being most similar to *A. mellifera* (15) and *Vespula vulgaris* (14), with two being closest to *Vespa crabro* and two to *Vespula germanica*.

Achromatic differences span a wider range of values than chromatic differences, and are usually larger than the latter (Fig. 5). The hoverflies generally show lower within-pattern contrast than the Hymenoptera (Fig. S4), but some model-mimic pairings were highly similar in achromatic terms, with five mimics differing from their closest model by $\Delta L < 1$ (Table 1). When mimics are allocated to models according to the lowest achromatic difference, we find twelve mimics of *Vespula vulgaris*, ten mimics of *A. mellifera*, ten of *Vespa crabro* and one of *Vespula germanica*. Agreement between the chromatic and achromatic measures is poor – the closest model in chromatic terms matches the achromatic for only 14 of the 33 mimics (Table 1).

We repeated the above analysis taking into account all eight sampled species of Hymenoptera, including those with very low abundance. Results in this re-analysis were very similar, with only nine of 33 hoverfly species having one of these rare species as their closest model (Table S2).

DISCUSSION

This study represents the first attempt to characterise, in detail, the colours of hoverflies and their hymenopteran models. The lack of such studies to date is somewhat surprising, given the availability of suitable methods (Vorobyev and Osorio 1998), the existence of similar studies in other mimetic taxa (Cheney and Marshall 2009; Kraemer and Adams 2014; Llaurens et al 2014) and speculation over the potential presence of colour signals that are not visible to the human observer (Cuthill and Bennett 1993).

From our measurements of insect specimens, we find no evidence that there are pattern elements in either hoverflies or their models that are invisible to the human eye. In particular, across the wide range of species sampled, all showed very low levels of reflectance in the UV region of the spectrum, which would be invisible to humans but visible to birds if present. At no point during the data collection did we detect a colour boundary that was not visible by human eye. Some colour signals, such as those involved in sexual selection in blue tits (Andersson et al 1998) are restricted to the UV range and thus are easily overlooked by humans unless they are specifically targeted for study. Others signals appear to have evolved specifically to have different properties depending on the observer (Llaurens et al 2014). It is therefore very useful to confirm that the mimetic patterns of hoverflies are not hiding a further signal that is invisible to us as humans, a fact which until now has only been indirectly inferred (Green et al 1999; Penney et al 2012).

Model and mimic colours were usually less distinct (smaller JND values) when calculated using the avian as opposed to the human visual model. This tallies well with recent behavioural data, which have shown that humans are at least as good at discriminating colours as chickens are, thanks to lower levels of receptor noise (Olsson et al 2015). Nonetheless, none of the mimics differs from its nearest model by less than one avian JND, which implies that, in the eyes of birds, any given mimic-model pair should in theory be distinguishable in terms of colour (Vorobyev and Osorio 1998).

A number of researchers have speculated that a threshold of one JND may not be realistic in a natural context, instead adopting higher threshold values in the range two to four (Feeney et al 2014; Limeri and Morehouse 2014; Siddiqi et al 2004). However, a recent behavioural experiment confirmed that chicks were able to discriminate reliably between two colour signals that are separated by only one JND (Olsson et al 2015). That experiment used the relatively natural behavioural context of the chicks choosing between food sources (containers) of different visual properties. It is likely, though, that temporal separation of the stimuli, as experienced by a predator learning to discriminate between models and mimics, would increase the difficulty of the task (Dyer and Neumeyer 2005), and that therefore a slightly higher threshold might indeed apply in this context.

Importantly, there is considerable variation among species in the levels of mimetic accuracy, with several showing chromatic contrast of less than three JNDs with their nearest model, and others with values of 10 or more. In their natural context, it is possible that the most accurate hoverflies are more or less “perfect” colour mimics. However, contrary to the “eye of the beholder” hypothesis (Cuthill and Bennett 1993), those mimics at the lower end of the accuracy scale should be clearly distinguishable from their models. If birds do indeed provide the main selective pressure on hoverfly colours, the observed variation in mimetic accuracy cannot be explained solely by the eye of the beholder hypothesis.

Our data do hint at an alternative explanation for at least some instances of mimetic inaccuracy. The model species that we sampled were all distinguishable in terms of their colours; even *Vespula vulgaris* and *V. germanica*, two very closely related wasps, differ by five JNDs. Rather than learn each species and its colour entirely separately, which would carry a high cognitive burden, predators may generalise over a range of colours (Richards-Zawacki et al 2013; Veselý et al 2013), even if they are in theory distinguishable. This generalisation would probably include colours of some of the mimics, although it is unlikely that the least accurate mimics would be similar enough to be included (Figs. S2 and S3).

It is interesting to note that, in the majority of cases, achromatic distances between model and mimic are larger than chromatic distances (Fig. 5 and Table 1). This implies that the chromatic properties of the colour pattern may be under stronger selection from predators than the achromatic properties. The same appears to be true of mimetic salamanders (Kraemer and Adams 2014), another system in which birds are thought to provide the main selective pressure on colours. Birds may find it difficult to compare achromatic signals that are separated in time and space given changing light conditions (Osorio et al 1999). Furthermore, a bird's sensitivity to achromatic contrasts is dependent on the spatial scale, with maximum sensitivity at a visual angle of around one degree (Ghim and Hodos 2006). Typical hoverfly or wasp patterns vary over a much smaller scale than this at realistic viewing distances (e.g. 1 mm stripes would have a visual angle of around 0.05° at a distance of one metre), so birds may be relatively insensitive to achromatic differences within the pattern when making a decision on whether or not to attack.

Numerous experiments have demonstrated that colour is an important stimulus for predators attempting to discriminate among prey items (Kazemi et al 2014; Morrell and Turner 1970; Svádová et al 2009; Veselý et al 2013). However, all of these experiments have used stimuli that are well separated in colour space, corresponding to different named colour categories. More behavioural studies are needed to establish predator responses to colour stimuli that differ by more subtle degrees, and to separate the response to achromatic and chromatic properties of the stimulus.

We have demonstrated considerable variation in the similarity of hoverflies to their models in terms of colour, and have shown that this variation is of a great enough magnitude to be detected by avian predators. Many hoverflies are indeed imperfect colour mimics in the eyes of their beholders.

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REFERENCES

Andersson MB (1994) Sexual Selection. Princeton University Press, Princeton, NJ

Andersson S, Örnborg J and Andersson M (1998) Ultraviolet sexual dimorphism and assortative mating in blue tits. Proc R Soc Lond B Biol Sci 265:445-450

Aronsson M and Gamberale-Stille G (2012) Colour and pattern similarity in mimicry: evidence for a hierarchical discriminative learning of different components. Anim Behav 84:881-887

Aronsson M and Gamberale-Stille G (2013) Evidence of signaling benefits to contrasting internal color boundaries in warning coloration. Behav Ecol 24:349-354

Azmeh S, Owen J, Sørensen K, Grewcock D and Gilbert F (1998) Mimicry profiles are affected by human-induced habitat changes. Proc R Soc Lond B Biol Sci 265:2285-2290

Bain RS, Rashed A, Cowper VJ, Gilbert FS and Sherratt TN (2007) The key mimetic features of hoverflies through avian eyes. Proc R Soc Lond B Biol Sci 274:1949-1954

Bates HW (1862) XXXII. Contributions to an Insect Fauna of the Amazon Valley. Lepidoptera: Heliconidæ. Trans Linn Soc Lond 23:495-566

Chen DM and Goldsmith TH (1986) Four spectral classes of cone in the retinas of birds. J Comp Physiol A 159:473-479

Cheney KL and Marshall NJ (2009) Mimicry in coral reef fish: how accurate is this deception in terms of color and luminance? Behav Ecol 20:459-468

Cuthill IC and Bennett ATD (1993) Mimicry and the eye of the beholder. Proc R Soc Lond B Biol Sci 253:203-204

Dittrich W, Gilbert F, Green P, Mcgregor P and Grewcock D (1993) Imperfect mimicry: a pigeon's perspective. Proc R Soc Lond B Biol Sci 251:195-200

Dlusski G (1984) Are dipteran insects protected by their similarity to stinging Hymenoptera. Byull Mosk O-Va Ispytatelei Prirody Otd Biol 89:25-40

Dyer AG and Neumeyer C (2005) Simultaneous and successive colour discrimination in the honeybee (Apis mellifera). J Comp Physiol A 191:547-557

Edmunds M (2000) Why are there good and poor mimics? Biol J Linn Soc 70:459-466

Endler JA (1990) On the measurement and classification of colour in studies of animal colour patterns. Biol J Linn Soc 41:315-352

Endler JA and Mielke PWJ (2005) Comparing entire colour patterns as birds see them. Biol J Linn Soc 86:405-431

Feeney WE, Stoddard MC, Kilner RM and Langmore NE (2014) "Jack-of-all-trades" egg mimicry in the brood parasitic Horsfield's bronze-cuckoo? Behav Ecol 25:1365-1373

Getty T (1985) Discriminability and the sigmoid functional response: how optimal foragers could stabilize model-mimic complexes. Am Nat 125:239-256

Ghim MM and Hodos W (2006) Spatial contrast sensitivity of birds. J Comp Physiol A 192:523-534

Gilbert F (2005) The evolution of imperfect mimicry. In: M. Fellowes, G. Holloway and J. Rolff (eds) Insect Evolutionary Ecology. CABI, Wallingford, UK, pp 231-288

Giurfa M, Vorobyev M, Brandt R, Posner B and Menzel R (1997) Discrimination of coloured stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A 180:235-243

Golding YC, Edmunds M and Ennos AR (2005) Flight behaviour during foraging of the social wasp *Vespula vulgaris* (Hymenoptera: Vespidae) and four mimetic hoverflies (Diptera: Syrphidae) *Sericomyia silentis*, *Myathropa florea*, *Helophilus* sp. and *Syrphus* sp. J Exp Biol 208:4523-4527

Green PR, Gentle L, Peake TM, Scudamore RE, McGregor PK, Gilbert F and Dittrich WH (1999) Conditioning pigeons to discriminate naturally lit insect specimens. Behav Process 46:97-102

Holloway G, Gilbert F and Brandt A (2002) The relationship between mimetic imperfection and phenotypic variation in insect colour patterns. Proc R Soc Lond B Biol Sci 269:411-416

Jones RT, Poul YL, Whibley AC, Mérot C, ffrench-Constant RH and Joron M (2013) Wing shape variation associated with mimicry in butterflies. Evolution 67:2323-2334

Kazemi B, Gamberale-Stille G, Tullberg Birgitta S and Leimar O (2014) Stimulus salience as an explanation for imperfect mimicry. Curr Biol 24:965-969

Kikuchi DW and Pfennig DW (2013) Imperfect mimicry and the limits of natural selection. Q Rev Biol 88:297-315

Kraemer AC and Adams DC (2014) Predator perception of Batesian mimicry and conspicuousness in a salamander. Evolution 68:1197-1206

Limeri LB and Morehouse NI (2014) Sensory limitations and the maintenance of colour polymorphisms: viewing the ‘alba’ female polymorphism through the visual system of male Colias butterflies. Funct Ecol 28:1197-1207

Lindström L, Alatalo RV and Mappes J (1997) Imperfect Batesian mimicry—the effects of the frequency and the distastefulness of the model. Proc R Soc Lond B Biol Sci 264:149-153

Llaurens V, Joron M and Théry M (2014) Cryptic differences in colour among Müllerian mimics: how can the visual capacities of predators and prey shape the evolution of wing colours? J Evol Biol 27:531-540

Maia R, Eliason CM, Bitton P-P, Doucet SM and Shawkey MD (2013) pavo: an R package for the analysis, visualization and organization of spectral data. Methods Ecol Evol 4:906-913

Marples NM, van Veelen W and Brakefield PM (1994) The relative importance of colour, taste and smell in the protection of an aposematic insect Coccinella septempunctata. Anim Behav 48:967-974

Morrell GM and Turner JRG (1970) Experiments on mimicry: I. The response of wild birds to artificial prey. Behaviour 36:116-130

Ödeen A and Håstad O (2003) Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. Mol Biol Evol 20:855-861

Olsson P, Lind O and Kelber A (2015) Bird colour vision: behavioural thresholds reveal receptor noise. J Exp Biol 218:184-193

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

Penney HD, Hassall C, Skevington JH, Abbott KR and Sherratt TN (2012) A comparative analysis of the evolution of imperfect mimicry. Nature 483:461-464

Poulton EB (1890) The colours of animals: their meaning and use especially considered int he case of insects. Kegan Paul, Trench, Trubner and Co., London

R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing

Richards-Zawacki CL, Yeager J and Bart HPS (2013) No evidence for differential survival or predation between sympatric color morphs of an aposematic poison frog. Evol Ecol 27:783-795

Richards OW (1980) Scolioidea, Vespoidea and Sphecoidea; Hymenoptera, Aculeata. Royal Entomological Society of London, London, UK

Rotheray GF and Gilbert F (2011) The Natural History of Hoverflies. Forrest Text, Cardigan, UK

Ruxton GD, Sherratt TN and Speed MP (2004) Avoiding Attack: The Evolutionary Ecology of Crypsis, Warning Signals, and Mimicry. Oxford University Press, Oxford

Siddiqi A, Cronin TW, Loew ER, Vorobyev M and Summers K (2004) Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. J Exp Biol 207:2471-2485

Stockman A and Sharpe LT (2000) The spectral sensitivities of the middle- and long-wavelength-sensitive cones derived from measurements in observers of known genotype. Vision Res 40:1711-1737

Stubbs AE and Falk SJ (2002) British Hoverflies: An Illustrated Identification Guide. British Entomological and Natural History Society, Reading, UK

Svádová K, Exnerová A, Štys P, Landová E, Valenta J, Fučíková A and Socha R (2009) Role of different colours of aposematic insects in learning, memory and generalization of naïve bird predators. Anim Behav 77:327-336

Veselý P, Luhanová D, Prášková M and Fuchs R (2013) Generalization of mimics imperfect in colour patterns: the point of view of wild avian predators. Ethology 119:138-145

Vorobyev M and Osorio D (1998) Receptor noise as a determinant of colour thresholds. Proc R Soc Lond B Biol Sci 265:351-358

Waldbauer G (1988) Asynchrony between Batesian mimics and their models. Am Nat S103-S121

Wyszecki G and Stiles WS (2000) Color Science: Concepts and Methods, Quantitative Data and Formulae. Wiley, New York, NY

SUPPLEMENTARY MATERIAL

Supplementary text: testing spatial resolution.

Fig. S1. Testing the accuracy of spectral readings at small spatial resolutions.

Fig. S2. Coloured patches of models and mimics plotted in 2D colour space.

Fig. S3. Black patches of models and mimics plotted in 2D colour space.

Fig. S4. Histograms showing the distribution of values for contrast between black and coloured areas, across all model and mimic species sampled.

Table S1. Comparison of spectral properties among the four most abundant model species.

Table S2. A comparison of achromatic and chromatic similarity values when rare Hymenoptera are either excluded or included as possible models.

FIGURE LEGENDS

Fig. 1. Examples of colour and pattern variation in hymenopteran (**a** and **b**) and hoverfly (**c-j**) abdominal patterns. Scale bars each show 1 mm. **a** – *Vesupla vulgaris*. **b** – *Apis mellifera*. **c** – *Eristalis tenax*. **d** – *Eristalis pertinax*. **e** – *Melangyna labiatarum*. **f** – *Sericomyia silentis*. **g** – *Syrphus ribesii*. **h** – *Sphaerophoria scripta*. **i** – *Episyrphus balteatus*. **j** – *Platycheirus albimanus*.

Fig. 2. Colour variation along a transect on an abdomen of *Helophilus hybridus*. **a** – The abdomen, with locations of spectral readings shown in red. **b** – All 22 spectra from the transect. **c** – Variation in spectral brightness along the transect.

Fig. 3. Reflectance spectra for all sampled species with N > 3. Solid and dashed lines show means for black and coloured patches respectively, shaded areas show standard error. For species abbreviations, see Table 1.

Fig. 4. Comparison of estimates of chromatic contrast as calculated in models based on avian and human vision. Each point represents a single model-mimic pairing. Values are in units of Just Noticeable Difference. The two panels show data on black and coloured patch types separately. For model abbreviations, see caption for Table 1.

Fig. 5. Comparison of chromatic and achromatic distances between mimic species and each of the four main model species. Each point represents a single model-mimic species pairing. For model abbreviations, see caption for Table 1.

Table 1. Achromatic and chromatic distances of each hoverfly species to its closest model. All values are given in units of Just Noticeable Differences.

Species	Abbrev.	Achromatic			Chromatic		
		Internal contrast	Closest model[1]	Dist[2]	Closest model[1]	Dist[3]	Patch colour
Chrysotoxum arcuatum	Car	41.2	Vvu	1.2	Vge	9.3	Coloured
Dasysyrphus albostriatus	Dal	55.7	Vcr	10.4	Vvu	8.5	Coloured
Dasysyrphus tricinctus	Dtr	49.3	Vcr	4.0	Vvu	11.4	Coloured
Epistrophe grossulariae	Egr	37.5	Vvu	2.5	Ame	3.8	Black
Episyrphus balteatus	Eba	25.9	Ame	4.0	Ame	1.3	Coloured
Eristalis arbustorum	Ear	29.6	Ame	7.7	Ame	3.5	Black
Eristalis horticola	Eho	35.5	Vvu	4.5	Vvu	2.9	Coloured
Eristalis interruptus	Eip	32.8	Vvu	7.2	Vcr	5.4	Coloured
Eristalis pertinax	Epe	31.3	Vvu	8.7	Ame	1.6	Coloured
Eristalis tenax	Ete	27.4	Ame	5.6	Vcr	3.6	Black
Eupeodes latifasciatus	Ela	52.4	Vcr	7.0	Vge	2.6	Coloured
Helophilus hybridus	Hhy	45.9	Vcr	0.6	Vvu	1.9	Coloured
Helophilus pendulus	Hpe	37.9	Vvu	2.1	Vvu	2.2	Black
Leucozona glaucia	Lgl	35.4	Vvu	4.6	Ame	16.2	Coloured
Melangyna labiatarum	Mla	53.5	Vcr	8.2	Vvu	10.7	Coloured
Melanostoma scalare	Msc	27.7	Ame	5.8	Ame	2.6	Coloured
Meliscaeva auricollis	Mau	34.6	Vvu	5.4	Vvu	4.4	Coloured
Meliscaeva cinctella	Mci	30.3	Ame	8.4	Ame	3.4	Coloured
Myathropa florea	Mfl	34.1	Vvu	5.9	Vvu	3.9	Coloured

Parhelophilus versicolor	Pve	43.0	Vge	1.0	Vvu	2.7	Coloured
Platycheirus albimanus	Pal	28.7	Ame	6.8	Ame	10.5	Coloured
Platycheirus clypeatus	Pcl	22.9	Ame	1.0	Ame	2.2	Black
Platycheirus occultus	Poc	21.5	Ame	0.3	Ame	13.0	Coloured
Sericomyia silentis	Ssi	63.9	Vcr	18.6	Vvu	3.1	Black
Sphaerophoria scalare	Ssc	38.5	Vvu	1.5	Vvu	2.7	Coloured
Syritta pipiens	Spi	22.3	Ame	0.5	Ame	4.5	Coloured
Syrphus ribesii	Sri	51.1	Vcr	5.8	Vvu	4.9	Coloured
Syrphus torvus	Sto	44.9	Vcr	0.5	Vvu	7.3	Coloured
Syrphus vitripennis	Svi	46.8	Vcr	1.5	Vvu	5.5	Coloured
Volucella inanis	Vin	44.9	Vcr	0.4	Ame	5.7	Black
Volucella pellucens	Vpe	32.9	Vvu	7.1	Ame	8.2	Coloured
Volucella zonaria	Vzo	38.6	Vvu	1.4	Ame	3.8	Black
Xylota segnis	Xse	15.4	Ame	6.5	Ame	5.7	Coloured

[1] Model abbreviations: Ame = *Apis mellifera*, Vcr = *Vespa crabro*, Vge = *Vespula germanica*, Vvu = *Vespula vulgaris*.

[2] Achromatic distances are calculated as the absolute difference between values of internal pattern contrast (that is, the achromatic distance between coloured and black patches) between the model and mimic.

[3] Chromatic distances are the ΔS values between model and mimic for whichever patch type (coloured or black, indicated in the ‘patch colour’ column) has the larger ΔS.

Figure 1

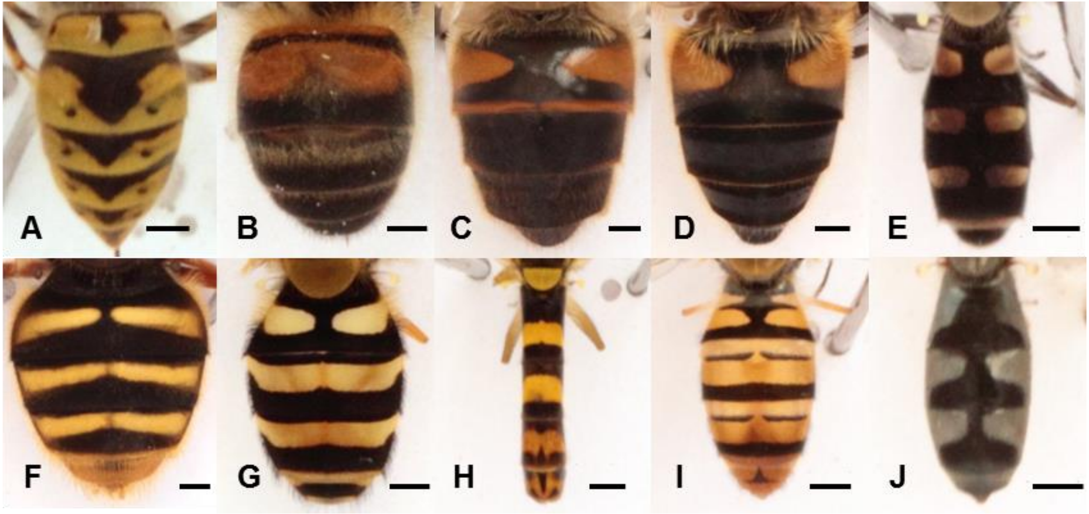


Figure 2

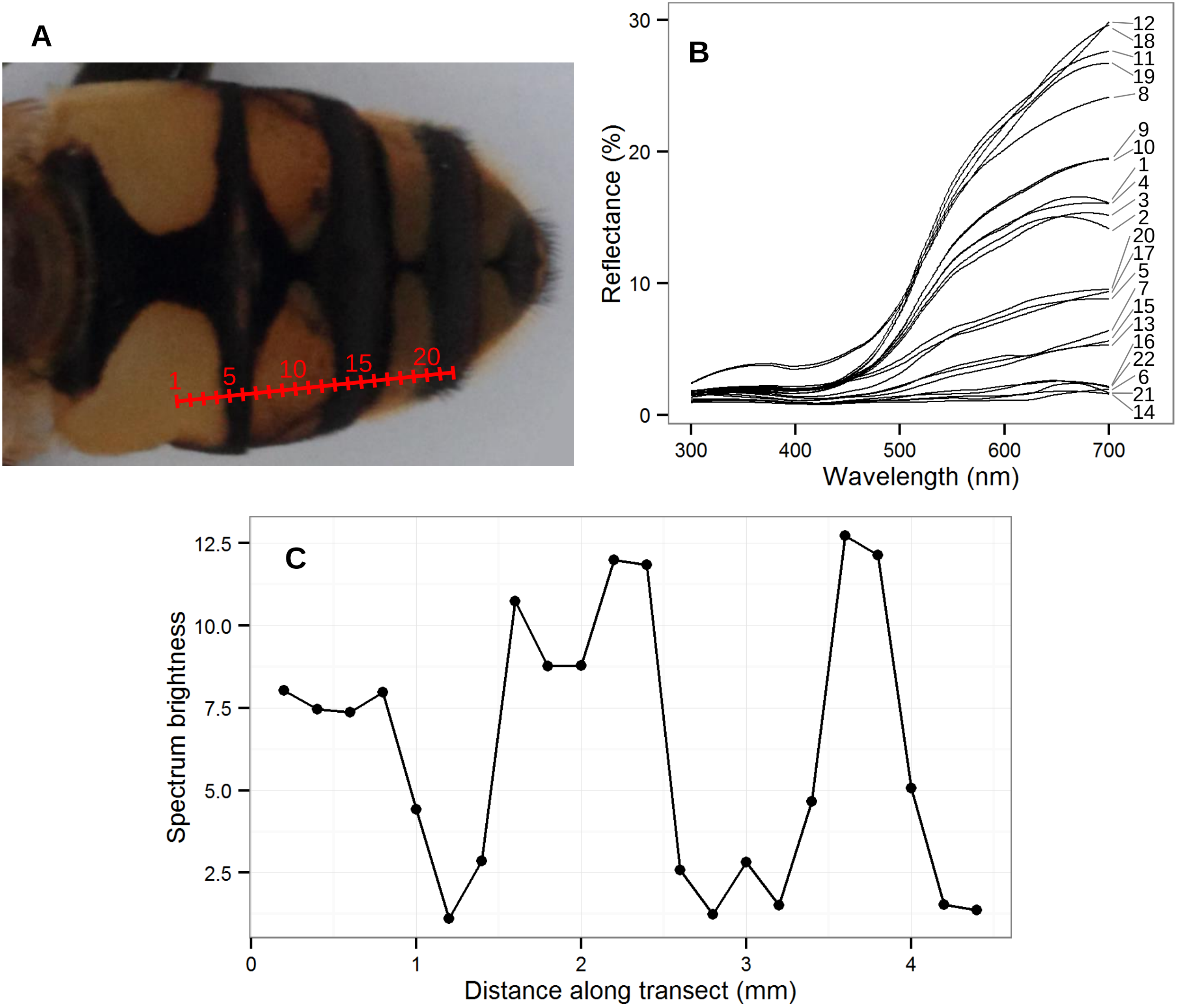

A
1
5
10
15
20
B
Reflectance (%)
30
20
10
0
300
400
500
600
700
Wavelength (nm)
12
18
11
19
8
9
10
1
4
3
2
20
17
5
7
15
13
16
22
6
21
14
C
Spectrum brightness
12.5
10.0
7.5
5.0
2.5
0
1
2
3
4
Distance along transect (mm)

Figure 3

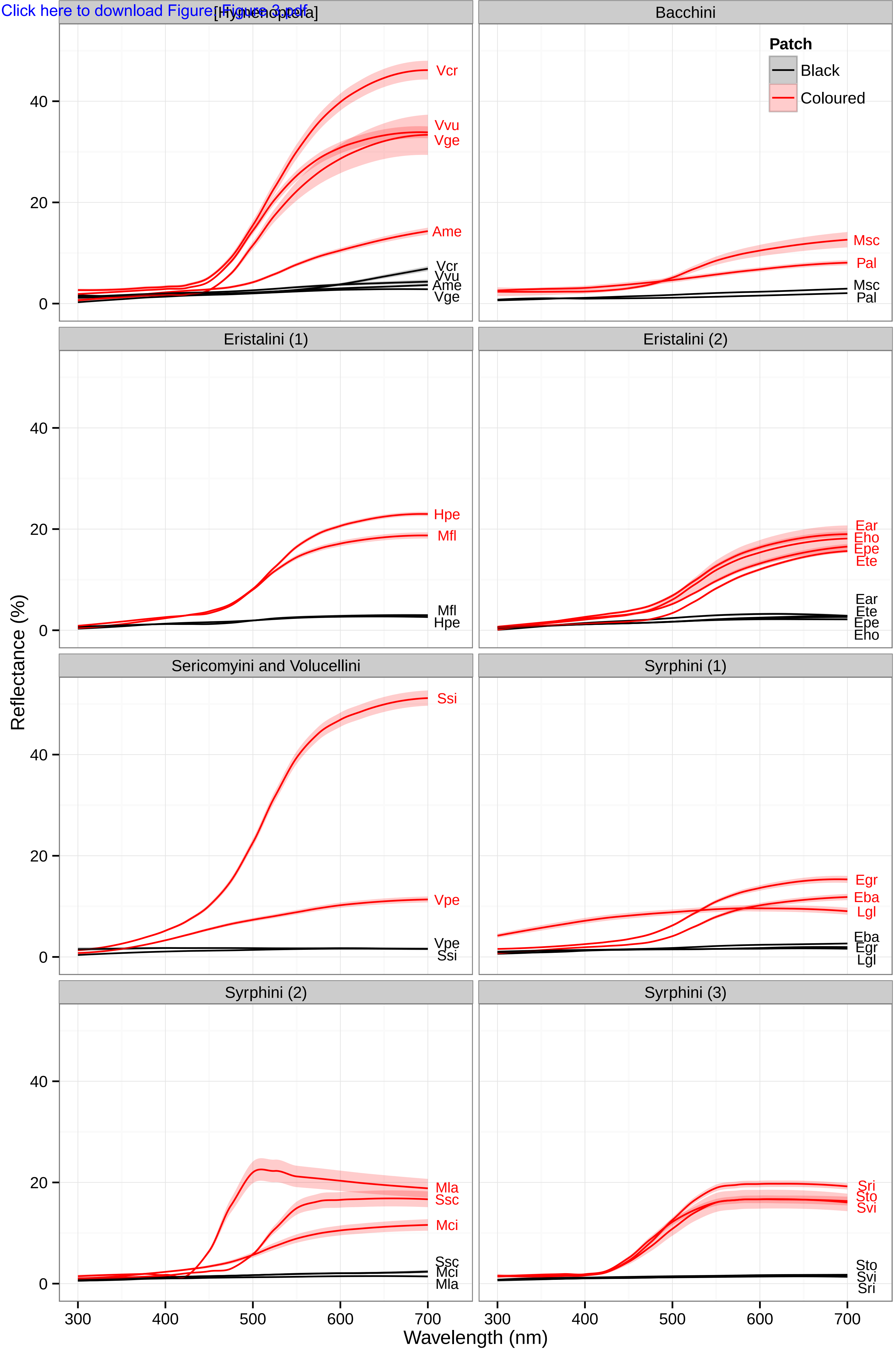

[Hymenoptera]
Vcr
Vvu
Vge
Ame
Vcr
Vvu
Ame
Vge
Bacchini
Patch
Black
Coloured
Msc
Pal
Msc
Pal
Eristalini (1)
Hpe
Mfl
Mfl
Hpe
Eristalini (2)
Ear
Eho
Epe
Ete
Ear
Ete
Epe
Eho
Sericomyini and Volucellini
Ssi
Vpe
Vpe
Ssi
Syrphini (1)
Egr
Eba
Lgl
Eba
Egr
Lgl
Syrphini (2)
Mla
Ssc
Mci
Ssc
Mci
Mla
Syrphini (3)
Sri
Sto
Svi
Sto
Svi
Sri
Reflectance (%)
0
20
40
300
400
500
600
700
Wavelength (nm)

Figure 4

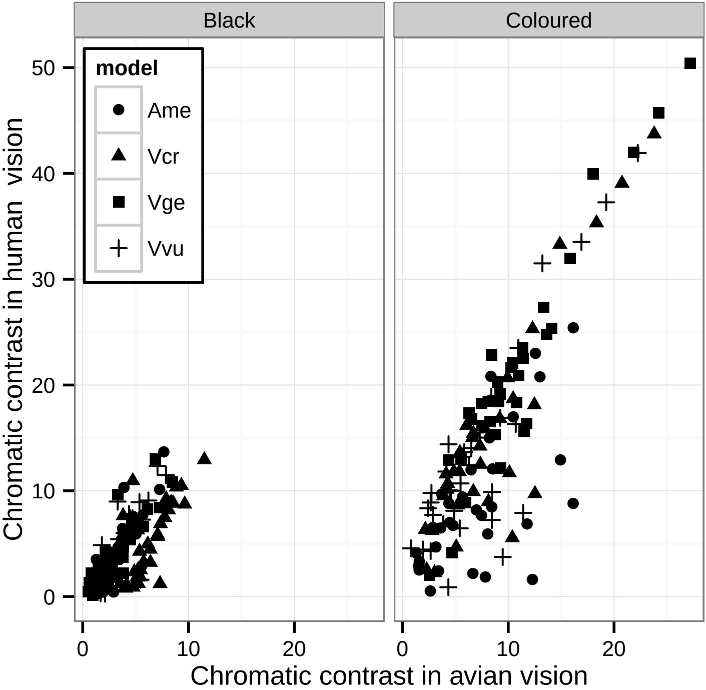
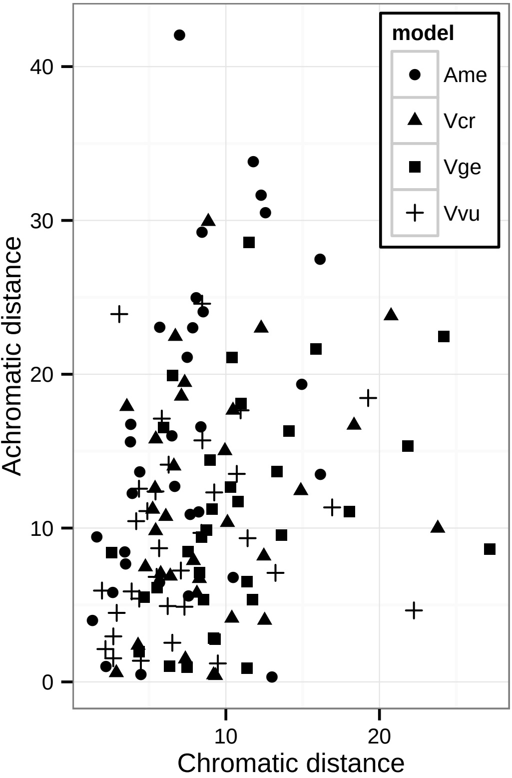

Black
Coloured
model
● Ame
▲ Vcr
■ Vge
+ Vvu
Chromatic contrast in human vision
0
10
20
30
40
50
0
10
20
0
10
20
Chromatic contrast in avian vision

Figure 5


model
● Ame
▲ Vcr
■ Vge
+ Vvu
Achromatic distance
0
10
20
30
40
Chromatic distance
10
20