



Reader, Tom (2016) Hoverflies are imperfect mimics of wasp colouration. *Evolutionary Ecology*, 30 (3). pp. 567-581. ISSN 1573-8477

**Access from the University of Nottingham repository:**

<http://eprints.nottingham.ac.uk/34272/1/Taylor%20et%20al%202016%20-%20Hoverfly%20and%20Wasp%20Colouration%20-%20for%20archiving.pdf>

**Copyright and reuse:**

The Nottingham ePrints service makes this work by researchers of the University of Nottingham available open access under the following conditions.

This article is made available under the University of Nottingham End User licence and may be reused according to the conditions of the licence. For more details see: [http://eprints.nottingham.ac.uk/end\\_user\\_agreement.pdf](http://eprints.nottingham.ac.uk/end_user_agreement.pdf)

**A note on versions:**

The version presented here may differ from the published version or from the version of record. If you wish to cite this item you are advised to consult the publisher's version. Please see the repository url above for details on accessing the published version and note that access may require a subscription.

For more information, please contact [eprints@nottingham.ac.uk](mailto:eprints@nottingham.ac.uk)

The final publication is available at Springer via <http://link.springer.com/article/10.1007%2Fs10682-016-9824-9>.

1 TITLE: Hoverflies are imperfect mimics of wasp colouration  
2  
3 CORRESPONDING AUTHOR: Christopher Taylor  
4 University of Nottingham  
5 School of Life Sciences, University Park, Nottingham, NG7 2RD  
6 christopher.taylor@nottingham.ac.uk  
7 AUTHORS: Christopher H. Taylor<sup>1</sup>, Tom Reader<sup>1</sup>, Francis Gilbert<sup>1</sup>  
8 <sup>1</sup>University of Nottingham, School of Life Sciences, University Park, Nottingham, NG7 2RD  
9 RUNNING TITLE: Hoverflies are imperfect mimics of wasp colouration  
10  
11 KEYWORDS: Spectrophotometry; Colour analysis; Visual model; Just Noticeable  
12 Difference; Batesian mimicry; Syrphidae  
13 WORD COUNTS: 4094 (total)  
14 1070 (introduction); 1257 (materials and methods); 534 (results); 1233 (discussion)  
15 NUMBER OF REFERENCES: 57  
16 FIGURES AND TABLES: 5 figures and 1 table.  
17 LIST OF APPENDICES: Supplementary text, Figs. S1-S4, Tables S1-S2.

18 ABSTRACT

19

20 Many Batesian mimics are considered to be inaccurate copies of their models, including a  
21 number of hoverfly species which appear to be poor mimics of bees and wasps. This  
22 inaccuracy is surprising since more similar mimics are expected to deceive predators more  
23 frequently and therefore have greater survival. One suggested explanation is that mimics  
24 which appear inaccurate to human eyes may be perceived differently by birds, the probable  
25 agents of selection. For example, if patterns contain an ultra-violet (UV) component, this  
26 would be visible to birds but overlooked by humans. So far, indirect comparisons have been  
27 made using human and bird responses to mimetic stimuli, but direct colour measurements of  
28 mimetic hoverflies are lacking. We took spectral readings from a wide range of hoverfly and  
29 wasp patterns. They show very low reflectance in the UV range, and do not display any  
30 human-invisible colour boundaries. We modelled how the recorded spectra would be  
31 perceived by both birds and humans. While colour differences between wasps and hoverflies  
32 are slightly more distinct according to human visual abilities, bird vision is capable of  
33 discriminating the two taxa in almost all cases. We discuss a number of factors that might  
34 make the discrimination task more challenging for a predator in the field, which could  
35 explain the apparent lack of selection for accurate colour mimicry.

36

37

38 INTRODUCTION

39

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

50

51 Some harmless organisms attempt to deceive predators by mimicking the display of a more  
52 dangerous “model”, in a process known as Batesian mimicry (Bates 1862). Mimetic displays  
53 can incorporate a range of different cues, including shape (Jones et al 2013), pattern (Bain et  
54 al 2007) and movement (Golding et al 2005), but among these, colour is thought to be  
55 particularly salient to predators (Marples et al 1994, Aronsson and Gamberale-Stille 2012,  
56 Kazemi et al 2014). Most experimental evidence suggests that Batesian mimics should gain  
57 the greatest protection by resembling their models as accurately as possible (Dittrich et al  
58 1993, Lindström et al 1997). However, to human eyes there is great variation in the degree of  
59 resemblance between mimics and models in nature, which raises the question of why the less  
60 accurate mimics persist in the face of predicted selection towards perfect resemblance  
61 (Edmunds 2000, Kikuchi and Pfennig 2013).

62

63 One proposed solution to the problem is that a perceived lack of mimetic accuracy as  
64 observed by humans might be specific to our particular visual abilities (Cuthill and Bennett  
65 1993). If mimicry is in the “eye of the beholder”, those mimics that we (as humans) consider  
66 to be inaccurate might be highly accurate when viewed by an observer with different sensory  
67 and cognitive capabilities. A key part of Cuthill and Bennett’s (1993) argument was that in  
68 systems with avian predators, the birds’ ability to detect UV light (Chen and Goldsmith 1986)  
69 might lead them to interpret patterns very differently to humans.

70

71 In their “eye of the beholder” hypothesis, Cuthill and Bennett (1993) make particular  
72 reference to hoverflies (Diptera: Syrphidae), which, together with their models, are a key  
73 study system for understanding the evolution of imperfect mimicry (see e.g. Dittrich et al  
74 1993, Azmeh et al 1998, Holloway et al 2002, Penney et al 2012). The family comprises a  
75 large number of species, many of which are abundant and widespread, ranging from non-  
76 mimetic to highly accurate mimics of various hymenopteran models (bees and wasps; Apidae  
77 and Vespidae), with a wide range of accuracy in between (Gilbert 2005, Rotheray and Gilbert  
78 2011).

79

80 Predation from birds is thought to provide the main selective pressure on hoverfly colour  
81 patterns (Waldbauer 1988, Gilbert 2005, Bain et al 2007). Bees and wasps are unpalatable to  
82 most birds due to their sting and tough cuticle (Mostler 1935), although a few specialists do  
83 prey on them despite these defences, usually by removing the sting prior to consumption  
84 (Birkhead 1974). Hoverflies do not appear to have any chemical or physical defence, being  
85 readily accepted and consumed by birds that have not previously encountered a model  
86 (Mostler 1935). Some models, such as *Apis mellifera* and *Vespula vulgaris*, are widespread in  
87 the Palearctic and overlap extensively with the ranges of many hoverfly species, while other

88 potential models (e.g. *Ectemnius cavifrons*) are more restricted geographically and may be  
89 present in only part of the range of a given mimic (Richards 1980). Most hoverflies, bees and  
90 wasps are also likely to overlap over a finer, microhabitat scale, with many being frequent  
91 flower visitors. In the UK, most species of hoverfly first emerge between March and May and  
92 remain active until at least September (Stubbs and Falk 2002), with workers of social  
93 Hymenoptera generally reaching peak abundance in July/August (Richards 1980).

94

95 Given the discrepancy between bird and human visual abilities, it is vital to consider avian  
96 perception of the mimetic signals (Cuthill and Bennett 1993). Suitable methods are well-  
97 developed for both collection of spectral data and its subsequent interpretation through the  
98 eyes of a particular observer (Endler 1990, Vorobyev and Osorio 1998, Endler and Mielke  
99 2005). These methods have been used to investigate mimetic accuracy in animals such as fish  
100 (Cheney and Marshall 2009), butterflies (Llaurens et al 2014) and salamanders (Kraemer and  
101 Adams 2014), but to our knowledge, detailed colour analysis is lacking for hoverflies and  
102 their hymenopteran models.

103

104 Most animals, including birds, are thought to perceive the chromatic (hue and saturation) and  
105 achromatic (brightness) components of colour separately, and the information in these  
106 different channels may be used in different contexts by the signal receiver (Giurfa et al 1997,  
107 Osorio et al 1999). Chromatic stimuli are useful for comparison among disparate objects, as  
108 the chromatic properties do not change much under different illumination conditions.

109 Achromatic signals are strongly affected by illumination, but are useful for detecting local  
110 changes in spectral properties, such as at the border between two colour patches (Osorio et al  
111 1999). From this, we predict two possible ways in which spectral properties could be used by  
112 an observer to discriminate between models and mimics. The absolute values of chromatic

113 stimuli could be important, as birds have been shown to learn and recognise particular colour  
114 combinations in potential prey (Svádová et al 2009, Aronsson and Gamberale-Stille 2012,  
115 Kazemi et al 2014). For achromatic stimuli, the absolute values are less likely to be relevant,  
116 since they are difficult to compare for samples that are separated in space and time (Osorio et  
117 al 1999). However, the achromatic contrast between colour patches within a single pattern  
118 will be easier to detect, and could form an important signal (Aronsson and Gamberale-Stille  
119 2013).

120

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

126

## 127 MATERIALS AND METHODS

128

### 129 **Specimens**

130

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



138

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

143

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

150

### 151 **Spectrophotometry**

152

153 Reflectance measurements were taken using a 100 µm bifurcating optic fibre probe (Ocean  
154 Optics, Dunedin, FL, USA, custom spec) with one fibre connected to a pulsed xenon light  
155 source (Ocean Optics PX-2) and the other to a spectrophotometer (Ocean Optics USB 2000+  
156 UV-VIS-ES). The probe was held steady and targeted using a micro-manipulator (Prior,  
157 Cambridge, UK). The probe was fixed at an angle of 45° to horizontal, and the patch under  
158 measurement was placed as close to horizontal as possible. A custom-made aluminium probe  
159 cover cut off at an angle of 45° aided with this alignment, and also helped to maintain a  
160 constant distance (approximately 2 mm) between the specimen and the probe (Endler 1990).  
161 The light source pulsed at a frequency of 50 Hz and spectral readings were integrated over 10  
162 pulses, or 200 ms. Measurements were recorded for wavelengths over the range 300 to 700

163 nm at intervals of 0.4 nm. Measurements were taken in relation to a white standard (Ocean  
164 Optics WS-1 Diffuse Reflectance Standard) and recalibrated to the standard after  
165 approximately every four specimens in order to account for lamp drift. All measurements  
166 were taken in a dark room with the xenon lamp being the only source of light.

167

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

175

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

184

## 185 **Spectral analysis**

186

187 Analysis was carried out in R version 3.1.2 (R Core Team 2014) making use of the package  
188 ‘pavo’ for spectral processing and visual models (Maia et al 2013). Noise was removed from  
189 the spectra using loess smoothing over a span of 0.4. Any smoothed spectra showing negative  
190 reflectance values, which can occasionally result from noise or a drift in calibration, were  
191 adjusted by adding a constant to the spectrum such that the minimum reflectance value was  
192 zero. “Brightness” was calculated as the mean reflectance value across the whole spectrum  
193 (300 to 700 nm).

194

195 Given that the main selective pressure on hoverfly mimicry is considered to come from  
196 passerine birds (Dlusski 1984, Gilbert 2005) but that no single species stands out as an  
197 obvious candidate, we modelled the colour perception on a generalised ‘UV-type’ retina, with  
198 four cone types (U, S, M and L) with peak sensitivity at 372, 456, 544 and 609 nm  
199 respectively (Ödeen and Håstad 2003, Maia et al 2013). Achromatic stimulation was based  
200 on a blue tit double cone with peak sensitivity of 566 nm. We used models of photon catch to  
201 calculate cone stimulation values for each spectrum (Vorobyev and Osorio 1998, Maia et al  
202 2013). Given the wide range of species included in this study, it is not possible to record  
203 precise illumination conditions that will be valid for all sampled individuals; however all  
204 were collected from locations with low canopy cover, and therefore we modelled illumination  
205 as ‘D65’, which is representative of daylight in open areas (Endler 1993). We then used  
206 receptor noise models to calculate the chromatic ( $\Delta S$ ) or achromatic ( $\Delta L$ ) contrast between a  
207 given pair of spectra, with units of ‘Just Noticeable Differences’ or JNDs (Vorobyev and  
208 Osorio 1998, Maia et al 2013), and based on a Weber fraction (a measure of signal to noise  
209 ratio) of 0.06 (Olsson et al 2015).

210

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

219

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

224

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

236  
237  
238  
239  
240  
241  
242  
243  
244  
245  
246  
247  
248  
249  
250  
251  
252  
253  
254  
255  
256  
257  
258  
259

## 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 ( $\Delta L$ ) 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 ( $\Delta S$ ) 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-

260 pattern achromatic contrast ( $\Delta L = 40\text{-}45$  JNDs), whereas contrast for *Apis mellifera* is much  
261 lower ( $\Delta L = 22$ ; Table 1).

262

263 All of the mimic species sampled are theoretically distinguishable from each of the four main  
264 model species in chromatic terms, although some have  $\Delta S$  values only just larger than one  
265 (e.g. *Episyrphus balteatus* differs from *A. mellifera* by  $\Delta S = 1.3$ ; Table 1, Figs. S2 and S3).

266 The species sampled are split roughly half and half between being most similar to *A.*  
267 *mellifera* (15) and *Vespula vulgaris* (14), with two being closest to *Vespa crabro* and two to  
268 *Vespula germanica*.

269

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

279

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

284

285 DISCUSSION

286

287 This study represents the first attempt to characterise, in detail, the colours of hoverflies and  
288 their hymenopteran models. From our measurements of insect specimens, we find no  
289 evidence that there are pattern elements or colour boundaries in either hoverflies or their  
290 models that are invisible to the human eye, a fact which until now has only been indirectly  
291 inferred (Green et al 1999, Penney et al 2012). Our estimates of colour similarity according to  
292 the sensory abilities of humans and birds correlated closely with one another.

293

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

301

302 A number of researchers have speculated that a threshold of one JND may not be realistic in a  
303 natural context, instead adopting higher threshold values in the range two to four (Siddiqi et  
304 al 2004, Feeney et al 2014, Limeri and Morehouse 2014). There is no behavioural evidence to  
305 support the choice of a particular threshold other than one (Olsson et al 2015) but there are a  
306 number of factors which might make the discrimination task more difficult for a predator in  
307 the wild than in the controlled laboratory settings on which JND values are based. Firstly,  
308 predators will rarely have the opportunity to compare two prey items side by side. Temporal

309 separation of the stimuli, as experienced by a predator learning to discriminate between  
310 models and mimics, will increase the difficulty of the task (Dyer and Neumeyer 2005).

311

312 Secondly, predators may not have the opportunity to view their prey from close range before  
313 deciding whether to attack, reducing the visual information available. It is not clear at what  
314 typical distance a bird might make its decision. Dlusski (1984) showed that hoverflies would  
315 not give flight until a mock predator approached to within 30 cm or less, suggesting that  
316 relatively close inspection might be possible on occasion, but he also observed that  
317 insectivorous birds could find prey from a distance of 10 m or more. The spatial resolution of  
318 the perceived pattern will decline with distance. Passerines can discriminate objects separated  
319 by a visual angle of about 1-3 arc minutes (0.017 to 0.05 °; Donner 1951). This would give a  
320 spatial resolution of about 0.2 mm at a distance of 30 cm, which would be enough to perceive  
321 the pattern of most hoverflies, but even at a distance of a few metres, very little detail would  
322 be visible (2 mm resolution at a distance of 3 m). At that range, rather than perceiving  
323 separate patches of colour, the bird would perceive a spectrum that is an average of the two  
324 spectra (weighted by area), but it might still be able to learn differences among species based  
325 on this colour information.

326

327 Thirdly, if the prey is moving, this may make the discrimination more difficult. Notably,  
328 motion creates blur which will make borders within the colour pattern less distinct, although  
329 (at least in humans) cognitive processing can remove much of the blur (Burr 1980). Hoverflies  
330 are fast fliers and highly active, but one might expect birds to target them when they are at  
331 rest on flowers or vegetation rather than in flight (Dlusski 1984), which would minimise the  
332 impact of blur. Motion blur would have a similar effect to the low resolutions described  
333 above, in that above a certain level, the pattern would not be visible, and colours of different



334 patches would become merged together. However, a strong colour difference would certainly  
335 still be visible under those circumstances.

336

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

346

347 Our data do hint at an alternative explanation for at least some instances of mimetic  
348 inaccuracy. The model species that we sampled were all distinguishable in terms of their  
349 colours; even *Vespula vulgaris* and *V. germanica*, two very closely related wasps, differ by  
350 five JNDs. Models and mimics do not segregate neatly according to their colour (Figures S2  
351 and S3). In contrast to other characteristics such as antenna length, in which models and  
352 mimics are consistently separated (Penney et al 2012), there is no simple rule that could be  
353 followed to reliably distinguish hoverflies from wasps on the basis of colour. A predator can  
354 therefore adopt one of two strategies: it can learn each species and its colour entirely  
355 separately, which would carry a high cognitive burden as well as requiring repeated sampling  
356 of potentially dangerous prey (Kikuchi and Sherratt 2015), or it may generalise over a range  
357 of colours (Richards-Zawacki et al 2013, Veselý et al 2013). In the latter strategy, a large area  
358 of colour space would be protected, including many (though perhaps not all) of the hoverfly

359 colours (Figs. S2 and S3), and this would explain the lack of selection for increased mimetic  
360 accuracy in those cases.

361

362 It is interesting to note that, in the majority of cases, achromatic distances between model and  
363 mimic are larger than chromatic distances (Fig. 5 and Table 1). This implies that the  
364 chromatic properties of the colour pattern may be under stronger selection from predators  
365 than the achromatic properties. The same appears to be true of mimetic salamanders  
366 (Kraemer and Adams 2014), another system in which birds are thought to provide the main  
367 selective pressure on colours. Birds may find it difficult to compare achromatic signals that  
368 are separated in time and space given changing light conditions (Osorio et al 1999). In  
369 addition, their sensitivity to achromatic contrast declines at small spatial scales ( $< 1^\circ$  visual  
370 angle; Ghim and Hodos 2006), meaning that they may exert little selection for accurate  
371 mimicry of luminance properties.

372

373 Numerous experiments have demonstrated that colour is an important stimulus for predators  
374 attempting to discriminate among prey items (Morrell and Turner 1970, Svádová et al 2009,  
375 Veselý et al 2013, Kazemi et al 2014). However, all of these experiments have used stimuli  
376 that are well separated in colour space, corresponding to different named colour categories.  
377 More behavioural studies are needed to establish predator responses to colour stimuli that  
378 differ by more subtle degrees, and to separate the response to achromatic and chromatic  
379 properties of the stimulus. Most importantly, future work on inaccurate mimicry must  
380 consider the natural history of the predator-prey interactions in order to take account of  
381 highly influential variables such as viewing distance and movement.

382

383 ACKNOWLEDGEMENTS

384 We would like to thank John Endler for advice on spectrophotometry methods, Mark  
385 Strickland for manufacture of our custom probe-cover, and two anonymous reviewers for  
386 invaluable comments on our manuscript. This research was partly funded by a small  
387 equipment grant from the University of Nottingham.

388

389

390 REFERENCES

391

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

393 Andersson S, Örnborg J, Andersson M (1998) Ultraviolet sexual dimorphism and assortative  
394 mating in blue tits. *Proc R Soc Lond B* 265(1395):445-450

395 Aronsson M, Gamberale-Stille G (2012) Colour and pattern similarity in mimicry: evidence  
396 for a hierarchical discriminative learning of different components. *Anim Behav* 84(4):881-  
397 887

398 Aronsson M, Gamberale-Stille G (2013) Evidence of signaling benefits to contrasting internal  
399 color boundaries in warning coloration. *Behav Ecol* 24(2):349-354

400 Azmeh S, Owen J, Sørensen K, Grewcock D, Gilbert F (1998) Mimicry profiles are affected  
401 by human-induced habitat changes. *Proc R Soc Lond B* 265(1412):2285-2290

402 Bain RS, Rashed A, Cowper VJ, Gilbert FS, Sherratt TN (2007) The key mimetic features of  
403 hoverflies through avian eyes. *Proc R Soc Lond B* 274(1621):1949-1954

404 Bates HW (1862) XXXII. Contributions to an Insect Fauna of the Amazon Valley.  
405 Lepidoptera: Heliconidæ. *Trans Linn Soc Lond* 23(3):495-566

406 Birkhead TR (1974) Predation by birds on social wasps. *Br Birds* 67(6):221-229

407 Burr D (1980) Motion smear. *Nature* 284(5752):164-165

408 Chen DM, Goldsmith TH (1986) Four spectral classes of cone in the retinas of birds. *J Comp*  
409 *Physiol A* 159(4):473-479

410 Cheney KL, Marshall NJ (2009) Mimicry in coral reef fish: how accurate is this deception in  
411 terms of color and luminance? *Behav Ecol* 20(3):459-468

412 Cuthill IC, Bennett ATD (1993) Mimicry and the eye of the beholder. *Proc R Soc Lond B*  
413 253(1337):203-204

414 Dittrich W, Gilbert F, Green P, Mcgregor P, Grewcock D (1993) Imperfect mimicry: a  
415 pigeon's perspective. Proc R Soc Lond B 251(1332):195-200

416 Dlusski G (1984) Are dipteran insects protected by their similarity to stinging Hymenoptera?  
417 Byull Mosk O-Va Ispyt Prir Otd Biol 89:25-40

418 Donner KO (1951) The visual acuity of some passerine birds. Acta Zool Fenn 66:1-40

419 Dyer AG, Neumeyer C (2005) Simultaneous and successive colour discrimination in the  
420 honeybee (*Apis mellifera*). J Comp Physiol A 191(6):547-557

421 Edmunds M (2000) Why are there good and poor mimics? Biol J Linn Soc 70(3):459-466

422 Endler JA (1990) On the measurement and classification of colour in studies of animal colour  
423 patterns. Biol J Linn Soc 41(4):315-352

424 Endler JA (1993) The color of light in forests and its implications. Ecol Monogr 63(1):2-27

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

427 Feeney WE, Stoddard MC, Kilner RM, Langmore NE (2014) “Jack-of-all-trades” egg  
428 mimicry in the brood parasitic Horsfield’s bronze-cuckoo? Behav Ecol 25(6):1365-1373

429 Getty T (1985) Discriminability and the sigmoid functional response: how optimal foragers  
430 could stabilize model-mimic complexes. Am Nat 125(2):239-256

431 Ghim MM, Hodos W (2006) Spatial contrast sensitivity of birds. J Comp Physiol A  
432 192(5):523-534

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

435 Giurfa M, Vorobyev M, Brandt R, Posner B, Menzel R (1997) Discrimination of coloured  
436 stimuli by honeybees: alternative use of achromatic and chromatic signals. J Comp Physiol A  
437 180(3):235-243

438 Golding YC, Edmunds M, Ennos AR (2005) Flight behaviour during foraging of the social  
439 wasp *Vespula vulgaris* (Hymenoptera: Vespidae) and four mimetic hoverflies (Diptera:  
440 Syrphidae) *Sericomyia silentis*, *Myathropa florea*, *Helophilus* sp. and *Syrphus* sp. J Exp Biol  
441 208(23):4523-4527

442 Green PR, Gentle L, Peake TM, Scudamore RE, McGregor PK, Gilbert F, Dittrich WH  
443 (1999) Conditioning pigeons to discriminate naturally lit insect specimens. Behav Processes  
444 46(1):97-102

445 Holloway G, Gilbert F, Brandt A (2002) The relationship between mimetic imperfection and  
446 phenotypic variation in insect colour patterns. Proc R Soc Lond B 269(1489):411-416

447 Jones RT, Poul YL, Whibley AC, Mérot C, ffrench-Constant RH, Joron M (2013) Wing  
448 shape variation associated with mimicry in butterflies. Evolution 67(8):2323-2334

449 Kazemi B, Gamberale-Stille G, Tullberg Birgitta S, Leimar O (2014) Stimulus salience as an  
450 explanation for imperfect mimicry. Curr Biol 24(9):965-969

451 Kikuchi DW, Pfennig DW (2013) Imperfect mimicry and the limits of natural selection. Q  
452 Rev Biol 88(4):297-315

453 Kikuchi DW, Sherratt TN (2015) Costs of learning and the evolution of mimetic signals. Am  
454 Nat 186(3):321-332

455 Kraemer AC, Adams DC (2014) Predator perception of Batesian mimicry and  
456 conspicuousness in a salamander. Evolution 68(4):1197-1206

457 Limeri LB, Morehouse NI (2014) Sensory limitations and the maintenance of colour  
458 polymorphisms: viewing the 'alba' female polymorphism through the visual system of male  
459 *Colias* butterflies. Funct Ecol 28(5):1197-1207

460 Lindström L, Alatalo RV, Mappes J (1997) Imperfect Batesian mimicry—the effects of the  
461 frequency and the distastefulness of the model. Proc R Soc Lond B 264(1379):149-153

462 Llaurens V, Joron M, Théry M (2014) Cryptic differences in colour among Müllerian  
463 mimics: how can the visual capacities of predators and prey shape the evolution of wing  
464 colours? *J Evol Biol* 27(3):531-540

465 Maia R, Eliason CM, Bitton P-P, Doucet SM, Shawkey MD (2013) pavo: an R package for  
466 the analysis, visualization and organization of spectral data. *Methods Ecol Evol* 4(10):906-  
467 913

468 Marples NM, van Veelen W, Brakefield PM (1994) The relative importance of colour, taste  
469 and smell in the protection of an aposematic insect *Coccinella septempunctata*. *Anim Behav*  
470 48(4):967-974

471 Morrell GM, Turner JRG (1970) Experiments on mimicry: I. The response of wild birds to  
472 artificial prey. *Behaviour* 36(1/2):116-130

473 Mostler G (1935) Beobachtungen zur frage der wespenmimikry [Observations on the  
474 question of wasp mimicry]. *Zoomorphology* 29(3):381-454

475 Ödeen A, Håstad O (2003) Complex distribution of avian color vision systems revealed by  
476 sequencing the SWS1 opsin from total DNA. *Mol Biol Evol* 20(6):855-861

477 Olsson P, Lind O, Kelber A (2015) Bird colour vision: behavioural thresholds reveal receptor  
478 noise. *J Exp Biol* 218(2):184-193

479 Osorio D, Miklósi A, Gonda Z (1999) Visual ecology and perception of coloration patterns  
480 by domestic chicks. *Evol Ecol* 13(7-8):673-689

481 Penney HD, Hassall C, Skevington JH, Abbott KR, Sherratt TN (2012) A comparative  
482 analysis of the evolution of imperfect mimicry. *Nature* 483(7390):461-464

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

485 Richards-Zawacki CL, Yeager J, Bart HPS (2013) No evidence for differential survival or  
486 predation between sympatric color morphs of an aposematic poison frog. *Evol Ecol*  
487 27(4):783-795

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

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

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

494 Siddiqi A, Cronin TW, Loew ER, Vorobyev M, Summers K (2004) Interspecific and  
495 intraspecific views of color signals in the strawberry poison frog *Dendrobates pumilio*. *J Exp*  
496 *Biol* 207(14):2471-2485

497 Stockman A, Sharpe LT (2000) The spectral sensitivities of the middle- and long-  
498 wavelength-sensitive cones derived from measurements in observers of known genotype.  
499 *Vision Res* 40(13):1711-1737

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

502 Svádová K, Exnerová A, Štys P, Landová E, Valenta J, Fučíková A, Socha R (2009) Role of  
503 different colours of aposematic insects in learning, memory and generalization of naïve bird  
504 predators. *Anim Behav* 77(2):327-336

505 Veselý P, Luhanová D, Prášková M, Fuchs R (2013) Generalization of mimics imperfect in  
506 colour patterns: the point of view of wild avian predators. *Ethology* 119(2):138-145

507 Vorobyev M, Osorio D (1998) Receptor noise as a determinant of colour thresholds. *Proc R*  
508 *Soc Lond B* 265(1394):351-358



509 Waldbauer G (1988) Asynchrony between Batesian mimics and their models. *Am Nat*  
510 131:S103-S121

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

513

514 SUPPLEMENTARY MATERIAL

515 Supplementary text: testing spatial resolution.

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

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

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

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

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

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

524 FIGURE LEGENDS

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

530

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

534

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

538

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

543

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

547

548

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

551

| 552 | Species                          | Abbrev. | Achromatic |         |                   | Chromatic |                   |        |        |
|-----|----------------------------------|---------|------------|---------|-------------------|-----------|-------------------|--------|--------|
| 553 |                                  |         | Internal   | Closest | Dist <sup>1</sup> | Closest   | Dist <sup>2</sup> | Patch  | Thorax |
| 554 |                                  |         | contrast   | model   |                   | model     |                   | colour | width  |
| 555 | <b>Mimics</b>                    |         |            |         |                   |           |                   |        |        |
| 556 | <i>Chrysotoxum arcuatum</i>      | Car     | 41.2       | Vvu     | 1.2               | Vge       | 9.3               | C      | 2.6    |
| 557 | <i>Dasysyrphus albostrigatus</i> | Dal     | 55.7       | Vcr     | 10.4              | Vvu       | 8.5               | C      | 2.4    |
| 558 | <i>Dasysyrphus tricinctus</i>    | Dtr     | 49.3       | Vcr     | 4.0               | Vvu       | 11.4              | C      | 2.5    |
| 559 | <i>Epistrophe grossulariae</i>   | Egr     | 37.5       | Vvu     | 2.5               | Ame       | 3.8               | B      | 3.2    |
| 560 | <i>Episyrphus balteatus</i>      | Eba     | 25.9       | Ame     | 4.0               | Ame       | 1.3               | C      | 2.2    |
| 561 | <i>Eristalis arbustorum</i>      | Ear     | 29.6       | Ame     | 7.7               | Ame       | 3.5               | B      | 3.2    |
| 562 | <i>Eristalis horticola</i>       | Eho     | 35.5       | Vvu     | 4.5               | Vvu       | 2.9               | C      | 3.5    |
| 563 | <i>Eristalis interruptus</i>     | Eip     | 32.8       | Vvu     | 7.2               | Vcr       | 5.4               | C      | 3.4    |
| 564 | <i>Eristalis pertinax</i>        | Epe     | 31.3       | Vvu     | 8.7               | Ame       | 1.6               | C      | 3.7    |
| 565 | <i>Eristalis tenax</i>           | Ete     | 27.4       | Ame     | 5.6               | Vcr       | 3.6               | B      | 4.4    |
| 566 | <i>Eupeodes latifasciatus</i>    | Ela     | 52.4       | Vcr     | 7.0               | Vge       | 2.6               | C      | 2.0    |
| 567 | <i>Helophilus hybridus</i>       | Hhy     | 45.9       | Vcr     | 0.6               | Vvu       | 1.9               | C      | 3.8    |
| 568 | <i>Helophilus pendulus</i>       | Hpe     | 37.9       | Vvu     | 2.1               | Vvu       | 2.2               | B      | 3.0    |
| 569 | <i>Leucozona glaucia</i>         | Lgl     | 35.4       | Vvu     | 4.6               | Ame       | 16.2              | C      | 2.6    |
| 570 | <i>Melangyna labiatarum</i>      | Mla     | 53.5       | Vcr     | 8.2               | Vvu       | 10.7              | C      | 1.8    |
| 571 | <i>Melanostoma scalare</i>       | Msc     | 27.7       | Ame     | 5.8               | Ame       | 2.6               | C      | 1.6    |
| 572 | <i>Meliscaeva auricollis</i>     | Mau     | 34.6       | Vvu     | 5.4               | Vvu       | 4.4               | C      | 2.0    |
| 573 | <i>Meliscaeva cinctella</i>      | Mci     | 30.3       | Ame     | 8.4               | Ame       | 3.4               | C      | 1.9    |
| 574 | <i>Myathropa florea</i>          | Mfl     | 34.1       | Vvu     | 5.9               | Vvu       | 3.9               | C      | 3.8    |
| 575 | <i>Parhelophilus versicolor</i>  | Pve     | 43.0       | Vge     | 1.0               | Vvu       | 2.7               | C      | 2.9    |

|     |                                   |     |      |     |      |     |      |   |     |
|-----|-----------------------------------|-----|------|-----|------|-----|------|---|-----|
| 576 | <i>Platycheirus albimanus</i>     | Pal | 28.7 | Ame | 6.8  | Ame | 10.5 | C | 1.8 |
| 577 | <i>Platycheirus clypeatus</i>     | Pcl | 22.9 | Ame | 1.0  | Ame | 2.2  | B | 1.7 |
| 578 | <i>Platycheirus occultus</i>      | Poc | 21.5 | Ame | 0.3  | Ame | 13.0 | C | 1.5 |
| 579 | <i>Sericomyia silentis</i>        | Ssi | 63.9 | Vcr | 18.6 | Vvu | 3.1  | B | 4.5 |
| 580 | <i>Sphaerophoria scalare</i>      | Ssc | 38.5 | Vvu | 1.5  | Vvu | 2.7  | C | 1.6 |
| 581 | <i>Syrpitta pipiens</i>           | Spi | 22.3 | Ame | 0.5  | Ame | 4.5  | C | 1.6 |
| 582 | <i>Syrphus ribesii</i>            | Sri | 51.1 | Vcr | 5.8  | Vvu | 4.9  | C | 2.8 |
| 583 | <i>Syrphus torvus</i>             | Sto | 44.9 | Vcr | 0.5  | Vvu | 7.3  | C | 2.9 |
| 584 | <i>Syrphus vitripennis</i>        | Svi | 46.8 | Vcr | 1.5  | Vvu | 5.5  | C | 2.4 |
| 585 | <i>Volucella inanis</i>           | Vin | 44.9 | Vcr | 0.4  | Ame | 5.7  | B | 4.8 |
| 586 | <i>Volucella pellucens</i>        | Vpe | 32.9 | Vvu | 7.1  | Ame | 8.2  | C | 4.9 |
| 587 | <i>Volucella zonaria</i>          | Vzo | 38.6 | Vvu | 1.4  | Ame | 3.8  | B | 6.1 |
| 588 | <i>Xylota segnis</i>              | Xse | 15.4 | Ame | 6.5  | Ame | 5.7  | C | 2.6 |
| 589 | <b>Models</b>                     |     |      |     |      |     |      |   |     |
| 590 | <i>Ancistrocerus trifasciatus</i> | Atr | 45.3 |     |      |     |      |   | 2.0 |
| 591 | <i>Apis mellifera</i>             | Ame | 21.9 |     |      |     |      |   | 3.6 |
| 592 | <i>Ectemnius cavifrons</i>        | Eca | 65.7 |     |      |     |      |   | 2.9 |
| 593 | <i>Ectemnius continuus</i>        | Eco | 65.9 |     |      |     |      |   | 2.8 |
| 594 | <i>Mellinus arvensis</i>          | Mar | 70.3 |     |      |     |      |   | 2.2 |
| 595 | <i>Vespa crabro</i>               | Vcr | 45.3 |     |      |     |      |   | 5.5 |
| 596 | <i>Vespula germanica</i>          | Vge | 44.0 |     |      |     |      |   | 3.6 |
| 597 | <i>Vespula vulgaris</i>           | Vvu | 40.0 |     |      |     |      |   | 3.1 |

598 <sup>1</sup> Achromatic distances are calculated as the absolute difference between values of internal  
599 pattern contrast (that is, the achromatic distance between coloured and black patches)  
600 between the model and mimic.

601 <sup>2</sup> Chromatic distances are the  $\Delta S$  values between model and mimic for whichever patch type  
602 (coloured or black, indicated in the 'patch colour' column) has the larger  $\Delta S$ .

