

1 **Convergent evolution of sexual deception via chromatic and achromatic**
2 **contrast rather than colour mimicry**

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14 Running title: "Sexual deception via contrast rather than colour mimicry"

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16 Keywords: colour pattern, orchid, pollinator, spectral reflectance, thynnine wasp,
17 visual signal

18 Total word count: 5362 (excluding references, tables and figures)

19 Introduction: 1019, Methods: 1607, Results: 944, Discussion: 1664,

20 Acknowledgements: 128, References: 2529, Number of cited references: 101

21 Number of tables: 1, figures: 10

22 Items to be published as online appendix: 3 tables, 6 figures,

23 Abstract (274w)

24 The Orchidaceae is characterised by the repeated evolution of sexual deception, one
25 of the most specialised pollination strategies. In orchids, sexual deception involves
26 long-range pollinator attraction via mimicry of female insect sex pheromones. At
27 close range, visual signals involving colour mimicry, contrast to the background, and
28 exploitation of pollinator sensory biases could attract pollinators, but remain largely
29 untested. Here we focus on a remarkable system in which species from two only
30 distantly related sexually deceptive orchid genera with strikingly different flowers
31 (*Drakaea livida* and three species of *Caladenia*) share the same pollinator, males of
32 the thynnine wasp *Zaspilothynnus nigripes*. We used spectral reflectance
33 measurements and modelling to investigate pollinator perception of colour, including
34 the first examination of overall colour patterns in flowers via colour pattern geometry
35 analyses. Rather than closely matching the colours of female *Z. nigripes*, these
36 orchids had strong chromatic and achromatic contrast against their backgrounds. For
37 *Caladenia*, the sepals and petals show high contrast, while in *D. livida*, which has
38 diminutive petals and sepals, it is the labellum that contrasts strongly against the
39 background. Despite varying in colour, the *Caladenia* species all had strong within-
40 flower contrast between a UV-bright central target (column and labellum) and a
41 corolla of radiating stripes (petals and sepals). The colour pattern geometry analyses
42 also indicated that the orchids' overall colour patterns are highly conspicuous against
43 their backgrounds. Contrast, UV, and target patterns could all enhance detection,
44 and exploit pollinators' innate preferences. Since colour contrast may function with a
45 range of colours and floral forms, attracting pollinators via contrast rather than visual
46 mimicry may be a critical but previously overlooked process facilitating the evolution
47 of sexual deception.

48 Introduction

49 Pollination by sexual deception is a major evolutionary trend in the Orchidaceae, with
50 multiple origins in several lineages across multiple continents (Bateman et al. 2003;
51 Inda et al. 2012; Johnson et al. 2013; Herberstein et al. 2014; Weston et al. 2014).
52 Sexually deceptive orchids mimic the sex pheromones of female insects and
53 pollination typically occurs during courtship or mating behaviour (Schiestl 2005;
54 Peakall et al. 2010; Ayasse et al. 2011). Sex pheromone mimicry means that sexual
55 deception is a highly specialised strategy with most orchid species restricted to a
56 single pollinator species (Paulus and Gack 1990; Phillips et al. 2009b; Peakall et al.
57 2010; Gaskett 2011). At close range, pollinator attraction and pollinia transfer may
58 rely on visual and tactile stimuli (Streinzer et al. 2009; Streinzer et al. 2010; Rakosy
59 et al. 2012). While floral odour is critical in the evolution and radiation of sexual
60 deceptive lineages (e.g. Xu et al. 2011; Peakall and Whitehead 2014), the role of
61 visual stimuli in facilitating the evolution of sexual deception remains comparatively
62 unexplored.

63 Many deceptive orchids attract pollinators via food deception, with colourful
64 displays that resemble rewarding flowers (Jersáková et al. 2009; Jersáková et al.
65 2012). In contrast, sexually deceptive orchids have repeatedly evolved dull-coloured
66 and morphologically reduced flowers (e.g. Peakall 1989, 1990; Bower 1996; Alcock
67 2000; Singer et al. 2004; Blanco & Barboza 2005; Ciotek et al. 2006; Phillips et al.
68 2009, 2014). Colour could be unimportant in sexual deception, or alternatively, floral
69 colour may function differently in food and sexual deception (Gaskett 2014).
70 Intriguingly, there are several orchid genera where there is no clear dichotomy
71 between brightly coloured food-deceptive species and dull-coloured sexually
72 deceptive species, e.g. European *Orchis* and Australian *Caladenia* (Bino et al. 1982;

73 Phillips et al. 2009a). While many sexually deceptive *Caladenia* are dull green and
74 red with reduced floral display, other species have prominent white, yellow and pink
75 flowers that look very different to female insects of their pollinator species (Phillips et
76 al. 2009). Multiple visual strategies appear to be involved in sexual deception, even
77 within a single orchid genus.

78 The colours of sexually deceptive orchids are often assumed to mimic female
79 insects (Schiestl 2005; Jersáková et al. 2006; Paulus 2006; Jersáková et al. 2009;
80 Gaskett 2011). However, objective colour comparisons or experimental tests of
81 pollinator perception of orchid and female insect colours are rare (though see
82 (Kullenberg 1961; Gaskett and Herberstein 2010; de Jager and Peakall 2015). In
83 sexual deception, colour may have other functions besides mimicry. For example,
84 ~30% of species in the sexually deceptive genus *Ophrys* have bright pink or white
85 sepals surrounding a darker central labellum (Streinzer et al. 2009). Experiments
86 with *Ophrys heldreichii* revealed that a pink perianth enhanced detection by
87 pollinators, leading to higher pollination success (Spaethe et al. 2007; Streinzer et al.
88 2009; Rakosy et al. 2012). Floral colours may also enhance detection or attraction by
89 functioning as a sensory trap to exploit insects' innate preferences or receiver biases
90 for certain wavelengths (e.g. yellow or UV; Shi et al. 2009; Kelly and Gaskett 2014).
91 Intraspecific variation in orchid colours and patterns may also inhibit pollinator
92 recognition and subsequent learned avoidance of orchids after a deceptive
93 encounter (Stejskal et al. 2015, but see Smithson et al. 2007).

94 Understanding and testing the functions of floral colour has so far relied on
95 analyses involving paired comparisons of colour patches (Streinzer et al. 2009;
96 Gaskett and Herberstein 2010; O'Hanlon et al. 2014). Methods making use of the
97 entire colour pattern provide a more realistic approach incorporating both spectral

98 reflectances and the arrangement of colour in patterns (Endler and Mielke 2005;
99 Endler 2012; Taylor et al. 2013). To our knowledge, these new methods, originally
100 developed for evolutionary studies of avian visual ecology, have yet to be applied to
101 floral mimicry, or indeed any other floral colour data.

102 Cases in which several sexually deceptive orchids share a pollinator provide a
103 powerful opportunity to investigate floral adaptation because they control for
104 phylogenetic differences in pollinator behaviour and morphology. While there are
105 several examples of congeners that share a pollinator (Gögler et al. 2009; Phillips et
106 al. 2009a; Gaskett 2011), a remarkable case of pollinator sharing between orchids
107 from different subtribes has recently been discovered. DNA barcoding and mark-
108 recapture studies show that four sexually deceptive orchid species, *Drakaea livida*,
109 *Caladenia decora*, *C. pectinata*, and *C. procera*, are all pollinated by males of a
110 single species of thynnine wasp, *Zaspilothynnus nigripes* (Phillips et al. 2009a;
111 Phillips et al. 2013; Phillips et al., unpublished data). While these *Caladenia* species
112 possess prominent, pink, red or green linear lanceolate tepals, *D. livida* has
113 drastically reduced flowers with diminutive tepals and a comparatively large hinged
114 insectiform labellum that is greenish-yellow and maroon, with dark maroon-black
115 spots (Fig. 1; Hopper and Brown 2007). When a male wasp attempts to fly off with
116 the labellum, his momentum swings him upside down and onto the column
117 (Stoutamire 1975; Peakall 1990; Fig. 1). The obvious differences in the appearance
118 of these *Caladenia* and *Drakaea* orchid species suggest considerable flexibility in the
119 colours and morphologies required for sexual deception, even for the same pollinator
120 species.

121 Here, we investigate the visual signalling adaptations of sexually deceptive
122 orchids to understand how floral colour could function in evolutionary transitions to

123 sexual deception. We model hymenopteran perception of floral colour in two orchid
124 genera with strikingly different floral displays: *Caladenia* which are various colours
125 and may retain traits of food-deceptive ancestors, and *Drakaea*, an entirely sexually
126 deceptive genus with highly reduced maroon and black flowers. Given the shared
127 pollinator of *C. decora*, *C. procera*, *C. pectinata* and *D. livida*, we predict similar floral
128 colour signals involved in pollinator attraction. This is likely due to convergent
129 evolution at the genus level for *Caladenia* and *Drakaea*, and shared ancestry when
130 considering the species within the genus *Caladenia*. Firstly, we test the hypothesis of
131 colour mimicry of female wasps of the pollinator species *Z. nigripes*, we then
132 examine evidence for other colour contrast or pattern signalling strategies by
133 applying new colour pattern geometry analyses. Finally, we investigate whether
134 these orchid species that share a single pollinator could have the same visual
135 signalling strategy.

136

137 **Methods**

138 *Study species*

139 *Drakaea* and *Caladenia* belong to different subtribes of the Diurideae (Drakaeinae
140 and Caladeniinae; Kores et al. 2001). No detailed phylogeny is available for
141 *Caladenia* below the level of subgenus (see Clements et al. 2015), but based on
142 morphology, our study species *Caladenia decora*, *C. procera* and *C. pectinata* are
143 believed to be closely related and may well be sister species (Hopper and Brown
144 2001; Fig. 1). Although *D. livida* is broadly sympatric with these *Caladenia* species,
145 the two genera rarely grow together due to different edaphic preferences (Hopper
146 and Brown 2001; Phillips et al. 2013). *Drakaea livida* occurs almost exclusively in

147 open microhabitats with well-drained, grey sandy soils (Hopper and Brown 2007; Fig.
148 1). The three *Caladenia* species all grow primarily in denser vegetation on loams,
149 clays or moist sandy soils (Hopper and Brown 2001). All are endemic to south-
150 western Australia and occur in a series of disjunct distributions from east to west
151 (Hopper and Brown 2001; Hopper and Brown 2007). *C. procera* has the most
152 easterly distribution and is entirely allopatric. *C. pectinata* occurs approximately
153 100kms westwards, and the distribution overlaps slightly with that of *C. decora* but
154 only in the region of the Fitzgerald River National Park. Our specimens were
155 collected 120-200kms away from this shared site (Table 2). To human eyes, *C.*
156 *decora* is mostly red-maroon or pink, *C. pectinata* predominantly reddish yellow, *C.*
157 *procera* is greenish/lemon yellow and *D. livida* is maroon, with dark maroon-black
158 spots (Fig. 1; Hopper and Brown 2001; Hopper and Brown 2007).

159 The pollinator, *Zaspilothynnus nigripes*, is widespread and common in higher
160 rainfall and sandplain habitats of south-western Australia (Phillips et al. 2014; Fig. 2).
161 Like other thynnine wasps, flightless females attract patrolling males with airborne
162 sex pheromones (Alcock 1981; Alcock and Gwynne 1987; Bohman et al. 2012).
163 Males carry the female *in copula* to feed on nectar (Menz et al. 2013; Brown and
164 Phillips 2014; Menz et al. 2015b). After mating, she burrows for scarab beetle larvae
165 in which to oviposit (Ridsdill Smith 1970). In our study region, *Z. nigripes* tend to fly
166 on sunny spring days of >18°C (Peakall 1990).

167 Flowers of *Caladenia decora* (n = 13), *C. pectinata* (n=13), *C. procera* (n = 6)
168 and *Drakaea livida* (n = 13) were collected from wild populations (online
169 supplementary Table 1). Female (n = 7) and male (n = 9) *Zaspilothynnus nigripes*
170 wasps were caught while feeding *in copula* using a hand net. Samples of the
171 soil/substrate were collected within 15cm of the base of the stem of each orchid.

172

173 *Spectral measurements*

174 For the *Caladenia* orchids, spectral reflectances were measured at the dorsal and
175 lateral sepals, the petals, column, labellum base and labellum tip, flower stem, and
176 leaf (Fig. 3). For *D. livida*, measurements were taken at the column, labellum base
177 and labellum tip, flower stem, and leaf. Some small elements were too diminutive to
178 measure separately with our probe, e.g. the protruding black tubercles on the
179 maroon labellum of *D. livida*. Background measurements were taken from the
180 substrate samples. Wasp spectra were measured at the head and dorsal abdomen
181 (Fig. 2b). Five measurements were taken per location and the averages were used
182 in analyses (300-700 nm; USB2000 spectrometer, PX-2 light source, Ocean Optics).

183

184 *Hymenopteran colour vision calculations*

185 Paired comparisons of colour patches

186 No photoreceptor spectral sensitivity data are available for thynnine wasps such as
187 our pollinator species *Z. nigripes*. However, despite this limitation, studies indicate
188 that all studied hymenoptera have largely similar photoreceptor sensitivities (Peitsch
189 et al. 1992; Briscoe and Chittka 2001). As for several other recent studies involving
190 non-bee hymenopteran pollinators (Streinzer et al. 2010; Milet-Pinheiro et al. 2015;
191 Ohashi et al. 2015; Stejskal et al. 2015), we have used a generic hymenopteran
192 vision model with data from the honeybee *Apis mellifera*, where spectral sensitivities
193 peak at 344nm (UV), 436nm (Blue), and 556nm (Green; Menzel and Blakers 1976;
194 Peitsch et al. 1992). A hymenopteran multi-species average ratio of the relative
195 abundances of each receptor type was used (UV:Blue:Green, 1:0.471:4.412; Defrize

196 et al. 2010). We used the receptor noise-limited colour-opponent model of
197 hymenopteran vision (Brandt and Vorobyev 1997; Vorobyev and Osorio 1998),
198 which has been validated with experimental data and used widely in the literature
199 (Hempel de Ibarra et al. 2014). The quantum catch Q of each receptor type i (UV,
200 blue, green) was calculated using equation (1) from (Kelber et al. 2003), integrated
201 across the hymenopteran visual range of 300-700nm. Quantum catches, chromatic
202 and achromatic colour contrast (in units of JND, Just Noticeable Differences) were
203 calculated according to Vorobyev and Osorio (1998), via the software Avicol v.5
204 (Gomez 2006). Theoretically, when JND is <1 , colours are likely to be
205 indistinguishable to the viewer, but this can vary between species, and higher
206 thresholds are likely when stimuli are viewed successively rather than
207 simultaneously (Renoult et al. 2015). Lower colour discrimination thresholds operate
208 when subjects have received training or stimuli are associated with costs. Ideally,
209 behavioural thresholds for colour vision should be tested experimentally, but these
210 data are not available for our study species so a generic threshold of ~ 1 was used
211 here. Triangle loci coordinates were calculated with equations A2-A5 from Kelber et
212 al. (2003; equivalent to equation 9 in Brandt and Vorobyev 1997). Although JNDs are
213 not an exact predictor of viewer perception of colour difference (Hempel de Ibarra et
214 al. 2014), and no precise threshold is known for thynnine wasps, JNDs do provide a
215 general and comparative sense of perceived colour difference.

216

217 Analysis of the overall colour patterns

218 To further investigate detectability and similarities between the orchids and the wasp,
219 we compared the entire colour patterns of the orchids and wasps against their
220 backgrounds using methods from Endler and Mielke (2005) and Endler (2012).

221 These methods combine the visual properties of spectra of each coloured patch with
222 the relative area, spacing and shape of that patch. As above, we used a honey bee
223 vision model. We estimated photoreceptor relative stimulation for each coloured
224 patch (taken from Figures 2 and 3, dorsal views for insects), after light-adapting (von
225 Kries correction) to the product of open/cloudy irradiance (Endler 1993) and the
226 mean of the visual background spectra from the substrates for each orchid species.
227 Hue and chroma were calculated for each colour from its polar coordinates in the
228 Maxwell Triangle positioned such that the gray point (equal photoreceptor
229 stimulation) is at position (0,0) and the maximum distance from the gray point to any
230 vertex is 1.0. Hue is the angle around the gray point (degrees) and chroma is the
231 distance from the gray point. We calculated the mean and standard deviation of hue
232 using circular statistics. These calculations excluded hue angles where chroma was
233 less than 0.06 because low chroma colour hues are very sensitive to measurement
234 noise. Means of these values, weighted by the relative area of each patch, were
235 used as a measure of contrast with the background (Table 1). The standard
236 deviations (SD) of these values, also calculated by weighting by relative areas,
237 indicate within-pattern contrast in all three of the measures (luminance, chroma and
238 hue; Endler and Mielke 2005). When calculating hue SD, a patch was classified as
239 achromatic and thus excluded when the chroma <0.06 .

240 The specific visual resolution of the pollinator at which colour patterns would
241 be detected is unknown. Field observations suggest that *Z. nigripes* first see orchids
242 when ~30cm away, when they stop flying in a zig-zag route within the odour plume,
243 and fly straight to the flower (Phillips, unpub. data). Here, we use the mean values
244 for hue, chroma and luminance to represent the viewer's perception of the object
245 when first detected. These mean values would not change even if the spatial

246 resolution angle of the wasp was as large as the diameter of the flower because low
247 resolution is merely a spatial average of the pattern patches. The SD values indicate
248 viewer detection of the within-pattern details. SD would increase as the wasp
249 approaches the flower and would equal our calculated values when the wasp is
250 close enough to resolve the patterns. As such, our SD values should be regarded as
251 the maximum possible contrasts.

252 We used adjacency analysis of colour patches (Endler 2012) to calculate a
253 range of values describing orchid and wasp colour patterns and their complexity
254 (Table 1). 'Colour diversity' (S_c) indicates the number of colours in a flower or wasp's
255 colour pattern. 'Transect diversity' (S_t) represents the frequency with which colours
256 are adjacent to each other. For example, for an orchid species with a three-colour
257 pattern in red, pink and white, a larger value of S_t indicates similar numbers of
258 transitions from red to pink, red to white, and white to pink. 'Complexity' is a measure
259 of the density of these transitions between colour classes, taking into account patch
260 sizes and shapes. 'Pattern aspect ratio' indicates the regularity of the pattern across
261 an axis where values < 1 indicate a simpler horizontal pattern, e.g. lateral stripes,
262 and values > 1 indicate a simpler vertical pattern, e.g. longitudinal stripes.

263 'Transitions at the body edge' is a measure of conspicuousness where transitions
264 between colours within the flower are divided by transitions between the flower and
265 background colours. Values < 0.5 indicate a flower with a simple pattern on a
266 complex background, and values > 0.5 indicate a flower with a complex pattern on a
267 simple background. When the value approaches 1, the flower colour patterns are
268 increasingly similar to the background and therefore more difficult to detect.

269 Pollinator viewing angle (and the images used in the analyses; Figs 2 and 3) can

270 affect some results (e.g. pattern aspect ratio), but not those most important for
271 assessing conspicuousness (e.g. transitions at the body edge).

272

273 **Results**

274 *Reflectance spectra*

275 For *Caladenia decora*, the petals, sepals and labellum tip reflected predominantly
276 red wavelengths (peak reflectance ~650nm), with no UV (~300-400nm; Fig. 4a). The
277 column reflected more evenly across the spectrum, including UV. For *Caladenia*
278 *pectinata*, petal, sepal and labellum tip reflectance also peaked in the red region (Fig.
279 4b). The column and labellum reflected UV. For *Caladenia procera*, petal and sepal
280 reflectance peaked in the yellow-green region (~550nm; Fig. 4c). Again, the column
281 and labellum reflected UV. The spectral reflectance of *Drakaea livida* differed from
282 the general pattern observed for the *Caladenia* species; reflectance was low for all
283 floral parts and the column did not reflect UV (Fig. 4d). The substrates reflected
284 evenly across the spectrum with some UV reflection for *Caladenia* substrates and
285 strong UV reflection for the *D. livida* substrate (Fig. 4). For the female wasps, the
286 head reflected in the green-red region of the spectrum (peak ~ 680nm) and no UV
287 (Fig. 4e). The female abdomen and both parts of the male wasp reflected evenly and
288 at a low level across the spectrum, including some UV.

289

290 *Hymenopteran colour vision calculations*

291 Paired comparisons of colour patches

292 Although the spectral reflectances of some *Caladenia* sepals and petals
293 appear superficially similar to the female wasp head and thorax (i.e. peak reflectance

294 in the red region and no UV), when modelled into the generic hymenopteran model
295 (Fig. 5), the orchids and wasps are different. Nearly all the orchid vs. wasp
296 comparisons generated high JND values for achromatic and chromatic contrast (>10;
297 Fig. 6, online supplementary Fig. 1. For orchid-male wasp comparisons see online
298 supplementary Fig. 2). The female wasp abdomen had low chromatic but high
299 achromatic contrast with the columns and labella of *C. pectinata* and *C. procera* (Fig.
300 6 chromatic contrast 1.7-3.9 JNDs; online supplementary Fig. 1 for achromatic
301 contrast 15-26 JNDs). Female and male wasps were clearly distinguishable from
302 each other for both achromatic and chromatic contrast (online supplementary Fig. 3).
303 However, for all these comparisons, there may be limitations associated with
304 applying a generic hymenopteran model to a thynnine wasp.

305 In terms of within-flower colour contrast, in all the *Caladenia* species, the
306 central column and labellum were similar, but contrasted strongly with the
307 surrounding petals and sepals chromatically (Fig. 7) and the labellum tip, petals and
308 sepals achromatically (online supplementary Fig. 4). In turn, these parts contrasted
309 strongly against the substrate chromatically (Fig. 8) and achromatically (online
310 supplementary Fig. 5). However, for *Drakaea livida* (which has drastically reduced
311 petals and sepals), it was the labellum and column that contrasted with each other
312 and the surrounding substrate both chromatically (Figures 8, 9) and achromatically
313 (online supplementary Figures 4 and 5). The heads of the female wasps contrasted
314 strongly with the backgrounds, while the abdomens contrasted more strongly with
315 the backgrounds of *C. procera* and *D. livida* than those of the other orchid species
316 (chromatic contrast Fig. 9, achromatic contrast online supplementary Fig. 6).

317 When intraspecific variation in the colour loci of the orchid labella and sepals
318 are compared (Fig. 10), for *Caladenia* species, the labella loci cluster closely

319 together but the lateral sepals are more variable. Conversely for *Drakaea livida*,
320 which has extremely reduced sepals, the labella loci are more variable. Interspecific
321 comparisons demonstrate all orchid species had similarly coloured labella (all mean
322 JNDs < 5, online supplementary table 2). The lateral sepals were similar for
323 *Caladenia decora* and *C. procera* (mean JND = 6.7), but *C. pectinata* differed (mean
324 JNDs > 15, online supplementary table 3).

325

326 Analysis of the overall colour patterns

327 Consistent with the paired comparisons of colour patches, analysis of all the
328 colour patterns on the orchids and the wasp provide no evidence of close visual
329 mimicry. Instead, various colour measurements reveal that the orchids would be
330 highly detectable against the background, especially the *Caladenia* species. The
331 mean values presented here represent perception when first detected and the SD
332 values indicate within-pattern details perceived once the viewer is near enough to
333 resolve them. For most measures, *D. livida* gave the most similar results to the
334 female wasp. Mean luminance was greatest for *C. procera*, then *C. pectinata* and *C.*
335 *decora*, then *D. livida* and the female wasp (both black-maroon; Fig. 1). The within-
336 pattern luminance contrast (as indicated by luminance SD values) was similar and
337 highest for the three species of *Caladenia*, then *D. livida*, the wasp and background
338 (Table 1). The mean chromas were similar for all the orchids and the female wasp,
339 but within-pattern chromatic contrast (indicated by chroma SD) is highest for the
340 wasp, then *Drakaea* and *C. procera*, and lowest for *C. decora* and *C. pectinata*. The
341 wasp mean hue is most similar to *Drakaea livida*, followed by *C. procera* and *C.*
342 *pectinata*, and differs considerably to *C. decora*. Within-pattern hue contrast

343 (indicated by hue SD) is greatest for *C. decora*, followed by *C. pectinata*, the female
344 wasp, *C. procera* and *D. livida*.

345 Adjacency analysis also indicates high detectability of the orchids against the
346 background, especially the low 'transitions at the body edge' values for all the orchid
347 species, indicating their colour patterns are highly conspicuous against the colour
348 patterns of their backgrounds. The other values calculated (Table 1) demonstrate
349 that the female wasp has lower colour and transect diversity (S_c and S_t) than the
350 orchids, reflecting its simpler overall colour patterning. The female wasp complexity
351 value is higher than for the orchids because she has more different patch sizes and
352 shapes than the flowers (see Fig. 2b *versus* Fig. 3). For all orchids and the wasp, the
353 pattern aspect ratio values are all <1 , indicating their patterns are all simpler
354 horizontally, that is, they all have horizontal rather than vertical bands of colour.

355

356 **Discussion**

357 It has long been assumed that sexually deceptive orchids mimic the colours of
358 female insects (Pouyanne 1917; Godfrey 1921; Coleman 1928; Kullenberg 1950),
359 but this has rarely been tested (e.g. Gaskett and Herberstein 2010; de Jager and
360 Peakall 2015). We found no evidence for precise colour mimicry of female wasps for
361 two distantly related lineages of sexually deceptive orchid when analysed with a
362 generic hymenopteran vision model. This seems consistent with the wide range of
363 floral colours in *Caladenia*, which bear no obvious resemblance to those of female
364 insects (Phillips et al. 2009a; Brown et al. 2013).

365 In our study system, the wasp abdomen had low chromatic contrast to the
366 column/labella of *C. pectinata* and *C. procera*, but there was high achromatic

367 contrast, and high chromatic and achromatic contrast for all other comparisons.
368 However, the orchid colours may still coarsely resemble female wasps at long-
369 distance when the spectra blend and the respective mean hue, chroma and
370 luminance would appear similar. Furthermore, in nature, male wasps are unlikely to
371 see females and orchids simultaneously as assumed in our analyses. Successive
372 viewing of stimuli results in poorer colour discrimination (Renoult et al. 2015). In our
373 system, this could impair recognition of mimics and relax selection for accurate
374 mimicry, although mimicry need not be perfect to achieve deception (Dalziell and
375 Welbergen 2016; de Jager et al. 2016).

376 The floral colours of our *Caladenia* and *Drakaea* species may enhance
377 detectability via pronounced contrast against their backgrounds. For *Caladenia*, the
378 colours of the central column and labella were similar between the species and to
379 the background, but had high chromatic and achromatic contrast to the surrounding
380 sepals and petals. This would produce a central target surrounded by radiating, high-
381 contrast stripes. Although we used a generic hymenopteran vision model and the
382 specific spatial visual resolution is unknown, the high contrast between the
383 columns/labella, petals/sepals, and backgrounds (all values >10), and the low
384 'transitions at the body edge' values indicate flowers are highly detectable against
385 the background. The interspecific similarity in labellum colour suggests some
386 selective constraint (or no directional selection) on this trait, whereas the interspecific
387 variation in sepal colour could indicate either selection for innovation in floral colour
388 or lack of stabilising selection. In some cases, diverse floral traits could be
389 advantageous if they impair pollinator recognition and subsequent avoidance of
390 orchids (Paulus 2006; Stejskal et al. 2015, but see Smithson et al. 2007; Juillet and

391 Scopece 2010). However, our three *Caladenia* species are allopatric, so pollinator
392 learning is probably not involved in their colour divergence.

393 For *Drakaea livida*, which has highly reduced sepals and petals, it was the
394 labellum itself that contrasted strongly against the unusual UV-bright sandy
395 background. The apparently similar visual signalling strategy of the *Caladenia*
396 species and *D. livida*, despite their differing substrates and morphologies, suggests
397 their shared single pollinator is driving convergent evolution for contrast and
398 detectability rather than precise visual mimicry. Convergent evolution is most likely at
399 the genus level. *Caladenia* and *Drakaea* are only distantly related, they are both
400 some of the most recently derived and diverse genera in two different and highly
401 separated subtribes of the Diurideae, the Caladeniinae (also including *Cyanicula*,
402 *Glossodia* and others) and the Drakaneinae (also including *Arthrochilus*, *Caleana*,
403 *Chiloglottis*, *Paracaleana* and *Spiculaea* and others; Weston et al. 2014).
404 Comprehensive surveys could be coupled with the existing Diurideae phylogenies to
405 test whether the many other species and diverse rewarding and deceptive pollination
406 systems in these genera or subtribes (or from more closely related sister subtribes)
407 share a similar, perhaps ancestral, contrast-based visual signalling strategy. Within
408 *Caladenia*, a detailed phylogeny is needed to test whether our three study species
409 have independently evolved this contrast-based strategy.

410

411 *Contrast and sexual deception*

412 If sexually deceptive orchids attract pollinators with high contrast flowers, their
413 pollinators' mate search behaviour likely also involves seeking contrasting objects,
414 rather than matching a coloured search image. Many pollinators of sexually

415 deceptive orchids, including *Z. nigripes*, will land on and attempt to mate with black
416 plastic beads spiked with synthetic semio-chemicals, even when the females are a
417 different colour (e.g. Peakall et al. 2010; Vereecken et al. 2010; Bohman and Peakall
418 2014; Bohman et al. 2014). Similarly, when *Ophrys insectifera* flowers are hidden,
419 pollinators are attracted to any nearby dark-coloured object (Kullenberg 1961).

420 Given the taxonomic diversity of insects and insect mating systems involved
421 in sexual deception (Gaskett 2011), the relative importance of contrast, visual
422 mimicry and sex pheromone mimicry may vary between insect taxa, and
423 correspondingly, the orchids they pollinate. For example, a coloured perianth that
424 contrasts with the labellum occurs in ~80% of *Ophrys* species pollinated by male
425 Eucerini bees that rely heavily on vision during mate searching, but in only 9% of
426 *Ophrys* pollinated by *Andrena* bees that rely primarily on sex pheromones (Spaethe
427 et al. 2010). Consistent with this, a coloured perianth on *O. arachnites* did not
428 enhance attraction of *Colletes cunicularis*, a bee relying on sex pheromones rather
429 than visual signals to find females (Vereecken and Schiestl 2009). Testing the roles
430 of contrast, visual and scent mimicry requires behavioural experiments, especially in
431 systems in which the semio-chemicals that mimic sex pheromones have been
432 identified (e.g. Peakall et al. 2010; Bohman et al. 2014). This is particularly important
433 given current reliance on generic vision models based on honeybees and flies (e.g.
434 Kelly and Gaskett 2014; de Jager et al. 2016), rather than specific orchid pollinating
435 taxa.

436

437 *Chromatic and achromatic contrast and colour patterns as floral strategies*

438 Maximising visual detectability is probably effective for most insect-pollinated
439 flowers, regardless of whether they are deceptive or rewarding. Detection of flowers
440 by hymenopterans is sometimes predicted to involve just short-range, chromatic
441 perception with all three receptor types, rather than longer-distance achromatic
442 contrast with only the L (green) receptor (Spaethe et al. 2001; Dyer et al. 2008).
443 However, laboratory and field experiments indicate both chromatic and achromatic
444 contrast are involved in pollinator attraction for both honeybees (Hempel de Ibarra et
445 al. 2000) and orchid pollinators (Eucerini solitary bees; Streinzer et al. 2009;
446 Streinzer et al. 2010). Here we found evidence of strong chromatic and achromatic
447 contrast, but behavioural experiments are required to determine whether one or both
448 types of contrast function in detectability for a thynnine wasp viewer.

449 As well as enhancing detectability, contrast also affects perception of the
450 edges of colour patches (Endler 2012). Although trichromats such as hymenoptera
451 typically detect patterns and edges achromatically with their L (green) receptor alone,
452 as for detection of an object against a background, colour pattern recognition can
453 employ both chromatic and achromatic detection, despite the lower spatial resolution
454 of the chromatic system in some taxa (e.g. *Apis mellifera*; Hempel de Ibarra et al.
455 2014); but see Dyer et al. 2008). Colour patterns such as nectar guides can
456 influence pollinator attraction (Hansen et al. 2012; Jersáková et al. 2012) and male
457 mate choice (in birds, Endler and Day 2006; in butterflies, Jiggins et al. 2001), and
458 thus are likely important in sexually deceptive pollination.

459

460 *Contrast, patterns, and sensory biases*

461 Sexual deception relies on exploiting pollinator sensory biases. The importance of
462 pre-adapted biases for olfactory signals in the evolution of sexual deception is now
463 recognised (Schiestl 2010; Schiestl and Dötterl 2012; Vereecken et al. 2012; Ayasse
464 and Dötterl 2014). Exploitation of colour and contrast biases is also likely, especially
465 when manipulating insects that rely on visual signals during foraging or mate
466 searching. UV contrast, dark centres, and radiating stripes (as we report here for
467 *Caladenia*) can all exploit pollinator sensory biases. Despite insects' strong, innate
468 attraction to UV, it is surprisingly uncommon in rewarding flowers (Chittka et al.
469 1994; Dyer 1996), but is reported for growing numbers of food, brood-site and
470 sexually deceptive orchids (Kullenberg 1961; Indsto et al. 2006; Paulus 2006;
471 Gaskett and Herberstein 2010; Kelly and Gaskett 2014; Bodley et al. 2016). Target
472 patterns and radiating stripes can be innately attractive (Lehrer et al. 1995; Lunau et
473 al. 2006), exploit sensory drive (Schaefer et al. 2004) or learned associations
474 between floral patterns and food rewards (Schiestl and Johnson 2013). UV markings
475 or dark centres can direct insects to nectar or shelter rewards, or into traps such as
476 pitcher plants or spider webs (Biesmeijer et al. 2005; Herberstein et al. 2009; Urru et
477 al. 2011; Vereecken et al. 2013).

478 In our study system, the pollinator may be pre-adapted to exploitation via
479 floral visual signals. *Zaspilothynnus nigripes* is nectivorous and visits mostly white,
480 cream, pale yellow and pale pink flowers (*Eucalyptus*, *Leptospermum*, *Xanthorea*
481 spp., *Hakea trifurcata*; Myrtaceae, Xanthoraceae, Proteaceae; Menz et al. 2013;
482 Menz et al. 2015a). These colours are also common for the flowers of sexually
483 deceptive *Caladenia* species pollinated by nectivorous thynnine species (Phillips et
484 al. 2009a; Menz et al. 2013; Brown and Phillips 2014; Menz et al. 2015a).
485 Conversely, dull-coloured orchids with reduced floral display are typically pollinated

486 by thynnines that rarely eat nectar and instead tend to feed on insect exudates
487 (Brown and Phillips 2014). Thus, although sexual deception exploits pollinators'
488 sexual behaviours, it may also involve sensory traps based on pre-existing foraging
489 sensory biases.

490

491 *Contrast and the evolutionary transition from food to sexual deception*

492 Attracting sexually deceived pollinators through contrast rather than colour mimicry
493 may facilitate the evolutionary transition from food to sexual deception. Food
494 deceptive orchids typically have brightly coloured flowers in a prominent visual
495 display that is readily detectable against the background (Jersáková et al. 2012;
496 Newman et al. 2012), suggesting that contrast is important for both food and sexual
497 deception. Importantly, the importance of contrast may explain how intermediate
498 forms could achieve pollination while evolving from food deceptive ancestors. Since
499 current phylogenies suggest pollination by nectar-foraging insects is the ancestral
500 state to the major known radiations of sexually deceptive orchids (Bateman et al.
501 2003; Weston et al. 2014), we hypothesise that pollinator attraction with achromatic
502 and chromatic contrast is a critical but overlooked process during the evolution of
503 sexual deception. Field behavioural experiments are now needed to test pollinator
504 responses to model flowers with varying contrast against the background, within-
505 flower patterns, and pollinator viewing angles.

506

507 Acknowledgements

508 ACG thanks those who provided childcare essential for the completion of the
509 analyses and manuscript: Greg Holwell, Val Gaskett, Rika Gaskett and Terry and Ian

510 Holwell. Orchids and wasps were collected under permits from the Department of
511 Parks and Wildlife, Western Australia. We thank John Rudolph for macros for
512 preparing spectral data for analyses and Vivian Ward for the botanical diagrams.
513 Funding for fieldwork and equipment was from a University of Auckland FRDF grant
514 to ACG and an Australian Orchid Foundation grant to RDP. The project was
515 completed while RDP was supported by an ARC Linkage grant (LP110100408) to
516 Rod Peakall, Kingsley Dixon and Celeste Linde and a Discovery Early Career
517 Research Award (DE150101720) and ACG was supported by University of Auckland
518 sabbatical and parental leave funding.

519

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773 isolation is the main reproductive barrier amongst closely related sexually deceptive
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775

776

777 Table 1: Geometric pattern analyses results for four sexually deceptive orchid
778 species and females of their shared pollinator species, *Zaspilothynnus nigripes*.
779 Pattern measures were not produced for the background. Values are mean \pm SD,
780 calculated for each species.

781

	n	Luminance	Chroma	Hue	Colour diversity (Sc)	Transect diversity (St)	Complexity	Pattern aspect ratio	Transitions at the body edge
Orchids:									
<i>Caladenia decora</i>	13	2.01 \pm 1.58	0.32 \pm 0.18	71.86 \pm 12.20	4.40	4.82	0.0031	0.75	0.21
<i>Caladenia pectinata</i>	13	2.42 \pm 1.85	0.37 \pm 0.18	82.57 \pm 26.19	3.87	3.82	0.0031	0.54	0.19
<i>Caladenia procera</i>	6	4.94 \pm 1.74	0.46 \pm 0.22	108.12 \pm 16.96	4.00	2.22	0.0062	0.58	0.34
<i>Drakaea livida</i>	13	0.94 \pm 0.52	0.45 \pm 0.26	113.82 \pm 4.12	3.74	1.90	0.0040	0.67	0.23
Female wasp	7	0.76 \pm 0.15	0.52 \pm 0.53	147.25 \pm 34.27	2.46	1.98	0.0160	0.32	0.11
Background	10	2.16 \pm 1.10	0.41 \pm 0.23	102.27 \pm 12.11					

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786 Online Supplementary Table 1: Collection sites for orchid and wasp specimens

Species	Location	Latitude/longitude
Orchids		
<i>Caladenia decora</i>	100 m W of Collier Rd on Keenan Rd, W of Esperance	33° 49' 20", 121° 50' 23'
<i>Caladenia pectinata</i>	Corner of Hassel Hwy and Kojaneerup Rd, SE side of the highway	34° 39' 06.9", 118° 20' 27.7"
<i>Caladenia procera</i>	Kings Park and Botanic Gardens glasshouse*	Location withheld*
<i>Drakaea livida</i>	Rainbow Cave Rd, SW of Margaret River	34° 00' 15", 115° 01' 18"
Wasps		
<i>Zaspilothynnus nigripes</i>	E of Dewar Rd on Watheroo Rd	30° 19' 05", 115° 35' 04"
<i>Zaspilothynnus nigripes</i>	Martlock Rd, N of Paganoni Rd	32° 22' 27", 115° 49' 26"

787 *original location withheld as a requirement of working on threatened flora. For the
788 original source population see voucher specimen at the Western Australian
789 herbarium (PERTH 08642966; RDP 0230)

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793 Online supplementary Table 2. Similar labella colour indicated by interspecific
 794 comparisons of colour contrast between four sexually deceptive orchids that share a
 795 single pollinator. All values are relatively low, thus colours are likely to be very similar
 796 or indistinguishable when viewed by hymenoptera. Values are mean \pm SE JNDs
 797 (Just Noticeable Differences) calculated according to (Vorobyev and Osorio 1998).

	<i>Drakaea livida</i>	<i>Caladenia procera</i>	<i>Caladenia pectinata</i>
<i>Caladenia decora</i>	2.88 \pm 0.11	4.50 \pm 0.24	3.12 \pm 0.22
<i>Caladenia pectinata</i>	3.52 \pm 0.22	4.61 \pm 0.30	
<i>Caladenia procera</i>	4.03 \pm 0.24		

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801 Online supplementary Table 3. Innovation or differences in lateral sepal colour
 802 indicated by interspecific comparisons of colour contrast between sexually deceptive
 803 orchids that share a single pollinator. Values are much higher than those for
 804 interspecific labella comparisons, thus colours are likely to be much more easily
 805 distinguished when viewed by hymenoptera. Values are JNDs (Just Noticeable
 806 Differences) calculated according to (Vorobyev and Osorio 1998).

	<i>Drakaea livida</i> *	<i>Caladenia procera</i>	<i>Caladenia pectinata</i>
<i>Caladenia decora</i>	-	6.7 ± 0.33	15.87 ± 2.09
<i>Caladenia pectinata</i>	-	17.7 ± 3.01	
<i>Caladenia procera</i>	-		

807 *no sepals

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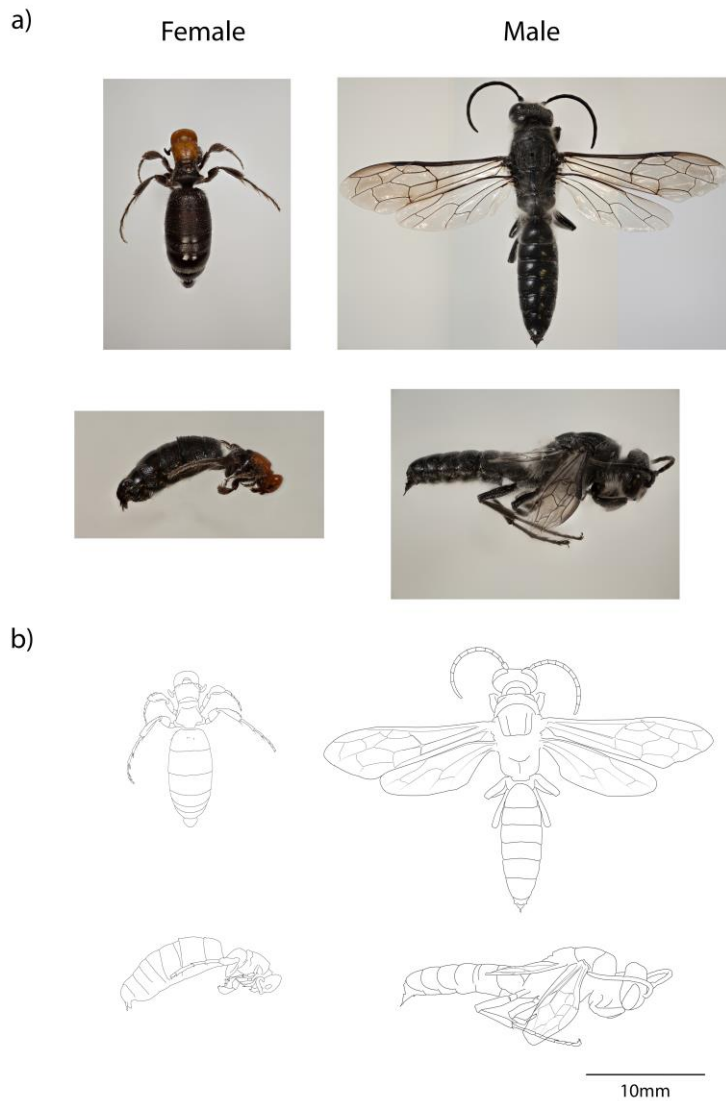
812 Fig. 1: Four sexually deceptive orchids pollinated by a single shared pollinator
 813 species. Top row from left: *Caladenia decora* (photo by Andrew Brown), *C. pectinata*
 814 (photo: Ryan Phillips), *C. procera* (photo: Andrew Brown), and *Drakaea livida* (photo:
 815 Ryan Phillips). Bottom row from left: Male thynnine wasps of *Zaspilothynnus nigripes*
 816 attracted to *Caladenia pectinata* orchid (photo: Christian Ziegler), a pair of
 817 *Zaspilothynnus nigripes in copula*, with the female below (photo: Keith Smith), and
 818 male *Zaspilothynnus nigripes* visiting *Drakaea livida* orchid, with the wasp in position
 819 to remove pollen (photo: Suzi Bond). *Drakaea livida* is distributed broadly across the
 820 range of the three *Caladenia* species, whereas the *Caladenia* species are almost
 821 entirely allopatric.

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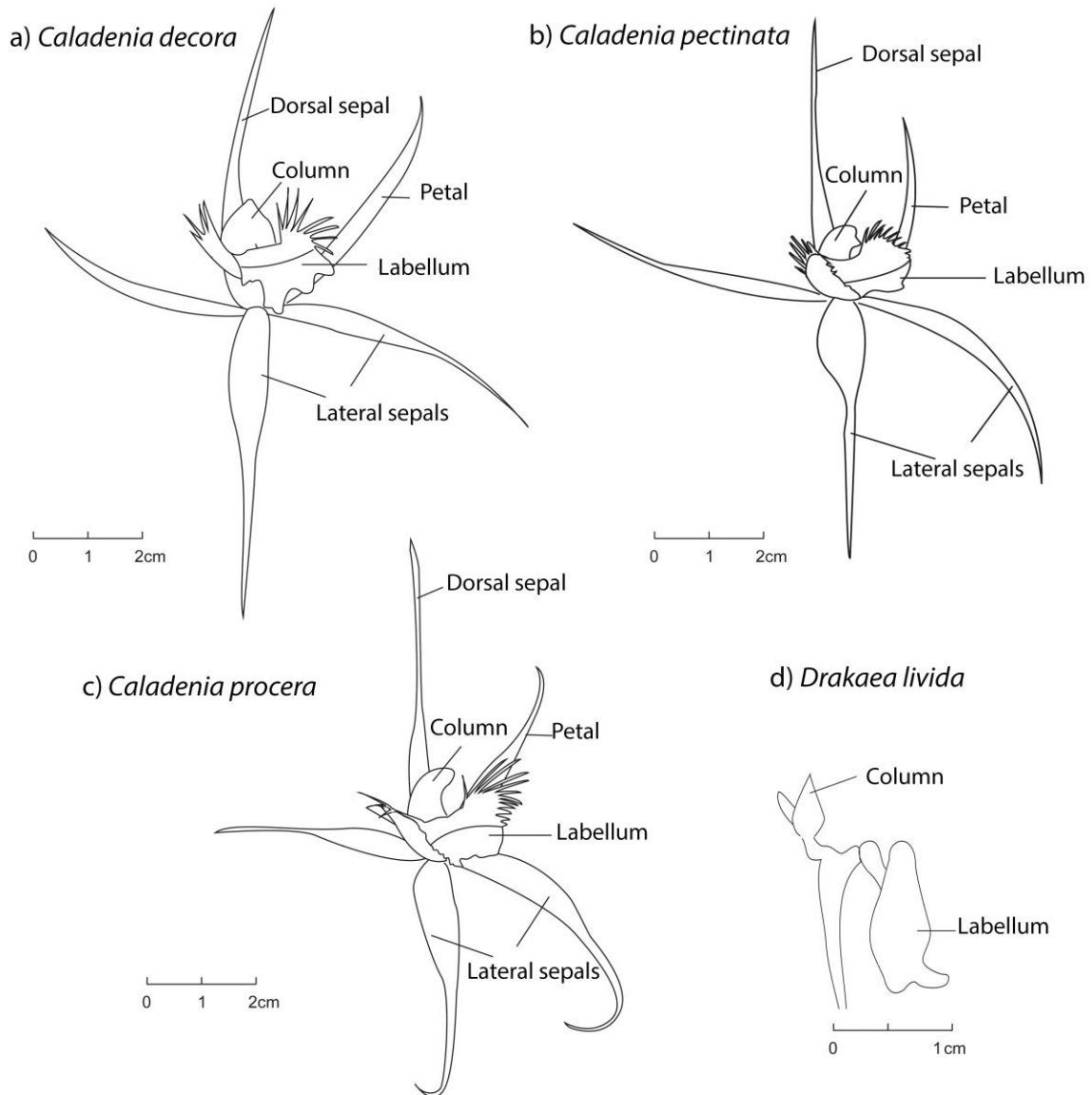


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827 Fig. 2: a) Female and male *Zaspilothynnus nigripes* (Tiphidae). Females are
828 flightless. Males pollinate sexually deceptive *Caladenia* and *Drakaea livida* orchids.

829 b) Line drawings used to calculate areas of colour patches in geometric analyses of
830 overall colour patterns. Photos by Corin Gardiner. Illustrations by Vivian Ward.

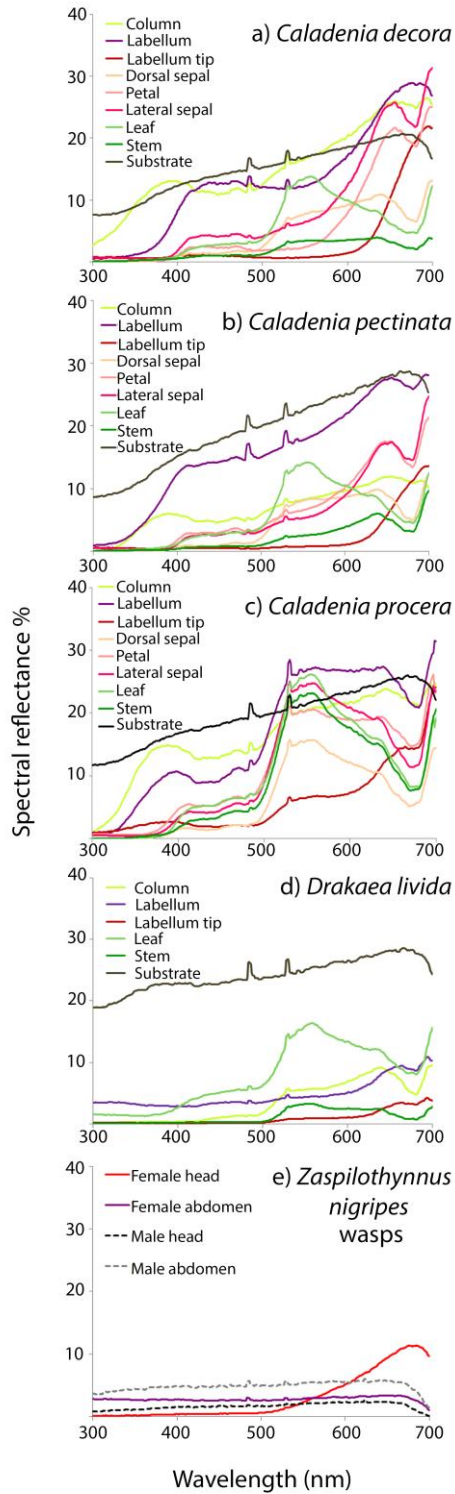
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834 Fig. 3: Line drawings of sexually deceptive orchids used to calculate areas of colour
835 patches for geometric analyses of overall colour patterns. Illustrations by Vivian
836 Ward.

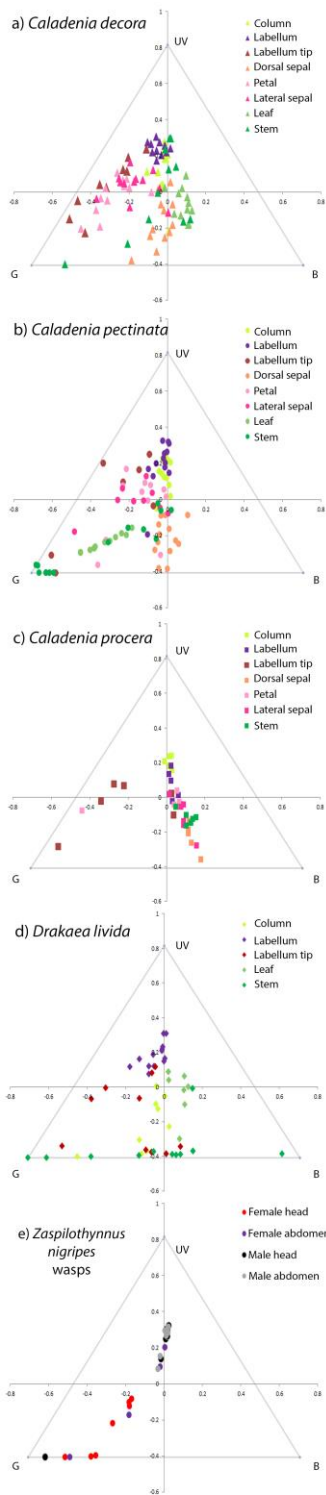
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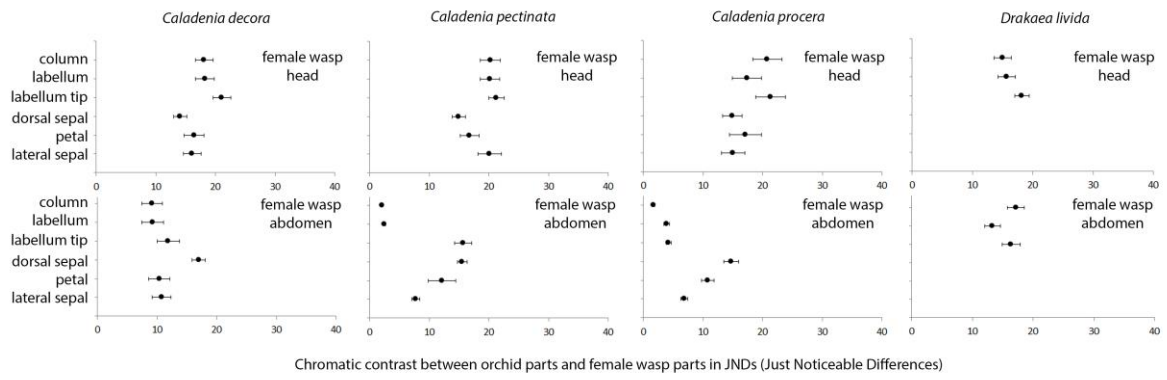
840 Fig. 4: Spectral reflectances of a) four sexually deceptive orchid species, and b)
841 females and males of their shared single pollinator species, *Zaspilothynnus nigripes*.

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 844 Fig. 5: Colour loci of sexually deceptive orchids *Caladenia decora*, *C. pectinata*, *C. procera*,
 845 *Drakaea livida*, and female and male of their pollinator species, *Zaspilothynnus nigripes*,
 846 modelled into a trichromatic hymenopteran colour space (Maxwell colour triangle),
 847 according to equations provided by Kelber et al. (2003).

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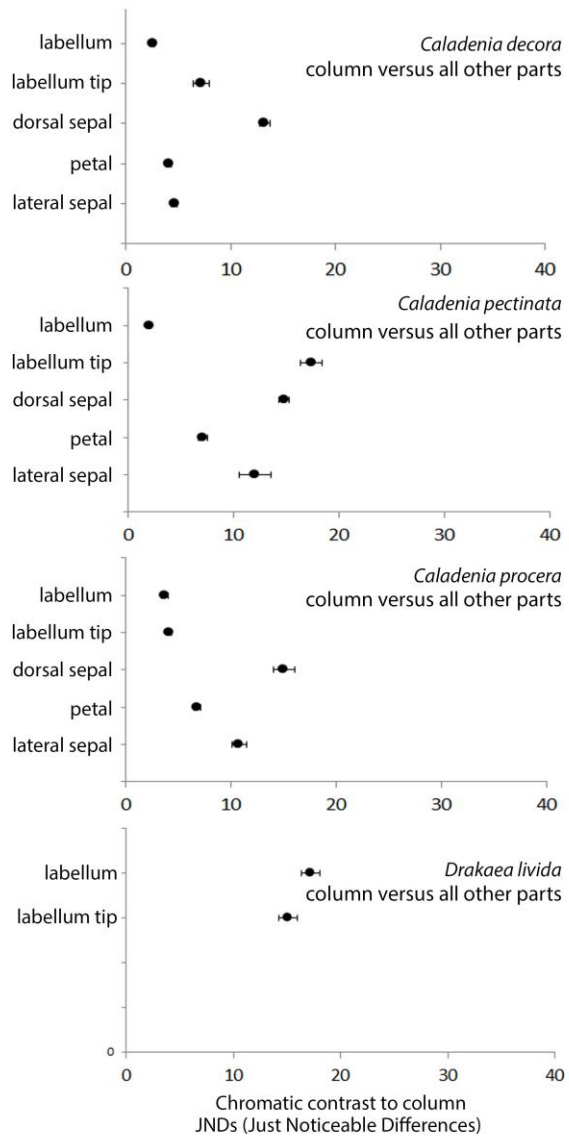
851

852 Fig. 6: Chromatic contrast between colours of orchids and females of their pollinator
 853 species. Units are Just Noticeable Differences (JNDs; according to Vorobyev &
 854 Osorio 1998).

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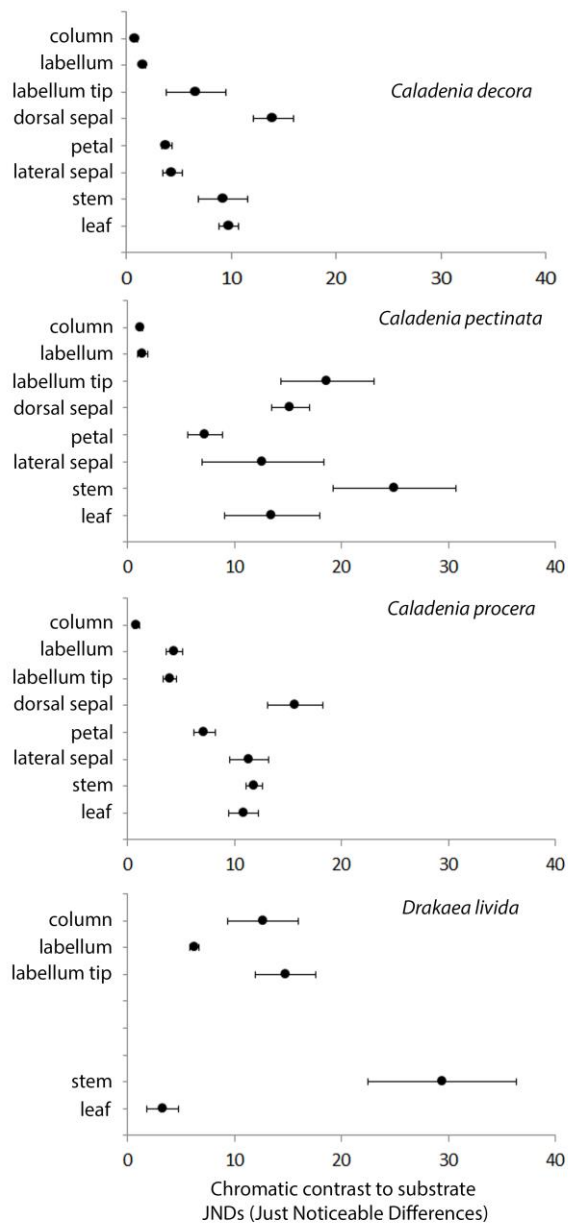
859

860 Fig. 7: Chromatic contrast within the floral parts of four species of sexually deceptive

861 orchid that share a pollinator.

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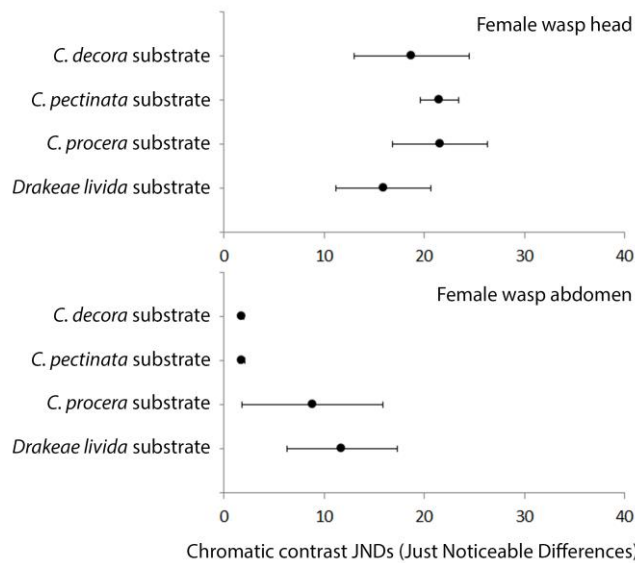


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866 Fig. 8: Chromatic contrast between four sexually deceptive orchids and their

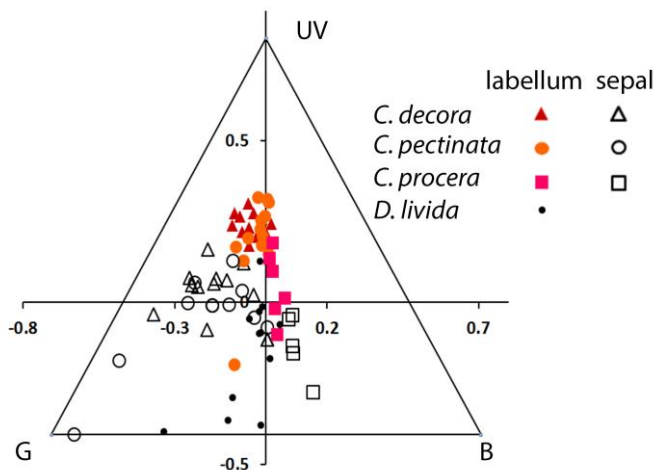
867 substrates.



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869 Fig. 9: Chromatic contrast between female wasps of *Zaspilothynnus nigripes* and
 870 substrates of four sexually deceptive orchids pollinated by male *Z. nigripes*.

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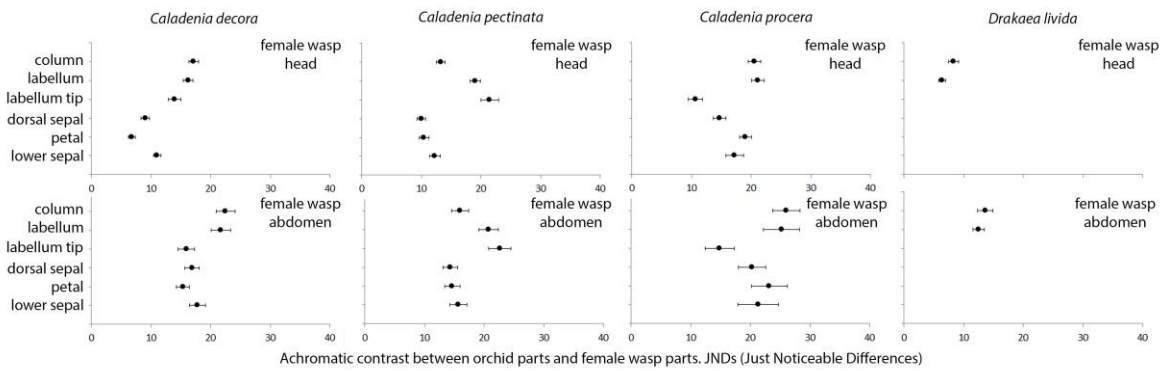


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 874 Fig. 10: Colour loci of the labella and lateral sepals of four sexually deceptive orchids
 875 *Caladenia decora*, *C. pectinata*, *C. procera*, and *Drakeae livida* (no petals) that share
 876 a single pollinator. Modelled into a trichromatic hymenopteran colour space (Maxwell
 877 colour triangle), according to equations provided by Kelber et al. (2003).

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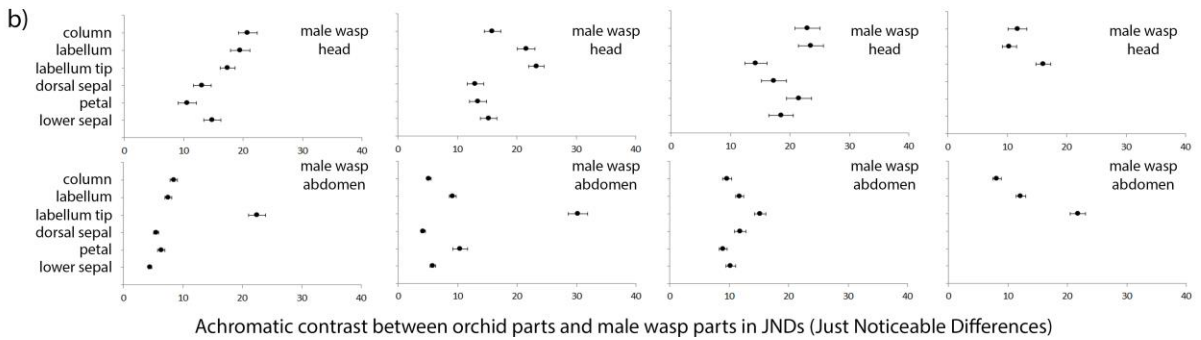
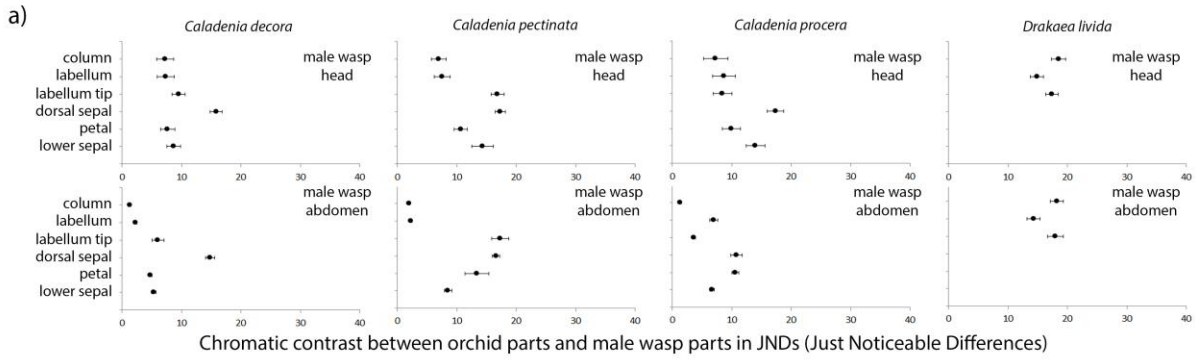
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882 Online supplementary Fig.1: Achromatic contrast between sexually deceptive
883 orchids and female wasps of the pollinator species, *Zaspilothynnus nigripes*. Units
884 are Just Noticeable Differences (JNDs; according to Vorobyev & Osorio 1998).

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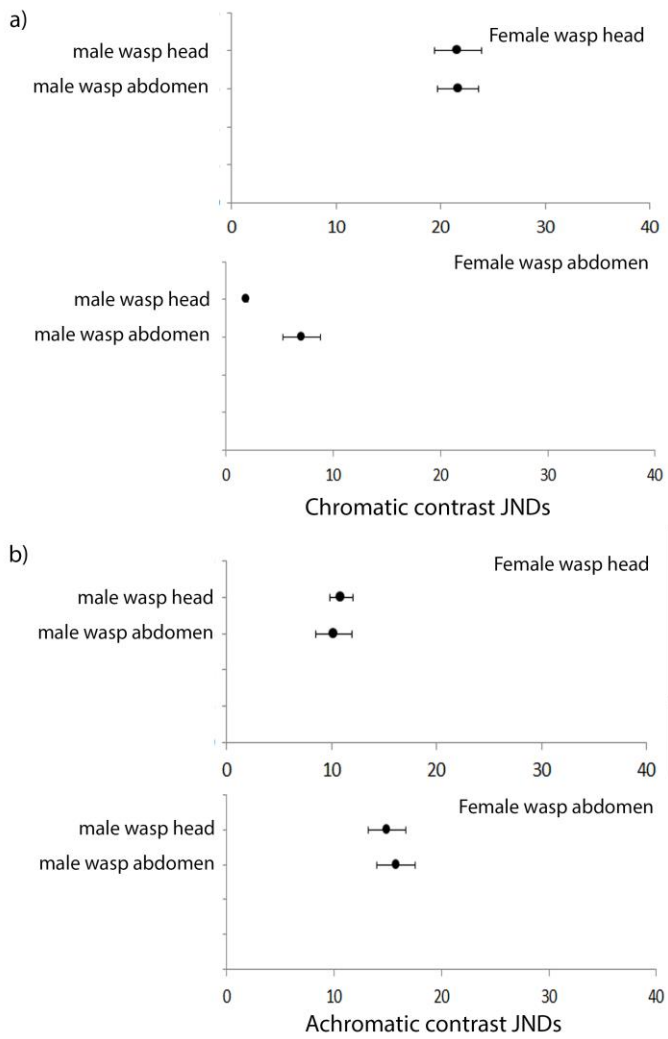
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888 Online supplementary Fig. 2: Contrast between sexually deceptive orchids and male
889 wasps of the pollinator species *Zaspilothynnus nigripes* for a) chromatic, b)
890 achromatic JNDs

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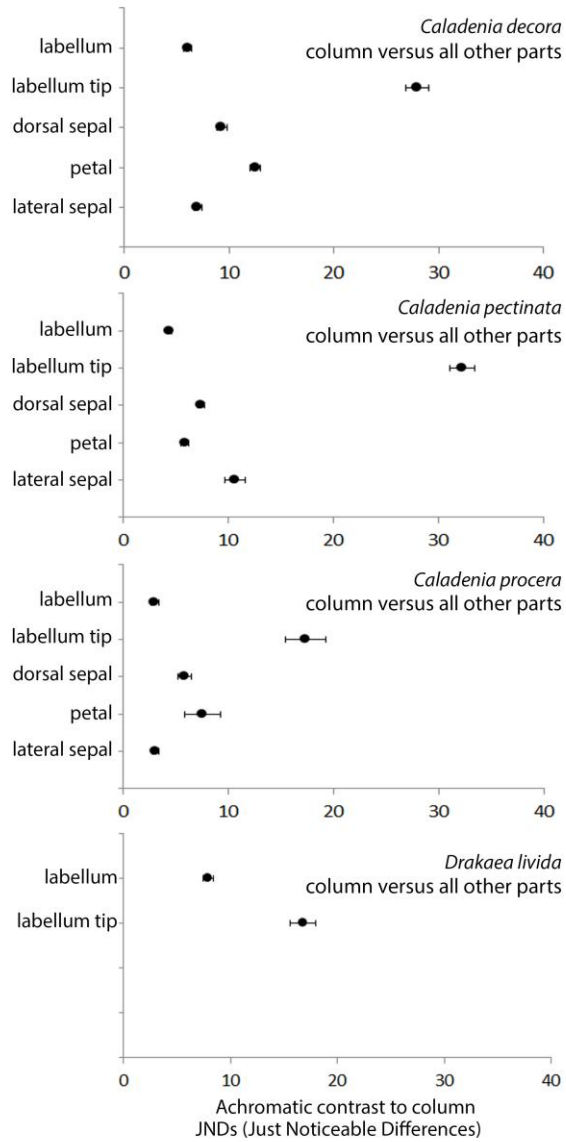
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894 Online supplementary Fig. 3: Colour contrast between female and male wasps of the
 895 sexually deceptive orchid pollinator species, *Zaspilothynnus nigripes* for a) chromatic,
 896 b) achromatic JNDs.

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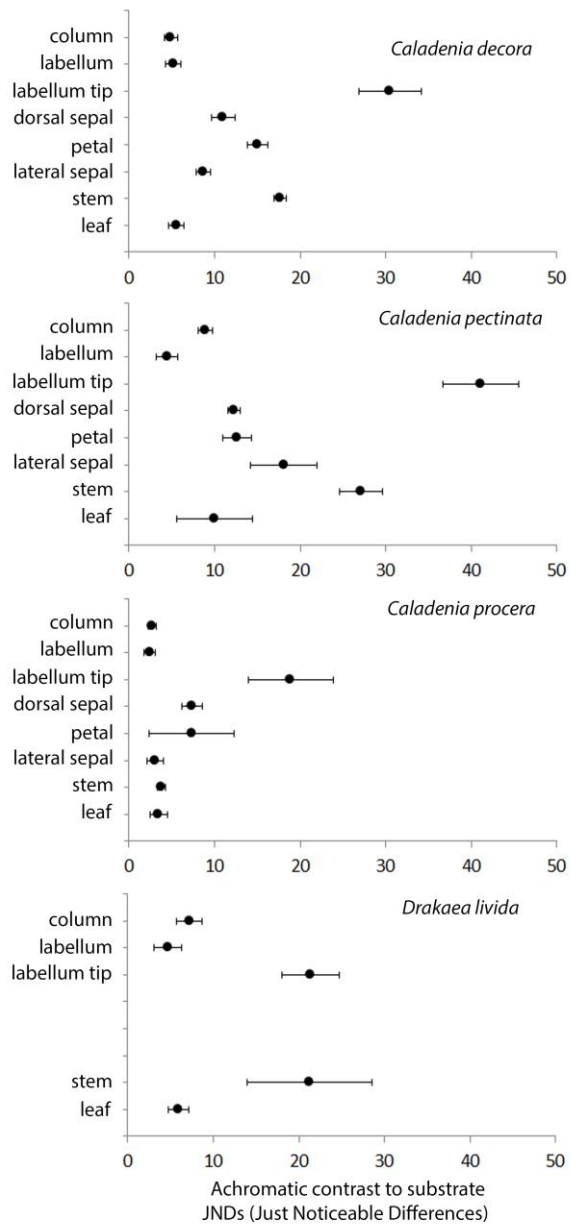
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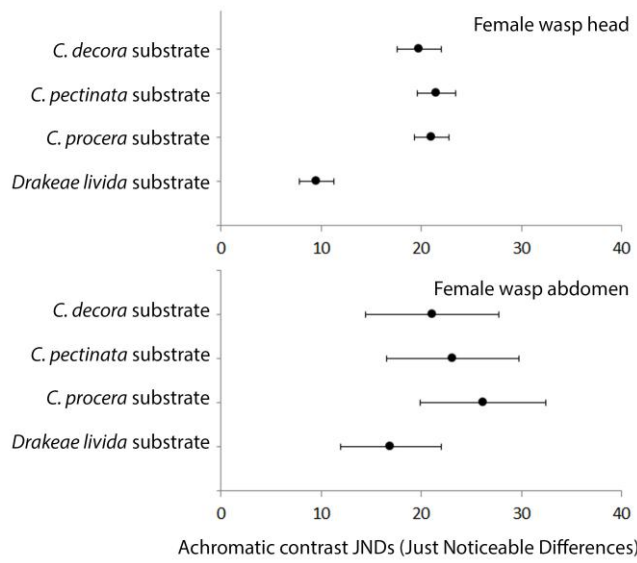
901 Online supplementary Fig. 4: Achromatic contrast between the floral parts within
902 each sexually deceptive orchid species.

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Online supplementary Fig. 5: Achromatic contrast between sexually deceptive orchids and their substrates



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912 Online supplementary Fig. 6: Achromatic contrast between female wasps of
913 *Zaspilothynnus nigripes* and substrates of sexually deceptive orchids.

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