

1 **Evidence for a food-deceptive pollination system using *Hylaeus* bees in *Caladenia***  
2 ***hildae* (Orchidaceae)**

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20 **Running head:** Pollination of *Caladenia hildae* by *Hylaeus* bees

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34 **Abstract**

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36 Numerous orchid species are pollinated by food deception, where rewardless flowers  
37 attract foraging pollinators through the mimicry of other flowers or the use of non-  
38 specific floral signals. Here we investigate the pollination of *Caladenia hildae*, a  
39 member of a diverse Australian genus containing species pollinated by sexual  
40 deception, and species pollinated by food foraging pollinators. Despite eight bee  
41 species occurring at the main study site, only food foraging bees of a single species of  
42 *Hylaeus* (Colletidae) were observed to remove and deposit pollen of *C. hildae*.  
43 Spectral reflectance of *C. hildae* flowers differed from co-flowering rewarding  
44 species in terms of both the wavelengths of light reflected, and the pattern of  
45 colouration. As such, there was no evidence that *C. hildae* uses a pollination strategy  
46 based on floral mimicry. However, the attraction of only a single bee species at this  
47 site suggests that *C. hildae* may use a deceptive strategy that exploits sensory biases  
48 or behaviours of that differ between *Hylaeus* sp. and the remainder of the bee  
49 community. While *Hylaeus* have been recorded visiting orchid flowers in several  
50 parts of the world, *C. hildae* may represent the first documented case of an orchid  
51 species specialised on pollination by *Hylaeus* bees.

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53 **Additional keywords:** orchid, pollination, deception, *Hylaeus*.

54

55 **Introduction**

56

57 Food deceptive orchids can be broadly categorised into those that attract pollinators  
58 via mimicry of the flowers of another plant species (e.g. Nilsson 1983b; Johnson  
59 1994, 2000; Gumbert and Kunze 2001; Peter and Johnson 2008; Jersakova *et al.*  
60 2012), and those that use a set of general signals that are of interest to food-foraging  
61 pollinators (generalised food deception) (e.g. Ackerman 1981; Nilsson 1983a; Steiner  
62 *et al.* 1998; Antonelli *et al.* 2009; Peter and Johnson 2013). Orchid species pollinated  
63 by mimicry of flowers tend to be reliant on one or few pollinator species, likely  
64 because they often exploit relatively specialised mutualisms between plant and  
65 pollinator (Johnson and Schieslt 2016). Alternatively, species pollinated by  
66 generalised food deception typically attract a broader range of pollinator species (e.g.  
67 Ackerman 1981; Nilsson 1983b; Fritz 1990; Henneresse and Tyteca 2016; Johnson

68 and Schiestl 2016; but see Peter and Johnson 2013). However, the gullet shaped  
69 flower of some orchids means that only visitors of a particular size may achieve  
70 pollination, even though a range of species are attracted (e.g. Li *et al.* 2008; Reiter *et*  
71 *al.* 2018).

72

73 *Caladenia* is a diverse Australian genus of terrestrial orchids containing  
74 approximately 330 species (Phillips *et al.* 2009a; Backhouse 2018). There is  
75 substantial variation in morphology, colour and floral odour in the genus (Backhouse  
76 2018). Many *Caladenia* species are pollinated by sexual deception of thynnine wasps,  
77 particularly those with red-green flowers or aggregations of calli on the labellum  
78 (Stoutamire 1983; Phillips *et al.* 2009b; 2017). Alternatively, the large number of  
79 brightly coloured species, which are typically white, cream, yellow or pink, are  
80 predicted to be primarily pollinated via nectar seeking insects (Stoutamire 1983;  
81 Phillips *et al.* 2009b; Phillips *et al.* 2011; though see Phillips *et al.* 2017 and Phillips  
82 and Peakall 2018 for some exceptions). Recent work on *Caladenia* that produce  
83 meagre nectar rewards has revealed specialised systems based on a species of  
84 thynnine wasp (Reiter *et al.* 2018, 2019b), a species of colletid bee (Reiter *et al.*  
85 2019a), and a more generalist system with visitation from both Hymenoptera and  
86 Diptera (Faast *et al.* 2009). Pollination by food deception has received comparatively  
87 little attention in *Caladenia*, though anecdotal reports from putatively food deceptive  
88 species suggest that a range of Hymenoptera, Diptera and Coleoptera are potentially  
89 involved (see Phillips *et al.* 2009b for review; Kuitert 2016). A recent study on  
90 *Caladenia nobilis*, a species with primarily nectarless flowers, revealed that  
91 pollination was by a single species of nectar-seeking thynnine wasp (Phillips *et al.*  
92 2020).

93

94 Here, we investigate pollination in *Caladenia hildae* Pescott & Nicholls, a member of  
95 *Caladenia* subgenus *Stegostyla*. At present very little is known about the pollination  
96 biology of this subgenus other than incidental records that suggest that bees in the  
97 genera *Hylaeus*, *Exoneura*, *Lasioglossum*, *Exoneurella*, and *Braunsapis* are likely to  
98 be among the pollinators of at least some species (Bates 1982; Kuitert 2016). There  
99 are no records of nectar production in *Caladenia* subgenus *Stegostyla*, or observations  
100 of glistening calli, which seems to be indicative of nectar secretion in some other  
101 *Caladenia* (e.g. Reiter *et al.* 2018). While there are no records of insect visitation to

102 *C. hildae*, the bright golden colouration (Figure 1), sweet floral odour, and lack of  
103 visible nectar suggest that pollination by food deception is the likely strategy. For *C.*  
104 *hildae*, we addressed three questions: (i) which insect species are involved in  
105 pollination? (ii) how specialised is the pollination system? (iii) based on floral traits of  
106 co-flowering plants, is there evidence that are pollinators attracted via mimicry of one  
107 or more model species?

108

## 109 **Materials and methods**

110

### 111 *Study species*

112

113 *Caladenia hildae* is patchily distributed in the southern part of the Australian Alps,  
114 primarily at subalpine elevations of 600 – 1400 m a.s.l. (Backhouse 2018). Most  
115 populations occur in open forest or woodland, often on drier slopes or ridge tops  
116 (Backhouse *et al.* 2016). Flowering time is variable between populations but occurs  
117 between October and December. Plants produce a single scape per flowering season  
118 (to 25cm), with one to four flowers (lateral sepals to 15mm in length; Backhouse  
119 2018). There is no evidence of self-pollination in *C. hildae* (Jones 2006).

120

### 121 *Study site*

122

123 The study was undertaken at two sites in the Alpine National Park, Victoria. The main  
124 study site was at a population of *Caladenia hildae* (voucher RDP 0477; submitted to  
125 the National Herbarium of Victoria) adjacent to Limestone Road near Native Dog Flat  
126 campground (NDF; 36° 53' 55"S 148° 05' 39" E). A secondary site, where *C. hildae*  
127 has been reported in previous years but was not seen flowering during the study, was  
128 also surveyed for pollinators. This site was further west along Limestone Road (LRW;  
129 36° 52' 24"S 148° 03' 00"E).

130

### 131 *Pollinator observations*

132

133 Pollinator observations were undertaken on the 18th –21st of November 2016, when  
134 *C. hildae* had just begun to flower at NDF. Attempts to make additional observations  
135 in subsequent years were abandoned due to dry conditions leading to low rates of

136 flowering of *C. hildae*. From an assessment of a 15 x 15 m quadrat in the centre of the  
137 population, 25 of 56 flowers were pollinated during the four days of the study period,  
138 confirming that pollinators were active at this site. Based on a preliminary assessment  
139 of pollinator activity, pollinator observations were undertaken when the temperature  
140 was above 18°C and there was no strong wind between the hours of 9:30 am and 4:30  
141 pm Eastern Daylight Savings Time.

142

143 While food-deceptive orchids are generally characterised by low visitation rates, it  
144 has recently been shown that pollinators can be attracted via a modification of the  
145 pollinator baiting method that has been used with much success for sexually deceptive  
146 orchids (Stoutamire 1983; Peakall 1990). In this method picked flowers are moved to  
147 a new part of the landscape, leading to the rapid attraction of deceived pollinators.

148 While baiting was initially thought to be ineffective for systems based on food-  
149 foraging pollinators, increasing the number of flowers and thereby the visual and  
150 chemical stimulus appears to lead to a much higher visitation rate (see Reiter *et al.*  
151 2018, 2019, 2020 for examples of species with a meagre nectar reward; see  
152 Scaccabarozzi *et al.* 2018, 2020; Phillips *et al.* 2020 for species that are food  
153 deceptive). Here, we used a bunch of 8 scapes of *C. hildae* (1-2 flowers per scape) all  
154 in the same vial to attract pollinators. A total of 99 6-minute baiting periods were  
155 undertaken at the NDF site and 9 baiting periods at the LRW site. For all insects  
156 attracted to the flower we scored whether they alighted on the flower, where they  
157 landed, if they contacted the labellum, if they contacted the column and if they  
158 removed or deposited pollinia. Given that many species of *Caladenia* are pollinated  
159 by sexual deception (e.g. Stoutamire 1983; Phillips *et al.* 2017), close attention was  
160 paid to the possibility of pollinators exhibiting sexual behaviour with the flower.

161

162 *Identifying the pool of potential pollinators*

163

164 Following preliminary evidence that *C. hildae* attracted bees as pollinators, we  
165 collected other members of the bee community at the NDF site to test if *C. hildae* was  
166 specialised on a subset of the locally available bee species. We focused on NDF  
167 because this was the site where we made the greatest number of pollinator  
168 observations, allowing for a more accurate comparison of the pool of potential  
169 pollinators with the number of actual pollinator species. In addition to collecting bees

170 seen opportunistically during pollinator observations (usually those feeding on  
171 *Daviesia latifolia*; Faboideae), we conducted a 1-hour period of sweep-netting  
172 through vegetation and in gaps within the shrub layer to capture species that were less  
173 easily observed on food plants. Voucher specimens were deposited in the Australian  
174 Museum.

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#### 176 *Floral spectral reflectance of Caladenia hildae and co-flowering plants*

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178 To test if there is any evidence of *C. hildae* visually mimicking members of the co-  
179 occurring plant community, we measured the floral spectral reflectance of *C. hildae*  
180 and the four species of rewarding plants also flowering at the NDF site (no additional  
181 species were flowering at LRW). For each species, flowers from six different  
182 individuals were used. For *C. hildae*, measurements were taken for the lateral sepal,  
183 petal and labellum tip. For *Goodenia hederacea* Sm. (Goodeniaceae) and *Epacris*  
184 *impressa* Labill. (Ericaceae), a measurement was made on one the petals, while for  
185 *Daviesia latifolia* R. Br. and *Daviesia ulicifolia* Andrews (Fabaceae) measurements  
186 were made on both the keel and the standard, which have different colours to the  
187 human eye.

188

189 Floral colour was quantified by spectrophotometry with an Ocean Optics (Dunedin,  
190 FL, USA) USB 4500 spectrometer and a UV–vis 400 fibre optic reflection probe, held  
191 at 5 mm from the middle of the surface of the floral tissue at 45°, with an integration  
192 time of 50 milliseconds. For each species the average of the reflectance for the six  
193 individuals was calculated. Spectral reflectance was analysed using the colour  
194 hexagon model of bee vision, which is based on the sensitivities of photoreceptors of  
195 the bee *Apis mellifera* (Chittka 1992; Chittka and Kevan 2005).

196

## 197 **Results**

198

### 199 *Pollinator observations*

200

201 With one exception, all the visitors to *C. hildae* belonged to an undescribed hylaeine  
202 bee species in the subgenus *Hylaeus* (*Prosopistemon*) (Colletidae; Figure 2). No other  
203 hylaeine bees were observed in the present study, so all references to *Hylaeus* sp.

204 refer to this species. Prior to this study, only 16 specimens of the bee had been  
205 identified in museum collections (two in the Australian National Insect Collection,  
206 two in the Queensland Museum, and twelve in the Australian Museum).

207

208 At the NDF site, a total of 41 floral visitors to bait flowers of *C. hildae* were  
209 observed, all of which were *Hylaeus* sp.. Of the specimens captured, six were males,  
210 and one was a female. At the LRW site, seven floral visitors to bait flowers of *C.*  
211 *hildae* were observed, all of which were *Hylaeus* sp. with the exception of one  
212 individual of *Melittosmithia* sp. (Colletidae). Of the total of 47 responses by *Hylaeus*  
213 sp. to *C. hildae*, seven individuals (14.9%) landed on the flower, five contacted the  
214 labellum and four contacted the column (8.5%). There were two cases of pollinia  
215 being removed, and two of pollen deposition. Of the individuals responding to the  
216 flowers, 9 were already carrying pollinia of *C. hildae*, the only *Caladenia* species  
217 flowering at the site. In all cases pollen of *C. hildae* was deposited on the dorsal side  
218 of the thorax. The single male *Melittosmithia* sp. attracted to the flower approached  
219 closely but did not land on the flower.

220

221 Rather than flying directly to the flower, visitors often showed some level of zig-  
222 zagging in flight as they approached. When landing on the flower, *Hylaeus* sp. tended  
223 to move directly to the labellum, though only some moved into the position needed  
224 for pollination. Those bees that moved onto the labellum always did so head first. In  
225 three instances, individuals moved to multiple flowers during the one visit. At least  
226 two individuals appeared to show nectar-seeking behavior on the labellum, but this  
227 was not possible to observe once they moved to the base of the column and were  
228 obscured from view. None of the bees made any attempt to collect pollen from the  
229 flower. Likewise, there was no evidence of the copulatory behaviour or sustained  
230 attraction of pollinators seen in sexually deceptive *Caladenia* (see Phillips *et al.* 2009;  
231 Phillips *et al.* 2017).

232

233 *Identifying the pool of potential pollinators*

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235 Opportunistic collections and sweep-netting yielded at NDF yielded a total of six  
236 additional species of native bee; *Exoneura* (*Exoneura*) *robusta*, *Exoneura*  
237 (*Brevineura*) sp. (Apidae), *Lasioglossum* (*Chilalictus*) *brunnesetum*, *Lasioglossum*

238 (*Austrevylaeus*) sp., *Lasioglossum* (*Parasphecodes*) *melbournense* (Halictidae), and  
239 *Megachile ordinaria* (Megachilidae). In addition, the introduced *Apis mellifera*  
240 (Apidae) was present at the site. While smaller species could not be as readily  
241 observed, some individuals of the *Lasioglossum* and *Exoneura* species, and *A.*  
242 *mellifera*, were foraging throughout the period in which pollinator observations were  
243 undertaken. However, no species other than *Hylaeus* sp. was observed carrying pollen  
244 of *C. hildae*.

245

#### 246 *Floral spectral reflectance of Caladenia hildae and the co-flowering plants*

247

248 The lateral sepals and labellum throat of *C. hildae* were highly reflective (Figure 3)  
249 and were in the blue-green sector of the colour hexagon bee vision model (Figure 4).  
250 Alternatively, the labellum was very dull and at the centre of the colour hexagon bee  
251 vision model, meaning it would be perceived as almost colourless. As such, the  
252 labellum tip provides a strong contrast with the remainder of the flower. The tubular  
253 flowers of *E. impressa* were highly reflective and in the blue-green sector of the  
254 colour hexagon, though were separated from the labellum throat of *C. hildae* by a  
255 Euclidean distance of 0.13, meaning that bees should readily be able to discriminate  
256 between the two colours (Dyer & Chittka 2004a,b; Garcia *et al.* 2017). Unlike *C.*  
257 *hildae*, the other flowers at the site all had a pronounced peak in the UV part of the  
258 spectrum and were in the UV and UV-green sectors of the colour hexagon.

259

#### 260 **Discussion**

261

262 Despite the occurrence of a community of at least eight species of native bee at the  
263 main study site, only the colletid bee *Hylaeus* sp. was regularly attracted to *C. hildae*  
264 and achieved pollination. The bees showed behaviour on the flower consistent with  
265 food-foraging behaviour, and there was no evidence of the sexual attraction seen in  
266 the numerous *Caladenia* pollinated by sexual deception of thynnine wasps  
267 (Stoutamire 1983; Phillips *et al.* 2009b). As such, at this site *C. hildae* seems to be  
268 using a food-deception strategy based on *Hylaeus* bees attempting to forage nectar.  
269 Despite *Hylaeus* being a diverse cosmopolitan genus (Michener 2000), and hylaeine  
270 species being recorded as visitors to several species of orchids (Bates 1982  
271 (Australia); Catling 1983 (North America); Slater and Calder 1988 (Australia);



272 Lehnebach and Robertson 2004 (New Zealand); Bänziger *et al.* 2008 (China);  
273 Henneresse and Tyteca 2016 (Europe); Kuitert 2016 (Australia); Sugiura 2017  
274 (Japan)), to our knowledge there are no known pollination strategies in the orchids  
275 involving specialisation on *Hylaeus*. As such, if *C. hildae* proves to be specialised on  
276 *Hylaeus* elsewhere in its geographic range, this strategy of pollination primarily by  
277 *Hylaeus* would be highly unusual among orchids.

278

279 Floral spectral reflectance measurements of the plant community that co-flowers with  
280 *C. hildae* provided strong evidence that pollinators are not attracted through visual  
281 mimicry. While some of the co-occurring flowers were also zygomorphic, both  
282 *Daviesia* species have the keel-flower floral form, while *G. hederacea* lacks the  
283 heavily modified petal (labellum) of orchids. Further, none of the co-flowering plants  
284 had similar floral colouration to *C. hildae*. In particular, *Goodenia* and *Daviesia* had  
285 pronounced peaks of UV reflectance, which was not the case in *C. hildae*. In addition  
286 to direct comparisons of floral colour, *C. hildae* also exhibited a different pattern to  
287 the other plant species, with the labellum tip, which is colourless in bee vision  
288 models, contrasting strongly with the adjacent labellum and tepals. It is possible that  
289 the dull colouration of the labellum tip of *C. hildae* plays a role in luring the pollinator  
290 to the labellum, and therefore the reproductive structures, through its pronounced  
291 colour contrast with the remainder of the flower.

292

293 While it is possible that observations in other years or at other sites may yield  
294 additional pollinator species, *Hylaeus* sp. was the only bee species attracted to *C.*  
295 *hildae* at the main study site despite the presence of eight co-occurring bee species.  
296 As such, in this system it appears that specialisation is achieved at the attraction  
297 phase, regardless of any secondary filters related to the size and behaviour required  
298 for pollination. In *C. hildae*, dietary specialisation by other bees is unlikely to explain  
299 avoidance of the orchid as, based on visitation data of these and/or related species,  
300 most are believed to be generalist nectar foragers (Hingston 1999; Sugden and Pyke  
301 1991; Walker 1995; Michener 2007; Batley 2019). However, some of these bees may  
302 be exhibiting floral constancy to the much more floriferous co-occurring rewarding  
303 plants (Grant 1950; Waser 1986) or ignoring *C. hildae* because its small flower may  
304 inhibit foraging by larger bees. Interestingly, at the LRW site, one case of visitation to  
305 *C. hildae* was observed by a male *Mellitostmithia* sp., a very poorly known genus

306 where there is preliminary evidence for a preference for Fabaceae (Houston 2018, M.  
307 Batley unpublished), supported by the possibility that the carina at the center of the  
308 clypeus is an adaptation for foraging on pea plants.

309

310 It is possible that pollinator attraction in *C. hildae* is achieved by exploiting visual or  
311 chemical cues that *Hylaeus* sp. finds more attractive than other bees, either through  
312 innate or learned behaviour (e.g. Dötterl and Vereecken 2010; Milet-Pinheiro *et al.*  
313 2012, 2013; Carvalho *et al.* 2014). For example, some bees show an innate preference  
314 for flowers that are UV absorbing white (Dyer *et al.* 2016, 2019), as is present on  
315 much of the labellum of *C. hildae*. Alternatively, bees may develop preferences  
316 through learning to associate particular floral traits with the provision of a food  
317 reward, though these preferences can extend to flowers without closely matching  
318 traits (Gumbert 2000; Dyer and Murphy 2009). In the case of *Hylaeus* sp., no flower  
319 visiting information is associated with the small number of known specimens, but two  
320 lines of evidence suggest that it is likely to be a generalist forager. Firstly, *Hylaeus* sp.  
321 has a broad geographic range having been collected in the Blue Mountains and  
322 Shoalhaven in NSW, Mount Buffalo and the study site in Victoria, and near Waratah,  
323 Tasmania (Houston, 1970; specimen labels in Australian Museum). Furthermore,  
324 specimens collected beyond the range of *C. hildae* in the Blue Mountains (NSW) by  
325 the late Norman Rodd have collection dates in August, October, November,  
326 December, April and May (specimens in the Australian Museum). Activity over such  
327 a large part of the year makes it unlikely that *Hylaeus* sp. visits a very limited number  
328 of flower species.

329

330 The present study is first systematic investigation of the pollination of a *Caladenia*  
331 outside of the ‘spider orchid’ clade (subgenera *Calonema*, *Phlebochilus* and  
332 *Drakonorchis*). Similarly, detailed studies of the pollination of related genera are  
333 mostly lacking, though Peakall (1987) showed that pollination of *Cyanicula gemmata*  
334 occurred through a combination of beetles congregating to mate and bees attempting  
335 to forage nectar. Based on incidental records, pollination by nectar foraging  
336 Hymenoptera, and to a less extent Diptera and Coleoptera, is likely to be typical for  
337 subgenera *Stegostyla*, *Elevatae* and *Caladenia* (Bates 1982; Phillips *et al.* 2009b;  
338 Kuiter 2016), and most other genera in the Caladeniinae (Rogers 1931; Erickson  
339 1965; Peakall 1987; Kuiter 2016). However, given the evidence for a specialised

340 pollination system in *C. hildae*, and the diversity of floral traits evident among  
341 putatively food deceptive Caladeniinae, there is likely to be diversity in the  
342 pollination niches occupied among these orchids, potentially including other species  
343 that exhibit specialisation on one or few pollinator species.

344

#### 345 **Conflicts of interest**

346

347 The authors declare no conflicts of interest.

348

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350

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610 **Figure 1:** *Caladenia hildae*. Photo by Colin Rowan.

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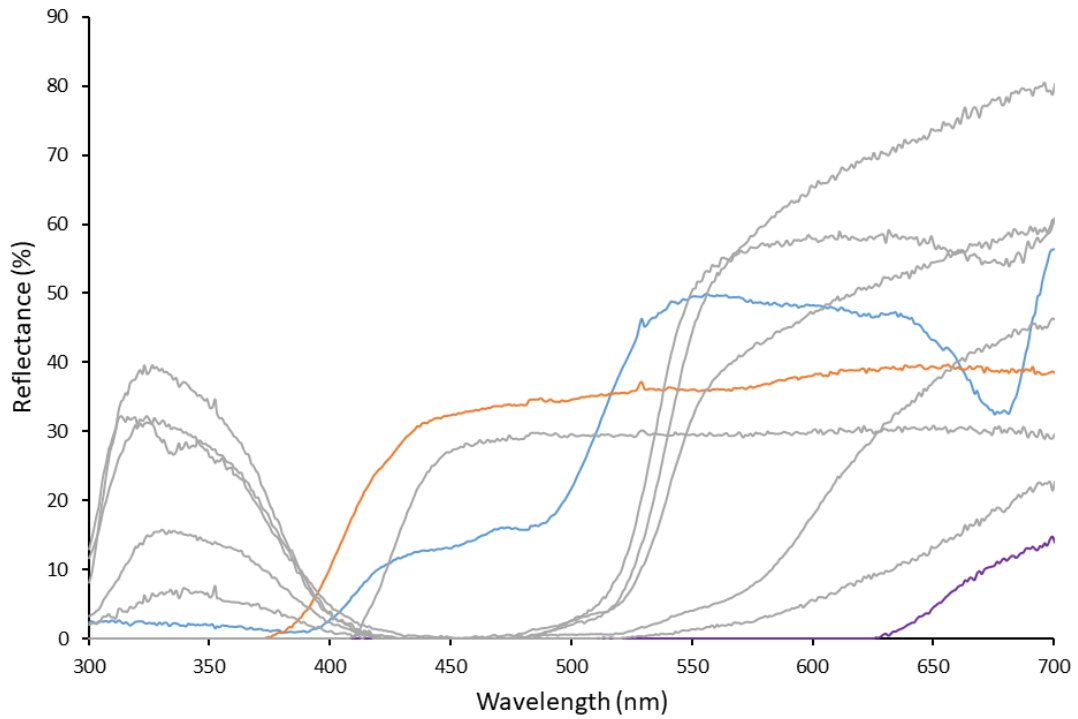
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614 **Figure 2:** A female *Hylaeus (Prosopistemon)* sp. collected carrying pollen of  
615 *Caladenia hildae* on its scutum. Photo by Michael Batley.

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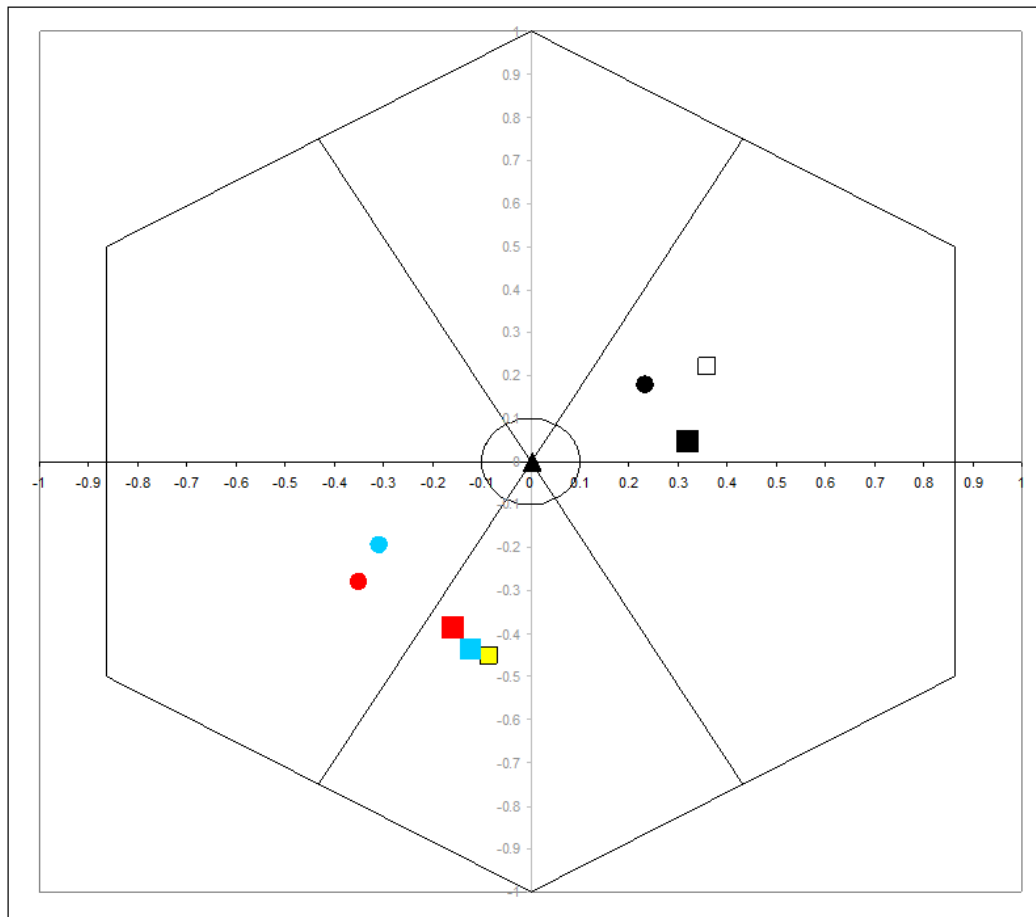


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619 **Figure 3:** Spectral reflectance curves of floral parts of *Caladenia hildae* compared to  
 620 the co-flowering plant community. Orange = underside of the labellum of *C. hildae*;  
 621 blue = lateral sepals of *C. hildae*; purple = tip of labellum of *C. hildae*; grey = other  
 622 members of the plant community.

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626 **Figure 4:** Mean colour loci for *Caladenia hildae* and co-flowering plant species in the  
 627 hexagon bee vision model. *C. hildae* underside of labellum: black circle; *C. hildae*  
 628 lateral sepals: black square; *C. hildae* labellum tip: black triangle; *Goodenia*  
 629 *hederacea*: yellow square; *Epacris impressa*: white square; *Daviesia latifolia* keel:  
 630 blue circle; *Daviesia latifolia* standard: blue square; *Daviesia ulicifolia* keel: red circle;  
 631 *Daviesia ulicifolia* standard: red square.