

UV reflectance but no evidence for colour mimicry in a putative brood-deceptive orchid *Corybas cheesemanii*

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Abstract Rewardless orchids attract pollinators by food, sexual, and brood-site mimicry, but other forms of sensory deception may also operate. Helmet orchids (*Corybas*, *Nematoceras* and related genera) are often assumed to be brood-site deceivers that mimic the colours and scents of mushrooms to fool female fungus gnats (Mycetophilidae) into attempting oviposition and pollinating flowers. We sampled spectral reflectances and volatile odours of an endemic terrestrial New Zealand orchid *Corybas cheesemanii*, and co-occurring wild mushrooms. The orchid is scentless to humans and SPME GC-MS analyses did not detect any odours, but more sensitive methods may be required. The orchids reflected strongly across all visible wavelengths (300–700nm) with peaks in the UV (~320nm), yellow-green (500–600 nm) and red regions (650–700 nm), whereas mushrooms and surrounding leaf litter reflected predominantly red and no UV. Rather than mimicking mushrooms, these orchids may attract pollinators by exploiting insects' strong sensory bias for UV. Modelling spectral reflectances into a categorical fly vision model and a generic tetrachromat vision model provided very different results, but neither suggest any mimicry of mushrooms. However, these models require further assessment and data on fly spectral sensitivity to red wavelengths is lacking – a problem given the predominance of red, fly-pollinated flowers worldwide [*Current Zoology* 60 (1): 104–113, 2014].

Keywords Diptera, Colour space, Fly pollination, Orchidaceae, Spectral reflectance, Visual modelling

Flowers typically attract pollinators by advertising rewards such as food (Chittka and Thompson, 2001; Raguso, 2004). Advertising usually involves floral odours and colours that attract pollinators, enhance the detectability of flowers, influence pollinator orientation on flowers, and interact with pollinator learning to generate behaviours such as floral constancy (Hempel De Ibarra et al., 2000; Gegeer and Laverty, 2001; Raguso, 2008; Wright and Schiestl, 2009). Floral odours and colours can even be tailored to pollinator physiology, sensory systems, and behavioural patterns, creating 'private channels' or pollinator syndromes that select for specific guilds or even single species of pollinators (Fenster et al., 2004; Schaefer et al., 2004; Johnson et al., 2006; Shuttlesworth and Johnson, 2010).

In rewardless or deceptive pollination systems, colour and scent signals may interact differently with pollinator behaviour than in rewarding systems, including additional functions such as mimicry, or sensory traps that exploit pollinators' pre-existing sensory biases (for reviews see Jersáková et al., 2009; Schaefer and Ruxton, 2009; Gaskett, 2011; Schiestl and Dötterl, 2012; Schiestl and Johnson, 2013). Deceptive pollination has evolved in a range of plants, but is particularly common

in the Orchidaceae, in which approximately one-third of species do not reward their pollinators (Jersáková et al., 2006). Most of these deceptive orchids exploit the foraging behaviour of their pollinators, both male and female insects, by resembling rewarding flowers but failing to provide food (Jersáková et al., 2009). Sexual deception, in which male insects are fooled into sexual behaviour with flowers, is less common than food deception and generally involves specialisation to a species-specific pollinator (Peakall et al., 2010; Gaskett, 2011; but see Phillips et al., 2013). For some sexually deceptive orchids, mimicry of female insect scents, colours and shapes can even cause pollinators to ejaculate on flowers and waste their sperm (Blanco and Barboza, 2005; Gaskett et al., 2008). A third and less well-studied form of deception by orchids is brood-site deception, in which flowers exploit primarily female insects by mimicking oviposition sites (Shi et al., 2009; Urru et al., 2011). This pollination mechanism may impose reproductive costs on female insects if they oviposit in flowers, and the eggs and larvae subsequently die (Kaiser, 2006).

Brood-site mimicry is best-known for the Araceae, Aristolochiaceae and Rafflesiaceae families, but is pre-

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sent in several other angiosperm families including Orchidaceae (Raguso, 2004; Kaiser, 2006; Urru et al., 2011; for a brief review of brood-site deception in orchids, see Kelly et al., 2013). In general, brood-site deceptive flowers tend to be fly- or beetle-pollinated and correspondingly, mimic oviposition sites such as carrion, dung, fermenting fruit or fungi, mainly through olfactory signals (Christensen, 1994; Jürgens et al., 2006; Kaiser, 2006). For example, across several flower families, carrion and carnivore dung mimicry often involves production of sulphide compounds such as dimethyl disulphide and dimethyl trisulphide, whereas herbivore dung mimics produce phenol, indole and *p*-cresol (Jürgens et al., 2006; Urru et al., 2011). Flowers can also attract flies by mimicking yeast and fungal odours (Stöckl et al., 2010; Goodrich, 2012). For example, the orchid *Dracula chesteronii* emits classic fungal compounds such as 3-octenol, 3-octenone, 1 octen-3-ol, and 1-octen-3-one (Kaiser, 2006; De Pinho et al., 2008).

Brood-site deception is also likely to involve visual signals. Many arums and stapeliads have red (“hematic”) colouration reminiscent of a carcass and sometimes a white or translucent centre that may draw pollinators into the flower (Raguso, 2004; Jürgens et al., 2006; Urru et al., 2011). The brood-deceptive orchid *Dracula chesteronii* resembles the gilled cap of a mushroom (Kaiser, 2006; Endara et al., 2010), and *Paphiopedilum dianthum* is patterned with small contrasting spots that resemble aphids; important prey for the larvae of its hoverfly pollinator (Shi et al., 2009).

Helmet orchids from the terrestrial Asian-Pacific genera *Corybas*, *Nematoceras*, *Corysanthes*, and *Anzybas* (Diurideae) are often considered specialist brood-site deceivers that mimic the colours and scents of mushrooms to fool female fungus gnats (Mycetophilidae) into acting as pollinators (Jones, 1970; Fuller, 1994; Pridgeon et al., 2001; Van Der Cingel, 2001; Jones, 2006; Scanlen, 2006; Clements et al., 2007; St George, 2007; Vereecken and McNeil, 2010). Data on pollinators is drawn largely from fortuitous anecdotal observations of Mycetophilids found on or inside flowers, or carrying orchid pollinia, and insect eggs in flowers presumably oviposited by duped pollinators (Scanlen, 1996; Tyler, 2005; Scanlen, 2006; St George, 2007; Scanlen, 2008). However, these reports often involve single observations or small sample sizes, and may be of considerable antiquity (e.g. Thomson, 1878; Miller, 1918). Conclusions drawn from these scarce data are further complicated by on-going taxonomic revisions. For example, all available descriptions of pollinator behaviour

or identity for the genus *Corybas* are for species that have now been reclassified as *Nematoceras*, *Corysanthes* or *Singularibas* (Thomson, 1878; Miller, 1918; Jones, 1970; Fuller, 1994; for revised names, see Clements et al., 2007).

After recent revisions, New Zealand retains just one helmet orchid in the genus *Corybas*, the diminutive, terrestrial, endemic spurred helmet orchid *Corybas cheesemanii*. This species is nectarless and therefore deceptive (Kelly et al., 2013), but as for most helmet orchids, it is unclear whether pollination is via brood-site mimicry and attraction of female insects that typically oviposit in fungi. Our recent attempts to identify the pollinator(s) of *C. cheesemanii* were inconclusive as a wide range of both male and female flies were attracted to the orchids and co-occurring mushrooms, mostly Mycetophilidae (*Mycetophila colorata*, *M. fagi*, *M. filicornis*, *M. nr. subspinigera* and *M. vulgaris* sp. group), Lauxaniidae (*Sapromyza* spp.) and Anisopodiidae (Kelly et al., 2013). This suggests a broader pollination strategy than previously assumed. Brood-deceptive flowers vary in the extent of pollinator specialisation, reflecting the range of insects attracted to the brood-sites they mimic. For example, pollinator specialisation in flowers that mimic fungi will vary according to the host-specificity of the fungus gnats they attract (Mycetophilidae; Jakovlev, 2012). True specialists attract only one pollinator (e.g. *Paphiopedilum barbigerum* pollinated by the syrphid *Episyrphus balteatus*; Shi et al., 2009), whereas others specialise at the genus or family level and could be termed functional group specialists (e.g. *Dracula* spp. attract drosophilids, especially *Zygothrica* spp.; Endara et al., 2010). Generalists such as many stapeliads attract a broad diversity of fly families (Jürgens et al., 2006).

Here we consider whether *C. cheesemanii*, exhibits visual and chemical signals consistent with pollination by brood-site deception. We sample the volatile odours and spectral reflectance of *C. cheesemanii* and sympatric basidiomycetes (mushrooms). To analyse how the colours of the orchid and mushroom might be perceived by potential pollinators, we model the spectral reflectances into two model visual systems incorporating Dipteran spectral sensitivities.

1 Materials and Methods

C. cheesemanii is a somewhat common but largely unstudied terrestrial species distributed patchily throughout New Zealand’s North and South Islands and offshore and subantarctic islands in lowland to montane

scrub forest amongst the leaf litter below kanuka, taraire and beech trees (*Kunzea ericoides*, *Beilschmiedia tarairi* and *Nothofagus* spp.; Smith, 2009; Scanlen and St George, 2010). *C. cheesemanii* produces only one flower and one heart-shaped leaf per plant. Pollinators are thought to enter and exit the flower via the single anterior opening formed by the tip of the hood or helmet (composed of the dorsal and lateral sepals) and the labellum (fused to the base of the anterior column and bearing a pair of closed nectarless spurs; Fig. 1; Pridgeon et al., 2001). We find the flowers are unscented when sniffed, although others report a ‘mushroom aroma’ (Scanlen and St George, 2010). The white/pale-pink dorsal surfaces of *C. cheesemanii* flowers are easily visible against the dark brown leaf litter and are sometimes described as having the appearance of pearls strewn across the forest floor (Smith, 2009). Flowers and leaves were collected from wild populations in Oratia, in the Waitakere district, south west of Auckland, New Zealand.

1.1 Scent analyses

Orchid flowers (singly [$n = 11$] and also in groups of five [$n = 5$]), wild Agaric (singly, $n = 5$) and store-bought portabella (*Agaricus bisporus*) mushrooms (singly, $n = 9$) were each placed into separate 5 ml glass beakers and sealed with polyethylene oven bags preheated to remove residual plastic odours. Controls were made from empty beakers sealed with oven bags. All beakers were sampled after being left overnight in the lab to allow scent accumulation (approximately 20 hours). The orchids in groups of five were also sampled

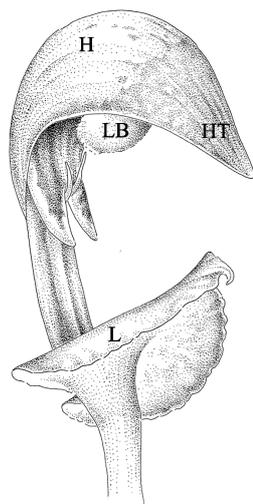


Fig. 1 The spurred helmet orchid *Corybas cheesemanii*. Spectral reflectance was measured for the helmet/dorsal sepal (H), helmet tip/dorsal sepal tip (HT), labellum (LB), and leaf (L). Illustration by Vivian Ward.

again after 48 hours (they maintain their condition well even when cut). Volatiles were sampled from each beaker for 30 min via SPME (solid phase microextraction) with a 65 μm PDMS/DVB fibre (polydimethylsiloxane/divinylbenzene; Supelco). After sampling, the fibre was inserted directly into a narrow glass inlet liner in the injection port of an Agilent 7890A gas chromatographer (GC) coupled to an Agilent 5975C mass spectrometer (MS). The column (DB-1701; 30 m plus 5 m retention gap \times 0.25 mm \times 0.15 μm) was held at 40°C for 3 min, then increased at 10°C/min to 260°C, then held for 10 min, resulting in a total run time of 26 min. The carrier gas was helium. Fibres were cleaned between each sample by desorption in the GC-MS injector port at 250°C for 10 min. Attempted identification of compounds was via injection of known standards and reference libraries (NIST05).

1.2 Spectral reflectance

We measured the spectral reflectance of another set of freshly collected *C. cheesemanii* flowers ($n=7$) and wild mushrooms ($n=5$), and leaf litter and soil ($n=10$), and the irradiance of the ambient light at the forest floor ($n=10$). Locations of reflectance measurements for the orchid included its leaf, the helmet (or dorsal sepal), helmet tip (or dorsal sepal tip above the entry to the flower) and the labellum below the entry to the flower (Fig. 1). Reflectance measurements for the mushrooms were taken at their gills, stalk and cap. Reflectance spectra for specimens were measured with an Ocean Optics USB2000 fibre optic spectrometer, coupled with a PX-2 pulsed xenon light source and a bifurcated optical fibre assembly. Measurements were taken at a 45° angle and at a standardised distance by using an Ocean Optics reflection probe holder as per Chittka and Kevan (2005) and Gaskett (2013). A white Spectralon reflectance block was used as a light standard, and black felt was used as the dark standard and as the background when measurements were taken. Each location on each specimen was measured 5 times, means for each location were calculated, and the data were interpolated to whole values within the range of 300 to 700 nm using Avicol (Gomez, 2006).

1.3 Modelling fly vision

To investigate how flies might perceive the orchids, mushrooms and backgrounds, the reflectance spectra were modelled into a fly-specific categorical vision model (Troje, 1993; as per Arnold et al., 2009 and Defrize et al., 2010) and a more generic tetrachromat colour opponency model (Vorobyev and Osorio, 1998; as per Brembs and Hempel de Ibarra, 2006). We used

fly photoreceptor spectral sensitivities from intracellular measurements conducted on the housefly *Musca domestica* indicating four types of central photoreceptors, typically referred to as R7p, R7y, R8p and R8y (Fig. 2; Hardie and Kirschfeld, 1983; Troje, 1993; Yamaguchi et al., 2010). R7p is sensitive to ultraviolet (UV) wavelengths less than 400 nm, and R7y to UV up to 500 nm (Hardie and Kirschfeld, 1983). R8p is sensitive to blue (peak ~460nm) and R8y to green (peak ~530nm). The fly-specific categorical vision model is based on behavioural experiments using blowflies *Lucilia* sp. that suggest they perceive only four colour categories and cannot distinguish between colours within a category (Troje, 1993). This model compares the differences in quantum catch between the receptors in two paired, antagonistic subsystems, R7p/R8p and R7y/R8y. This results in four colour categories: fly-UV (p+ y+), fly-blue (p- y+), fly-yellow (p- y-) and fly-purple (p+ y; Hardie and Kirschfeld, 1983, Defrize et al., 2010). The more generic tetrachromat model compares quantum catch for all four receptor types (Vorobyev and Osorio, 1998). This Receptor Noise Limited colour opponency model uses spectral sensitivities and the relative numbers of each photoreceptor type to calculate the perceived colour contrast between objects (ΔS), expressed in units of JNDs (Just Noticeable Differences), and predict the threshold spectral sensitivity (ΔS^f), at which this colour contrast becomes distinguishable without requiring behavioural tests (Kelber et al., 2003; Brembs and Hempel de Ibarra, 2006). The relative proportions of each photoreceptor type was calculated to be 1:2:1:2 (for R7p:R7y:R8p:R8y; from Kirschfeld et al., 1978; Earl and Britt, 2006), the Weber fraction used was 0.1 (Brembs and Hempel de Ibarra, 2006) and quantum

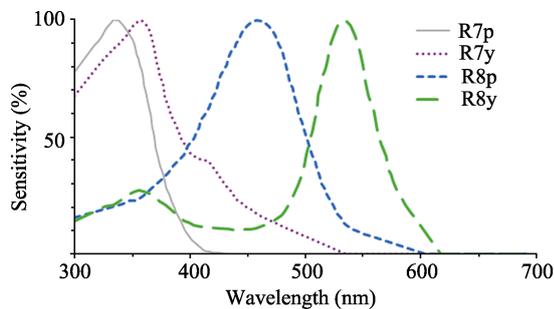


Fig. 2 Normalised spectral sensitivity of four photoreceptor subtypes in the fly *Musca domestica* (Muscidae) used here for modelling fly perception of orchid and mushroom spectral reflectances

Data extracted from figure provided by Hardie & Kirschfeld (1983). R = rhabdomere. p = pale, y = yellow (from the appearance of the rhabdomeres under microscopy).

catch and ΔS calculations were made using Avicol (Gomez, 2006). Conversion of quantum catches into coordinates for the tetrahedral colour space was performed using equations A8-A12 from Kelber et al. (2003).

2 Results

2.1 Scent analyses

SPME and GC-MS analyses found no known fungal volatiles in any of the specimens and only trace amounts of compounds that were hard to identify and extremely variable between individual flowers and mushrooms.

2.2 Spectral reflectance & modelling fly vision

The orchids reflected across a range of visible wavelengths (300–700 nm; Fig. 3). The dorsal surface of the helmets reflected most strongly in the UV (~320 nm), yellow-green (500–600 nm) and red regions (650–700 nm). The helmet tip above the entry to the flower reflected only red wavelengths, whereas the labellum be-

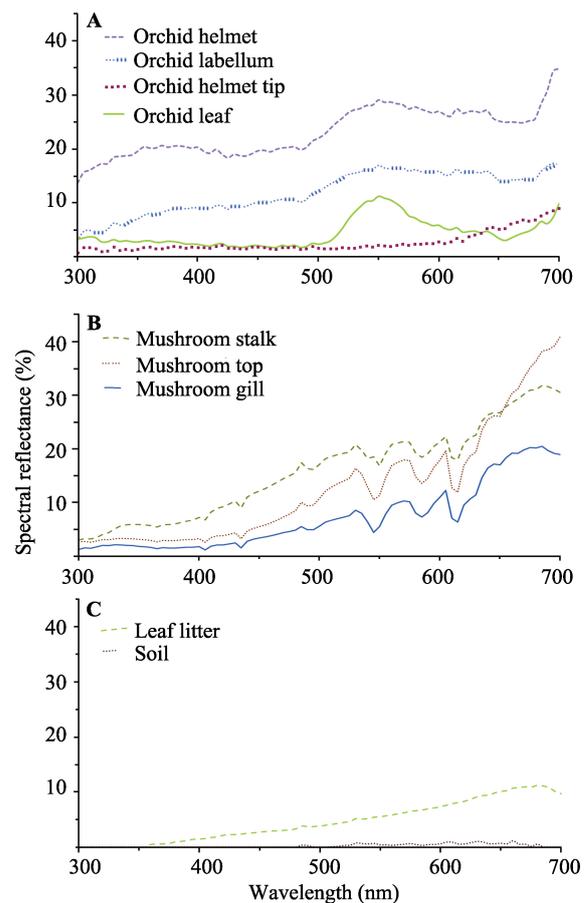


Fig. 3 Average spectral reflection of (A) helmets (dorsal sepals), helmet tips (anterior of dorsal sepals), labella and leaves of *C. cheesemanii* orchids, (B) gills, caps and stalks of wild Agaric mushrooms, and (C) background leaf litter and soil

low the entry to the flower reflected a similar range of peaks to the dorsal helmet, but less strongly.

The mushrooms and the surrounding leaf litter reflected predominantly red wavelengths and no UV (Fig. 3). The mushroom stalks had similar but slightly more intense reflectance than both the gills and the caps. The soil reflected very little light across the entire spectrum. Modelling the orchid, mushroom and background reflectances into the categorical fly vision model (Troje, 1993) suggested that flies were unlikely to discriminate between any of the specimens because all the specimens plotted into the same colour category, p- y- or fly-yellow (Fig. 4). Different results were obtained for the more generic Receptor Noise Limited tetrachromat model (Vorobyev & Osorio, 1998; Fig. 5), where the colours of all the orchid parts and the mushrooms contrasted strongly with each other and the surrounding leaf litter and soil (all JND values > 1; Table 1).

3 Discussion

Here we found little evidence for visual mimicry of fungi by *Corybas cheesemanii*. Anecdotal evidence and our own pollinator trapping data (Jones, 1970; Fuller, 1994; St George, 2001; Scanlen, 2006; St George, 2007; Smith, 2009; Kelly et al., 2013) suggest *Corybas* could well be pollinated by fungus gnats, and may well be brood-site deceptive, but if so, in *C. cheesemanii* this

deceit does not appear to rely on accurate visual mimicry of mushrooms.

Our preliminary GC-MS analyses also produced no evidence that the orchids produced any generalised fungal scent compounds (e.g. 1-octenol, 3-octenol, 1-octene, 1-octen-3-ol, 3-octanone). If these compounds are present, they are at very low concentrations. Despite our attempts to increase the concentration of our scent samples by sampling from multiple orchids at once and over longer time periods, the low abundances in both the orchid and mushroom samples and the individual variability suggest the sample sizes and methods we used were inadequate. The long extraction times did result in some condensation on the bags, which may have affected sampling efficacy. Future attempts to sample the odours of *Corybas cheesemanii* could use extraction of the labella in solvents such as hexane or dichloromethane, as used successfully for *Ophrys* and other sexually deceptive orchids from Australia such as *Chiloglottis* (Poldy et al., 2008; Peakall et al., 2010; Vereecken et al., 2010; Xu et al., 2011). These techniques extract volatile as well as surface compounds so other volatile-only methods may provide better results. e.g. sampling with capillary type trapping tubes filled with absorbent material such as tenax or porepak and fitting the GC with a ChromatoProbe (Dötterl et al., 2005; Gaskett et al., 2005; Jürgens et al., 2006).

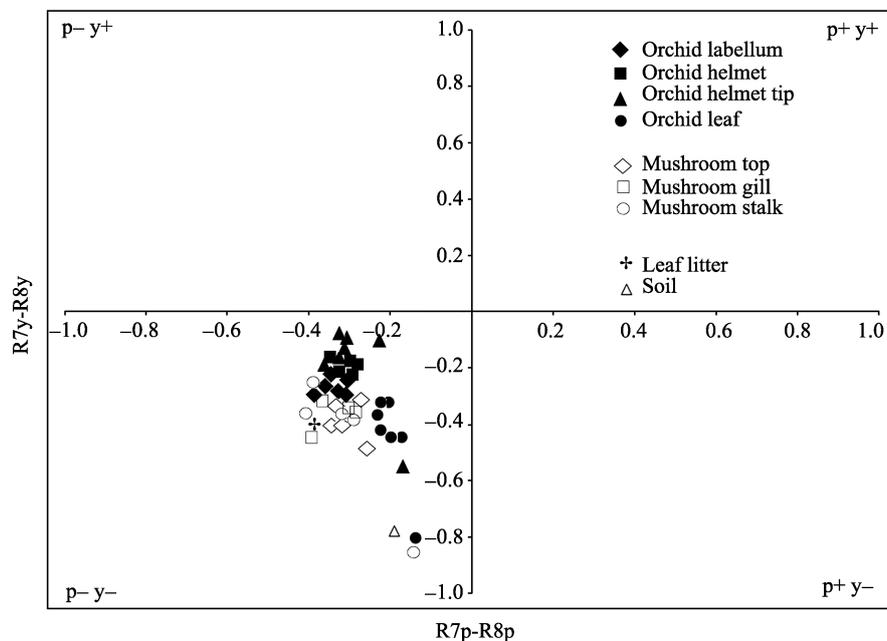


Fig. 4 Colours of *Corybas cheesemanii* flower parts plotted into a categorical fly colour space model according to Troje (1993), which compares the differences in quantum catch between the R7 and R8 photoreceptors in two paired subsystems

Black symbols represent orchid parts. White symbols represent wild Agaric mushroom parts. A fly is unlikely to be able to discriminate between colours of objects that plot in the same quadrant, however spectral sensitivity data is lacking for wavelengths in the red region, as often reflected by fly-pollinated flowers, including *C. cheesemanii*.

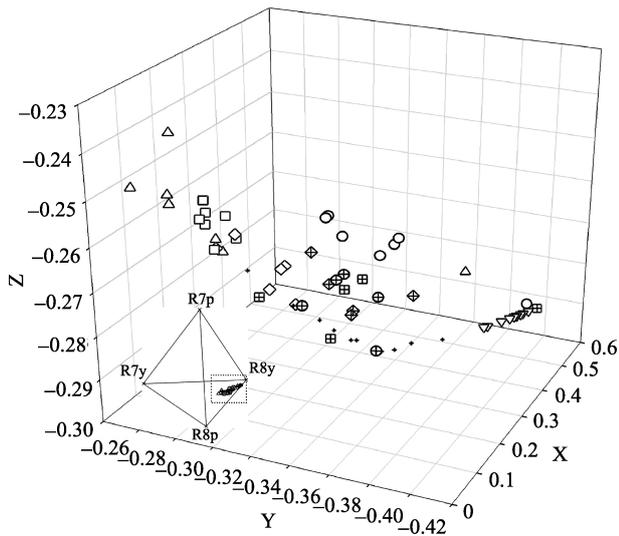


Fig. 5 A. Colours of *Corybas cheesemanii* plotted into a tetrachromat colour space model according to Vorobyev and Osorio (1998). B. Enlargement of relevant region of tetrahedron to show data points

Open squares are orchid helmets (dorsal sepals), open triangles are helmet tips (anterior of dorsal sepals), open diamonds are labella, open circles are leaves of *C. cheesemanii* orchids. Mushroom caps (diamond crossed), mushroom gills (square crossed), and mushroom stalks (circle crossed) are from wild Agaric mushrooms. Small black diamonds are background leaf litter and black inverted triangles are soil.

Our failure to detect any strong odours could be a methodological issue, but it might also be that odour signals are not a primary insect attractant for *Corybas cheesemanii*, much as in some brood-deceptive *Paphiopedilum* species (Shi et al., 2007; Shi et al., 2009). *Paphiopedilum barbigerum* is also scentless to humans and GC-MS analyses failed to detect any volatiles. Shi J et al. (2009) concluded that the pollinator (female

hoverflies, Syrphidae) was attracted by the orchid's bright yellow staminode. Yellow wavelengths are strongly and innately attractive to range of insects (Goulson et al., 2007), suggesting a possible sensory trap based on visual rather than olfactory signals.

Deceptive pollination systems need not involve mimicry. They each exploit different aspects of pollinator foraging and sexual behaviours. Correspondingly, the signals that attract pollinators are likely to involve a range of sensory modes and deceptive strategies. Analyses of deceptive orchid shapes, colours, and scents do suggest mimicry, but also demonstrates non-mimicked elements that vary between individuals or even sympatric orchid species with the same pollinator (e.g. in sexually deceptive *Cryptostylis* species; Gaskett and Herberstein, 2010; Gaskett, 2012). These non-mimicked elements could be 'imperfect mimicry' that exploits pollinators' preferences for novelty (Vereecken and Schiestl, 2008; Vereecken et al., 2010), or variation that impairs pollinator learned avoidance of deceptive orchids (Ackerman et al., 2011; but see Juillet and Scopce, 2010). An alternative function for non-mimicked elements may be 'sensory traps' that exploit animals' pre-existing biases towards signals or cues that generally provide fitness benefits, e.g. colours and scents associated with food (Schaefer, 2010).

In the present study, neither vision model suggested colour mimicry - *C. cheesemanii* orchid colours were not more similar to mushrooms than to the backgrounds. However, the orchid's pearlescent helmet (dorsal sepal) reflected UV and generated the strongest colour contrast values against the background (Table 1; according to Vorobyev and Osorio's Receptor Noise Limited tetra-

Table 1 Colour contrast values (ΔS) for the spectral reflectances of *Corybas cheesemanii* orchids and sympatric mushrooms, expressed as JND (Just Noticeable Differences) according to the Receptor Noise Limited tetrachromat vision model (Vorobyev and Osorio, 1998)

	Backgrounds		Mushroom			Orchid		
	Soil	Leaf litter	Cap	Stalk	Gill	Leaf	Helmet tip*	Helmet [†]
Orchid labellum	50.87	15.96	7.10	4.31	6.40	6.66	4.78	3.44
Orchid helmet	57.11	19.30	10.50	7.70	9.80	8.48	1.58	
Orchid helmet tip	39.33	20.33	11.88	8.87	11.17	10.04		
Orchid leaf	39.47	16.69	6.79	7.53	6.49			
Mushroom gill	40.96	10.74	0.72	3.06				
Mushroom stalk	51.30	11.74	3.67					
Mushroom cap	45.62	10.23						
Leaf litter	37.78							

* Dorsal sepal tip. [†]Dorsal sepal.

When JND < 1, the perceiver is unlikely to be able to distinguish between the colours (in bold).

achromat vision model). This contrast value already takes into account the low availability of UV on the forest floor because ambient light measurements were taken at orchid sites. This contrast between the bright orchid helmets and the dim background may be sufficient to lure pollinators as tests with other insects (honeybees) indicate this is very attractive (Hempel de Ibarra et al., 2000).

Alternatively, the bright UV and yellow orchid surface may attract flies by operating as a sensory trap. Many insects are strongly attracted to UV and have photoreceptors tuned to UV wavelengths (Hardie and Kirschfeld, 1983; Tovée, 1995; Kelber and Osorio, 2010). Two of the four fly colour receptor types, R7p and R7y, are targetted towards UV and flies have strong innate preferences for these shorter wavelengths (Yamaguchi et al., 2010). UV receptors can be much more sensitive than other photoreceptor types, so a small amount of UV reflectance can stimulate a strong sensory response (Chittka et al., 1994). For example, in choice trials, wild type *Drosophila* are 57 times more strongly attracted to UV than blue lights of equal intensity (Yamaguchi et al., 2010). Similarly, in choice trials comparing insect attraction to flowers, predators, and human-made objects such as wind turbines, UV reflectance is highly attractive (Heiling et al., 2005; Long et al., 2010; O'Hanlon et al., 2014). Furthermore, in insects, UV wavelengths are the most common releasers of wavelength-specific fixed behaviours such as phototaxis and more complex routines associated with foraging and reproduction (Goldsmith, 1994). These types of fixed behaviours are the ones most commonly exploited by other deceptive orchids.

Despite the strong general and scientific interest in UV markings and nectar guides on flowers, very few rewarding flowers actually reflect UV (Chittka et al., 1994; Dyer, 1996). Correspondingly, UV reflectance is also rare in food-deceptive orchids (e.g. Johnson, 2000; Johnson et al., 2003, but see Indsto et al., 2006). Many rewarding *Dendrobium* orchid species have moderate to high near-UV reflectance, often in a 'bulls-eye' pattern (Indsto and Weston, 2000). Some sexually deceptive orchids such as *Cryptostylis* species have UV-reflective patches (Gaskett and Herberstein, 2010), and manipulations with UV filters affected pollinator attraction to sexually deceptive *Ophrys heldreichii* and *O. vernixia* (Paulus, 2006). However, it is unclear whether UV reflectance by these orchids is mimicry (of female insect wings) or a sensory trap (Gaskett, 2013). For the orchid investigated in our current study, the function (if any) of

UV or other often attractive wavelengths such as yellow could be investigated by monitoring pollination success in manipulative experiments in which wavelengths are blocked with filters or sunscreen (as per Johnson and Andersson, 2002; Paulus, 2006) and behavioural tests of fungus gnat attraction to UV and other wavelengths.

The different results obtained by the two vision models used here suggest further experimental testing and adjustments are required before they can be applied to flies. Firstly, the fly spectral sensitivity data from Hardie & Kirschfeld (1983) is only available up to 600 nm, which excludes red wavelengths that typically range from 620–740 nm. This has strong implications for this study and all others attempting to model fly perception of colour using these data (Arnold et al., 2009; Defrize et al., 2010). Red is a very common colour for many fly-pollinated brood-site deceptive flowers including arums, stapeliads and *Dracula* orchids (Raguso, 2004; Jürgens et al., 2006; Endara et al., 2010; Urru et al., 2011). In addition, this categorical model may not be accurate because studies with *Drosophila* spp. suggest they can discriminate between blue and green colours that would plot within the same category (Brembs and Hempel de Ibarra, 2006; Yamaguchi et al., 2010). The hoverfly *Episyrphus balteatus* is also able to distinguish between very similarly coloured yellow, green and yellow-green artificial flowers (Sutherland et al., 1999). However, the second model we used, Vorobyev and Osorio's (1998) Receptor Noise Limited tetrachromat model, may also have limited applicability to fly vision. This model compares quantum catch from different receptors in an additive manner, but Yamaguchi et al. (2006) show that *Drosophila* preferences are not additive; converting the much more abundant green-sensitive R8y photoreceptors into blue-sensitive R8p photoreceptors (via genetic manipulations) did not enhance attraction to blue. Also, it is untested whether the default colour contrast threshold ($\Delta S^l = 1$ JND) is appropriate for fly vision. Finally, for both models used here, the spectral sensitivities are drawn from the only behavioural study of fly colour discrimination, which used the housefly, *Musca domestica* (Muscidae; Troje, 1993). The pollinators of *C. cheesemani* are likely to be fungus gnats (Mycetophilidae), which may have very different visual systems (Kelly et al., 2013). Further spectral sensitivity and behavioural data for flies are urgently needed, but these may be very difficult to achieve because unlike honeybees, flies are likely to be very difficult to train to colour stimuli.

In conclusion, we found no evidence for mimicry of

fungal colours or strong fungal scents in a putatively brood-site deceptive orchid. Instead, we propose a deceptive pollination system based on exploitation of pollinator visual biases because the contrasting reflectance of *C. cheesemanii* orchids may be attractive against the dim background, or function as a sensory trap. Spectral modelling into the only available fly vision models produced different results and the limitations of these models suggest they should be used with caution.

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