

Breaking the rules: Discovery of sexual deception in *Caladenia abbreviata* (Orchidaceae), a species with brightly coloured flowers and a non-insectiform labellum

Ryan D. Phillips^{1,2,3*} & Rod Peakall¹

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¹Ecology and Evolution, Research School of Biology, The Australian National University, Canberra, ACT, 0200, Australia

²Kings Park and Botanic Garden, The Botanic Garden and Parks Authority, West Perth, 6005, Western Australia, Australia

10 ³Department of Ecology, Environment & Evolution, La Trobe University, Victoria, 3086, Australia

***corresponding author:** Ryan.Phillips@anu.edu.au

Running head: sexual deception in *Caladenia*

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Summary text for table of contents: *Caladenia* is unique among orchids in that it contains both species pollinated by food foraging insects, and species pollinated by sexual deception, providing the opportunity to investigate the evolution of sexual mimicry. Here we report a new case of pollination by sexual deception in the *Caladenia filamentosa* complex, a group with brightly coloured, scented flowers previously thought to be pollinated by food deception. Our findings demonstrate that sexual deception can be achieved without dull coloured flowers and insectiform labella.

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35 **Abstract**

Caladenia is exceptional among orchid genera in that it contains both species pollinated by sexual deception and species pollinated by food-foraging insects. While pollination strategies have been elucidated in some species complexes, others groups have received limited
40 attention, hampering our ability to understand the evolution of the exceptional diversity of species and floral traits in *Caladenia*. Here we conducted the first detailed pollination study of a member of the *Caladenia filamentosa* complex. Based on the brightly coloured and scented flowers exhibited by many species, it has been assumed that most members of the group are food-deceptive. However, we show that *Caladenia abbreviata* Hopper & A.P. Br.
45 is pollinated by an undescribed species of *Rhytidohynnus* thynnine wasp via sexual deception. Floral dissections revealed that the terminal glands on the sepals and petals are the primary source of the sexual attractant, supporting observations from intact flowers that pollinators frequently copulated with these floral parts. Interestingly, contact with the plant's reproductive structures was not associated with attempted copulation. Our findings highlight
50 that sexual deception can evolve in lineages without dull-coloured flowers and insectiform labellum structures. Given that the orchid is apparently dependent on a single pollinator species, efforts to conserve this rare species will be dependent on the availability of the pollinator in suitable orchid habitat.

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Introduction

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Pollination by sexual deception involves the sexual attraction of male pollinators through chemical and/or physical mimicry of a female insect (Schiestl *et al.* 1999; Schiestl *et al.* 2003; Johnson and Schiestl 2016). As a by-product of mimicking specific insect sex pheromones, sexually deceptive species are highly specialised, with often just a single

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pollinator species (Paulus and Gack 1990; Peakall *et al.* 2010; Phillips *et al.* 2017). New cases of pollination by sexual deception continue to be discovered, with the strategy now confirmed for at least 22 genera of orchids (Phillips *et al.* 2014b; Arakaki *et al.* 2016;

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Bohman *et al.* 2016), and single genera in the Asteraceae (Ellis and Johnson 2010) and Iridaceae (Vereecken *et al.* 2012). Australia is a centre of diversity for sexually deceptive orchids, with over 100 species spanning 11 genera confirmed to use this strategy (Gaskett

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2011; Phillips *et al.* 2014a,b, Phillips *et al.* 2017; Swarts *et al.* 2014). Collectively, these Australian sexually deceptive orchids exploit four families of Hymenoptera (Coleman 1928; Peakall 1989; Stoutamire 1974, 1983; Alcock 2000) and at least one family of Diptera (Phillips *et al.* 2014b).

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Caladenia is a diverse (over 370 taxa), primarily Australian genus with a large number of species pollinated by sexual deception of thynnine wasps (Tiphidae:Thynninae; Phillips *et al.* 2009a; Phillips *et al.* 2009b). Based on present knowledge, *Caladenia* is the only predominantly sexually deceptive genus that also contains a number of species apparently

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pollinated by food foraging insects (Faast *et al.* 2009; Phillips *et al.* 2009b; Kuitert 2016), providing a unique opportunity to investigate the evolution of sexual mimicry (Weston *et al.* 2014; Clements *et al.* 2015). Like most other sexually deceptive orchids, the flowers of sexually deceptive *Caladenia* are usually unscented to the human nose, diminutive in size, dull red/green in colour, and have labella with clusters of maroon-coloured calli that give

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them an insectiform appearance to human eyes (Stoutamire 1983; Phillips *et al.* 2009b). In contrast, the flowers of *Caladenia* species visited by food-foraging insects tend to be scented, with larger and more colourful flowers (Phillips *et al.* 2009b). While there has been increasing research into the pollination of *Caladenia* (Phillips *et al.* 2013a, 2015b, 2017;

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Swarts *et al.* 2014; Bower 2015; Bohman *et al.* 2017; Reiter *et al.* 2017; Xu *et al.* 2017),

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some species complexes have received very little attention, particularly those that appear to be dominated by food-deceptive species. This gap in our knowledge of the pollination

systems of *Caladenia* represents a substantial impediment to a better understanding of the evolution of the species diversity and floral traits of this extraordinary genus of orchids.

105 The *Caladenia filamentosa* complex contains approximately 45 species (Hopper and Brown
2001; Brown and Brockman 2015), with the main taxonomic and morphological diversity of
the group centred in semi-arid areas of south-western Australia (Phillips *et al.* 2009a). The
flowers have long, filamentous petals and sepals, which are typically white, yellow or red in
colouration. In many cases, the flowers produce a faint scent variously described as ‘like
110 burnt metal’, ‘sweet’ or ‘fetid’ depending on the species (Hopper and Brown 2001). This
combination of traits led Phillips *et al.* (2011) to the prediction that most members of the *C.*
filamentosa complex are pollinated by food-deception. However, until now, there have been
no detailed pollination studies of any member of this group. Anecdotal observations of
visitation by apparently nectar-seeking bees and thynnine wasps have been reported for *C.*
115 *denticulata*, *C. capillata*, *C. nobilis* and *C. horistes* (Erickson 1965; Kuitert 2016; RDP
unpublished observations). However, there is also one unpublished observation of sexual
attraction of an individual thynnine wasp in *C. filamentosa* (by C. Bower cited in Phillips *et*
al. 2009b), though this behaviour was not observed for other wasp individuals of this species
that were observed visiting the flower. There are also reports of multiple male thynnine
120 wasps perching overnight on several deep-red flowered species closely allied to *C.*
filamentosa (e.g. *C. pulchra*; Hopper and Brown 2001; *C. dundasiae*; Backhouse 2011;
Phillips *et al.* 2017), but these floral visitors have not been confirmed as pollinators.

Caladenia abbreviata Hopper & A.P.Br. (Fig. 1), is a rare member of the *C. filamentosa*
125 complex with floral morphology typical of a food deceptive species. In particular, *C.*
abbreviata has conspicuous cream-coloured tepals and lacks insectiform floral structures
such as dense aggregations of calli on the labellum (Hopper and Brown 2001). However,
preliminary observations by the first author revealed the attraction of an unidentified species
of male thynnine wasp, possibly via sexual deception. Therefore, we investigated if this
130 species is indeed sexually deceptive, following the criteria outlined by Phillips *et al.* (2014b)
for confirmation of this pollination strategy. Our results revealed pollination by males of a
single species thynnine wasp, which exhibited courtship behaviour and attempted copulation
with the flower. Thus, we conclude that *C. abbreviata* represents an intriguing case of sexual
deception in the absence of the floral traits typically associated with this pollination strategy.

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Materials and methods

Study species

140 *Caladenia abbreviata* is a rare species restricted to consolidated coastal dunes from
Gracetown to Walpole in south-western Australia (Hopper and Brown 2001). A single
flowering scape is produced per plant per year, typically with only a solitary flower.
Flowering occurs from November to December. Due to a dry habitat, and a late flowering
145 period compared to co-occurring *Caladenia* (Hopper and Brown 2001), in years with low
winter and spring rainfall many buds abort prior to flowering (R. Phillips, pers obs.). Further,
the flowering period is often brief compared to other *Caladenia*. The combination of these
factors leads to restricted opportunities for pollination studies in this species.

Thynnine wasps are a diverse group of solitary wasps (over 1600 known species), which
150 reach their highest diversity in Australia (Brown 2009). The flightless females use a sex
pheromone to attract males, usually while perched in a prominent position in vegetation or on
the soil surface (Alcock 1981). The males, which engage in patrolling flights searching for
females, respond rapidly to newly emerged females and vigorously compete to achieve
copulation with the female (Alcock & Gwynne 1987). The males then carry the female *in*
155 *copula*, either feeding the female directly or allowing her to feed from a food source (Alcock
1981; Alcock & Gwynne 1987) such as nectar or exudates from scale insects (Brown and
Phillips 2014).

Study site

160 The study was conducted at a site east of Augusta, Western Australia. Precise details are
withheld for conservation reasons. The population is represented in the Western Australian
Herbarium by the record PERTH 08739463 RDP 0352. Field studies were undertaken on the
10th and 17th of November 2014, and the 28th of October and 10th-11th of November 2015.

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Pollinator observations and experiments

Following preliminary evidence that *C. abbreviata* is potentially pollinated by sexual
deception, pollinator observations were conducted in 2014 and 2015 using the pollinator

170 baiting method (Stoutamire 1983; Peakall 1990). In this method, picked flowers are moved to
a new part of the landscape, often leading to the rapid attraction of sexually deceived
pollinators. Flowers were picked immediately before the use, with only one flower used as
bait at any given time. Pollinator observations were restricted to periods of sunny weather
with temperatures above 20°C. A total of 100 4-minute baiting trials were conducted across
175 four days, using five replicate flowers. For each floral visitor to a bait flower, it was recorded
if they alighted, where they landed, if they contacted the labellum and/or sepal tips during the
visit, if they attempted copulation and where, and if they contacted the column (as per
Phillips *et al.* 2013). Attempted copulation was recognised by vigorous attempts to grasp the
flower and probing of the flower with the tip of the abdomen. Floral visitors were collected
180 from flowers of *C. abbreviata*, and later identified by Graham Brown from the Museum and
Art Gallery of the Northern Territory.

Following confirmation that all bait flowers readily attracted pollinators, floral dissections
were conducted using three of the five bait flowers to determine the source of the sexual
185 attractant (experiment undertaken in 2014). Each of the different floral parts were presented
separately, pinned to the top of wooden skewer by pins with a black plastic head. Following
preliminary evidence that the tips of petals and sepals were attractive, we undertook
sequential choice trials (following Bower 1996; Phillips *et al.* 2013). In phase 1, the column,
labellum and sepal plus petal remains (termed the floral display) were presented for 3
190 minutes. In phase 2, the petal and sepal tips were added to the choice experiment for an
additional 3 minutes. In these experiments, the first phase provides a test of whether the
remaining floral parts are attractive, while the second phase acts as a control to confirm that
pollinators were present and responding during the trial. A total of five trials were undertaken
for each of the three individual experiments, with a different flower used in each experiment.

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Results

In both years of the study, an undescribed species of *Rhytidothygnus* sp. (Thynninae) was the
only species observed to visit the intact bait flowers of *C. abbreviata* (Supplementary video
200 1). A total of 86 observations of floral visitation by *R.* sp. were recorded at intact flowers
across the 100 trials. Three of these wasps were observed carrying pollinia of *Caladenia* on
the dorsal side of the thorax. During the November study period no other *Caladenia* species
were flowering at this site, thus we are confident these pollen loads were from *C. abbreviata*.

205 All responding individuals exhibited the characteristic zig-zag approach flight that is typical
of thynnine wasps tracking a sex pheromone (Stoutamire 1983, Peakall 1990). A total of 21
individuals attempted copulation with the flower (Supplementary video), on every occasion
with the glandular tips of the petals and/or sepals. Of the 86 approaching wasps, 72 alighted
(83.7%) but only 5 contacted the column (5.8%), the behaviour necessary for pollen
210 deposition and removal. Of these five wasps, three also attempted copulation during the visit,
but always with the glands of the sepals and petals rather than the labellum. One of the
individuals contacting the column appeared to attempt to feed from the surface of the
labellum. Interestingly, *Rhytidothynnus* sp. was not recorded during the October observation
period, suggesting that the flying season of these male wasps did not begin until later in the
season.

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A total of 105 individuals of *Rhytidothynnus* sp. were attracted to the floral parts during the
dissection experiments. During the initial phase of the experiments, a small number of wasps
were attracted to the floral display (12 attracted, 7 landing), but none were attracted to either
the column or the labellum. The second phase of these experiments, where all floral parts
220 were presented simultaneously, confirmed that the glandular tips of sepals and petals were the
dominant source of attraction, with 68 wasps being attracted to the sepal tips (44 landing), 23
to the petal tips (16 landing), 2 to the floral display (2 landing), and none to the remaining
floral parts (Fig. 2). Attempted copulation was most frequently observed with the sepal tips
(20 occasions), but was also observed with the petal tips (4 occasions, Fig. 2). The results
225 were consistent across the three flowers dissected, with the sepal tips always being the
primary source of attractant.

Discussion

230 Here we provide the first compelling evidence of pollination by sexual deception in a
member the *Caladenia filamentosa* complex. *Caladenia abbreviata* attracted a single
undescribed species of thynnine wasp in the genus *Rhytidothynnus*, which routinely
attempted copulation with the terminal glands on the sepals and petals (21 out of the 86
individuals attracted to the flower). Both pollen removal and contact with the column, the
235 behaviour needed to achieve pollination, were observed. Although three wasps were
observed carrying pollen from wild *C. abbreviata* plants, pollen deposition was not witnessed
during our experiments. Our failure to observe pollen deposition is likely due to the

combination of the scarcity of flowering plants at this site available to donate pollen, and to the small proportion of wasps observed contacting the column.

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Given that sexually deceptive *Caladenia* typically only have a single pollinator species at any given location (Phillips *et al.* 2017), and the large number of floral visitors observed during this study, it is likely that *Rhytidothygnus* sp. is the exclusive pollinator of *C. abbreviata* at this site. Interestingly, this is the first record of a member of the tribe Rhagigasterini as a
245 pollinator of a *Caladenia*. However, two lines of evidence suggest that pollination by members of the Rhagigasterini is likely to be rare in sexually deceptive *Caladenia*. Firstly, members of the Thynnini have been identified as pollinators for representative species of all of the major species complexes of sexually deceptive *Caladenia* in south-western and south-eastern Australia (Phillips *et al.* 2009, 2017). Secondly, while the Rhagigasterini genera
250 *Rhytidothygnus* and *Rhagigaster* are moderately diverse (e.g. 16 and 10 known species respectively in south-western Australia; Phillips *et al.* 2017), the Rhagigasterini contains far fewer genera and species when compared with the Thynnini.

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The mating behaviour of thynnine wasps, where the flightless females attract the winged males by emitting sex pheromones, is common across all subfamilies of thynnine wasp (Alcock 1981). Alternatively, studies of the sex pheromone chemistry of thynnine wasps have revealed a diversity of chemical systems (Peakall *et al.* 2010; Bohman *et al.* 2014; Bohman *et al.* 2017; Xu *et al.* 2017). As such, pollination by a member of the Rhagigasterini suggests that *C. abbreviata* may well be using a different chemical system to sexually attract its
260 pollinator to those documented for *Caladenia* species using members of the tribe Thynnini (e.g. Bohman *et al.* 2017; Xu *et al.* 2017). These species have been shown to use (methylthio)-phenols (*C. crebra*; Bohman *et al.* 2017), and a blend of (*S*)- β -citronellol and 2-hydroxy-6-methylacetophenone (*C. plicata*; Xu *et al.* 2017), while pyrazines and
chiloglottones are involved in the attraction of sexually deceived thynnine wasps in *Drakaea*
265 and *Chiloglottis* respectively (Peakall *et al.* 2010; Bohman *et al.* 2014). We predict that exploitation of the Rhagigasterini by Australian sexually deceptive orchids may have been limited by biochemical constraints on the mimicry of the sex pheromones of this group.

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Our unexpected discovery of pollination by sexual deception in *C. abbreviata*, a cream-coloured member of the *C. filamentosa* complex, provides another independent opportunity

to investigate transitions in pollination strategy within *Caladenia*. Indeed, this case of sexual deception within subgenus *Phlebochilus* parallels findings in *Caladenia* subgenus *Calonema* where there are now several examples of sexually deceptive species with bright floral colouration such as white (e.g. *C. hastata* – Reiter *et al.* 2017), yellow (e.g. *C. xanthochila* – (Swarts *et al.* 2014) and pink (e.g. *C. gardneri* – Phillips *et al.* 2017). Our observations add to increasing evidence that colouration, and the absence of insectiform floral structures, may not place a strong constraint on the ability of *Caladenia* to achieve pollination by the sexual deception of male thynnine wasps. While evidence from floral traits and anecdotal pollinator observations suggest that food deception is likely to be the main pollination strategy in the *C. filamentosa* complex, investigations of unusual members of this group, particularly those with dark colouration or relatively short tepals, may reveal other cases of sexual deception.

Another interesting parallel to some members of *Caladenia* subgenus *Calonema* (see Phillips *et al.* 2013) is that in *C. abbreviata* the sexual attractant is produced primarily from the terminal glands on the petals and sepals. In most sexually deceptive orchids outside of some *Caladenia*, the labellum is the primary source of the sexual attractant (e.g. Kullenberg and Bergstrom 1976; Phillips *et al.* 2013, 2014b; De Jager and Peakall 2016; though see Singer 2002), providing the benefit of bringing the pollinator regularly into close proximity to the column. However, in *C. abbreviata* attempted copulation with the labellum was not needed to contact the column, a similar result to that seen in *C. pectinata* from *Caladenia* subgenus *Calonema* (Phillips *et al.* 2013). As such, the unexpected strategy of producing attractant from the sepal tips but not the labellum appears to be more widespread in *Caladenia* than previously appreciated.

Our discovery of pollination by sexual deception in *C. abbreviata* may have important implications for the management of this rare species. Like most sexually deceptive orchids, *C. abbreviata* is likely to be reliant on a single primary pollinator species (for some interesting exceptions see Breitkopf *et al.* 2013; Menz *et al.* 2015; Phillips *et al.* 2015a) meaning that the availability of the pollinator could limit the potential sites where reproduction of the orchid could occur (Phillips *et al.* 2014a; Phillips *et al.* 2015b). Further, the apparent late spring-early summer flight time of the wasp may mean that the orchid experiences a trade-off between greater pollinator availability later in the season and the likelihood of resource limitation following pollination. As such, if selecting sites for conservation translocations (see IUCN 2013 for definition), it will be essential to choose

305 locations based on both the presence of the pollinator (e.g. Reiter *et al.* 2017), and the
likelihood that the environmental conditions will sustain *C. abbreviata* through to flowering
and fruit set. Furthermore, given the trends towards warmer temperatures and decreased
autumn, winter and spring rainfall across south-western Australia (Suppiah *et al.* 2007),
appropriate site selection should also consider future climate predictions.

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Conflicts of interest

320 The authors declare no conflicts of interest.

References

Alcock J (1981) Notes on the reproductive behaviour of some Australian thynnine wasps
325 (Hymenoptera: Tiphidae). *Journal of the Kansas Entomological Society* **54**, 681–693.

Alcock J (2000) Interactions between the sexually deceptive orchid *Spiculaea ciliata* and its
wasp pollinator *Thynn Turneria* sp (Hymenoptera : Thynninae). *Journal of Natural History*
34, 629-636.

330

Alcock J, Gwynne DT (1987) Courtship feeding and mate choice in thynnine wasps
(Hymenoptera: Tiphidae). *Australian Journal of Zoology* **35**, 451–458.

Arakaki N, Yasuda K, Kanayama S, Jitsuno S, Oike M, Wakamura S (2016) Attraction of
335 males of the cupreous polished chafer *Protaetia pryeri pryeri* (Coleoptera: Scarabaeidae) for
pollination by an epiphytic orchid *Luisia teres* (Asparagales: Orchidaceae). *Applied*
Entomology and Zoology **51**, 241-246.

- 340 Backhouse G (2011) 'Spider-orchids — the Genus *Caladenia* and its Relatives in Australia.'
(Gary Backhouse: Melbourne).
- Bohman B, Flematti GR, Barrow RA, Pichersky E, Peakall R (2016) Pollination by sexual deception - it takes chemistry to work. *Current Opinion in Plant Biology* **32**, 37-46.
- 345 Bohman B, Phillips RD, Flematti GR, Barrow RA, Peakall R (2017) The spider orchid *Caladenia crebra* produces sulfurous pheromone mimics to attract its male wasp pollinator. *Angewandte Chemie* **129**, 1-5.
- Bohman B, Phillips RD, Menz MHM, Berntsson BW, Flematti GR, Barrow R, Dixon KW,
350 Peakall R (2014) The discovery of alkyl- and hydroxyl pyrazines as insect sex pheromones and orchid semiochemicals. *New Phytologist* **203**, 939-952.
- Bower CC (1996) Demonstration of pollinator-mediated reproductive isolation in sexually deceptive species of *Chiloglottis* (Orchidaceae: Caladeniinae). *Australian Journal of Botany*
355 **44**, 15-33.
- Bower CC (2015) Pollinator sharing by the sexually deceptive Greencomb Spider Orchids, *Caladenia parva*, *C. phaeoclavia* and *C. villosissima* (Orchidaceae: Caladeniinae): taxonomic considerations. *Telopea* **18**, 309-323.
- 360 Breitkopf H, Schluter PM, Xu S, Schiestl FP, Cozzolino S, Scopece G (2013) Pollinator shifts between *Ophrys sphegodes* populations: might adaptation to different pollinators drive population divergence? *Journal of Evolutionary Biology* **26**, 2197-2208.
- 365 Brown AP, Brockman G (2015) New taxa of *Caladenia* (Orchidaceae) from south-west Western Australia *Nuytsia* **25**, 45-123.
- Brown GR (2009) Description of two new pseudoposematic species with a review of defensive adaptations in the subfamily Thynninae (Hymenoptera: Thynnidae). *The Beagle, Records of the Museums and Art Galleries of the Northern Territory* **25**, 71–78.
- 370

- Brown GR, Phillips RD (2014). A review of the diet of flower wasps (Hymenoptera: Thynnidae: Thynninae). *Northern Territory Naturalist* **25**, 50–63.
- 375 Clements MA, Howard CG, Miller JT (2015) *Caladenia* revisited: results of molecular phylogenetic analyses of Caladeniinae plastid and nuclear loci *American Journal of Botany* **102**, 581-597.
- Coleman E (1928) Pollination of an Australian orchid by the male Ichneumonid *Lissopimpla semipunctata*, Kirby. *Transactions of the Entomological Society of London* **2**, 533–539.
- 380
- De Jager ML, Peakall R (2016) Does morphology matter? An explicit assessment of floral morphology in sexual deception. *Functional Ecology* **30**, 537-546.
- 385 Ellis AG, Johnson SD (2010) Floral mimicry enhances pollen export: The evolution of oollination by sexual deceit outside of the Orchidaceae. *American Naturalist* **176**, E143-E151.
- Erickson R (1965) 'Orchids of the west.' (Paterson Brokenshaw: Perth).
- 390
- Faast R, Farrington L, Facelli JM, Austin AD (2009) Bees and white spiders: unravelling the pollination syndrome of *Caladenia rigida* (Orchidaceae). *Australian Journal of Botany* **57**, 315-325.
- 395 Gaskett AC (2011) Orchid pollination by sexual deception: pollinator perspectives. *Biological Reviews* **86**, 33-75.
- Hopper SD, Brown AP (2001) Contributions to Western Australian orchidology: 2. New taxa and circumscriptions in *Caladenia*. *Nuytsia* **14**, 27-307.
- 400
- IUCN (2013) 'Guidelines for reintroduction and other conservation translocations.' (IUCN Species Survival Commission: Gland).
- Johnson SD, Schiestl FP (2016) 'Floral mimicry.' (Oxford University Press: Oxford).
- 405

- Kuiter RH (2016) 'Orchid pollinators of Victoria.' (Aquatic Photographics: Melbourne).
- Kullenberg B, Bergstrom G (1976) The pollination of *Ophrys* orchids. *Botaniska Notiser* **129**, 11-19.
- 410
- Menz MHM, Phillips RD, Anthony JM, Bohman B, Dixon KW, Peakall R (2015) Ecological and genetic evidence for cryptic ecotypes in a rare sexually deceptive orchid, *Drakaea elastica*. *Botanical Journal of the Linnean Society* **177**, 124-140.
- 415
- Paulus HF, Gack C (1990) Pollination of *Ophrys* (Orchidaceae) in Cyprus *Plant Systematics and Evolution* **169**, 177-207.
- Peakall R (1989) The unique pollination of *Leporella fimbriata* (Orchidaceae) – pollination by pseudocopulating male ants (*Myrmecia urens*, Formicidae). *Plant Systematics and*
- 420 *Evolution* **167**, 137-148.
- Peakall R, Ebert D, Poldy J, Barrow RA, Francke W, Bower CC, Schiestl FP (2010) Pollinator specificity, floral odour chemistry and the phylogeny of Australian sexually deceptive *Chiloglottis* orchid: implications for pollinator-driven speciation. *New Phytologist*
- 425 **188**, 437-450.
- Phillips RD, Backhouse G, Brown AP, Hopper SD (2009a) Biogeography of *Caladenia*, with special reference to the Southwest Australian Floristic Region. *Australian Journal of Botany* **57**, 259-275.
- 430
- Phillips RD, Bohman B, Anthony JM, Krauss SL, Dixon KW, Peakall R (2015a) Mismatch in the distribution of floral ecotypes and pollinators: insights into the evolution of sexually deceptive orchids. *Journal of Evolutionary Biology* **28**, 601-612.
- 435
- Phillips RD, Brown AP, Dixon KW, Hopper SD (2011) Orchid biogeography and the factors associated with rarity in a biodiversity hotspot: the Southwest Australian Floristic Region. *Journal of Biogeography* **38**, 487-501.

440 Phillips RD, Brown GR, Dixon KW, Hayes C, Linde CC, Peakall R (2017) Evolutionary relationships among pollinators and repeated pollinator sharing in sexually deceptive orchids. *Journal of Evolutionary Biology* **30**, 1674-1691.

445 Phillips RD, Faast R, Bower CC, Brown GR, Peakall R (2009b) Implications of pollination by food and sexual deception on pollinator specificity, fruit set, population genetics and conservation of *Caladenia*. *Australian Journal of Botany* **57**, 287-306.

450 Phillips RD, Peakall R, Hutchinson HF, Linde CC, Xu T, Dixon KW, Hopper SD (2014a) Specialized ecological interactions and plant species rarity: The role of pollinators and mycorrhizal fungi across multiple spatial scales. *Biological Conservation* **169**, 285-295.

Phillips RD, Peakall R, Retter BA, Montgomery K, Menz MHM, Davis BJ, Hayes C, Brown GR, Swarts ND, Dixon KW (2015b) Pollinator rarity as a threat to a plant with a specialized pollination system. *Botanical Journal of the Linnean Society* **179**, 511-525.

455 Phillips RD, Scaccabarozzi D, Retter BA, Hayes C, Brown GR, Dixon K, Peakall R (2014b) Caught in the act: pollination of sexually deceptive trap-flowers by fungus gnats in *Pterostylis* (Orchidaceae). *Annals of Botany* **113**, 629-641.

460 Phillips RD, Xu T, Hutchinson MF, Dixon KW, Peakall R (2013) Convergent specialisation - the sharing of pollinators by sympatric genera of sexually deceptive orchids. *Journal of Ecology* **101**, 826-835.

465 Reiter N, Vlcek K, O'Brien N, Gibson M, Pitts D, Brown GR, Bower CC, Phillips RD (2017) Pollinator rarity limits reintroduction sites in an endangered sexually deceptive orchid (*Caladenia hastata*): implications for plants with specialized pollination systems. *Botanical Journal of the Linnean Society* **184**, 122-136.

470 Schiestl FP, Ayasse M, Paulus HF, Lofstedt C, Hansson BS, Ibarra F, Francke W (1999) Orchid pollination by sexual swindle. *Nature* **399**, 421-422.

Schiestl FP, Peakall R, Mant JG, Ibarra F, Schulz C, Franke S, Francke W (2003) The chemistry of sexual deception in an orchid-wasp pollination system. *Science* **302**, 437-438.

- 475 Singer RB (2002) The pollination mechanism in *Trigonidium obtusum* Lindl (Orchidaceae :
Maxillariinae): Sexual mimicry and trap-flowers. *Annals of Botany* **89**, 157-163.
- Stoutamire WP (1974) Australian terrestrial orchids, thynnid wasps, and pseudocopulation.
American Orchid Society Bulletin **43**, 13-18.
- 480 Stoutamire WP (1983) Wasp-pollinated species of *Caladenia* (Orchidaceae) in southwestern
Australia. *Australian Journal of Botany* **31**, 383-394.
- Suppiah, R, Hennessy KJ, Whetton PH, McInnes K, Macadam J, Bathols J, Ricketts J, Page
CM (2007) Australian climate projections derived from simulations performed for the IPCC
485 4th assessment report. *Australian Meteorological Magazine* **56**, 131-152.
- Swartz ND, Clements MA, Bower CC, Miller JT (2014) Defining conservation units in a
complex of morphologically similar, sexually deceptive, highly endangered orchids.
Biological Conservation **174**, 55-64.
- 490 Vereecken NJ, Wilson CA, Hotling S, Schulz S, Banketov SA, Mardulyn P (2012) Pre-
adaptations and the evolution of pollination by sexual deception: Cope's rule of specialization
revisited. *Proceedings of the Royal Society B-Biological Sciences* **279**, 4786-4794.
- 495 Weston PH, Perkins AJ, Indsto JO, Clements MA (2014) Phylogeny of the Orchidaceae tribe
Diurideae and its implication for the evolution of pollination systems. In 'Darwin's Orchids:
Then and Now. (Eds R Edens-Meier and P Bernhardt) pp. 91-154. (The University of
Chicago Press: Chicago).
- 500 Xu H, Bohman B, Wong DCJ, Rodriguez-Delgado C, Scaffidi A, Flematti GR, Phillips RD,
Pichersky E, Peakall R (2017) Complex sexual deception in an orchid is achieved by co-
opting two independent biosynthetic pathways for pollinator attraction. *Current Biology* **27**,
1867-1877.

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Figure captions:



510 **Fig. 1:** Comparison of the flower of *Caladenia abbreviata* with other members of *Caladenia*
subgenus *Phlebochilus* that are either food-deceptive or sexually deceptive. The labellum
calli, tepal size and colouration of *C. abbreviata* (left) are more similar to food-deceptive
species such as *C. denitculata* (centre) than other sexually deceptive species such as *C.*
cristata (right). Note that in *C. abbreviata* the glandular tips of the petals and sepals are the
515 source of the chemical sexual attractant.

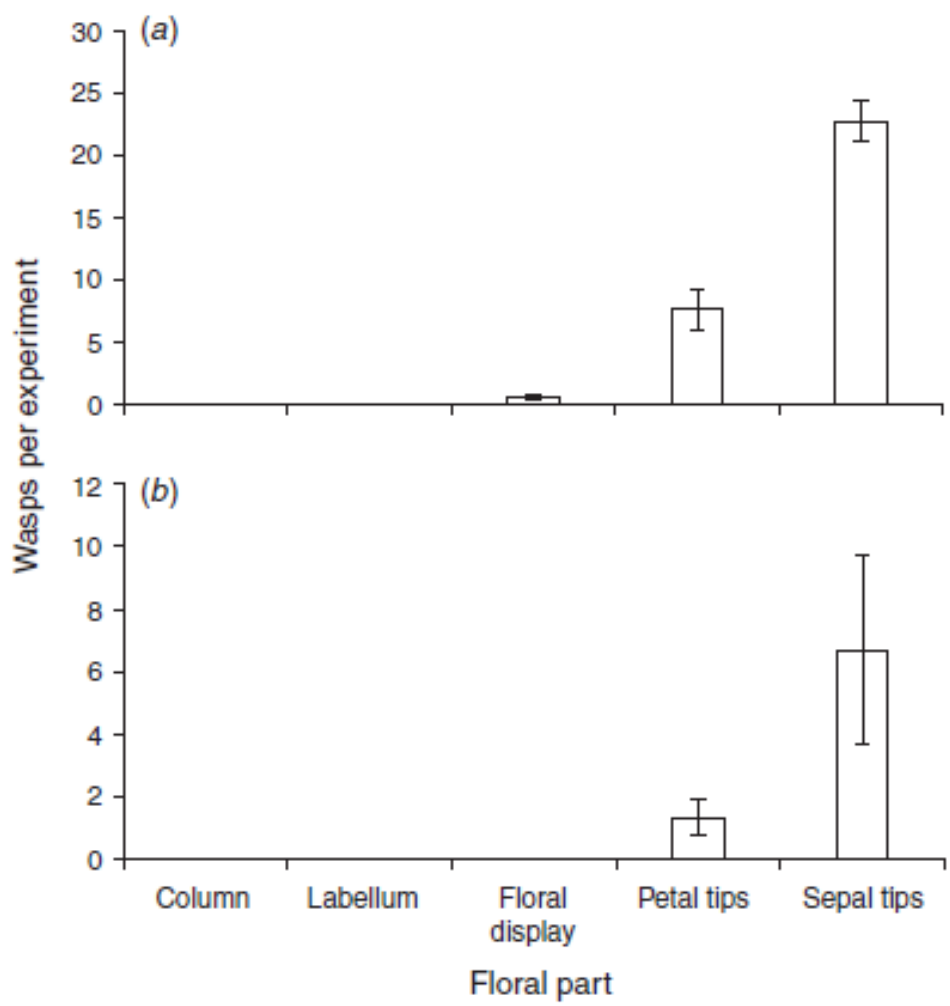


Fig. 2: The number of thynnine wasps (A) attracted to, and (B) attempting copulation with, 520 dissected floral parts of *Caladenia abbreviata*. Bars represent means with standard errors.