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*Pollination Ecology and Pollination Evolutionary Processes with Relevance in  
Ecosystem Restoration –*

**Pollination Biology of *Diuris*:**

**Testing for Batesian Mimicry in Southwestern Australia**



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## **Declaration**

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## Abstract

Mimicry is based on the interaction between a mimic, a model and a receiver. While there is increasing recognition of Batesian floral mimicry in plants, there are few confirmed cases where mimicry involves more than one model species.

The Australian orchid genus *Diuris* has been long hypothesised to engage in guild mimicry of a range of co-occurring pea plants (Faboideae). Some clades of *Diuris* are superficially similar in both colour and shape to those of a guild of yellow and brown pea plants (Faboideae). Here, we test for pollination via mimicry of pea plants in *Diuris* (Orchidaceae). Additionally, we test for further ecological interactions (non-model plants, pollination limitation, habitat size and plant frequency) in order to assess the reproductive success of the orchids. For addressing these hypothesis we select two study species, occurring in different habitat: *Diuris brumalis* (Jarrah forest) and *Diuris magnifica* (Banksia woodland), the latter occurring in fragmented habitat. We test for floral mimicry criteria in both of the species. In order to frame the pollination ecology of the putative model plants, we verify the type of pollinator interactions (generalised vs specialised) occurring in four communities of pea plants in the southwestern Australian Floristic Region (SWAFR).

*D. brumalis*, *D. magnifica* and the pea plants showed strong flower similarity and were likely to be perceived as the same by pollinators, native bees (*Trichocolletes*; Colletidae). However, in *D. brumalis* the orchid reproductive success increased with the local abundance of the model species (*Daviesia* spp.), while in *D. magnifica* the reproductive success wasn't in relation to the putative models. Alternatively, *D. magnifica* reproductive success was influenced by a non-model pea plant (*Hardenbergia*) which is locally abundant and widespread in all the study sites. Additionally, habitat size and orchid plant frequency influenced the orchid reproductive success.

Pea plant species were visited by between one and four genera of native bees, indicating variation in levels of specialisation of the pollination systems of Faboideae. Several pea plant species showed specialised interactions with bee genera attracted. Unexpectedly, some pea plant species frequently attracted beetles that may play an important role in pollination.

Evidence for mimicry of multiple models suggests that *D. brumalis* and *D. magnifica* may be engaged in guild mimicry system. Interestingly, *D. brumalis* and *D. magnifica*

belongs to a complex of species with similar floral traits, suggesting that this represents a useful system for investigating speciation in lineages that employ mimicry of food plants. Furthermore, the study on pollination of Faboideae species of SWAFR, offers a pivotal research for next investigations on pollinator webs and syndromes of Australian pea plants scarcely documented until now.

## General introduction

The present work aims to investigate a case of floral mimicry in South Western Australia where multiple ecological plant-pollinator interactions occur, including between co-occurring flowering plants. An understanding of floral mimicry, focused on orchids and legumes, is vital for unravelling pollination processes relevant to restoration ecology.

A restored ecosystem must be supported by a solid network of pollinators in order to ensure plant reproductive continuity (Memmot et al., 2007; Klein et al., 2007). However, re-activation of pollination network within a restored ecosystem is a complex dynamic that needs to be framed by previous pollination studies (Furup et al., 2008).

For example, in Australia the urban restoration programmes have neglected the inclusion of pollinator network due to the lack of knowledge of local pollination interactions (Dixon, 2009).

To put this study of mimicry in Australian orchids of the genus *Diuris* (Orchidaceae) and co-occurring legumes in context, a general introduction to floral mimicry, pollination and diversity of the Australian pea plants is provided below. The study species / aims are presented, followed by the thesis outline.

### 1. Floral mimicry

Mimicry was first recognised in animals by Henry Walter Bates in 1862, who discovered that palatable butterflies were imitating the wing pattern of unpalatable butterflies in order to avoid predation. However, the discovery of floral mimicry predated Bate's work on butterfly mimicry. Sprengel in 1793, interpreted the carrion odour emanated by the succulent South African plant *Stapelia hirsuta*, as a deceit pollination system to attract flies.

Vane-Wright (1980) defined mimicry as a phenomenon in which the mimic, the model and the operator interact with each other, causing a cognitive misclassification and behavioural response by the operator that leads to a fitness benefit for the mimic. The mimicry can be achieved through various signals,

including visual, acoustic, chemical, tactile and possibly electrical (Norman et al., 2001; de Jager and Peakall, 2016; Barbero et al., 2009; Schiestl and Johnson, 2013; Gaskett, 2011; Stoddard, 1999). Adaptive resemblance is a basal criterion occurring in all cases of mimicry (Johnson and Schiestl, 2016).

While mimicry in animals has been well studied, in plants the phenomenon has rarely been reported and was largely controversial (Ruxton et al., 2004) for about 200 years. During the last three decades, there has been an increase in floral mimicry studies, suggesting that the phenomena may be widespread in some plant families (Johnson and Schiestl, 2016).

The general criteria for floral mimicry (Roy and Widmer, 1999; Johnson and Schiestl, 2016) are: I) mimic and models are sympatric, have overlapping phenologies and interact with the same operators; II) the mimic resembles the model, making difficult for the operator to distinguish between them; III) behaviour of the operator on the mimic depends on the experience with the model; IV) the fitness of the mimic is higher when the model occurs than it is absent; V) individuals where the resemblance with the model is more pronounced benefit in terms of fitness, than individuals less similar to their model; VI) mimic fitness is higher with model abundance.

The target of mimicry in plants is to attract insects, rather than repel predators, as happens in animals (Little, 1983). It varies along a gradient from generalised deception to a specific resemblance towards a definite phenotype with specific floral traits (Batesian mimicry; Johnson and Schiestl, 2016).

In food deception, which is the most common form of deception in non-rewarding plants, the receiver is attracted by a ‘general search image’ of surrounding rewarding plants, rather than by a given model with specific floral traits (Van der Cingel, 2001; Johnson and Schiestl, 2016).

In Batesian food-source the mimic resembles a defined model phenotype (Peter and Johnson, 2008; Jersáková et al., 2012). There are only a few examples of Batesian food-source mimicry in plants that have fully tested the predictions above (Johnson and Schiestl, 2016).

Instead, ‘Guild mimicry’ consists of a mimic sharing pollinators with multiple model species (Brown and Brown, 1979; Dafni and Bernhardt, 1990).

Generalised food deceptive systems (GFD) are distinguishable by specific food mimicry systems. Generalised food deceptive plants usually lack in floral traits that confer a similarity to a specific model plant (Dafni, 1984; Nilsson, 1992), and as a consequence of the general nature of signals they tend to have a wide group of pollinators (Nilsson, 1993, Cozzolino et al., 2005). Furthermore, general food deceptive orchids, such as the European orchid genera *Dactylorhiza* (Nilsson, 1980; Lammi and Kuitunen, 1995) and *Anacamptis* (Nilsson, 1984; Johnson et al., 2003; Johnson et al., 2004), are based on an innate pollinator behaviour rather than being conditioned by the mimic-model similarity. In fact, in GDF the innate preference of pollinators towards a 'general flower image', may independently occur by the conditioned effect of signals by the model (Schaefer and Ruxton, 2009).

## **2. Pollination and diversity of Australian pea plants**

The Leguminosae are the third largest Angiosperm family (Mabberley, 1997; Christenhusz et al., 2017), consisting of three subfamilies (Faboideae, Caesalpinioideae and Mimosoideae) with 730 genera and over 19 400 species (Lewis et al., 2005). Among the three subfamilies, the Faboideae (hereafter referred to as pea plants), exhibit a typical keel-flower floral form (Westerkamp, 1997). With the exception of some members of the tribe Amorphaeae, the flowers of the pea plants are characterised by zygomorphic symmetry, and a papilionaceous corolla formed by a standard petal (vexillum), two wing petals (alae) and two keel petals (carina).

In the pea plants, pollinator pressure causes “tripping” (Arroyo, 1981; Galloni et al., 2006; Aronne et al., 2012) consisting with the stamens emerging from the keel and coming in contact with the pollinator abdomen. As this mechanism necessitates an appropriate pollinator size, and in some cases strength of the pollinator to pull down the petal wings and contact the stamens, some pea plants species may be specialised on particular pollinator types (Córdoba and Cocucci, 2011).

The pea plants in Australia (approximately 1500 species and 136 genera; Crisp, 2009) often are a dominant element of the understorey in many plant communities. Based on incidental records, often relating to specimens cited in bee taxonomic papers, the majority of Australian pea plants are likely to be pollinated primarily by bees (e.g. Rayment, 1936; Houston, 2000; Batley and Houston, 2012; Popic et al., 2013). Detailed studies of pollination of Australian pea plants are few but support these observations (Gross, 1992; 2001; Ogilvie et al., 2009).

Over 540 species of pea plants occur in the southwestern Australian Floristic Region (SWAFR *sensu* Hopper and Gioia, 2004) but there have been no comprehensive pollination studies in this area (Phillips et al., 2010). However, observational studies (e.g. Hopper, 1981; Houston, 2000) suggest that bees are likely to play an important role in the pollination of many species of pea plants that display a wide range of floral colours (Barrett and Pin Tay, 2005).

### **3. Evidence of likely mimicry in *Diuris***

Predominantly consisting of mimetic species (Van der Pijl and Dodson 1966; Ackerman, 1986), orchids are an iconic and unusually deceptive group among flowering plants. Nearly one third of orchid species, between 6500 and 9000 species, do not offer a reward and are believed to deceive their pollinators (Dafni 1984, Ackerman, 1986). To attract pollinators, non-rewarding orchids utilise a diversity of mechanisms including food and sexual deception (Coleman, 1928; Schiestl et al., 1999; Schiestl et al., 2003), brood site mimicry (Van der Niet et al., 2011; Martos et al., 2015) and shelter site imitation (Dafni and Iveri, 1980).

The predominance of deceptive orchids lure their operator by imitating floral rewards (Ackerman, 1996).

After the pollination has being firstly documented by Rayment (1936), the Australian genus *Diuris* has long been hypothesised to engage in guild mimicry (Dafni and Bernhardt, 1990) and tends to be specialised in faboideae legumes (Edens-Meier and Bernhardt, 2014). While *Diuris* shows considerable interspecific variation in colour, including species that are predominantly yellow, white or pink (Jones, 2006; Hoffmann and Brown, 2011), some species groups exhibit a superficially similar colour and shape to those of a guild of yellow and brown ‘pea plants’ (Faboideae). The first study of floral mimicry by *Diuris* was on

the eastern Australian species *Diuris maculata* (Beardsell et al., 1986) where bees in the genera *Leioproctus* and *Trichocolletes* pollinated both the *Diuris* and pea plants in the genera *Daviesia* and *Pultenaea scabra*.

*Diuris alba* represents a further pollination study within this genus in which generalist *Exoneura* bees, but also the wasps *Eurys pulcher* and a *Paralastor* species are involved (Indsto et al., 2007).

Indsto et al. (2006) showed that not only do pea plants and *D. maculata* share pollinator species, but that both plants share similar floral colouration when it is quantified using reflectance spectrophotometry and analysed according to a bee visual model (Houston, 2000). Whereas some records in *Diuris* show pollination evidence of genera like *Leioproctus* and *Exoneura*, they are polylectic bees foraging on a range flowers (Houston, 2000). Alternatively, *Trichocolletes* bees are observed to primarily forage on Faboideae flowers (Rayment, 1929, 1936; Houston, 2000). Currently, 25 species of *Trichocolletes* are known from southwestern Australia (Batley and Houston, 2012). Nectar production in *Diuris* has been firstly documented by Rayment (1936) and subsequently disproved by Beardsell et al. (1986). Additional investigations on *Diuris* of eastern Australia spotted the presence of nectar in the studied species (Indsto et al., 2006; 2007).

#### **4. The study**

**The main aim** of the project is to resolve the mimicry system in the orchid species *Diuris brumalis* and *D. magnifica* that co-occur with pea plants, reputed putative models for the orchids.

*Diuris* is an Australian genus comprising about 100 species, primarily widespread in South Australia, with 22 species occurring in outhern Western Australia. It includes species with different floral colors – (predominantly yellow with brown and purple notes, but also white and pink), distinguished by analogous floral morphology (Jones, 2006; Hoffmann and Brown, 2011). *Diuris brumalis* is a yellow-brown flowering orchid, flowering between July and August in South Western Australia (Brown et al., 2013), occurring with a range of Faboideae species with similar colour patterns. *Diuris magnifica* exhibits yellow, orange and purple flowers and flowers from August to September (Brown et al., 2013). It is distributed along the southern Western Australian coast and grows in *Banksia* and

Sheoak woodland (Brown et al., 2013) which are characterised by an abundance of co-flowering Faboideae species.

Specific hypotheses for the two orchid study species match the criteria for floral mimicry (see Roy and Widmer, 1999; Johnson and Schiestl, 2016). We tested the following predictions:

- (1) that the colour and morphology overlap between models and mimic, but not with the remainder of the floral community;
- (2) that the flowering phenology of the proposed mimic overlaps with the models;
- (3) that the pollinator exhibits with the mimic a deceived behaviour normally only associated with the model;
- (4) that the fitness of the mimic is greater in the presence of the models; and
- (5) that the fitness of the mimic increases with the number of flowers of the model species.

Additionally, for *D. magnifica* we tested if:

- (6) the reproductive success of *D. magnifica* increases with more non-model food plants;
- (7) the orchid's reproductive success decreases with more orchid frequency; and
- (8) the orchid's reproductive success is lower in small habitat remnants.

Secondly, for 15 pea plants in the SWAFR the study tests if co-occurring pea plants in four regions (Perth hills, Perth coastal plains, Margaret River-Augusta, Waroona) share pollinator species. The study species, flowering throughout July and December, were *Bossiaea aquifolium*, *B. disticha*, *B. eriocarpa*, *B. linophylla*, *Daviesia decurrens*, *D. divaricata*, *D. horrida*, *D. rhombifolia*, *Hardenbergia comptoniana*, *Hovea chorizemifolia*, *H. pungens*, *Isotropis cuneifolia*, *Jacksonia sternbergiana*, *Mirbelia dilatata*, *Viminaria juncea*.

Specifically, we addressed the following questions:

- (1) do different species of pea plant have different species of potential pollinators?;
- (2) how do different visitors behave when foraging?;
- (3) is the length of the stamens related to the size of visiting bees?; and
- (4) does the nectar vary in sugar composition and volume between pea plants with different groups of potential pollinators?

Lastly, the work validated an effective methodology for enhancing the attraction of insects in deceptive orchids, testing if more insects were attracted by using arrays of orchid flowers, than by carrying out observations on orchid in their natural habitat.

## **5. Thesis outline**

### **Chapter 1: ‘Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid’**

In this chapter, we tested for pollination via mimicry in *Diuris brumalis* (Orchidaceae); *Diuris* is an Australian genus hypothesised to attract pollinators via imitation of a range of co-occurring pea plants (Faboideae). We addressed the fundamental criteria for floral mimicry between the mimic orchid and the putative models (pea plants): sharing of pollinators, similarity of floral traits and colour reflectance, flowering period overlap and relation between the orchid reproductive success and the presence of co-occurring pea plants. We found that *Diuris brumalis* is pollinated via mimicry of co-occurring congeneric Faboideae species (*Daviesia* spp.), suggesting that this may represent a guild mimicry system. As *D. brumalis* belongs to a complex of species with similar floral traits, this case is an ideal system for investigating speciation in lineages that employ food-source mimicry.

### **Chapter 2: ‘Pea plants in the southwestern Australia biodiversity hotspot: pronounced differences in potential pollinators between co-occurring species’**

We selected 15 species of pea plants (Faboideae) from four communities in the SWAFR for investigating the pollination biology and a preliminary evidence of ecological specialisation between pea plants and their pollinators. In particular, we tested if co-occurring pea plants showed differences in pollinator species, in behaviour on the flower, and whether floral traits as stamen length or nectar composition indicated a sort of specialisation level with some genera of pollinators. Overall, pea plant species showed a variation in levels of specialisation of the pollination systems. In pea plant species with more specialised interactions, co-

occurring pea plants showed pronounced differences in the bee genera attracted. Some pea plant species frequently attracted beetles, suggesting their involvement in the pollination of pea plants. The examined floral traits did not reveal any evidence of specialisation with pollinator type. We found that the introduced honeybee *Apis mellifera* visited all pea species studied, suggesting that honey bees may be both a pollinator and potential competitor for resources with native pollinators.

### **Chapter 3: ‘A general pea flower image? Ecological factors affecting reproductive success in an orchid that exhibits imperfect floral mimicry**

In the course of this chapter, we tested the floral mimicry of *Diuris magnifica* (Orchidaceae) towards co-occurring pea plants. Additionally, we tested if the orchid success depended on other ecological interactions decoupled from the evolution of mimicry: surrounding food plants, pollinator occurrence, and habitat size.

We found evidence of floral mimicry between *D. magnifica* and yellow-red co-occurring pea plants, based on pollinator and behavioural observations, spectral reflectance, and superficial overlap of floral traits.

However, we did not find any evidence that the orchid reproductive success was facilitated by the presence of putative model pea plants. Alternatively, the orchid reproductive success was enhanced by a food non-model pea plant and by bushland remnant size. Unexpectedly, *D. magnifica* reproductive success was independent by the primary pollinator occurrence, due to the likely contribution of sub-optimal pollinators (*Apis* and beetles). Concluding, the attention on the bushland remnant size, the presence of model and non-model pea plants may be essential for supporting the reproductive success in *D. magnifica*, offering new scenarios within conservation of orchid species in urban context.

### **Chapter 4: Rotating arrays of orchid flowers: a simple and effective methodology for studying pollination in deceptive plants**

In this methodology chapter, we validated a novel methodology to assess pollination effectiveness in food deceptive orchids. We tested the methodology on the mimic

orchid, *Diuris brumalis* (Orchidaceae) and putative model plants belonging to the genus *Daviesia* (Fabaceae), rotating arrays of orchid flowers in proximity to model plants. This simple method resulted in effective attraction of pollinators. The methodology has universal application to other food deceptive pollination syndromes in plants with relevance to developing more effective conservation assessments regarding pollinator capability.

The thesis provides ecological insights into the floral food mimicry within two specialised orchid pollination systems. This work is also relevant for understanding pollinator-driven speciation in other similar deceptive systems. Furthermore, the study on pollination of Fabaceae species of S-W Australia, offers a pivotal research for potential investigations on pollinator webs and on syndromes of Australian pea plants until now poorly explored.

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## Chapter 1

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### **Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid**

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## Chapter 1

### Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid

#### ABSTRACT

*Background and Aims:* While there is increasing recognition of Batesian floral mimicry in plants, there are few confirmed cases where mimicry involves more than one model species. Here, we test for pollination by mimicry in *Diuris* (Orchidaceae), a genus hypothesized to attract pollinators via mimicry of a range of co-occurring pea plants (Fabaceae).

*Methods:* Observations of pollinator behaviour were made for *Diuris brumalis* using arrays of orchid flowers. An analysis of floral traits in the co-flowering community and spectral reflectance measurements were undertaken to test if *D. brumalis* and the pea plants showed strong similarity and were likely to be perceived as the same by bees. Pollen removal and fruit set was recorded at 18 sites over two years to test if fitness of *D. brumalis* increased with the abundance of the model species.

*Key Results:* *Diuris brumalis* shares the pollinator species *Trichocolletes capillosus* and *T. leucogenys* (Hymenoptera: Colletidae) with co-flowering Fabaceae from the genus *Daviesia*. On *D. brumalis*, *Trichocolletes* exhibited the same stereotyped food-foraging and mate-patrolling behaviour that they exhibit with *Daviesia*. *Diuris* and pea plants showed strong morphological similarity compared to the co-flowering plant community, while the spectral reflectance of *Diuris* was similar to *Daviesia* spp. Fruit set and pollen removal of *D. brumalis* was highest at sites with a greater number of *Daviesia* flowers.

*Conclusions:* *Diuris brumalis* is pollinated by mimicry of co-occurring congeneric Fabaceae species. Evidence for mimicry of multiple models, all of which share pollinator species, suggests that this may represent a guild mimicry system. Interestingly, *D. brumalis* belongs to a complex of species with similar floral traits, suggesting that this represents a useful system for investigating speciation in lineages that employ mimicry of food plants.

**Key words:** *Diuris brumalis*, *Daviesia*, Fabaceae, Colletidae, mimicry, food deception specialization, pollination, pollinator behaviour, plant fitness

## INTRODUCTION

Batesian mimicry represents an interaction between model, mimic and operator (the signal receiver), in which the operator mistakes the mimic for the model leading to a fitness benefit for the mimic (Vane-Wright, 1980). Mimicry can be achieved through a diversity of cues, including visual, acoustic, chemical, tactile and possibly electrical (Stoddard, 1999; Norman *et al.*, 2001; Barbero *et al.*, 2009; Schiestl and Johnson, 2013; Bohman *et al.*, 2018). Despite mimicry in animals being well supported in multiple systems, the phenomenon remained rarely confirmed and largely controversial in plants (Ruxton *et al.*, 2004). Only in the last three decades has evidence been presented suggesting that floral mimicry may be widespread in some plant families (Johnson and Schiestl, 2016).

Orchids (Orchidaceae) are an unusual group among flowering plants in that approximately one-third of known species (6500–9000 species) are believed to attract their pollinators via deception (Dafni 1984; Ackerman 1986). Non-rewarding orchids exhibit a range of mechanisms to attract pollinators, including sexual deception (Coleman, 1928; Schiestl *et al.*, 1999; Schiestl *et al.*, 2003), brood site mimicry (Van der Niet *et al.*, 2011; Martos *et al.*, 2015) and alarm pheromone imitation (Brodmann *et al.*, 2009). However, the majority of deceptive orchids attract pollinators by falsely advertising floral rewards to pollinators (Ackerman, 1986), using traits such as inflorescence shape and architecture, flower color and brightness, scent, nectar guides and pollen marks (Kunze and Gumbert, 2001; Galizia *et al.*, 2005; Jersáková *et al.*, 2012). The most common form of food-deception is generalised food deception, where food-seeking animals are attracted by general floral signals rather than the traits of any particular rewarding species (Van der Cingel, 1995, 2001; Jersáková *et al.*, 2006). Alternatively, deceptive orchids that exhibit similar floral traits to those of a particular rewarding flower are predicted to be using Batesian mimicry to attract pollinators (Jersáková *et al.*, 2006), where the mimic receives a benefit from co-flowering plant species through increased reproductive success (Jersáková *et al.*, 2006).

The most comprehensive evidence to date for Batesian mimicry in orchids comes from research undertaken on the South African flora. For example, Peter and Johnson (2008) employed UV-manipulation experiments to show that *Eulophia zeyheriana* mimics the floral colour of nectar rewarding *Wahlenbergia cuspidata* (Campanulaceae) to attract

*Lipotriches* (Halictidae) bees. Similarly, Jersáková *et al.* (2012) demonstrated that *Disa pulchra* attracts long-proboscid tabanid flies by mimicking the rewarding iris *Watsonia lepida* through closely matching the floral reflectance spectra of both species. In these cases, and most others where floral Batesian mimicry has been hypothesised, in any given population there is evidence for mimicry of a single model species (Dafni *et al.*, 1981; Nilsson, 1983; Johnson, 2000; Benitez-Vieyra *et al.*, 2007). However, in orchids there is some evidence for guild mimicry, where a rewardless species mimics a range of model species that have similar floral traits and share the same pollinator species (Brown and Brown, 1979; Dafni and Bernhardt, 1990; Johnson and Schiestl, 2016). For example, the European orchid *Traunsteinera globosa* attracts pollinators by mimicking the colour and inflorescence shape of representatives of three morphologically similar co-occurring genera in the Dipsacaceae and Caprifoliaceae (Juillet *et al.*, 2007; Jersáková *et al.*, 2016). This strategy may be advantageous over other more specialized forms of Batesian mimicry as the mimic may receive a fitness benefit from co-flowering with a wider range of model plants.

The Australian orchid genus *Diuris* has been long hypothesised to engage in guild mimicry (Dafni and Bernhardt, 1990). Some clades of *Diuris* are superficially similar in both colour and shape to those of a guild of yellow and brown pea plants (Faboideae). Floral mimicry of Faboideae was first tested in the eastern Australian species *D. maculata* (Beardsell *et al.*, 1986; Indsto *et al.*, 2006), where it was shown that *Diuris* and some Faboideae share pollinators and have similar floral colouration according to a bee visual model. While *Diuris* encompasses a range of floral shapes and colourations, this yellow and brown Faboideae like flower has evolved at least twice within the genus (Indsto *et al.*, 2009), suggesting that these traits could be adaptations to the mimicry of Faboideae (see argument of Johnson *et al.*, 2003). However, to tease apart if this is truly a pollination strategy based on mimicry, or convergent evolution of floral signals that are attractive to bee pollinators, requires comparison with the floral traits of the broader plant community (de Jager *et al.*, 2016), observations of pollinator behavior on model and mimic, and data on reproductive success of the orchid in relation to the abundance of the model (Roy and Widmer, 1999; Peter and Johnson, 2008). Further, a compelling line of evidence for the existence of mimicry would be if the pollinator is deceived into engaging in the same

specific behaviours with the putative mimic that it typically exhibits only with the model species. Such behavioural evidence confirms that the orchid is functioning as a mimic, regardless of whether some of its floral traits originally evolved through selection for mimicry, or independently to exploit the foraging behaviour of the bee.

We tested the mimicry hypothesis in *Diuris brumalis*, an orchid species that co-occurs with a range of Faboideae species that exhibit similar yellow-brown colour patterns. Having identified candidate model species based on the diet of the bee species involved in pollination of *D. brumalis*, we tested the following predictions: (1) that the colour and morphology overlap between models and mimic, but not with the remainder of the floral community (2) that the flowering phenology of the proposed mimic overlaps with the models (3) that the pollinator exhibits with the mimic a deceived behaviour normally only associated with the model (4) that fitness of the mimic is greater in the presence of the models and (5) that fitness of the mimic increases with the number of flowers of the model species. Further, to investigate if this mimicry system operates with more than one model species, observations of pollinator behavior were undertaken in habitats that differed in the pea plant species present.

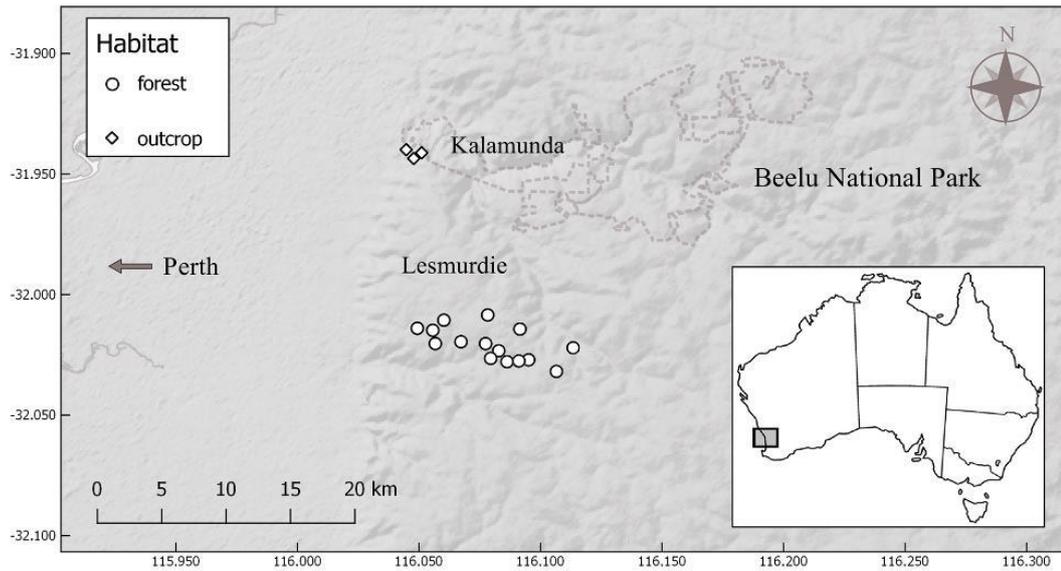
## MATERIALS AND METHODS

### *Study species*

*Diuris* is a primarily Australian genus comprising of approximately 100 species, with centers of diversity in south-western and south-eastern Australia (Jones, 2006). *Diuris* are terrestrial geophytes, with a solitary scape produced per plant in any given year (Jones, 2006). Most species of *Diuris* appear to be capable of clonal reproduction through vegetative multiplication of daughter tubers (Dixon, 1989). *Diuris brumalis* produces yellow-brown nectarless flowers during July and August, with between three and 15 flowers per inflorescence (Brown *et al.*, 2013). A vector is required for pollination, and the flowers are self-compatible [Supplementary data, Appendix S1]. Pollination within a given flowering season is primarily pollen limited, with most or all flowers on a scape forming fruit after pollination by hand (Elliott & Ladd, 2002; Supplementary data, Appendix S1). *Diuris brumalis* occurs in a range of habitats, which differ in their community of winter flowering Faboideae species. Unlike *D. brumalis*, these Faboideae produce floral nectar [Supplementary data, Appendix S1].

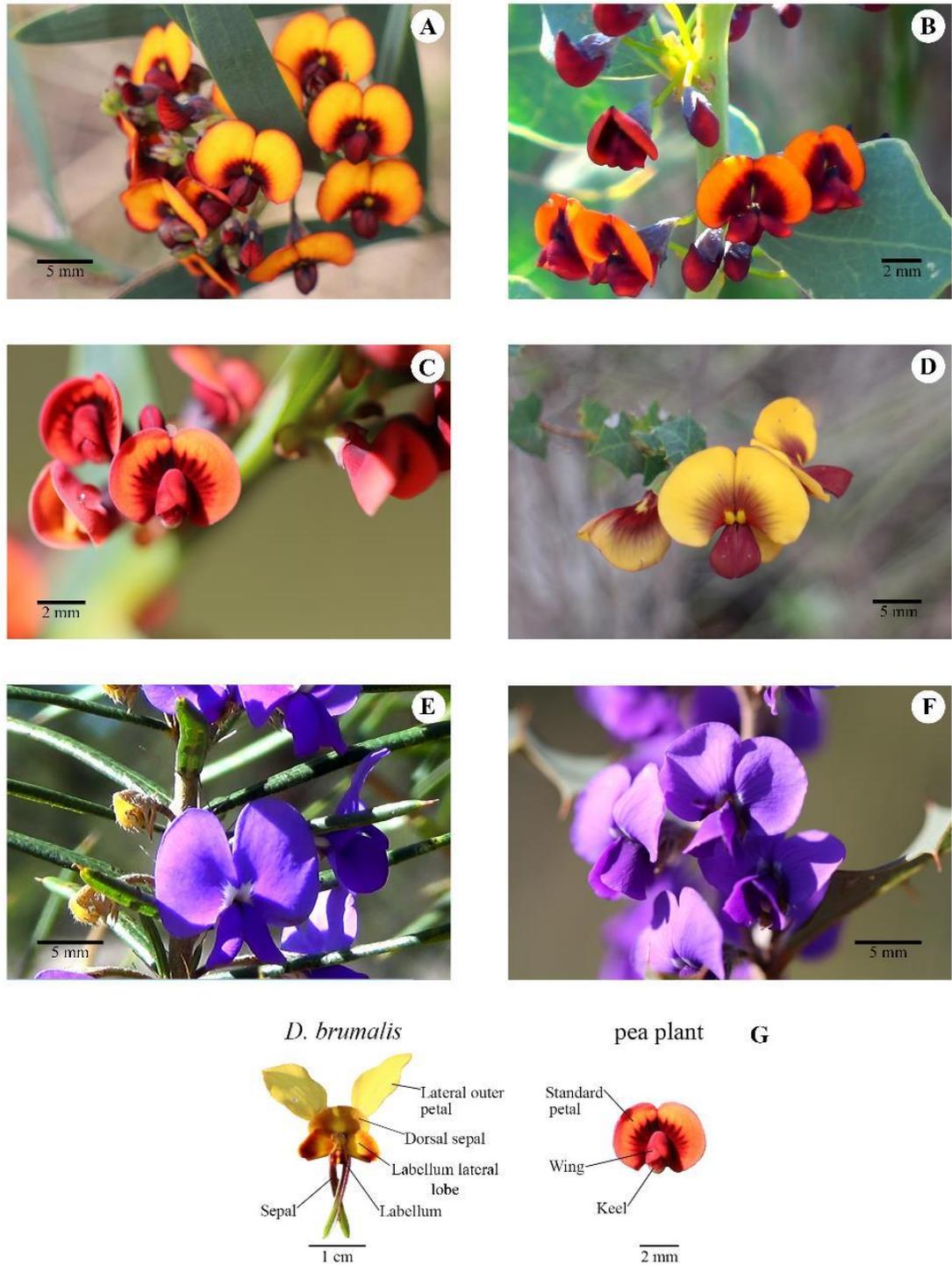
### *Study sites*

Data were collected from *D. brumalis* populations in the Darling Range, near Perth, Western Australia during 2016 and 2017 (Fig. 1). The populations were selected across two different habitat types (Fig. 1; Supplementary data, Table S1): Jarrah forest (hereby referred to as ‘forest’; 15 sites) and heathland surrounding granite outcrops (hereby referred to as ‘outcrop’; 3 sites). No other species of *Diuris* was observed flowering at any site during the study period.



**Fig. 1** Distribution of the 18 *Diuris brumalis* study sites in the Darling Range, Western Australia. Fifteen sites were in Jarrah forest and three in granite outcrops.

*Diuris brumalis* frequently co-occurs with several species of Faboideae (Fabaceae; Marshall, 1995). Six species of flowering Faboideae, commonly referred to as pea plants, were identified at the study sites (Fig. 2, A-F), namely *Daviesia decurrens*, *D. horrida*, *D. rhombifolia*, *Hovea chorizemifolia*, *H. pungens*, and *Bossiaea aquifolium* [Supplementary data, Table S1]. While *D. decurrens*, *D. rhombifolia*, *H. chorizemifolia*, *H. pungens* and *B. aquifolium* were present at forest sites and only *D. horrida* and *H. pungens* were present at outcrop sites. Voucher specimens of all studied species were collected and accessioned at Herbarium of Western Australia in Perth [Supplementary data, Table S2].



**Fig. 2** Fabaceae co-occurring with *Diuris brumalis*: A *Daviesia horrida*; B *D. rhombifolia*; C *D. decurrens*; D *Bossiaea aquifolium*; E *Hovea pungens*; F *Hovea chorizemifolia*; G Pea-like floral morphology of *D. brumalis* formed by two lateral petals, the dorsal sepal, labellum lateral lobe, labellum and two basal sepals (Hoffman and Brown, 2011).

### *Observation of pollinators on Diuris brumalis*

To identify the pollinators of *D. brumalis* and quantify their behaviour, observations of pollinator visitation to orchid flowers were undertaken at three sites (F1, F2 and O3) between 13 July to 15 August 2016 and 12 July to 13 August 2017. A total of 191, 15-minute observation periods were conducted (for a total of 2865 minutes observation), with insect behaviour recorded using an EOS M video camera (Canon, Tokyo, Japan) for subsequent viewing in slow motion. Observations were conducted between 10.00 to 15.30 when temperatures were higher than 17°C (temperature ranged between 8 °C and 19 °C, as measured with a Smartsensor AR827, set 20 cm above the ground). Arrays of orchid flowers were designed to replicate the colony forming habit of *D. brumalis* and were comprised of multiple inflorescences that had been cut and placed in glass vials (two inflorescences per vial, each with 4–6 flowers). For each observation period, three vials were spaced 10–20 cm apart to create a conspicuous floral display, with vials placed 1–2 meters from flowering individuals of *Daviesia decurrens*, *D. rhombifolia*, or *D. horrida*. While artificial arrays of flowers were used as the basis for pollinator observations, naturally occurring *D. brumalis* were common at each of these three sites where observations were undertaken.

For each individual insect visiting a flower of *Diuris* and pea plants, the behaviour was recorded and categorised as follows: (I) the insect approached the flower (II) the type of behaviour exhibited upon approaching the flower: zig-zag flight = moving side to side in flight as they approached the flowering plant; direct flight = flying in a straight line as they approached the flower; aligned = body of visitor aligned along the midpoint of the tri-lobed labellum/keel during attempts to forage; patrolling = appearing to inspect multiple flowers around the plant; searching = the bee approaches a flower closely (<5cm) but then chooses to alight on a different flower (III) the insect was carrying pollinaria of *D. brumalis* (IV) if the insect landed on the flower (V) length of time spent on the flower (if >1 second) (VI) if the insect attempted to forage on the flower, either attempting to manipulate the labellum/keel by opening the wings, or attempting to feed on nectar (Fig. 2, G) (VII) if the insect removed or deposited pollen of *D. brumalis* or pea plant (based on filament contact with the insect) (VIII) if the insect visited additional *D. brumalis* or pea plant flowers [Supplementary data, Table S3, S4]. Behaviour was only recorded for the first flower visited, as due to the very

rapid movement of pollinators, it was often impossible to accurately quantify visits to subsequent flowers.

To determine whether pollinator behaviour differs in response to flowers of *Diuris* compared with *Daviesia decurrens* and *Daviesia rhombifolia*, we compared the proportion of floral visitors exhibiting food-foraging behaviours between pea plants and *Diuris* using a Generalised Linear Model with a Bernoulli distribution of the response variable. Plant species was the fixed effect and was treated as categorical variable. Specifically, we tested if between *Diuris* and pea plants (*Daviesia decurrens* and *D. rhombifolia* in the forest habitat) (i) there is a difference in the proportion of bees landing on the flower ii) among the bees landing on the flower, is there a difference in the proportion of bees that exhibit foraging behaviour, either by manipulating the labellum/keel or attempting to forage on nectar.

#### *Observation of pollinators on co-flowering plants*

To determine if *Diuris brumalis* shares pollinators with co-flowering pea plants, pollinator observations were undertaken at two forest sites (F1, F2) and one outcrop site from 13 July to 6 September in 2016 and from 11 July to 9 September 2017. Observations were made between 11.00 and 15.00 daily, with the same video camera set up as described above. A total of 32 observation periods were undertaken for *B. aquifolium*, *D. decurrens*, *D. horrida*, *D. rhombifolia*, *H. chorizemifolia* and *H. pungens* individuals, each of 15 minutes, yielding a total of 480 minutes of observation for each plant species. The pollinator behaviours recorded corresponded to those used for visitors to the *Diuris* (see above), to enable a formal behavioural comparison. To test if bees that visited *D. brumalis* also visited members of the plant community other than pea plants, additional ten minute observation periods were undertaken for other dominant co-flowering species: *Acacia pulchella*, *Adenanthos barbiger*, *Calothamnus sanguineus*, *Hakea lissocarpha*, *Hibbertia hypericoides* and *Hypocalymna robustum* (from four to five observation periods per species, for a total of 270 minutes).

#### *Identification of pollinators*

Pollinators observed on *D. brumalis* and pea plant flowers (particularly individuals carrying the distinctive white pollinaria of *D. brumalis*) were collected for identification. All collected insect pollinators were sent to the Western Australian

Museum as voucher specimens [Supplementary data, Table S5]. Native bee pollinators observed were sexed and identified according to Batley and Houston (2012) based on behavioural (patrolling – males; collecting pollen on the abdomen - females) and morphological features including differences in antennae length (generally longer in males), body size (larger in females), abdomen width (larger in females), and number of hairs on the head (more abundant on males).

#### *Quantification of pollen loads of floral visitors*

As a complementary approach to resolving the food plants of the floral visitors, pollen was identified from the bodies of bees caught [Supplementary data, Table S5] visiting *D. brumalis* and pea plants. Pollen observed on the tibiae or abdomen of pollinators during identification was removed by washing the insect with distilled water, acetolysed following the methods of Erdtmann (1960), and mounted on glass microscope slides. All pollen samples were identified under high magnification (Olympus-BX 51 microscope with Olympus–DP71 camera, Olympus, Tokyo, Japan) by comparison with acetolysed mounted pollen samples from herbarium specimens of *D. brumalis*, *B. aquifolium*, *D. decurrens*, *D. horrida*, *D. rhombifolia*, *H. chorizemifolia*, *H. pungens*, and other commonly co-flowering plant taxa.

#### *Morphological evaluation of floral traits and spectral reflectance*

To test if *D. brumalis* shows greater overlap in floral morphology with the candidate model species than the remainder of the plant community, a morphological evaluation of the floral traits of the dominant co-flowering plant species, including functional pollinators traits, was conducted at three forest sites (F1, F2 and F3). Morphological evaluation was conducted on *D. brumalis* and 20 co-flowering species from 11 families in accordance with the descriptions in Marchant *et al.* (1987). The traits included were corolla symmetry (zygomorphic, actinomorphic), corolla shape (rotate, papilionaceous, bilabiate, ligulate), flower width and length (in mm), flower orientation (pendant, upright, horizontal), plant height (in centimetres), petal protrusion as a platform for pollinators (presence or absence), anther position (exposed or not exposed), and inflorescence type (umbel, raceme, spike, panicle, solitary) [Supplementary data, Table S6]. Morphological similarity of floral traits was evaluated using non-metric multi-dimensional scaling (NMDS) following the methodology of Jolles (2015).

To test if the floral colour of *D. brumalis* flowers is likely to be distinguishable by bees from the co-flowering pea plants species (*D. decurrens*, *D. horrida*, *D. rhombifolia*, *H. chorizemifolia*, *H. pungens* and *B. aquifolium*), we took spectral reflectance measurements and analysed them using the Chittka (1992) model of bee vision. In addition, spectral reflectance was also measured for two yellow-flowered species that occurred at all sites, *Hibbertia hypericoides* (Dilleniaceae) and *Acacia pulchella* (Fabaceae), to test if other yellow flowered species could also be part of the same guild as the pea plants. Spectral reflectance was measured on two flowers per plant for six randomly selected individuals of each species using a spectrometer (Jaz, DH-2000 UV-VIS-NIR Light source) with an integration time of 50 nm. For *D. brumalis*, measurements of spectral reflectance were taken from the outer lateral petals, the central dorsal sepal, the labellum, and lateral labellum lobe; for pea plant species measurements were taken from the standard and wing petals (Fig. 2, G) and for *H. hypericoides* and *A. pulchella* from the most conspicuous part of the floral display (the corolla and stamens respectively). Spectral reflectance was analysed using the colour hexagon model, which is based on the sensitivities of photoreceptors of the bee *Apis mellifera* (Chittka, 1992; Chittka and Kevan, 2005). For quantifying similarity of spectral reflectance, the distance between colour loci coordinates was measured as the Euclidean distance.

#### *Comparative flowering phenology of Diuris brumalis and candidate model species*

To test the prediction that mimics overlap in flowering period with their proposed models, the extent of flowering across the study period was quantified for *D. brumalis* and the co-occurring pea plants (*D. decurrens*, *D. horrida*, *D. rhombifolia*, *H. chorizemifolia*, *H. pungens*, and *B. aquifolium*). For each species, weekly counts of open flowers were undertaken in 30 x 30 meter quadrats at intact forest sites (sites F1, F2 and F3) from 28 June to 11 October 2017. For pea plants, due to the high number of flowers, we scored the total number of flowers per quadrat as (binned category): (1) 1-100, (2) 101-200, (3) 301-400, (4) 401-500 and so on, up to 2000 for a total of 19 categories (1 - 19). However, in the case of *H. chorizemifolia* and *D. brumalis*, due to the paucity of flowers per inflorescence, the exact number of flowers on each plant was counted. The average number of flowers (or binned category) was calculated as the measure of flowering during any given week.

### *Reproductive success of the mimic in relation to the abundance of the model*

To test if the fitness of *D. brumalis* [Supplementary data, Table S7 A,B] increased with the number of flowers of the putative model species, the proportion of flowers with pollen removal and the proportion of flowers with fruit formation was quantified at 18 populations. In 2016 (15 sites) and 2017 (18 sites), we delimited a single 30 x 30 meter quadrat per site, and at the peak flowering period for *D. brumalis* we recorded: (i) the number of pea plants of each species within the quadrat; (ii) the estimated number of flowers for each pea plant species; (iii) how many *D. brumalis* plants and flowers were present (counted at the end of the flowering season in August). Variable (ii) was estimated by counting the number of flowers on ten stems per pea plant to enable calculation of a mean, then multiplying this value by the total number of stems on the plant. Following evidence that the pollinators of *D. brumalis* fed almost entirely on *Daviesia*, this variable was modified to be the estimated number of flowers of *Daviesia*. In both years, at the end of the flowering period of *D. brumalis* we collected data on the number of flowers without pollinaria and the number of fruits produced. The proportion of flowers with pollinaria removal was used as a proxy for male fitness, while the proportion of flowers setting fruit was used as a proxy for female fitness.

Pollinaria removed and fruit set were analysed by Generalized Linear Mixed Models (GLMM) using the package `glmmTMB` in R Studio (Version 3.3.2). Firstly, we tested if pollinaria removal and fruit set were greater at sites where *Daviesia* was present. Secondly, we tested if pollinaria removal and fruit set increased when there were more *Daviesia* flowers. In these latter models we included the abundance of *Daviesia* flowers, habitat, and year all as fixed effects. In each model, site was treated as a random effect, as pollinaria removal and fruit set was quantified at the same sites in two field seasons. Because pollinaria removal and fruit set were analysed as proportions of the total number of flowers, they were assumed to be binomially distributed. However, when using a binomial distribution, the models for pollinaria removed showed overdispersion (overdispersion parameters: 5.833 for the model of presence-absence and 3.897 for the model testing the effect of *Daviesia* flowers abundance, habitat and year; see Zuur *et al.* 2013 for calculations) necessitating a switch to a betabinomial distribution. Evaluation of which model was most strongly supported by the data was undertaken using the AICc index, which dropped 120 and 57 points respectively for the two models by switching to the betabinomial distribution. Models testing the effect of the covariates (see above) on fruit set were not over-dispersed. Therefore, fruit set was

confirmed to be a binomially distributed response variable. The abundance of *Daviesia* flowers was log-transformed in order to improve the fit of the fruit set model. The improvement of the model following a log transformation was confirmed by the AICc index, which dropped 6.5 points, and verified using the “anova” R function, ( $\chi^2_{33,4} = 6.371$ ,  $p < 0.001$ ). For all models we undertook the checks suggested by Zuur *et al.* (2013) to confirm that the underlying assumptions of the model are not violated.

## RESULTS

### *Pollinators of Diuris brumalis*

During baiting experiments a total of 132 insects were observed visiting *D. brumalis*. Of these, 102 visits were by *Trichocolletes* spp. (Colletidae) and 25 by *Apis mellifera* (Apidae). Other visitors included Syrphidae (3) and *Leioproctus* sp. (2; Colletidae). Only *Trichocolletes* spp. and *Apis mellifera* were observed with orchid pollinaria attached, in each case to the frontal region of the head (Fig. 3). However, only in the case of *Trichocolletes* was deposition of pollinia observed, with parts of the pollinia deposited in visits to subsequent flowers.



**Fig. 3** Pollinators of *Diuris brumalis* and *Daviesia* spp.: A Inflorescence of *D. brumalis* (Orchidaceae). B Female of *Trichocolletes leucogenys* with pea plant pollen (orange in colour) on the abdomen and posterior legs, feeding on *Daviesia rhombifolia* by positioning the abdomen over the keel of the flower. C Male *Trichocolletes capillosus* carrying *Diuris brumalis* pollinaria on the head.

A total of 32 insects were caught for identification during baiting experiments and observations of pea plants [Supplementary data, Table S5]. In 2016 a total of 14 *T. capillosus*, two *T. leucogenys* and one *T. dives* were caught, while seven *T. leucogenys* were caught in 2017. A total of 14 *Trichocolletes* were observed carrying pollen of *D. brumalis*, eight while visiting *D. brumalis*, and six while foraging on *Daviesia* spp. (see example in Supplementary video). Of the eight individuals observed carrying pollinia while visiting *D. brumalis*, two arrived at the plant already carrying pollinaria, and six removed pollinaria while being observed. As the six individuals removing pollinaria were all captured for identification, the remaining bees must have all sourced pollinaria from wild *D. brumalis*, independent of our artificial arrays. The identification of captured visitors and/or pollinators shows that the *Trichocolletes* spp. individuals caught on *D. brumalis* and on pea plants with orchid pollinaria included

both females (4) and males (6). On two occasions *Apis mellifera* were collected with attached *D. brumalis* pollinaria. Of the *Trichocolletes* collected during the study, ten carried on their hind legs pollen of the same colour as seen in pea plants (yellow-orange). *Trichocolletes capillosus* was recorded in 2016 in the habitat forest, whereas *T. leucogenys* was recorded both in 2016 and 2017 in the habitats forest and outcrop [Supplementary data, Table S5].

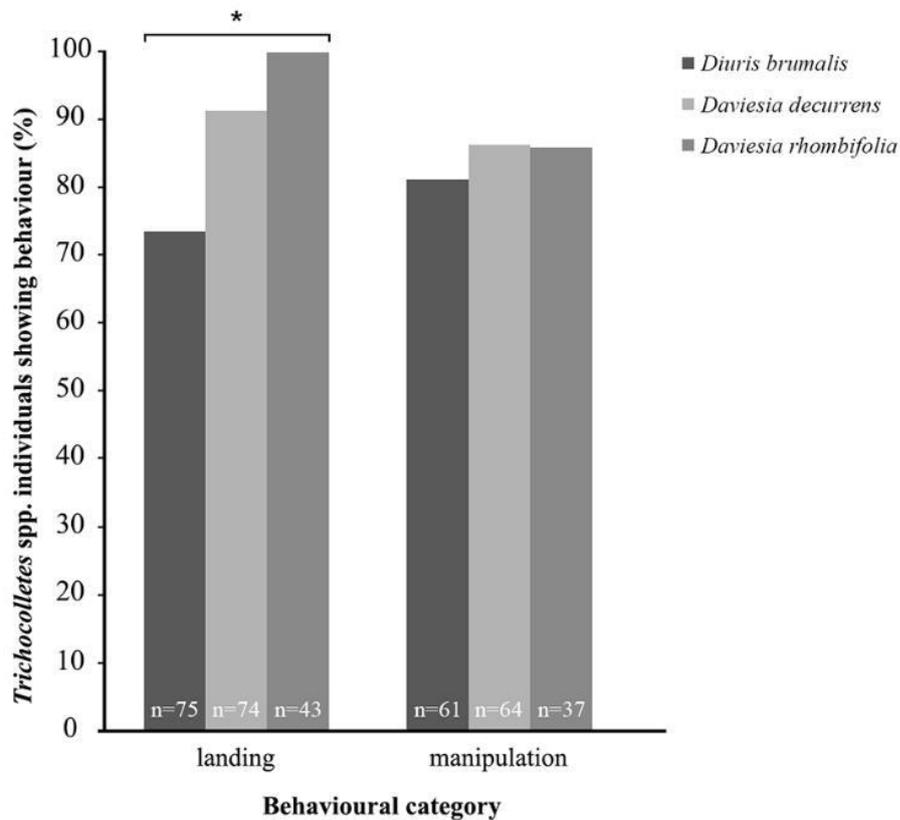
#### *Pollinators of co-occurring pea plants*

Based on observations of contact with the reproductive structures, *Daviesia decurrens* and *D. rhombifolia* (occurring at only the forest sites) were pollinated by both *Trichocolletes capillosus* and *T. leucogenys*, while *D. horrida* (occurring only at the outcrop sites) was pollinated only by *T. leucogenys*. *Apis mellifera* was also observed to pollinate all three species as well as *Hovea pungens* and *Bossiaea aquifolium* [Supplementary data, Table S4]. No *Trichocolletes* species was observed visiting other pea plants or other plant species in the community.

#### *Quantification of pollinator behaviour*

Of the 102 *Trichocolletes* spp. visiting *D. brumalis*, 74.6% alighted on the flower. In each case the insect aligned along the labellum with its head facing the column. Those individuals that flew around the flowers without landing (25.6%) were mostly ‘patrolling’ and could be visually distinguished as males by longer antennas and smaller body size, suggesting mate-searching behaviour (Barrows, 1976). Occasionally, males behaving in this fashion were observed mating with females that had been located while foraging on *Daviesia* spp. Both male and female of *Trichocolletes* spp. landed on the flowers of *D. brumalis* for 1–2 seconds. However, we weren’t able to record the behaviour of *Trichocolletes* that landed for less than one second due to the rapidity of visits. Of the bees alighting on the flower, 81.3% attempted to manipulate the labellum by articulations of the anterior legs and/or pushing of the abdomen onto the labellum, as seen when foraging on nectar and pollen from *Daviesia* spp (Fig. 4). On 50.8% of occasions, attempting to manipulate the labellum resulted in pollinaria removal, with 29.5 % attributable to females and 21.3 % males. After the visit, 19.3 % of insects extracting orchid pollinaria visited other orchid flowers within the clump, with the remaining 80.7% of bees going on to forage on *Daviesia* spp. flowers.

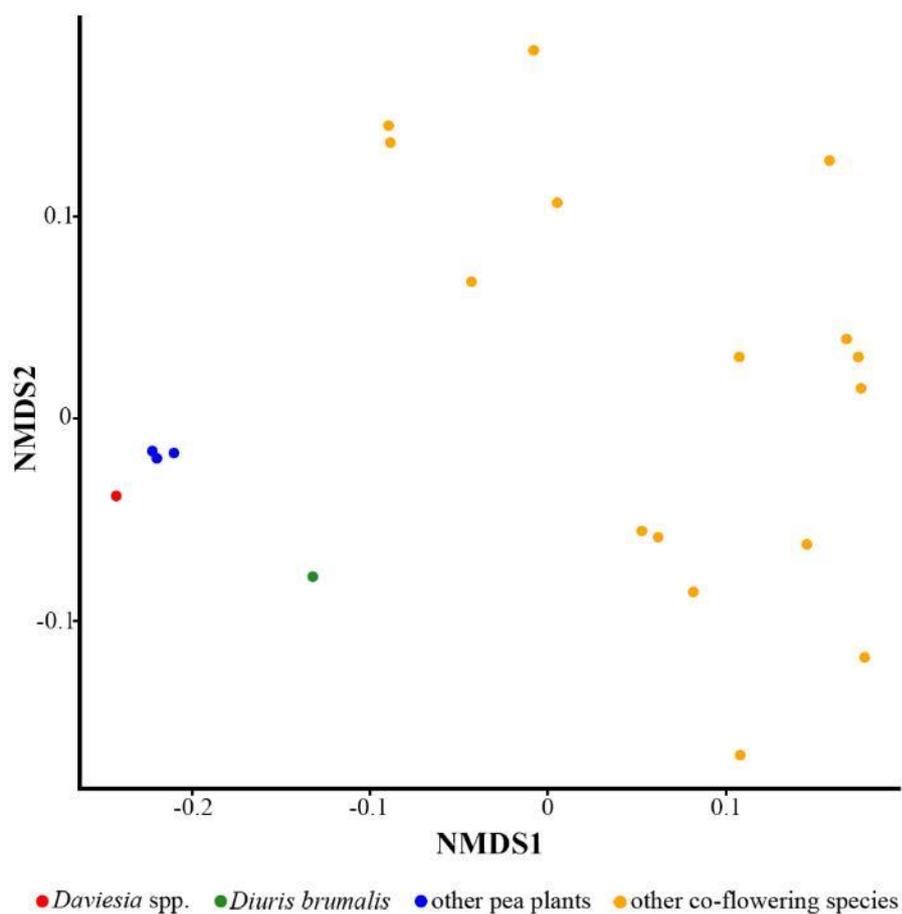
The behaviour exhibited by *Trichocolletes* spp. on *D. brumalis* is characterized by similar behaviour as seen when foraging on the flowers of *Daviesia* spp. in the forest habitat (*D. decurrens* and *D. rhombifolia*). However, significantly more visitors landed on the *Daviesia* spp. than on *D. brumalis* (*D. brumalis* 74.2%,  $n = 102$  vs *Daviesia rhombifolia* 100%,  $n = 43$ ,  $p = 0.009$ ; *D. brumalis* vs *D. decurrens* 91.3%,  $n = 74$ ,  $p = 0.004$ ). Alternatively, among the bees that landed, there was no difference in how frequently the bees attempted to forage on the flower (*D. brumalis* 81.3%,  $n = 75$  vs *D. rhombifolia* 86%,  $n = 37$ ,  $p = 0.513$ ; *D. brumalis* vs *Daviesia decurrens* 86.4%,  $n = 64$ ;  $p = 0.394$ ).



**Fig. 4** Comparison of the foraging behavior of *Trichocolletes* spp. (*Trichocolletes capillosus*, *T. leucogenys*) on *Diuris brumalis* and *Daviesia* spp. (*Daviesia decurrens*, *D. rhombifolia*, *D. horrida*). Bars represent the proportion of individuals engaging in: 1 **Landing**: flying directly to the flower and alighting on keel or labellum; 2 **Manipulation**: attempted to manipulate the flower as part of foraging behaviour for either nectar or pollen. \*: indicates a significant difference

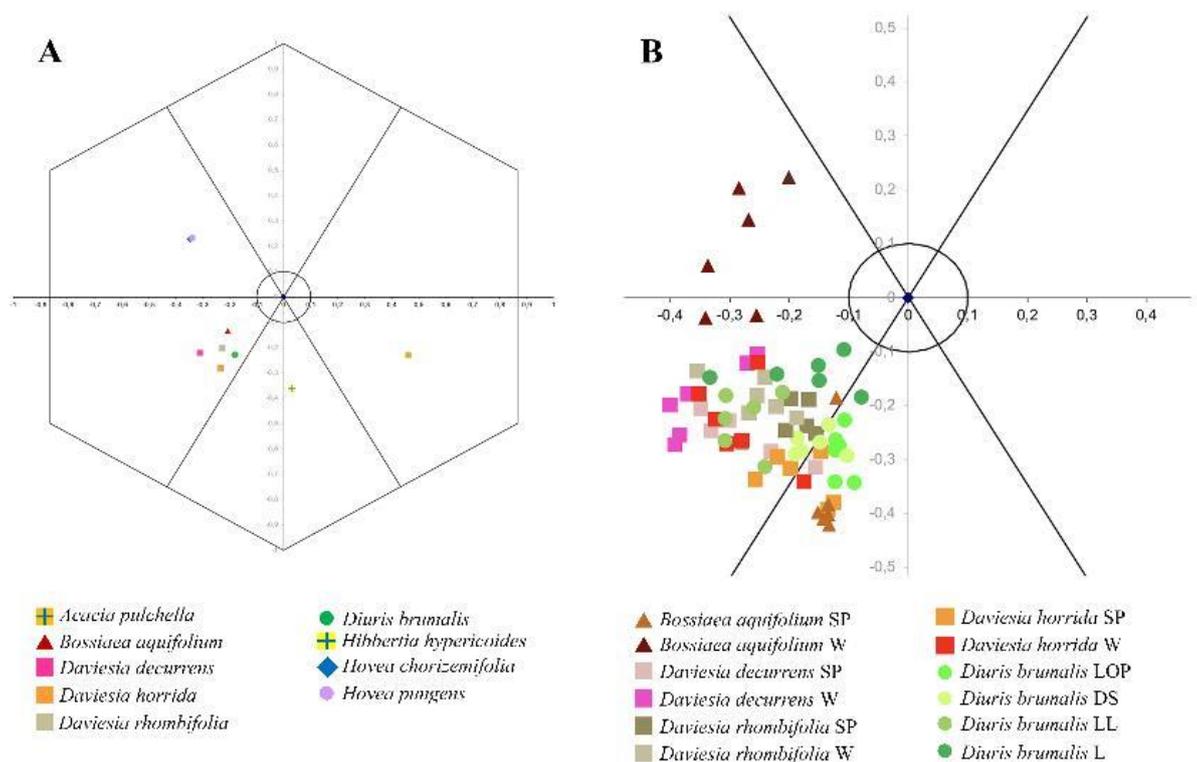
### *Floral similarity of mimics and models*

A NMDS plot shows that all pea plants are morphologically similar, and formed a tight cluster, with a pronounced similarity of *Daviesia* spp. that overlap in the plot (Fig. 5). *Diuris brumalis* is more similar to pea plants than the remainder of the co-flowering plant community, but does not overlap with the morphology of peas in the NMDS plot (Fig. 5). Investigation of the species by trait matrix reveals that *D. brumalis* matches pea plants for all morphological traits except for height of plant and flower size. In the case of flower size, *D. brumalis* is much larger because of the prominently projecting lateral sepals (Fig. 5).



**Fig. 5** Non-metric multi-dimensional scaling plot of floral traits for *Diuris brumalis* and co-flowering species in the forest habitat. *Diuris brumalis* and co-occurring pea plants (Fabaceae) form a distinct site cluster reflecting strong morphological similarity compared to the remainder of the plant community.

Analysis of spectral reflectance using the hexagon bee vision model (Chittka, 1992; Chittka and Kevan, 2005) showed that the average colour loci of *D. brumalis*, all three *Daviesia* spp. and *Bossiaea aquifolium*, corresponded to the UV-region. The colour loci for *Hovea* spp. fell in the UV-blue region (Fig. 6, A), and the colour loci *Acacia pulchella* and *Hibbertia hypericoides* were located in the UV-green and green region respectively. The mean distance of the colour loci measured on flower parts between *D. brumalis* and *Daviesia decurrens*, *D. rhombifolia*, *D. horrida* and *Bossiaea aquifolium* is 0.12, 0.05, 0.06 and 0.1 respectively [Supplementary data, Table S9]. Colour loci for individual plants of *Daviesia* spp., distributed in the coordinates range  $y: [-0.39; -0.10]$   $x: [-0.12; -0.40]$  overlap the visual space of *D. brumalis* individuals ranging across the positions  $y: [-0.34; -0.09]$   $x: [-0.33; -0.08]$  (Fig. 6, B). In *Bossiaea aquifolium* the overlap of colour space of individual colour loci with *D. brumalis* is limited to the dorsal petals, as the keel is in the UV-blue region (Fig. 6, B).



**Fig. 6** (A) Mean values of colour loci calculated between floral parts for the species *Daviesia decurrens*, *D. horrida*, *D. rhombifolia*, *Hovea chorizemifolia*, *H. pungens* and *Bossiaea aquifolium*. In addition, colour loci are presented for two commonly occurring yellow-flowered species present at all sites, *Hibbertia hypericoides* (Dilleniaceae) and *Acacia pulchella* (Fabaceae), to test model similarity based on floral colour.

(B) Distribution of color loci most similar to the *D. brumalis* color signal. Measurements of spectral reflectance were taken for *D. brumalis*: LOP = lateral outer petal; DS = dorsal sepal; LL = labellum lateral lobe L = labellum; for pea plant species (Fabaceae): SP = standard petal; W = wing petals. The calculations were made using the Hexagon colour model of bee vision (Chittka, 1992).

#### *Quantification of the pollen load of floral visitors*

Pollen counts [Supplementary data, Table S8] showed that the pollen assemblage carried by four *Trichocolletes* specimens consisted of almost 100% *Daviesia* pollen with traces (<10 pollen grains in the scanned slide) of Myrtaceae spp. and *Grevillea*. One specimen of *T. leucogenys* from the outcrop habitat [Supplementary data, Table S8, no. 2 ], which was caught on *Daviesia horrida*, contained 97.5% *Daviesia* pollen and traces of pollen of Myrtaceae (2%) and *Hovea* (0.5%). The amount of *Daviesia* pollen in samples from *Apis mellifera* specimens caught foraging on *Daviesia* plants was variable (80-98%), and also they contained pollen of *Banksia*, *Acacia* and Myrtaceae (1-20%). On the *Apis mellifera* specimen caught foraging on *Bossiaea aquifolium* [Supplementary data, Table S8, no. 10], *Bossiaea* pollen comprised 97.5% of the assemblage with 1.5% *Banksia* pollen, 1% Myrtaceae pollen and traces of *Acacia* pollen.

#### *Flowering phenology of target species*

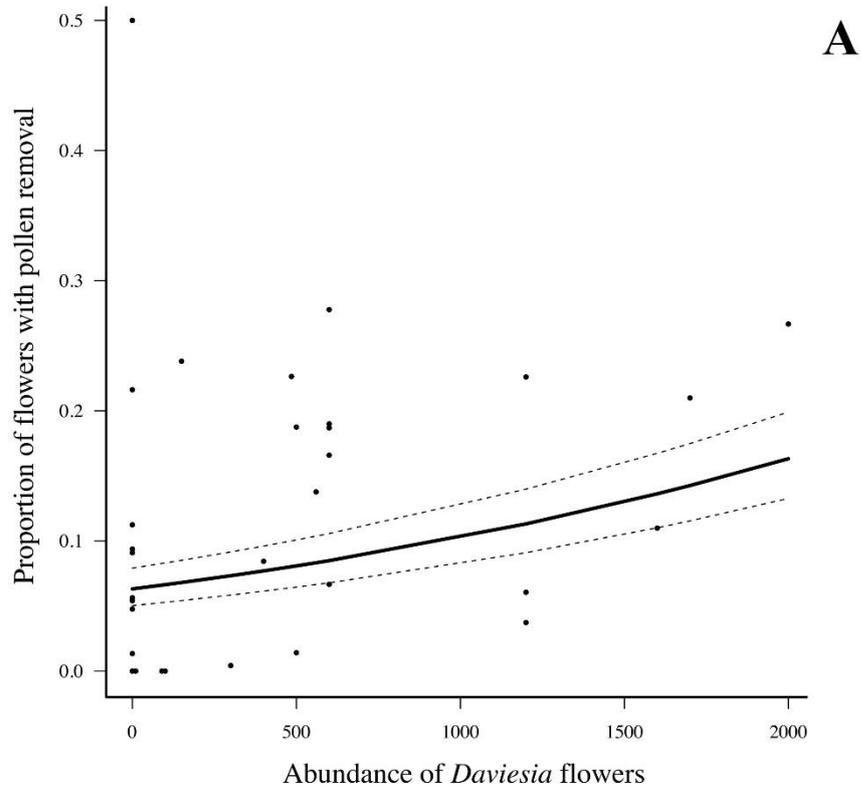
There was pronounced variation in the overlap of the flowering periods of *D. brumalis* and the co-occurring pea plants [Supplementary data, Fig. S1]. Among the species that are frequently visited by *Trichocolletes* spp., flowering of *Daviesia decurrens* and *D. rhombifolia* peaked two weeks and 5 weeks respectively after the peak of *D. brumalis*. Flowering of *H. chorizemifolia* peaks two weeks before *D. brumalis*, while the peak of *H. pungens* corresponded to the peak of *D. brumalis*. Peak flowering for *Bossiaea aquifolium* occurred near the end of the *D. brumalis* flowering period.

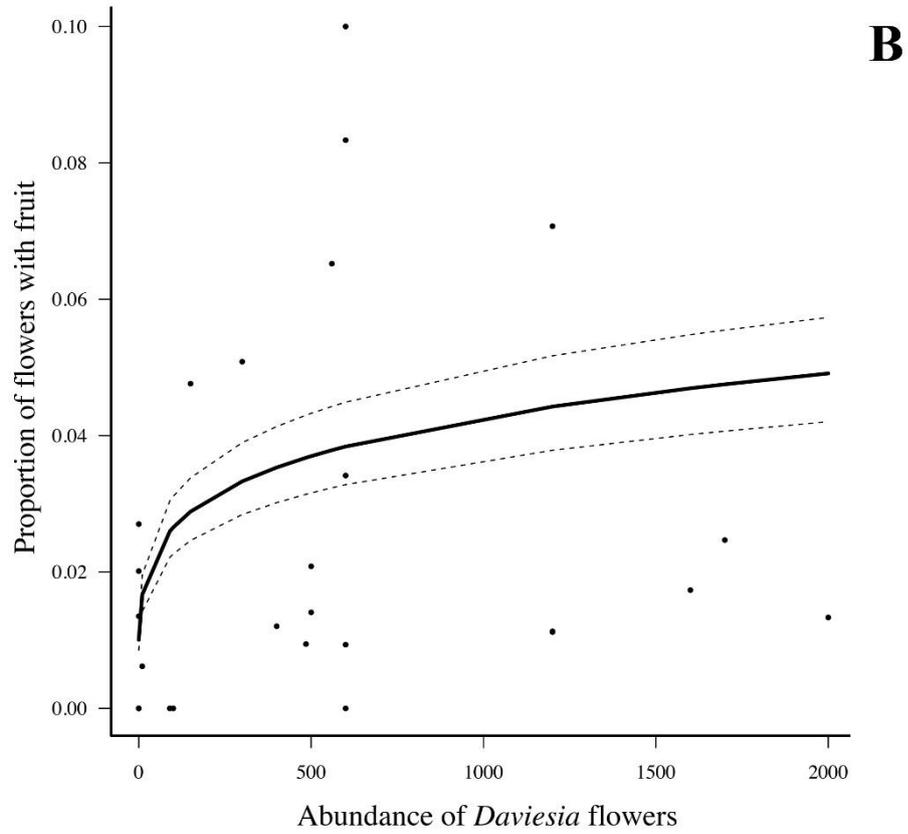
#### *Reproductive success of the mimic in relation to the abundance of the model*

Pollinaria removed did not show any significant difference between sites where *Daviesia* spp. were present (marginal mean of  $0.119 \pm <0.001$  S.E) or absent ( $0.1 \pm <0.001$ ,  $p =$

0.592). However, fruit set was higher in the presence of *Daviesia* spp. ( $0.027 \pm 0.003$ ) than in their absence ( $0.008 \pm 0.001$ ,  $p = 0.049$ ).

The proportion of pollinaria removed exhibited a positive relationship ( $m = 0.2982 \pm 0.1237$ ,  $p = 0.016$ ) with the abundance of *Daviesia* flowers (Fig. 7, A). Year also had a significant effect on the proportion of pollinaria removed from *D. brumalis* flowers (2017 =  $0.146 \pm 0.013$ ; 2016 =  $0.069 \pm 0.006$ ,  $p = 0.019$ ). The proportion of pollinaria removed was marginally higher in the jarrah forest ( $0.123 \pm 0.011$ ) compared to the outcrop habitat ( $0.057 \pm 0.015$ ,  $p = 0.068$ ), though the difference was non-significant. Fruit set showed a positive relation with the number of *Daviesia* flowers (log-transformed  $m = 0.21398 \pm 0.08328$ ,  $p = 0.01$ ) (Fig. 7, B). Fruit set was significantly different between years (2016 =  $0.031 \pm 0.006$ ; 2017  $0.01 \pm 0.002$ ,  $p < 0.001$ ), but did not differ between the forest ( $0.021 \pm 0.004$ ) and outcrop habitats ( $0.017 \pm 0.006$ ,  $p = 0.692$ ). The relationship between the number of *Daviesia* flowers and both pollen removal and fruit set was influenced by several sites where *Daviesia* spp. did not occur, and there was very little or no reproductive success in *D. brumalis*.





**Fig. 7** The proportion of flowers with pollen removal (A) and fruit set (B) of *Diuris brumalis* as a function of the number of flowers of *Daviesia* spp.

## DISCUSSION

### *Pollinator sharing between models and mimic*

One of the most fundamental criteria to assess the occurrence of floral mimicry is to establish whether the proposed model and mimic species share the same pollinators (Roy and Widmer, 1999; Johnson and Schiestl, 2016). Data from this study indicates that *Diuris brumalis* shares the same pollinators (the bees *Trichocolletes capillosus* and *T. leucogenys*) with *Daviesia decurrens* and *D. rhombifolia* in jarrah forest, and *D. horrida* in heathland with granite outcrops. Additionally, observations of pollinator foraging, and analysis of pollen collected from the bodies of pollinators, revealed that in the study areas both *T. capillosus* and *T. leucogenys* feed primarily on *Daviesia decurrens*, *D. rhombifolia* and *D. horrida* [Supplementary data, Table S8]. *Trichocolletes capillosus* individuals were observed and caught only in 2016 in forest sites, while *T. leucogenys* were observed and caught in both 2016 and 2017 in forest and outcrop sites. Previous observations suggest that depending on seasonal conditions, the numbers of *Trichocolletes* that emerge at particular sites can vary from year to year, and in some years none may emerge (T Houston, Western Australian Museum, AUS, ‘pers. comm.’) *Apis mellifera* and *Trichocolletes dives* are potential pollinators as they have been observed to extract orchid pollinaria, but they were not observed to deposit pollen on *D. brumalis*. However, given their ability to remove and carry pollen, they may be responsible for occasional pollination events.

### *Behavioural evidence for mimicry*

Being deceived into exhibiting specific behaviours typically associated with the model species provides strong evidence that the proposed mimic has deceived the operator. In the present study, *Trichocolletes capillosus* and *T. leucogenys* exhibit very similar foraging behavior on *D. brumalis* and *Daviesia* spp. (Fig. 4) [Supplementary data, Video], suggesting that the orchid is sufficiently similar to the model to deceive pollinators. For all three *Daviesia* species observed, *Trichocolletes* spp. show abdomen bending around the keel when attempting to collect nectar/pollen on *Daviesia*. Further, female bees part the keel using their hind legs to collect pollen from the filaments. Our observations of *T. capillosus* and *T. leucogenys* individuals on *D. brumalis* flowers suggested that they attempt to use the same stereotyped foraging behavior. Both species landed along the midline of the labellum and push their abdomen upon it,

unsuccessfully attempting to open it using their anterior legs in a similar fashion to the pollen collecting behavior they exhibit on *Daviesia* [Supplementary data, Video]. Crucially, *Trichocolletes* have only been recorded exhibiting this keel-parting behavior on pea plants, meaning that this behavior indicates mimicry of Faboideae, not other plant groups. Interestingly, other insects observed visiting *Daviesia* (particularly *Apis mellifera* and *Leioproctus* spp.) that have broader foraging preferences beyond the Faboideae were seen to land on the flowers and probe the keel with the body oriented in different directions, not necessarily along the keel.

When visiting *D. brumalis* flowers, some male *T. capillosus* and *T. leucogenys* individuals appeared to exhibit patrolling behavior, where the male searches for females in specific landmarks or rendezvous places that can be resourced-based (Haas, 1960; Barrows, 1976; Paxton, 2005). In *Trichocolletes* landmarks are represented by flowering *Daviesia* bushes, where males often approach closely without landing, likely searching for females engaged in foraging behaviour. Males exhibiting this same apparent patrolling behaviour were observed occasionally to mate with females foraging on *Daviesia* plants. Exhibiting this ‘patrolling’ behavior provides evidence that male *Trichocolletes* confuse *D. brumalis* with *Daviesia* food sources, even though courtship or patrolling behavior is not directly involved in pollination.

#### *Physical similarity between the mimic and the models*

A multi-variate analysis of floral morphological traits indicated that *D. brumalis* is more similar to species of Faboideae than any other co-flowering species in the studied communities (Fig. 5). While *D. brumalis* did not overlap with the Fabaceae in the NMDS plot, all of the characters scored were matching between pea plants and *D. brumalis* except for plant height and overall flower size. Among the pea plants, the spectral reflectance of *D. brumalis* was most similar to that of the *Daviesia* species on which *Trichocolletes* feed (Fig. 6, A, B). The similarity of colour loci between *D. brumalis* and *Daviesia* appeared particularly pronounced between the standard (model) and lateral outer petal /dorsal petal (mimic), and between the wing (model) and labellum lateral lobe (mimic), suggesting a level of colour matching between morphologically corresponding floral parts (Fig. 2, G; Fig 6, B). Between *Daviesia* species and *D. brumalis* the distances between mean colour loci (averaged across floral parts) ranged between 0.05 and 0.10. In some individuals, the coloration of *Daviesia rhombifolia*, *D. decurrens* and *D. horrida* overlapped in colour

space with *D. brumalis*, and was less than the 0.06 units whereby bumble bees and honey bees cannot distinguish colours (Dyer and Chittka 2004 a, b; Giurfa, 2004). However, due to only partial overlap of colour loci of individual plants in *Daviesia* and *D. brumalis*, colours of model species and mimic are likely to often be distinguishable by pollinators. Further, it is likely that precise colour patterns differ between *D. brumalis* and the *Daviesia*. Nonetheless, mimics do not have to be identical, as long as they are perceived as similar by the pollinator (Dalziell and Welbergen, 2016). Indeed, *Diuris* may benefit from an unspecific mimicry of a range of pea plants, rather than appearing identical to a single species, as this may enable them to function effectively with multiple model species.

While the labellum, dorsal sepal/outer petals and labellum lateral lobe of *D. brumalis* appear respectively to replicate the keel, dorsal and wing petals of *Daviesia*, the prominently projecting external petals and the curved sepals in *D. brumalis* appear a component of floral architecture that is absent in *Daviesia* spp. (Fig 2, G). However, it may be possible that some floral parts are involved in the mimicry of pea plants while others are not essential for mimicry and are free to vary. For example, in some genera of sexual deceptive orchids, where the role of floral traits in pollinator attraction is well studied, mimicry of the sex pheromone of the pollinator is often precise (Peakall *et al.*, 2010; Bohman *et al.*, 2018), while colour is not a close match to the female (Gaskett *et al.*, 2016). Similarly, parts of the flower involved in positioning of the pollinator may be under stronger selection than morphologically inactive parts (Rakosy *et al.*, 2017; de Jager & Peakall 2018). In *Diuris* flowers, selection may operate through a dual mechanism, where floral traits involved in mimicry, such as colour and shape of the labellum and column wings, have evolved to resemble pea plants whereas the projecting outer petals may have evolved exaggerated size to increase long distance attraction of pollinators. Indeed, there is a large body of supporting evidence suggesting that a greater floral display increases pollinator visitation rates (e.g. Peter and de Jong, 1990; Karron *et al.*, 2004).

#### *Overlap between mimics and models in flowering phenology*

An overlap in flowering phenology between mimic and model is another key requirement of floral mimicry (Roy and Widmer, 1999; Johnson and Schiestl, 2016). Here, we have shown that the flowering periods of *D. brumalis* and *Daviesia* spp. overlap, but that the

flowering peak of *D. brumalis* precedes the peak of the model species (two weeks before *D. decurrens* and five weeks before *D. rhombifolia*) [Supplementary data, Fig. S1]. In *Trichocolletes*, males often emerge several days prior to females (observations by T Houston, Western Australian Museum, AUS, unpubl. res.), meaning that *Diuris* may take advantage of early emerging males that are searching for females and nectar on pea plants. This interpretation was supported in the present study, where most observations at the start of the flowering period were of males, but the number of females increased as the *Daviesia* came into flower. The exploitation of naïve pollinators appears to be a common characteristic of food-deception systems. Species that use generalized food deception often flower when naïve pollinators emerge (Pellisier *et al.*, 2010) and are yet to learn that the orchid flowers are rewardless (Internicola and Harder, 2012). Alternatively, pollinators can exhibit an innate sensory bias to certain colours and shapes and, following emergence, automatically searching for food sources with these traits (Çakmak and Wells, 1995; Lanau and Maier, 1995). In the case of *D. brumalis*, pollinators may attempt to forage on the mimetic *Diuris* through either naivety or an innate preference for pea like flowers, even though flowering individuals of the model pea plants may be scarce at the time of emergence.

*Does fitness of the mimic increase in the presence of the model?*

Adaptive resemblance between mimic and model species is achieved when pollinators are not able to distinguish between them, and this ‘misclassification’ behaviour enhances the fitness of the mimic (Endler, 1981; Skelhorn and Ruxton, 2010). As such, it is expected that in mimicry systems the fitness of the mimic should be greater when the model is more abundant (Anderson & Johnson, 2006). However, in practice it is difficult to separate the effects on fitness of reduced pollinator learning in the presence of the more model flowers, and greater pollinator abundance in the presence of more model flowers. This challenge applies to *D. brumalis*, as the *Trichocolletes* species foraged primarily on the model flowers, making pollinator abundance likely to be highly correlated with abundance of the model. For example, fruit set was lowest at sites where *Trichocolletes* were not observed and *Daviesia* were almost absent. Further, fruit set increased with the number of *Daviesia* flowers, with this relationship likely to be influenced by sites where there were few or no *Daviesia*, and thus very low reproductive success of *D. brumalis*. As expected under pollinator learning, rates of pollinaria removal increased when there were greater numbers of *Daviesia* flowers, though this could also potentially be attributable to greater numbers of

pollinator at these sites. To resolve this issue, it would be of interest to compare the response of *Trichocolletes* to experimentally presented *Diuris* flowers in areas with and without natural populations of *Diuris*.

Interestingly, even at sites where *Trichocolletes* were not observed and *Daviesia* were largely absent, occasional cases of pollen removal and deposition occurred. These events may be partly attributable to the introduced honey bee *Apis mellifera*, which was frequently observed foraging on co-occurring flowering plants in both habitats, including sites where *Trichocolletes* was not observed. However, forest sites without *Daviesia* exhibited a level of fruit set approaching zero, despite some level of pollen removal, suggesting that honeybees may fail to complete pollination through pollen deposition. At present, there is very little information on the potential negative or positive effects of *A. mellifera* on pollination of Australian orchids (e.g. Adams and Lawson, 1993; Phillips *et al.*, 2009), though given the occasional visitation witnessed here, *Diuris* may represent an interesting study genus to tackle this issue.

#### *Is there evidence for guild mimicry in Diuris brumalis?*

While pollination via mimicry of flowering plants usually involves a particular model species, there is evidence that some plants mimic a guild of plant species rather than a specific model (Jersáková *et al.*, 2016). Plant guilds are recognised by both sharing a particular pollinator (or group of related pollinators) and having very similar floral traits (Manning and Goldblatt, 1996), which are likely to represent adaptations to the particular pollinator(s) (Johnson, 2010). Based on some sharing of pollinators and their striking resemblance, *Diuris* have been hypothesised to mimic a guild of pea plants (Beardsell *et al.*, 1986; Dafni and Bernhardt, 1990; Indsto *et al.*, 2006). The present study shows that while *Daviesia* spp. share pollinators and may form the basis of a guild, this does not extend to all pea plant species in the community. However, based on behavioural observations and floral traits, we provide evidence that mimicry functions with different *Daviesia* species in different habitats (*D. decurrens*, *D. rhombifolia* in jarrah forest; *D. horrida* in the outcrop heath). As such, through the use of more than one model species the *Diuris-Daviesia* mimicry system may meet some of the conditions for guild mimicry.

While the guild mimicry hypothesis has received support from observational studies in orchids (e.g. Jersakova *et al.*, 2016 and the present study), at present experimental tests are lacking. A complimentary approach to conducting field observations in different habitats would be to move experimental arrays of orchids between pea plant communities, thereby testing if any given population of *D. brumalis* can attract pollinators in the presence of other pea plant species. In addition, it would be of interest to investigate the breadth of phenotypes that can achieve mimicry through the use of models or manipulated *Diuris* flowers. Alternatively, experiments with bees conditioned on different species of pea plant could be used understand the full range of models that *D. brumalis* can mimic. However, the outcomes of such experiments would also be partly affected by whether the bees learn to associate rewards with particular pea plants, or if the attraction is innate. If the attraction is innate it is possible that *D. brumalis* may be attractive to pollinators regardless of prior experience with food plants.

### *Conclusions*

Here we present evidence that *D. brumalis* achieves pollination by mimicking the flowers of multiple co-flowering species of *Daviesia*. In addition to meeting the criteria for sharing pollinators and flowering times, pollinators exhibited pea plant-specific foraging behaviour on the *Diuris*, providing strong evidence that the mimic had successfully deceived the pollinator. This evidence was further supported by data on morphology and colour, showing that not only are *Diuris* and *Daviesia* spp. very similar compared to the remainder of the co-flowering community, but that based on bee vision models, in many cases the colour of *Diuris* and the proposed model species will not be readily distinguishable to pollinators. Fruit set and pollen removal of *D. brumalis* was more frequent in the presence of *Daviesia*, though evidence suggests that this is likely through some combination of both learning, and greater pollinator abundance at sites where the model is present. The diversity of species related to *D. brumalis* with pea-like floral traits (*Diuris corymbosa* complex) suggests that this may be an effective system for understanding diversification in lineages that use floral Batesian mimicry.

## SUPPLEMENTARY DATA

Supplementary data are available online at [www.aob.oxfordjournals.org](http://www.aob.oxfordjournals.org) and consist of the following. Fig. S1: phenology of *Diuris brumalis* and co-occurring Faboideae, Table S1: habitat assigned with description; Table S2: plant species vouchered at the WA Herbarium; Table S3: observations of floral visitors to *Diuris brumalis*; Table S4: observations of floral visitors to Faboideae; Table S5: insects caught on *Diuris brumalis* and co-occurring Faboideae; Table S6: Floral traits of *Diuris brumalis* and the 20 most abundant co-flowering species; Table S7 A,B: Populations and reproductive data of *Diuris brumalis*; Table S8: composition of pollen loads; Table S9: Means and standard deviation of colour loci of *D. brumalis* and pea plants. Appendix S1: floral biology of *Diuris brumalis* and co-occurring Faboideae; Video on line <https://doi-org.dbgw.lis.curtin.edu.au/10.1093/aob/mcy166> in Supplementary data: showing *Trichocolletes* behaviour on *Daviesia decurrens* (model) and *Diuris brumalis* (mimic). Key behaviours illustrated: ‘patrolling’, courtship behaviour by males looking for females, keel (model) or labellum (mimic) ‘manipulation’, ‘foraging’ behaviour by females, including searching for sources without landing. All video is presented in slow motion.

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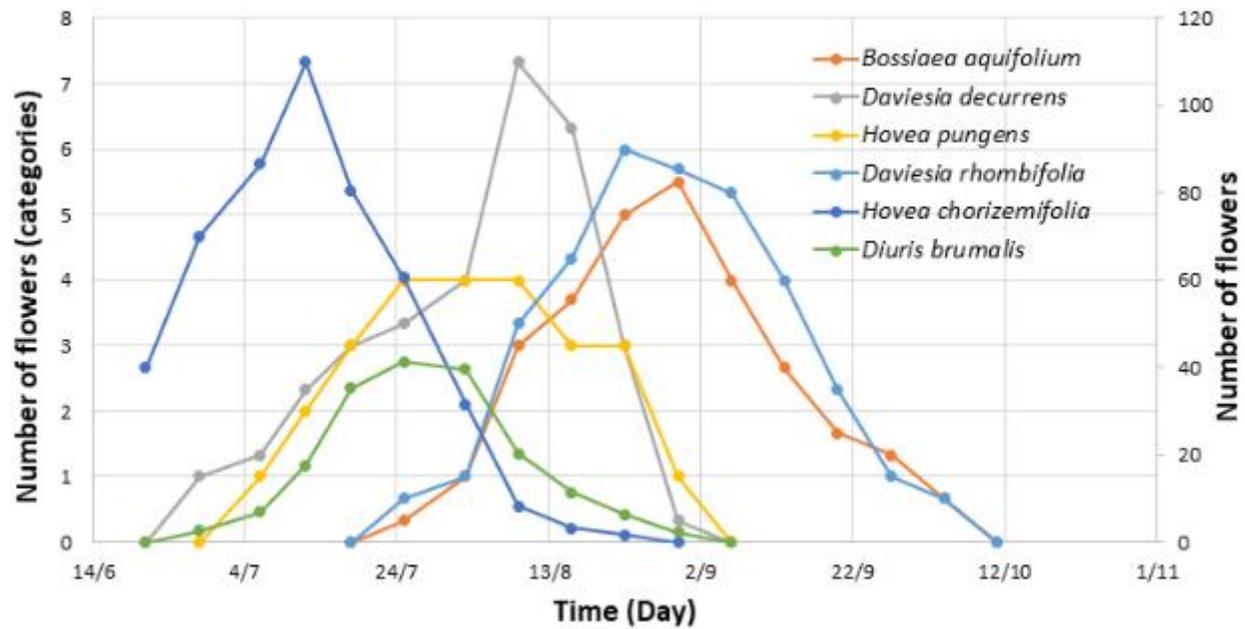
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**SUPPLEMENTARY DATA, Fig. S1.** Flowering phenology of *Diuris brumalis* and co-occurring Faboideae species at three sites in the forest habitat. Phenology data was collected in a single 30 x 30 metre quadrat per site. Due to the high number of flowers for *Daviesia decurrens*, *D. horrida*, *D. rhombifolia*, *Hovea pungens* and *Bossiaea aquifolium*, we estimated the total number of flowers and assigned categories (primary y axis): (1) 1-100, (2) 101-200, (3) 301-400, (4) 401-500... to 2000 for a total of 19 categories. For *H. chorizemifolia* and *D. brumalis* the number of flowers per quadrat (secondary y axis) was directly scored.



## **SUPPLEMENTARY DATA, Appendix S1.** Observations of the floral biology of *Diuris brumalis* and co-occurring Faboideae

### **Background**

While *Diuris brumalis* produces no visible nectar, and the co-occurring pea plants (Faboideae) are all assumed to provide a nectar reward, this has not been tested. Further, it has not been tested if *D. brumalis* produces fruit by autogamy and if it is self-compatible. All of these issues needed to be addressed prior to undertaking a study of floral mimicry and its consequences for plant fitness.

### **Methods**

#### *Nectar production*

To test for nectar production in *D. brumalis*, and quantify nectar production in the proposed models (*D. decurrens*, *D. horrida*, *D. rhombifolia*, *H. chorizemifolia*, *H. pungens* and *B. aquifolium*), nectar content in flowers of each species was determined in July-August 2016. One inflorescence per individual was bagged for 10 randomly selected individuals of each species at three sites (F1, F2, O3). Inflorescences were bagged in the afternoon, with nectar collected the following day during the warmest hours (from 11.00 to 14.00) to ensure maximum nectar production (Corbet *et al.*, 1995). Nectar was collected from two flowers on each inflorescence using a 2  $\mu$ l microcapillary tube (Drummond Microcaps, Broomall; Pa., USA), with nectar volume calculated by measuring capillary length of the column of liquid (Corbet, 2003) using a digital calliper.

#### *Testing for autogamy and self-compatibility in Diuris brumalis*

To test for autogamy, in July 2017, inflorescences with newly-opened flowers and no observed pollinia deposited on stigma were covered with a fine, insect proof nylon bag until floral senescence (ca. four weeks). To test for self-compatibility, one flower on each inflorescence was manually pollinated with pollinia from a different flower on the same inflorescence before the pollinated flower was covered with a fine, insect proof nylon bag until senescence or fruit formation (ca. four weeks). Six individuals

were randomly selected at each of the three largest populations (F4, F5 and F6) for each test, with one inflorescence selected per individual for a total of eighteen inflorescences tested.

## Results

### *Nectar content*

Measurements of nectar content in species of pea plants revealed that the following average amount of nectar was produced by flower: *Daviesia decurrens* ( $0.15 \mu\text{l} \pm 0.05$  s.d), *D. rhombifolia* ( $0.1 \mu\text{l} \pm 0.04$  s.d.), *D. horrida* ( $0.1 \mu\text{l} \pm 0.05$  s.d.), *Hovea chorizemifolia* ( $0.14 \mu\text{l} \pm 0.06$  s.d.), *H. pungens* ( $0.21 \mu\text{l} \pm 0.15$  s.d.) and *Bossiaea aquifolium* ( $0.14 \mu\text{l} \pm 0.09$  s.d.). No nectar was produced by any part of studied *Diuris* flowers.

### *Testing for autogamy and self-compatibility in Diuris brumalis*

None of the bagged flowers produced fruits, demonstrating that *D. brumalis* requires a vector to achieve pollination. Experimental hand pollination revealed that *D. brumalis* is able to produce seed capsules through self-pollination, with 88% (n=18) of flowers forming a capsule.

## Conclusions

As expected, *D. brumalis* is nectarless, while all of the pea plant species produced small amounts of nectar on the upper surface at the base of the standard. *Diuris brumalis* was shown to require a vector for pollination, and to be self-compatible.

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**SUPPLEMENTARY DATA, Table S1.** General habitat assigned with detailed description. All the sites correspond with populations of *Diuris brumalis*, surveyed in both 2016 and 2017, except for the ones noted\*, which were surveyed only in 2017. Each habitat is characterized by a variable range of pea plants (Fabaceae).

Habitat	Vegetation description	Sites surveyed	Co-occurring faboideae
Forest	Jarrah forest on lateritic soils, with an overstorey of <i>Eucalyptus marginata</i> and <i>Corymbia calophylla</i> over a diverse understorey dominated by <i>Acacia pulchella</i> , <i>Bossiaea aquifolium</i> , <i>Daviesia</i> spp., <i>Hibbertia hypericoides</i> and <i>Hovea</i> spp. <i>Banksia sessilis</i> occurs discontinuously among sites.	F1, F2, F3, F4, F5, F6, F7, F8*, F9*, F10*, F11, F12, F13, F14, F15	<i>Bossiaea aquifolium</i> , <i>Daviesia decurrens</i> , <i>D. rhombifolia</i> , <i>Hovea chorizemifolia</i> , <i>H. pungens</i>
Outcrop	Open shrubland on granite outcropping and lateritic slopes, dominated by <i>Trymalium ledifolium</i> with <i>Grevillea</i> spp., <i>Hakea</i> spp. and <i>Petrophile</i> spp.	O1, O2, O3	<i>Daviesia horrida</i> , <i>Hovea pungens</i>

**SUPPLEMENTARY DATA, Table S2.** List of the plant species per family collected in the field sites in different habitat, subsequently vouchered at the Herbarium of Western Australia, Perth.

Number	Family name	Specimens collected in field and vouchered at Herbarium	Voucher	Habitat	Latitude; Longitude
1	Fabaceae	<i>Hovea chorizemifolia</i>	DS 1	forest	31°29' 01.2" S; 115° 57' 55.0" E
2	Fabaceae	<i>Hovea pungens</i>	DS 7	forest	31°56' 28.6" S; 116° 02' 52.7" E
3	Fabaceae	<i>Daviesia rhombifolia</i>	DS 3	forest	31°29' 01.2" S; 115° 57' 55.0" E
4	Orchidaceae	<i>Diuris brumalis</i>	DS 4	forest	31°29' 01.2" S; 115° 57' 55.0" E
5	Fabaceae	<i>Bossiaea aquifolium</i> subsp. <i>aquifolium</i>	DS 5	forest	31°29' 01.2" S; 115° 57' 55.0" E
6	Fabaceae	<i>Daviesia decurrens</i> subsp. <i>decurrens</i>	DS 6	forest	31°29' 01.2" S; 115° 57' 55.0" E
8	Fabaceae	<i>Daviesia horrida</i>	DS 8	outcrop	31°56' 28.6" S; 116° 02' 52.7" E
9	Orchidaceae	<i>Diuris brumalis</i>	DS 9	outcrop	32°00' 56.1" S; 116° 03' 36.3" E
10	Orchidaceae	<i>Diuris brumalis</i>	DS 10	forest	32°00' 56.1" S; 116° 03' 36.3" E

**SUPPLEMENTARY DATA, Table S3.** Observations of floral visitors to *Diuris brumalis*. Eight behavioural categories were distinguished to reflect the pollination process. For Category (II), the types of behaviour for insects approaching the flower were: zig-zag flight = moving side to side in flight as they approach the flowering plant; direct flight = flying in a straight line as they approach the flower; aligned = body of visitor aligned along the midpoint of the labellum/keel during attempts to forage; patrolling = appearing to inspect multiple flowers around the plant; approach but choose another flower = the bee approaches a flower closely (<5cm) but then chooses to alight on a different flower. In addition, it was recorded if males were observed patrolling for females around the flower. M = male, F = female.

Behaviour categories		Syrphidae	<i>Leioproctus</i> sp.	<i>Apis mellifera</i>	<i>Trichocolletes capillosus</i>	<i>Trichocolletes leucogenys</i>	<i>Trichocolletes dives</i>
(I)	N insects approaching the flower	3	2	25	39F, 41M	13F, 8M	1M
(II)	Behaviour when approaching the flower	zig zag flight	zig zag flight	slow zig zag flight	direct flight and aligned on labellum/ patrolling/searching	direct flight and aligned on labellum/ patrolling/ searching	direct flight and aligned on labellum
(III)	N insects carrying orchid pollen on arrival	0	0	0	7F, 5M	1F	0
(IV)	N insects landing on the flower	0	2	18	29F, 30M	10F, 5M	1M
(V)	Visiting time $\geq$ 1s	0	0	18	28F, 22M	8F, 4M	1M
(VI)	N insects attempting labellum manipulation	0	0	16	28F, 21M	8F, 3M	1M
(VII a)	N insects removing pollen	0	0	4	14F, 10M	4F, 2M	1M
(VII b)	N insect depositing pollen	0	0	0	1 F, 1 M	1 F	0
(VIII)	N insects visiting another orchid flower	0	0	4	2F, 3M	1M	-0

**SUPPLEMENTARY DATA, Table S4.** Observations of floral visitors to pea plants (Faboideae; *Bossiaea aquifolium*, *Daviesia decurrens*, *D. horrida*, *D. rhombifolia*, *Hovea pungens*). Eight behaviour categories and descriptions were distinguished as for *Diuris brumalis*. M = male, F = female.

<b><i>Bossiaea aquifolium</i></b>			
	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Trichocolletes sp.</i>
(I)	N insects approaching the flower	85	1 F
(II)	Behaviour typology approaching the flower	flying slowly zig zag	flying straight on keel
(III)	N insects carrying orchid pollen on arrival	-	-
(IV)	N insects landing on the flower	85	1 F
(V)	Visiting time $\geq$ 1s	85	1 F
(VI)	N insects attempting keel manipulation	68	1 F
(VII)	N insect collecting pollen	67	1 F
(VIII)	N insects visiting to another flower of pea plant	65	1 F

<b><i>Daviesia decurrens</i></b>				
	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Trichocolletes capillosus</i>	<i>Trichocolletes leucogenys</i>
(I)	N insects approaching the flower	24	30 F, 26 M	14 F, 11 M
(II)	Behaviour typology approaching the flower	flying slowly zig zag	flying straight and aligned on keel/ patrolling/ searching	flying straight and aligned on keel/ patrolling/ searching
(III)	N insects carrying orchid pollen on arrival	2	3 F, 1 M	2 F
(IV)	N insects landing on the flower	24	30 F, 22 M	14 F, 8 M
(V)	Visiting time $\geq$ 1s	24	30 F, 20 M	13 F, 4 M
(VI)	N insects attempting keel manipulation	14	30 F, 18 M	13 F, 3 M
(VII)	N insect collecting pollen	10	15 F	8 F
(VIII)	N insects visiting to another flower of pea plant	24	14 F	8 F

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***Daviesia horrida***

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	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Trichocolletes leucogenys</i>
(I)	N insects approaching the flower	81	55 F, 28 M
(II)	Behaviour typology approaching the flower	flying slowly zig zag	flying straight and aligned on keel/ patrolling/ searching
(III)	N insects carrying orchid pollen on arrival	1	2 F
(IV)	N insects landing on the flower	81	55 F, 20 M
(V)	Visiting time $\geq$ 1s	81	50 F, 12 M
(VI)	N insects attempting keel manipulation	58	49 F, 15 M
(VII)	N insect collecting pollen	68	45 F
(VIII)	N insects visiting to another flower of pea plant	81	43 F

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***Daviesia rhombifolia***

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	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Trichocolletes capillosus</i>	<i>Trichocolletes leucogenys</i>
(I)	N insects approaching the flower	20	23 F, 5 M	10 F, 5 M
(II)	Behaviour typology approaching the flower	flying slowly zig zag	flying straight and aligned on keel/ patrolling/ searching	flying straight and aligned on keel/ patrolling/ searching
(III)	N insects carrying orchid pollen on arrival	-	2 F, 1 M	1 F
(IV)	N insects landing on the flower	20	23 F, 5 M	10 F, 5 M
(V)	Visiting time $\geq$ 1s	20	21 F, 2 M	10 F, 4 M
(VI)	N insects attempting keel manipulation	11	21 F, 2 M	10 F, 4 M
(VII)	N insect collecting pollen	17	15 F	10 F
(VIII)	N insects visiting to another flower of pea plant	19	14 F	9 F

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***Hovea pungens***

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<b>Behaviour categories</b>	<b><i>Apis mellifera</i></b>
(I) N insects approaching the flower	88
(II) Behaviour typology approaching the flower	flying slowly zig zag
(III) N insects carrying orchid pollen on arrival	-
(IV) N insects landing on the flower	88
(V) Visiting time $\geq$ 1s	88
(VI) N insects attempting keel manipulation	32
(VII) N insect collecting pollen	-
(VIII) N insects visiting to another flower of pea plant	28

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**SUPPLEMENTARY DATA, Table S5.** List of insects caught on *Diuris brumalis* above and co-occurring pea plants below. All the insects were sexed and identified at the Western Australia Museum, where possible at species level. Sex column: F: female; M: male; W: worker. Pollen column: o: carrying orchid pollinaria; p: pea plant pollen carried on legs and abdomen. \*: Insect carrying orchid pollenia, arriving on orchids for depositing the pollen, coming from natural fashion

Insects caught on <i>Diuris brumalis</i> (using artificial arrays)									
Number	Code	Plant species	Date	Area	Habitat	Genus (subgenus)	Species (subspecies)	Sex	Pollen
1	DB01-16	<i>Diuris brumalis</i>	14/07/2016	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	M	o
2	DB02-16	<i>Diuris brumalis</i>	14/07/2016	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	F	o
3	DB03-16	<i>Diuris brumalis</i>	18/07/2016	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	M	o
4	DB04-16	<i>Diuris brumalis</i>	19/07/2916	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	M	o
5	DB05-16	<i>Diuris brumalis</i>	19/07/2916	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	F	o
6	DB06-16	<i>Diuris brumalis</i>	19/07/2916	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	F	o*
7	DB07-16	<i>Diuris brumalis</i>	20/07/2016	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>capillosus</i>	M	o*
8	DB08-16	<i>Diuris brumalis</i>	26/07/2016	Lesmurdie	Forest	<i>Tricocholletes</i>	<i>dives</i>	M	o

**Insects caught on pea plants with orchid pollinaria or pea plant pollen**

9	DC07-16	<i>Daviesia decurrens</i>	26/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	M	o
10	P09-17	<i>Daviesia decurrens</i>	18/08/2017	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	o
11	P11-17	<i>Daviesia rhombifolia</i>	18/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	o
12	P14-17	<i>Daviesia rhombifolia</i>	25/08/2017	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	o
13	DC01-16	<i>Daviesia decurrens</i>	13/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	M	-
14	DC02-16	<i>Daviesia decurrens</i>	13/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	M	-
15	DC03-16	<i>Daviesia decurrens</i>	20/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	F	-
16	DC04-16	<i>Daviesia decurrens</i>	18/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	M	-
17	DC05-16	<i>Daviesia decurrens</i>	18/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	M	-
18	DC08-16	<i>Daviesia decurrens</i>	26/07/2016	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>capillosus</i>	F	p
19	DH01-16	<i>Daviesia horrida</i>	02/08/2016	Kalamunda	Outcrop	<i>Trichocolletes</i>	<i>leucogenys</i>	M	-
20	DH02-16	<i>Daviesia horrida</i>	02/08/2016	Kalamunda	Outcrop	<i>Apis</i>	<i>mellifera</i>	W	p
21	DH03-16	<i>Daviesia horrida</i>	02/08/2016	Kalamunda	Outcrop	<i>Trichocolletes</i>	<i>leucogenys</i>	F	p
22	HP01-16	<i>Hovea pungens</i>	03/08/2016	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	-
23	BA02-16	<i>Bossiaea acquifolium</i>	11/08/2016	Lesmurdie	Woodland	<i>Apis</i>	<i>mellifera</i>	W	p
24	P01-17	<i>Daviesia decurrens</i>	07/08/2017	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	p
25	P02-17	<i>Daviesia decurrens</i>	08/08/2017	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	p
26	P03-17	<i>Daviesia decurrens</i>	11/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	-
27	P04-17	<i>Daviesia rhombifolia</i>	11/08/2017	Lesmurdie	Forest	<i>Apis</i>	<i>mellifera</i>	W	p
28	P05-17	<i>Daviesia decurrens</i>	11/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	p
29	P06-17	<i>Daviesia rhombifolia</i>	11/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	-
30	P10-17	<i>Daviesia rhombifolia</i>	18/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	-
31	P12-17	<i>Daviesia horrida</i>	23/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	p
32	P13-17	<i>Daviesia rhombifolia</i>	24/08/2017	Lesmurdie	Forest	<i>Trichocolletes</i>	<i>leucogenys</i>	F	p

**SUPPLEMENTARY DATA, Table S6.** Principal floral traits of *Diuris brumalis* and the 20 most abundant co-flowering species in the habitat forest (F1, F2, F3 sites). The floral traits, following Marchant *et al.*, (1987), represent potential traits for attracting pollinators. They are coded in each column as **corolla symmetry:** zygomorphic (z) actinomorphic (a); **corolla shape:** tubular (t); rotate (r); papilionaceous (p); bilabiate (b); ligulate (l); **flower width** (mm); **flower length** (mm); **flower orientation:** pendant (p); upright(u); horizontal (h); **maximum height** (cm); **petal projection as a platform for pollinators:** yes or no; **prominent parts of the flowers** (a) anthers (t) tepals; **anthers exposure:** enclosed (c); exposed (e); **inflorescence:** umbel (u), raceme (r), spike (s), panicle (p), solitary (so).

Number	Family	Species	Corolla Symmetry	Corolla shape	Flower Width (mm)	Flower Length (mm)	Flower orientation	Max Height (cm)	Petal projection as platform for pollinators	Prominent parts of flower	Anthers exposure	Inflorescence
1	Ericaceae	<i>Astroloma foliosum</i>	a	t	1	25	u	100	no	t	c	r
2	Fabaceae	<i>Acacia pulchella</i>	a	r	6.5	6.5	o	200	no	a	e	r
3	Proteaceae	<i>Adenanthos barbigera</i>	z	t	12	25	u	100	no	t	e	r
4	Fabaceae	<i>Bossiaea aquifolium</i>	z	p	18.25	19.2	o	250	yes	t	c	r
5	Colchicaceae	<i>Burchardia umbellata</i>	a	r	25	25	u	44	no	t	e	u
6	Myrtaceae	<i>Calothamnus sanguineus</i>	z	l	6.5	26.5	p	150	no	t	e	r
7	Fabaceae	<i>Daviesia decurrens</i>	z	p	5.4	4.75	o	100	yes	t	c	r
8	Fabaceae	<i>Daviesia rhombifolia</i>	z	p	5.6	4.7	o	80	yes	t	c	r
9	Orchidaceae	<i>Diuris brumalis</i>	z	p	12.95	26	o	50	yes	t	e	r
10	Dilleniaceae	<i>Hibbertia hypericoides</i>	a	r	20	20	o	70	no	t	e	r

Number	Family	Species	Corolla Symmetry	Number	Family	Species	Corolla Symmetry	Number	Family	Species	Corolla Symmetry	Number
11	Proteaceae	<i>Hakea lissocarpa</i>	z	r	3.5	3.5	u	300	no	t	e	u
12	Fabaceae	<i>Hovea chorizemifolia</i>	z	p	12.95	10.85	o	60	yes	t	c	r
13	Fabaceae	<i>Hovea pungens</i>	z	p	13.05	12.25	o	150	yes	t	c	r
14	Myrtaceae	<i>Hypocalymma robustum</i>	a	r	12.5	12.5	o	100	no	t	e	r
15	Proteaceae	<i>Isopogon dubius</i>	a	r	22.5	22.5	u	120	no	t	e	s
16	Goodeniaceae	<i>Lechenaultia biloba</i>	z	b	21	21	u	60	no	t	c	r
17	Asparagaceae	<i>Lomandra nigricans</i>	a	r	2	2	o	70	no	t	e	p
18	Iridaceae	<i>Orthrosanthus laxus</i>	a	r	55	55	u	60	no	t	e	r
19	Iridaceae	<i>Patersonia umbrosa</i>	a	r	60	53.5	u	90	no	t	e	so
20	Rutaceae	<i>Philotheca spicata</i>	a	r	3.75	3.75	o	60	no	t	e	r
21	Asparagaceae	<i>Sowerbaea laxiflora</i>	a	r	11.5	11.5	o	45	no	t	e	u

**SUPPLEMENTARY DATA, Table S7, A** Above list of the 15 populations sites of *Diuris brumalis* surveyed in 2016 with the population code indicating the habitat (F: forest; O: outcrop), as presented in Table 1. All the values are related to plants surveyed within 30 x 30 meter. **B** Below list of the 18 populations sites of *Diuris brumalis* surveyed in 2017. The populations surveyed only in 2017 were F8, F9, F10.

Population number	Pop code	Site	Latitude, longitude	N plants of <i>D. brumalis</i>	N flowers <i>D. brumalis</i>	N flowers <i>D. brumalis</i> without pollinaria	N pollinated flowers of <i>D. brumalis</i>	<i>D. brumalis</i> Male Fitness (proxy)	<i>D. brumalis</i> Female Fitness (proxy)	N total flowers <i>Daviesia</i> spp. plants	N 'other pea plants' flowers
1	F1	Lesmurdie - Canning Rd	32°01'45.6" °S, 116°06'01.3" °E	8	36	10	3	0.28	0.08	300	0
2	F2	Lesmurdie - Canning Rd	32°01'41.9" °S, 116°05'41.7" °E	26	106	24	1	0.23	0.01	485	0
3	F3	Lesmurdie - Canning Rd	32°01'43.8" °S, 116°05'12.1" °E	33	138	19	9	0.14	0.07	484	0
4	F4	Lesmurdie - Canning Rd	32°01'43.2" °S, 116°04'51.9" °E	48	268	10	3	0.04	0.01	1200	0
5	F5	Lesmurdie - Canning Rd	32°01'39.8" °S, 116°04'45.3" °E	24	99	6	7	0.06	0.07	1200	0
6	F6	Lesmurdie - Canning Rd	32°01'34.0" °S, 116°04'34.3" °E	8	42	10	2	0.24	0.05	90	18
7	F7	Lesmurdie - Canning Rd	32°01'39.1" °S, 116°04'42.8" °E	25	120	8	12	0.07	0.10	90	0
8	O1	Kalamunda - Zig Zag Point	31°56'35.3" °S, 116°02'46.5" °E	48	236	1	12	0.00	0.05	300	0
9	O2	Kalamunda - Zig Zag Point	31°56'30.4" °S, 116°02'37.4" °E	17	64	0	0	0.00	0.00	90	0
10	O3	Kalamunda - Zig Zag Point	31°56'28.6" °S, 116°02'54.5" °E	5	21	0	0	0.00	0.00	0	0
11	F8	Lesmurdie - Pomeroy Rd	32°00'33.3" °S, 116°04'47.0" °E	20	74	4	1	0.05	0.01	0	192
12	F9	Lesmurdie - Pomeroy Rd	32°00'48.6" °S, 116°03'15.0" °E	30	149	2	3	0.01	0.02	0	0
13	F10	Lesmurdie - Pomeroy with Prutni Rd	32°00'38.4" °S, 116°03'24.8" °E	40	162	0	1	0.00	0.01	10	801
14	F11	Lesmurdie - Reid Rd	32°00'48.8" °S, 116°03'01.0" °E	16	71	4	0	0.06	0.00	0	325
15	F12	Lesmurdie - Moffet Rd	32°01'03.0" °S, 116°03'26.6" °E	18	73	4	0	0.05	0.00	0	124

Population number	Pop code	Site	Latitude, longitude	N plants of <i>D. brumalis</i>	N flowers <i>D. brumalis</i>	N flowers <i>D. brumalis</i> without pollinaria	N pollinated flowers of <i>D. brumalis</i>	<i>D. brumalis</i> Male Fitness (proxy)	<i>D. brumalis</i> Female Fitness (proxy)	N total flowers <i>Daviesia</i> spp. plants	N 'other pea plants' flowers
1	F1	Lesmurdie - Canning Rd	32°01'45.6" °S, 116°06'01.3" °E	25	81	17	2	0.21	0.02	1700	100
2	F2	Lesmurdie - Canning Rd	32°01'41.9" °S, 116°05'41.7" °E	25	83	7	1	0.08	0.01	400	24
3	F3	Lesmurdie - Canning Rd	32°01'43.8" °S, 116°05'12.1" °E	5	12	0	0	0.00	0.00	100	0
4	F4	Lesmurdie - Canning Rd	32°01'43.2" °S, 116°04'51.9" °E	36	100	19	0	0.19	0.00	600	20
5	F5	Lesmurdie - Canning Rd	32°01'39.8" °S, 116°04'45.3" °E	50	205	34	7	0.17	0.03	600	0
6	F6	Lesmurdie - Canning Rd	32°01'34.0" °S, 116°04'34.3" °E	13	48	9	1	0.19	0.02	500	50
7	F7	Lesmurdie - Canning Rd	32°01'39.1" °S, 116°04'42.8" °E	35	150	40	2	0.27	0.01	2000	7
8	O1	Kalamunda - Zig Zag Point	31°56'35.3" °S, 116°02'46.5" °E	115	519	57	9	0.11	0.02	1600	0
9	O2	Kalamunda - Zig Zag Point	31°56'30.4" °S, 116°02'37.4" °E	30	107	20	1	0.19	0.01	600	0
10	O3	Kalamunda - Zig Zag Point	31°56'28.6" °S, 116°02'54.5" °E	0	0	0	0	0.00	0.00	0	0
11	F11	Lesmurdie - Pomeroy Rd	32°00'33.3" °S, 116°04'47.0" °E	4	11	1	0	0.09	0.00	0	56
12	F12	Lesmurdie - Pomeroy Rd	32°00'48.6" °S, 116°03'15.0" °E	23	74	16	2	0.22	0.03	0	0
13	F13	Lesmurdie - Pomeroy with Prutni Rd	32°00'38.4" °S, 116°03'24.8" °E	19	89	10	0	0.11	0.00	0	300
14	F14	Lesmurdie - Reid Rd	32°00'48.8" °S, 116°03'01.0" °E	1	2	1	0	0.50	0.00	0	20
15	F15	Lesmurdie - Moffet Rd	32°01'03.0" °S, 116°03'26.6" °E	9	32	3	0	0.09	0.00	0	20
16	F8	Lesmurdie - Canning Rd	32°01'39.6" °S, 116°06'11.8" °E	44	177	40	2	0.23	0.01	1200	20
17	F9	Lesmurdie - Walshpole Rd	32°00'58.9" °S, 116°03'47.6" °E	20	71	1	1	0.01	0.01	500	0
18	F10	Lesmurdie - Walshpole Rd	32°00'56.1" °S, 116°03'36.3" °E	7	21	1	0	0.05	0.00	0	0

**SUPPLEMENTARY DATA, Table S8.** Composition of pollen loads of insect species that visit *Diuris brumalis* and co-flowering Faboideae. Pollen loads were collected from the tibiae or abdomen. Pollen percentages occurrence for each individual insect was calculated by scanning 200 pollen grains. The remaining grains were then scanned for pollen in trace amounts (X = <10 pollens grains per slide).

No.	Insect species	Habitat	Where caught	Pollen percent occurrence (%)						
				<i>Daviesia</i>	<i>Bossiaea</i>	<i>Acacia</i>	<i>Banksia</i>	<i>Grevillea</i>	<i>Hovea</i>	Myrtaceae
1	<i>Trichocolletes capillosus</i>	forest	<i>Daviesia decurrens</i>	100	-	-	-	X	X	X
2	<i>T. leucogenys</i>	outcrop	<i>D. horrida</i>	97.5	-	-	-	-	0.5	2
3	<i>T. leucogenys</i>	forest	<i>D. decurrens</i>	100	-	-	-	-	-	X
4	<i>T. leucogenys</i>	outcrop	<i>D. horrida</i>	100	-	-	-	-	-	X
5	<i>T. leucogenys</i>	forest	<i>D. rhombifolia</i>	100	-	-	-	-	-	-
6	<i>Apis mellifera</i>	forest	<i>D. decurrens</i>	98	-	-	1	-	-	1
7	<i>A. mellifera</i>	forest	<i>D. decurrens</i>	98	-	-	1	-	-	1
8	<i>A. mellifera</i>	forest	<i>D. decurrens</i>	96.5	-	1	1.5	-	-	1
9	<i>A. mellifera</i>	forest	<i>D. rhombifolia</i>	80	-	-	-	-	-	20
10	<i>A. mellifera</i>	forest	<i>Bossiaea aquifolium</i>	-	97.5	X	1.5	-	-	1

**SUPPLEMENTARY DATA, Table S9.** Means (averages across flower parts) and standard deviation of colour loci coordinates for each plant species measured: *Diuris brumalis*, pea plants (Fabaceae) species (*Bossiaea aquifolium*, *Daviesia decurrens*, *D. rhombifolia*, *D. horrida*, *Hovea chorizemifolia* and *H. pungens*) and other yellow-flowered species present at sites, *Acacia pulchella* and *Hibbertia hypericoides*. Means are based on colour measurements for six individuals. Colour loci were calculated using the Hexagon colour model of bee vision (Chittka, 1992).

<b>Species</b>	<b>Mean (x)</b>	<b>Mean (y)</b>	<b>SD (x)</b>	<b>SD (y)</b>
<i>Acacia pulchella</i>	0.46	-0.23	0.01	0.02
<i>Bossiaea aquifolium</i>	-0.21	-0.14	0.08	0.26
<i>Daviesia decurrens</i>	-0.31	-0.22	0.26	0.21
<i>Daviesia horrida</i>	-0.23	-0.28	0.08	0.08
<i>Daviesia rhombifolia</i>	-0.23	-0.21	0.06	0.04
<i>Diuris brumalis</i>	-0.18	-0.23	0.08	0.07
<i>Hibbertia hypericoides</i>	0.03	-0.36	0.08	0.03
<i>Hovea chorizemifolia</i>	-0.34	0.23	0.07	0.05
<i>Hovea pungens</i>	-0.34	0.23	0.09	0.05

## Chapter 2

This Chapter has been sent to *Plant Ecology* and is currently in review.

### **Pea plants in the southwestern Australia biodiversity hotspot: pronounced differences in potential pollinators between co-occurring species**

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## Chapter 2

### Pea plants in the southwestern Australia biodiversity hotspot: pronounced differences in potential pollinators between co-occurring species

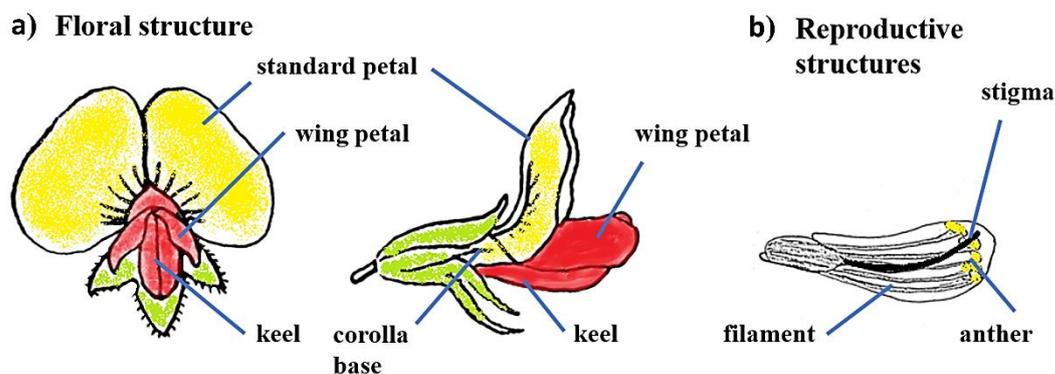
#### Abstract

The Faboideae is a species-rich group of flowering plants, most of which exhibit the keel-flower floral structure. In the south-western Australian Floristic Region (SWAFR), a recognised biodiversity hotspot, the Faboideae (pea plants) exhibit a range of floral colours and forms, which is suggestive of adaptation to different groups of pollinators. For four communities of pea plants in the SWAFR we tested if co-occurring pea plants share pollinator species, if they show differences in behaviour on the flower, and whether variation in stamen length or nectar composition is associated with pollinator type. With the aid of a video camera, we recorded which floral visitors contacted the reproductive structures of pea plants. We measured stamen length of pea plants, nectar volume by bagging flowers and nectar composition by gas chromatography-mass spectrometry. Pea plant species were visited by between one and four genera of native bees, indicating variation in levels of specialisation of the pollination systems. In pea plant species with more specialised interactions, co-occurring pea plants showed pronounced differences in the bee genera attracted. Unexpectedly, some pea plant species frequently attracted beetles that may play an important role in pollination. There was no evidence for an association between stamen length and pollinator size, or sugar composition and pollinator type. In addition to native pollinators, the introduced honey bee *Apis mellifera* visited all pea species studied, suggesting that honey bees may be both a pollinator and potential competitor for resources with native pollinators.

**Key-words:** *Apis*, bees, Faboideae, pollination, insect behaviour, Scarabaeidae

## Introduction

Among the Leguminosae, the Faboideae subfamily (hereafter referred to as pea plants) have the most morphologically specialised flowers, typically exhibiting the keel-flower floral form (Westerkamp 1997; Lewis et al. 2005; Fig. 1). Pollen deposition in pea plants occurs via a mechanism known as “tripping” (Arroyo 1981; Galloni et al. 2008; Aronne et al. 2012), where the pollinator alights on the wing petals, exerting pressure on the keel and causing the stamens to emerge from the keel and make contact with the ventral side of the pollinator (Fig. 1). Since this mechanism requires the appropriate size, behaviour, and in some cases strength of the pollinator to part the keel and contact the stamens, some pea plants species may be specialised on relatively few species of pollinator (Cordoba and Cocucci 2011).



**Fig. 1** a) Pea plant flower structure adapted from Woolcock (1989) and b) the reproductive structures enfolded between the keel petals.

Most species of pea plant, particularly in the comparatively well-studied temperate regions of the northern hemisphere, appear to be pollinated primarily by bees, as they are the only floral visitors capable of routinely contacting the reproductive structures (Green and Bohart 1975; Frankie et al. 1976; Gross 1992; Navarro 2000; Aronne et al. 2012; Galloni et al. 2008; Carleial et al. 2015). However, some pea plant species with red flowers and highly elongated keels are pollinated by birds (Feinsinger et al. 1979; Bruneau 1997; Agostini et al. 2006), and others are even pollinated by mammals (Barker, 1970; Letten and Midgely 2009; Kobayashi et al. 2015). Interestingly, while insect-pollinated pea plants retain a similar overall floral morphology, the flowers exhibit considerable variation in colour, which could be associated with shifts in the group of pollinating insects. Further, while some pea plants attract a range of bees (e.g. Green and Bohart 1975; Frankie et al. 1976), in some species the precise distance between the anthers

and the nectar source means that a level of specialisation could also arise via a morphological fit. Given the potential for morphological specialisation and differences in pollinator attraction between species, pollination could potentially drive diversification in the pea plants, either through pollinator shifts facilitating speciation (Stebbins 1970; Van der Niet et al. 2014) or through aiding in the co-existence of diverse assemblages of pea plants (Pauw 2013).

Pea plants are a conspicuous, widespread and highly diverse component of the Australian flora (approximately 1500 species and 136 genera; Crisp 2009). Despite their diversity and the potential for specialised pollination systems, pollination of Australian pea plants has received surprisingly little attention. Based on the few detailed studies of pollination of Australian pea plants (Gross 1992, 2001; Ogilvie et al. 2009; though see Popic et al. 2016), and incidental records of bees (e.g. Rayment 1936; Houston 2000; Batley and Houston 2012; Maynard 2014), the majority of Australian pea plants are probably pollinated primarily by bees (though see Popic et al. 2013). Ogilvie et al. (2009) showed that, while a diversity of insect species visited flowers of *Pultanaea villosa*, the pollinators were a range of solitary bee species. Alternatively, Gross (1992, 2001) found some *Dillwynia* and *Pultanaea* species to be somewhat specialised on the colletid bee genus *Trichocolletes*. As such, in the Australian pea plants there is some evidence for ecologically specialised pollination systems (where there are one or few pollinator species; *sensu* Armbruster 2017), but it remains unknown whether such specialisation plays any role in the origin or maintenance of diverse pea plants communities.

The southwestern Australian Floristic Region (SWAFR *sensu* Hopper and Gioia 2004) is recognised as a biodiversity hotspot, primarily due to its diverse and threatened flora (Myers et al. 2000). There have been no detailed pollination studies of pea plants in the region (Phillips et al., 2010) despite the occurrence of over 540 species (Hopper and Gioia 2004). However, observational studies suggest that bees are likely to play an important role in the pollination of many species of pea plants (e.g. Hopper 1981; Houston 2000; Scaccabarozzi et al. 2018). In the SWAFR, pea plants often occur in communities with multiple co-flowering genera, encompassing a considerable range of floral colours such as violet, blue, yellows, red/yellow, orange and pink (e.g. Barrett and Pin Tay 2005). To test for differences in floral preference and behavioural patterns of potential pollinators, the present study focuses on 15 pea plant species in the SWAFR, occurring across four subregions with different plant communities. We address the following questions: (I) Do different species of pea plant have different species of potential pollinators? (II) How do different visitors behave when foraging? (III) Is the length of the

stamens related to the size of visiting bees? (IV) Does the nectar vary in sugar composition and volume between pea plants with different groups of potential pollinators? In addition, we quantify the proportion of visits contributed by the introduced bee *Apis mellifera* (Apidae) relative to native pollinators, as this species visits numerous species of native plant in southwestern Australia (Wills *et al.*, 1990; Paton, 1993), and is known to pollinate at least some species of native Australian pea plants (Gross, 2011).

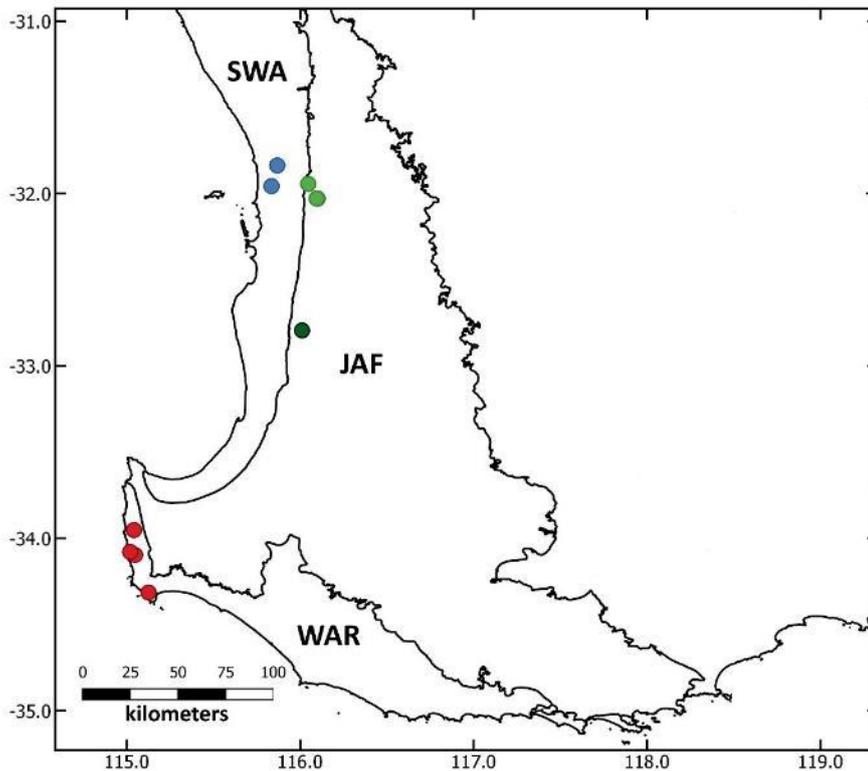
## Materials and Methods

We studied 15 species from eight genera of pea plants endemic to the SWAFR (Table 1, Table S1). All of the study species are common, and most are widely distributed in the SWAFR. Within the SWAFR, four study regions were selected, each with a different community of pea plants (PH: Perth hills; PC: Swan Coastal Plain; W: Waroona; MA: Margaret River-Augusta; Table 1, Fig. 2). For each species, pollinator observations were undertaken in either one or two sites (Table 1). All the study species in each region overlapped extensively in flowering period (Marshall 1995; Table 1). While most species are late winter to spring flowering (when observations were undertaken), one species, *Jacksonia sternbergiana*, flowers throughout the year.

Species		Flowering time	Region	Co-occurring species
<i>Bossiaea aquifolium</i>		A-S	PH	<i>H. chorizemifolia</i> , <i>H. pungens</i> , <i>D. decurrens</i> , <i>D. rhombifolia</i>
<i>Bossiaea disticha</i>		S-N	MA	<i>B. linophylla</i>
<i>Bossiaea eriocarpa</i>		JL-O	PC	<i>D. divaricata</i> , <i>I. cuneifolia</i> , <i>J. sternbergiana</i>
<i>Bossiaea linophylla</i>		JL -D	MA	<i>B. disticha</i> , <i>V. juncea</i>
<i>Daviesia decurrens</i>		JN-A	PH	<i>D. rhombifolia</i> , <i>B. aquifolium</i> , <i>H. chorizemifolia</i> , <i>H. pungens</i>
<i>Daviesia divaricata</i>		JL-N	PC	<i>B. eriocarpa</i> , <i>H. comptoniana</i> , <i>J. sternbergiana</i> , <i>I. cuneifolia</i>
<i>Daviesia horrida</i>		JL-S	PH	<i>H. pungens</i>
<i>Daviesia rhombifolia</i>		J-A	PH	<i>D. decurrens</i> , <i>B. aquifolium</i> , <i>H. chorizemifolia</i> , <i>H. pungens</i>
<i>Isotropis cuneifolia</i>		A-O	PC	<i>B. eriocarpa</i> , <i>H. comptoniana</i> , <i>J. sternbergiana</i>

Species		Flowering time	Region	Co-occurring species
<i>Hardenbergia comptoniana</i>		JL-O	PC	<i>B. eriocarpa</i> , <i>D. divaricata</i> , <i>I. cuneifolia</i> , <i>J. sternbergiana</i>
<i>Hovea chorizemifolia</i>		JN-S	PH	<i>H. pungens</i> , <i>D. decurrens</i> , <i>D. rhombifolia</i> , <i>B. aquifolium</i>
<i>Hovea pungens</i>		J-N	PH	<i>D. horrida</i>
<i>Jacksonia sternbergiana</i>		J-D	PC	<i>B. eriocarpa</i> , <i>D. divaricata</i> , <i>H. comptoniana</i> , <i>I. cuneifolia</i>
<i>Mirbelia dilatata</i>		S-J	W	-
<i>Viminaria juncea</i>		O-J	MA	<i>B. linophylla</i>

**Table 1** A summary of the study species of pea plants with information on flowering time (information from Marchant et al. 1997), co-occurring species, and location of study sites. Four regions are represented: PH = Perth hills; PC = Swan Coastal Plain; W = Waroona; MA = Margaret River-Augusta. Flowering time JL, JN, A, S, O, N, J: July, June, August, September, October, November, January



**Fig. 2** Study site locations: Swan Coastal Plain (PC; blue circles), Margaret River / Augusta (MA; red circles), Perth Hills (PH; paler green circles), and Waroona (W; dark green circle). The irregular polygons represent Interim Bio regionalisation of Australia (IBRA) ecoregions described by Environment Australia (2000): SWA = Swan Coastal; WAR = Warren; JAF = Jarrah Forest

### *Insect observations*

We undertook observations of floral visitors to test whether different species of pea plant were likely to be pollinated by different genera of native bees, and to quantify the frequency of visitation by *Apis mellifera*. Only floral visitors (grouped at genus level) that were observed to contact the floral reproductive structures were recorded. While further experiments are needed to confirm the role of any given floral visitor in pollination, species that contacted the reproductive structures and carried pollen (see below) were considered to be potential pollinators. Observations of floral visitors were carried out in the four study regions over different periods to cover slightly different peak flowering seasons. Observations in the PH were taken from 9<sup>th</sup> July until 6<sup>th</sup> September 2016 and from 8<sup>th</sup> July to 9<sup>th</sup> September 2017, on the PC from 26<sup>th</sup> August until 14<sup>th</sup> October 2015 and 29<sup>th</sup> August until 14<sup>th</sup> October 2016, at W from 22<sup>th</sup> to 23<sup>th</sup> November 2015, and in the MA region from 13<sup>th</sup> to 20<sup>th</sup> November 2015 and from 8<sup>th</sup> to 20<sup>th</sup> November 2016.

We recorded the number of insects landing on the flowers of the focal pea species during 20-minute periods between 7.00 a.m. and 5.30 p.m. The air temperature was measured with a Smartsensor

AR827 digital thermometer and hygrometer (Arco Electronics Ltd. Dongguan City, China) set 20 cm above the ground. Pollinator observations were undertaken while the weather was sunny or partly cloudy, in all cases avoiding exceptionally windy conditions. During the observation periods the temperature varied from 11 °C to 30 °C, but most observations were undertaken when the temperature was higher than 17 °C. In total, across the four regions we conducted 176 observation periods of 20 minutes in 2015 and 140 in 2016 (6320 minutes of total observation). For each pea plant species, pollinator observations were conducted from two to ten days, covering between 320 and 640 min, from 16 to 32 observation periods per species. Observations for any given species covered three to eight different plants (depending on availability), which were observed on rotation, with one individual plant observed per 20 minute trial. Observations were made over two years for all species with the exception of *Mirbelia dilatata*, for which observations were carried out only in 2015. Visitation rates were calculated as mean ( $\pm$  SD) of total number of visiting insects on the number of observation periods.

#### *Comparative behaviour of potential pollinators*

To quantify the foraging behaviour of different insect genera, two behavioural categories were recorded for each potential pollinator: (I) foraging on nectar inferred by the insect repeatedly probing the area at the corolla base (tongue extension was not always obvious), and (II) whether the body of the insect contacted the reproductive structures of the plant. Further detailed description of the behaviour was made to identify distinctive behavioural traits displayed by different genera of potential pollinators. Quantification of insect behaviour was carried out through direct observation in the field and by watching slow-motion video records with an EOS M video camera (Canon, Tokyo, Japan). Observations of pollinator behaviour were carried out for the first flower on a given plant visited by the insect. We compared between genera the proportion of insect visitors feeding on nectar and contacting the reproductive structures using *G*-tests in GenAlEx 6.5 (Peakall and Smouse 2006, 2012). To account for the effects of floral traits on insect behaviour, and that not all pollinator genera visited all pea plants, comparisons were only made between pollinator genera when they visited the same species of pea plant. Pollinator genera were only included for any given comparison if there were more than 40 observations on that particular pea plant species.

### *Identification of potential pollinators*

Of the insects observed visiting pea plant flowers (particularly females carrying pollen loads), 110 were caught for identification (Table S2). The observations were supplemented with video (EOS M video camera; Canon, Tokyo, Japan) to aid in identifying the bees to genus. All collected insects were submitted as vouchers to the Western Australian Museum. Bees that were recorded visually or photographically, but not caught as a voucher specimen, were only identified to genus.

### *Pollen loads on potential pollinators*

To test whether insects were carrying pollen of the pea plant that they were visiting, and therefore were likely to act as pollinators, pollen was identified from the bodies of potential pollinators (also including corbicular pollen in bees). For each pea plant species, between one and 12 observed insect specimens were caught and their pollen load analysed. Pollen observed on the tibiae or abdomen of pollinators during specimen identification was removed by washing the insect with distilled water, acetolysed following the methods of Erdtmann (1960), and permanently mounted on glass microscope slides. Pollen was identified under high magnification (Olympus-BX 51 microscope with Olympus-DP71 camera, Olympus, Tokyo, Japan) by comparison with acetolysed mounted pollen samples from herbarium specimens at the Curtin University Palynology Laboratory, Bentley (WA, Australia).

### *Stamen length and bee size*

To test whether stamen length of pea plants was correlated with size of the visiting bees, for the PH and PC regions we measured the total body length of bees and the length of the filament-stamens of each pea plant using digital callipers (Prowin, China). Due to the stamens overlap, the measurements were taken on a random filament-stamen included in the aggregation of stamens. We measured both male and female bees and calculated separate means to account for sexual dimorphism. Stamens of 10 individual flowers of different plants from 11 species were measured. Generally stamen length did not vary greatly within a flower, so a single measurement was taken per flower. However, for *Jacksonia sternbergiana* the stamen length showed pronounced variation within individual flowers, so the minimum and maximum stamen lengths were measured for each flower. The relationship between stamen length and bee size was assessed by Spearman's rank correlation coefficient ( $\rho$ ), using JMP statistical software. The value for bee body length was taken from all individuals, regardless of which pea species

that they were collected from. As such, this same value was used for each of the different pea plant species that a bee species visited.

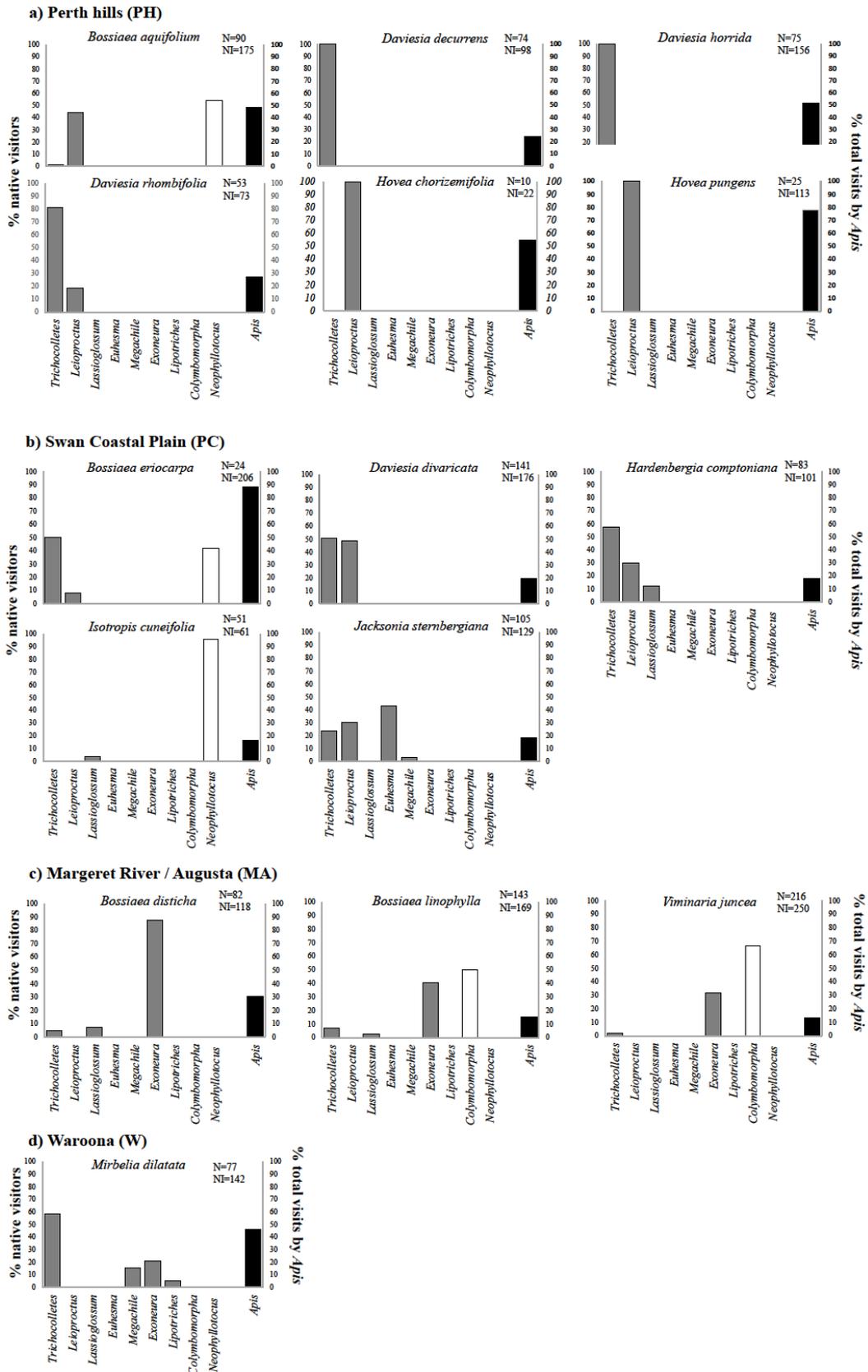
#### *Nectar volume and composition*

We conducted a pollinator exclusion experiment to test the assumption that the study species produce nectar, and to enable comparison of nectar composition between species. Ten randomly selected individuals of each species of pea plants occurring in the regions PH and PC had one inflorescence covered with an organza bag to exclude pollinators. Inflorescences were bagged in the afternoon, with nectar collected the following day when the temperature exceeded 17°C between 11.00 a.m. to 2 p.m. to ensure maximum nectar production (Corbet et al. 1995). Nectar was collected at the base of the corolla (Fig. 1) from two flowers on each inflorescence using a 2 µl microcapillary tube (Drummond Microcaps, Broomall; Pa., USA), and the volume of nectar estimated by measuring the length of the column of liquid along the microcapillary tube (Corbet 2003) using digital callipers. Gas chromatography-mass spectrometry (GC-MS) was used to determine the proportion of fructose, glucose, and sucrose in the nectar of each species (Reiter et al. 2018; Appendix S1). Kruskal-Wallis non-parametric comparisons were made to compare the nectar volumes and proportions of sugars (sucrose, glucose and fructose) between all species, with Wilcoxon post-hoc comparisons made for pairs of species within each community. Both the analyses were run using JMP statistical software.

## Results

### *Potential pollinators of pea plants*

Ten species of pea plants were predominantly visited by bees (*B. disticha*, *D. decurrens*, *D. divaricata*, *D. horrida*, *D. rhombifolia*, *H. comptoniana*, *H. chorizemifolia*, *H. pungens*, *J. sternbergiana* and *M. dilatata*), and three species were visited by a combination of bees, and *Neophyllotocus* (Scarabaeidae) and/or *Colymbomorpha* (Scarabaeidae) beetles: *B. aquifolium*, *B. eriocarpa*, *B. linophylla*. Both female and male bees were visitors, though based on the captured specimens there appears to be a bias towards visitation by females. Two species (*I. cuneifolia* and *V. juncea*) were visited principally by beetles, comprising more than 50 % of the total visits from native insects (Fig. 3; Table S3). Most species of pea plant were visited by two or three genera. In the PH region, the three *Daviesia* species were primarily visited by *Trichocolletes* (Colletidae), while *Hovea* was visited predominantly by *Leioproctus* (Colletidae), and *Bossiaea* by *Leioproctus* and *Neophyllotocus* beetles. In the PC region, *Daviesia* and *Hardenbergia* were frequently visited by *Trichocolletes* and *Leioproctus*. *Jacksonia* was unusual in that it had a large number of visits by *Euhesma* (Colletidae) and was also visited by native bees shared with other pea species. Unlike other pea plant species in the study, almost all potential pollinators of *I. cuneifolia* were *Neophyllotocus* beetles (97%). In the MA region, bees in the genus *Exoneura* (Apidae) were the main potential pollinators of all three pea plant species, but *B. linophylla* and *V. juncea* attracted both bees and beetles. In the W region, *M. dilatata* was visited predominantly by *Trichocolletes* bees.



**Fig. 3** Proportions of visits by potential pollinators (i.e. those species where some individuals contact the reproductive structures) per each species of pea plant in **a)** the Perth Hills (PH), **b)**

the Swan Coastal Plain (PC), **c**) the Margaret River / Augusta (MA) and **d**) the Waroona (W). Grey bars indicate the proportion of visits by native Hymenoptera out of all native insect visitors, white bars indicate the proportion of visits by native beetles of all native insect visitors, and black bars represent the proportion of total insect visits (native and non-native insect visitors) by the introduced *Apis mellifera*. N is the total number of visits from native potential pollinators while NI is the number of visits from potential pollinators including native species and the introduced honey bees *Apis mellifera*

*Trichocolletes* bees visited the greatest diversity of pea plant species in the PH, PC and W regions, and accounted for more than 50% of visits by native potential pollinators to seven species of pea plants. In the MA region, the greatest diversity of pea plant species was visited by *Exoneura* bees, which accounted for more than 30% of visits by native potential pollinators to three species of pea plants. In the W region, *Exoneura* bees account for 20% of native pollinator visits to *M. dilatata*.

Across the entire set of study species, *Apis mellifera* was the most prolific potential pollinator, visiting all of the pea plant species studied in each region. In the PH region, for two *Hovea* spp. and *D. horrida*, more than 50% of the total visits were by *A. mellifera*. In the PC region *B. eriocarpa* was predominantly visited by *A. mellifera* rather than native insects, with *A. mellifera* accounting for 90% of all visits. In the W region, *A. mellifera* represented over 50% of total visits to *M. dilatata*.

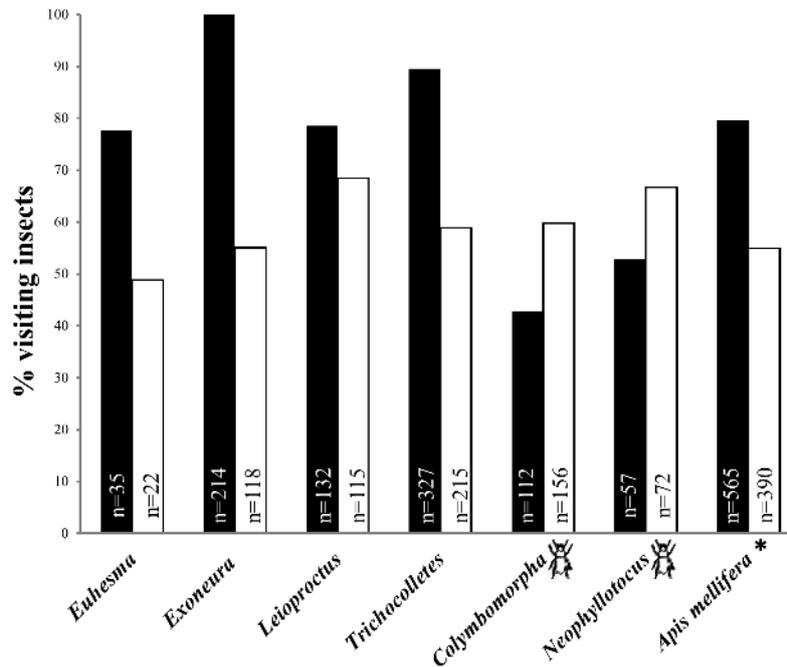
#### *Pollen loads of potential pollinators*

Pollen analysis confirmed the presence of pea plant pollen on the body of bee specimens (including corbicular pollen), and therefore the potential role as pollinators for 10 bee taxa across 11 pea plants (Table S4). For 37 out of 40 individual bees, the most abundant pollen type on the bee matched the plant on which the bee was observed feeding. The only exceptions were specimens of *Lipotriches australica* and *Trichocolletes* sp. in the W region that mainly carried pollen from Myrtaceae, despite being caught on *M. dilatata*, and an *A. mellifera* caught on *D. divaricata*, but carrying predominantly pollen from *Oxalis* (Table S4).

#### *Comparative behaviour of potential pollinators*

All genera of potential native bee pollinators foraged on nectar and contacted the reproductive structures. For native bees, the frequency of individuals attempting to forage on nectar varied between 78% and 100%, while contact with the reproductive structures occurred on 49% - 69% of

the occasions (Fig. 4). Interestingly, while *Exoneura* bees visiting *B. disticha*, *B. linophylla* and *V. juncea* parted the keel during foraging, on *M. dilatata* they only opened the petal wings and contacted the anthers after they had been left exposed by other visitors. As with the native bees, all honeybees were observed foraging on nectar and contacting the reproductive structures. Contact with the reproductive structures was observed on nearly 55% of visits by *A. mellifera*, a frequency exceeded by all other insect genera except *Euhesma* (Fig. 4; Table S5).



**Fig. 4** Frequency of behaviours displayed by insect visitors: (I) foraging on nectar, as inferred by the insect repeatedly probing the area at the corolla base (white bars); and (II) contacting the reproductive structures (grey bars). 🐞 beetle genera \* introduced honeybee

Unlike bees, beetles frequently entered inside the parted keel and were observed exiting the flower covered in pollen. While beetles of the genera *Neophyllotocus* and *Colymbomorpha* frequently foraged on nectar (Fig. 4), they were also observed consuming pollen once inside the keel. In addition to feeding, beetles were often seen mating while they visited pea plant flowers (Table 2). The visits often lasted several minutes and occurred on various parts of the flower, where the beetles sometimes made contact with the anthers.

Pollinator taxon	Pollinator group	Landing	Time of visit	Abdomen	Additional behaviour
<i>Apis mellifera</i>	HB	DD	>2 s	F	-
<i>Colymbomorpha</i>	B	DD	>2 s	-	Mating, pollen eating
<i>Euhesma</i>	NB	S	>2 s	F	-
<i>Exoneura</i>	NB	S	>2 s	F	-
<i>Lassioglossum</i>	NB	S	≤2 s	F	-
<i>Leioproctus</i>	NB	DD	>2 s	F, B	-
<i>Megachile</i>	NB	S	≥2 s	U	-
<i>Neophyllotocus</i>	B	DD	>2 s	-	Mating, pollen eating
<i>Trichocolletes</i>	NB	S	<2 s	B, F	-

**Table 2** Behaviour of genera of potential pollinators during visits to pea plants. “Landing” was categorised based on orientation relative to the keel: body straight (S), body not straight (DD). The abdomen position was categorised as: flexing (F), curving around the keel (B) and curving upward (U). ‘Time of visit’ refers to the approximate time spent on an individual flower based on video recordings. HB: honeybees; B: beetles; NB: native bees

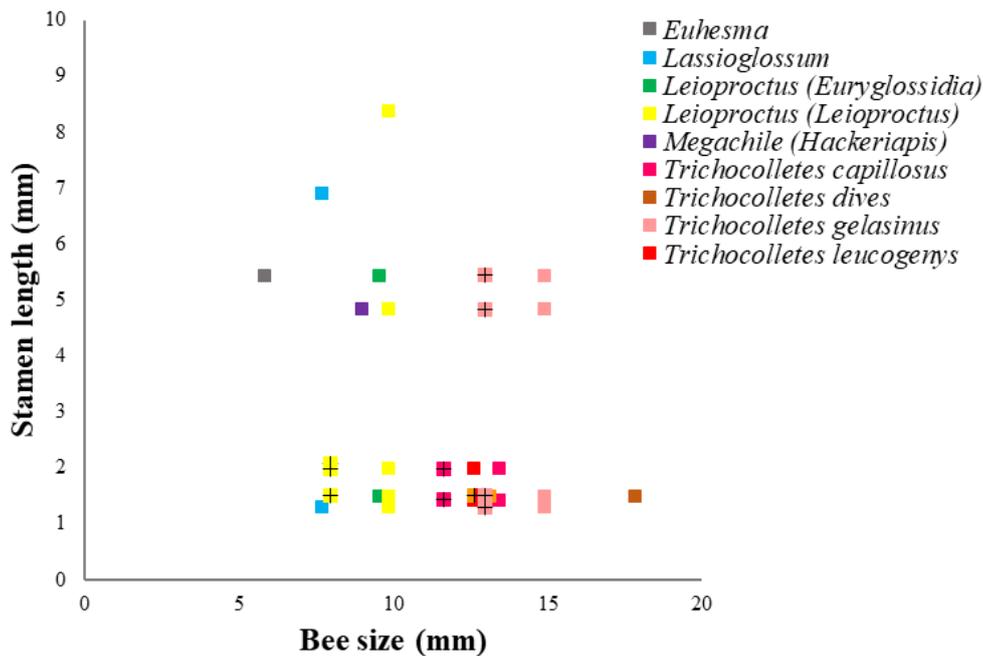
Comparison of the behaviour of pollinators visiting particular pea plants revealed significant differences in the frequency of nectar foraging and the frequency with which they contacted the reproductive structures (Table 3). In both comparisons involving beetles, they fed on nectar significantly less often than native bees, but in *V. juncea* the *Collymbomorpha* beetles contacted the reproductive structures more frequently. In the three comparisons involving *Apis* and native bees, on each occasion there was no difference in the frequency of visits in which they foraged on nectar. However, in both occasions involving *Trichocolletes*, *A. mellifera* contacted the reproductive structures less frequently, but in the case involving *Leioproctus*, there was no significant difference.

Pea plant	Comparison	Nectar foraging (G <sub>df</sub> , p)	Contacting reproductive structures (G <sub>df</sub> , p)
<i>Bossiaea aquifolium</i>	<i>Leioproctus</i> > <i>Neophyllotocus</i>	11.68 <sub>1</sub> ***	ns
	<i>Leioproctus</i> - <i>Apis</i>	ns	ns
<i>Daviesia divaricata</i>	<i>Leioproctus</i> > <i>Trichocolletes</i>	6.89 <sub>1</sub> **	4.31 <sub>1</sub> *
<i>Daviesia horrida</i>	<i>Trichocolletes</i> < <i>Apis</i>	ns	11.39 <sub>1</sub> ***
<i>Mirbelia dilatata</i>	<i>Trichocolletes</i> > <i>Apis</i>	ns	13.16 <sub>1</sub> ***
<i>Viminaria juncea</i>	<i>Exoneura</i> > <i>Colymbomorpha</i>	101.56 <sub>1</sub> ***	-
	<i>Exoneura</i> < <i>Colymbomorpha</i>	-	10.55 <sub>1</sub> **

**Table 3** Results of *G*-tests comparing the frequency of nectar foraging behaviour and contact with reproductive structures of different pollinator genera when visiting the same species of pea plant. Significance values: \* <0.05 \*\*<0.01 \*\*\*<0.001. ns: not significant

#### *Stamen length and bee size*

Based on a clear disjunction in average stamen length between species, pea plants could be classified into two broad groups. The species with short stamens (< 2.1 mm) were: *H. comptoniana*, *D. rhombifolia*, *D. horrida*, *D. divaricata*, *D. decurrens*, *H. chorizemifolia*, and *H. pungens*. The species with the long stamens (> 4.8 mm) were *B. aquifolium*, *B. eriocarpa*, *J. sternbergiana* and *I. cuneifolia* (Table S6). Spearman's rank correlation coefficient showed no relationship between stamen length of pea plants and the size of the visiting bees ( $\rho = 0.27$ ;  $p = 0.109$ ; Fig. 5; Table S7).



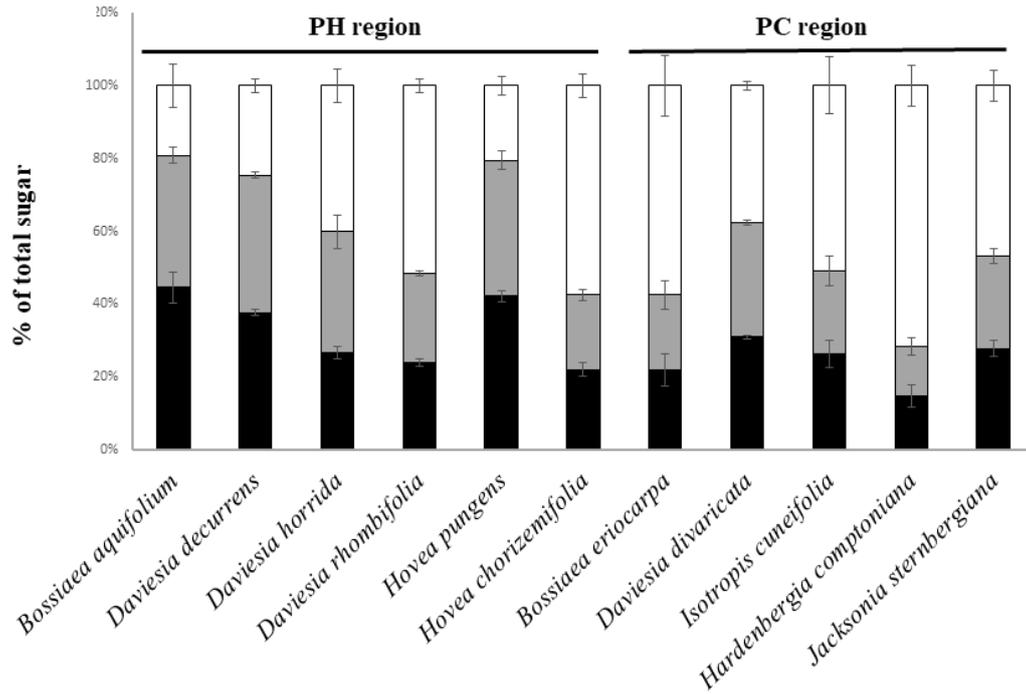
**Fig. 5** The relationship between stamen length of pea plants (y) and the body length of bee pollinators (x). +: indicates males, females are represented by plain squares. Potential pollinators are reported by genus or subgenus (in brackets) and when possible by species.

*Nectar: volume and sugar ratio*

Nectar volumes ranged from 0.1  $\mu$ l for *D. rhombifolia* and *D. horrida* to 0.36  $\mu$ l for *J. sternbergiana*, varying significantly between species ( $X^2_{10} = 41.05$ ;  $p < 0.0001$ ; Table 4; Table S8). Sucrose, glucose and fructose were present in the nectar of each species of pea plant and relative proportions showed significant variation between species for fructose ( $X^2_{10} = 62.63$ ;  $p < 0.0001$ ), glucose ( $X^2_{10} = 59.83$ ;  $p < 0.0001$ ) and sucrose ( $X^2_{10} = 62.99$ ;  $p < 0.0001$ ; Table 4). The proportion of sucrose ranged from 20% to 70%, with the remaining sugar comprised of similar proportions of glucose and fructose (Fig. 6).

Region	Comparison	Volume	Sugar ratio		
			Fructose	Glucose	Sucrose
		$X^2_{10} = 41.0$	$X^2_{10} = 62.63$	$X^2_{10} = 59.83$	$X^2_{10} = 62.99$
PH	DH-BA	-	DH<BA*	-	DH>BA*
	DH-DD	DD>DH*	DH<DD**	DH<DD*	DH>DD*
	DH-DR	-	-	DH>DR*	DH<DR*
	DR-BA	-	DR<BA**	DR<BA**	DR>BA**
	DR-DD	DR<DD*	DR<DD**	DR<DD**	DR>DD**
	HCH-BA	-	HCH<BA**	HCH<BA**	HCH>BA**
	HCH-DD	-	HCH<DD**	-	HCH>DD**
	HCH-DH	-	HCH<DH*	HCH<DH*	HCH>DH*
	HCH-DR	-	-	HCH<DR*	-
	HCH-HP	-	HCH<HP**	-	-
	HP-DH	HP>DH*	HP>DH**	HP>DH*	DH>HP*
	HP-DR	HP>DR*	HP>DR**	HP>DR*	HP<DR**
	HP-HCH	-	-	HP<HCH**	HP<HCH**
PC	DDI-BE	-	DDI>BE*	DDI>BE*	DDI<BE*
	DDI-IC	-	DDI>IC*	DDI>IC*	-
	DDI-HC	DDI>HC*	DDI>HC*	DDI>HC**	HC>DDI**
	IC-HC	-	IC>HC*	-	-
	IC-DDI	IC<DDI*	-	-	IC>DDI*
	IC-JS	IC<JS*	-	-	-
	JS-HC	-	JS>HC*	JS>HC**	HC>JS*

**Table 4** For nectar volume and sugar ratios, non-parametric comparison for each species pair within regions (PH and PC) using Kruskal-Wallis tests, reporting only the significant Wilcoxon comparisons. BA: *Bossiaea aquifolium*; DD: *Daviesia decurrens*; DH: *D. horrida*; DR: *D. rhombifolia*; HP: *Hovea pungens*; HCH: *Hovea chorizemifolia*; BE: *Bossiaea eriocarpa*; DDI: *Daviesia divaricata*; HC: *Hardenbergia comptoniana*; IC: *Isotropis cuneifolia*; JS: *Jacksonia sternbergiana*. p-value: \*<0.05 \*\*<0.001 \*\*\*<0.0001.



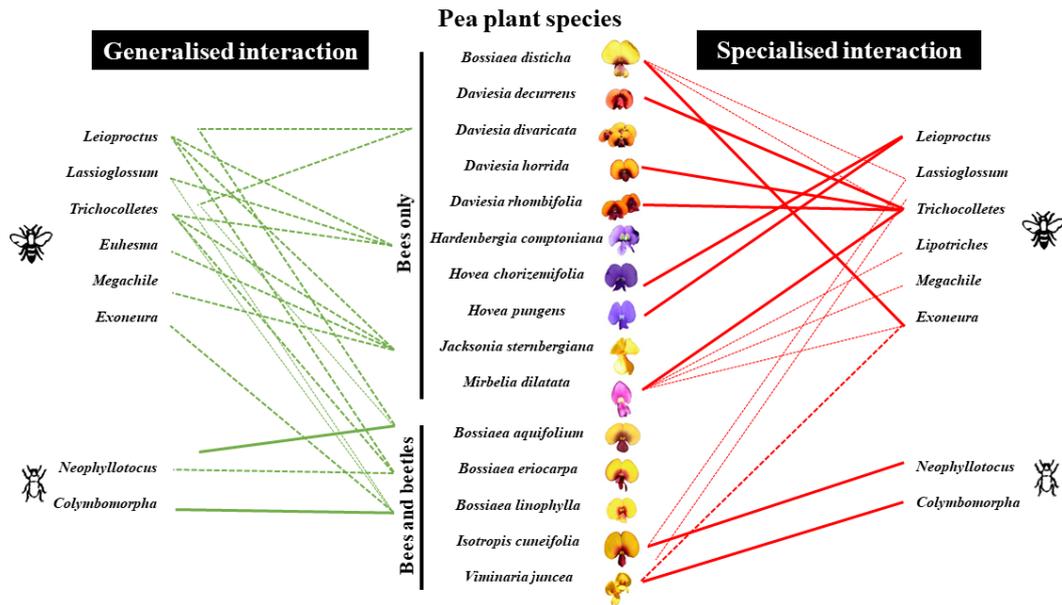
**Fig. 6** Mean proportions ( $\pm$  SD) of sugars (black = fructose, grey = glucose, white = sucrose) in the nectar of pea plant species in the Perth hills (PH) and Swan Coastal Plain (PC)

## Discussion

### *Generalised versus specialised pollination systems in pea plants*

Based on the presence of pollen loads from pea plants, and observations of stigmatic contact, it is likely that all bee species observed in this study are pollinators of the pea plants on which they forage. Our observations showed that native bees are the main potential pollinators of at least 10 of the investigated species of pea plants (Table S4). However, *B. aquifolium*, *B. eriocarpa* and *B. linophylla* were visited frequently by both bees and beetles, while *I. cuneifolia* and *V. juncea* were visited predominantly by beetles. In these cases, to assess the relative effectiveness of the two different groups of potential pollinators requires direct tests of fruit formation after pollinator visits (e.g. Gross 2001; Etcheverry et al. 2012).

The apparent reliance on bees as the main potential pollinators conforms to expectations based on observations of other Australian pea plants with relatively short keels (Gross 1992, 2001; Ogilvie et al. 2009). Nevertheless, it is interesting that most pea plant species were visited by only a subset of bee genera (Fig. 7). Several studies of bee pollination of pea plants outside of Australia have found that multiple bee genera are generally involved (e.g. Green and Bohart 1975; Frankie et al. 1976; Wainwright 1978; Navarro 2000; Galloni et al. 2008; Aronne et al. 2012; though see Carleial et al. 2015). As such, the *Daviesia* and *Hovea* species we observed, where pollination was mostly or entirely by a single genus, may exhibit unusually high levels of ecological specialisation in their pollination system compared with other pea plants visited by bees.



**Fig. 7** A summary of pea plant-insect interactions at the level of pollinator genus. Pea plants species with more generalised pollination systems are in green, while in pink are pea plants that have more specialised pollination systems. The weight of the arrows indicates the strength of the relationship based on number of visits. Here, pea plants were only considered to have specialised interactions when a single potential pollinator genus represented over 50% of visits of the insect genera that contacted the reproductive structures.

#### *Behaviour of native bees foraging on pea plants*

All observed bees regularly foraged on both nectar and pollen. Interestingly, in *Daviesia divaricata* the two genera of native bee that frequently visited the plant showed differences in the frequency with which they foraged nectar and contacted the reproductive structures, indicating that native bee genera could vary in their effectiveness as pollinators. Additionally, there was the potential for different bee species to harvest pollen from pea plants with either short or long stamens. For example, genera such as *Leioproctus* and *Lassioglossum* collected pollen from pea plant species presenting various stamen lengths. While most bee species were able to part the keel of their own accord, when visiting *M. dilatata*, *Exoneura* bees were observed to only weakly manipulate the keel. These bees could open the petal wings with resultant contact with the reproductive structures only in flowers that had been previously manipulated by another visiting species (e.g. Lopez 1999; Aronne et al. 2012).

### *Mechanisms underpinning the apparent specialisation of bee pollination systems*

In the present study, several species of pea plant attracted a distinct subset of the community of bee genera. In general, the attraction of a specific group of pollinators can arise through visual or olfactory signals that may be particularly detectable or attractive (Jersáková et al. 2012; Peakall et al. 2010), a morphology that favours efficient foraging by only a particular pollinator species (Abrahamczyk and Kessler 2015), or a reward that is of value to only few species of potential pollinators (Houston et al. 1993; Pauw 2006). In our study case, even though there was a significant variation of sugar composition between co-occurring pea plant species, the same pollinator genera (and species where identified) foraged on multiple pea plants with varied sugar composition, suggesting that the sugar composition is unlikely to represent an adaptation to particular pollinator species. Alternatively, given the wide range of floral colours within the pea plant community, it is possible that visual signals could be important in driving differences in the attraction of functional groups of visitors. Manipulating the colour of artificial flowers (e.g. Jersáková et al. 2012) would provide a tractable approach to experimentally test the role of colour in pollinator attraction in these systems.

### *Is stamen length correlated with the size of the bee?*

While we predicted that stamen length would be positively correlated with the body length of the primary bee genus visiting each pea plant, such a relationship was not evident. Nonetheless, the generalist *J. sternbergiana* may represent an interesting example of adaptation in stamen length, with the stamens exhibiting a 3 mm variation in length within the same flower. *Jacksonia sternbergiana* was visited by a broad spectrum of bee species, ranging from small bees such as *Euhesma* ( $5.82 \pm 0.01$  mm body length) to the larger *Leioproctus* ( $9.72 \pm 1.66$  mm) and *Megachile* ( $8.98 \pm 0$  mm). Variation in stamen length may be useful for achieving pollination via a wide range of bee species, particularly as *J. sternbergiana* flowers throughout the year and will be encountered by bee species with a range of flight times (see Houston 2000). Due to the short spring observation period compared with the year-long flowering of *J. sternbergiana*, it is likely that our observations have underestimated the full range of bee species (with different body sizes) that pollinate this plant.

### *The potential role of beetles as pollinators*

Beetles of the family Scarabaeidae were found to be potential pollinators in five species of pea plants, making up between 40% and 98% of all visits by native pollinators. Unlike bees, beetles typically foraged primarily on pollen rather than on nectar, and often displayed prolonged mating behaviour within and outside the keel of any given flower. While the beetle species

appeared to consume pollen, they nonetheless had pollen on the body after visiting a flower, suggesting that they are likely to act as pollinators. Given that in the present study beetles frequently foraged on pollen, it remains to be tested whether they are as effective a pollinator as bees. In the particular case of *Isotropis cuneifolia*, *Neophyllotocus* represented 98% of the floral visitors, suggesting that beetles may be the primary pollinators for this species.

While beetles are reported to visit pea plants in other regions, they generally fail to make contact with the reproductive structures and have been considered unlikely to readily achieve pollination (e.g. Galloni et al. 2008). Indeed, we are unaware of any specialised beetle pollination system in pea plants, suggesting that the relationship between *I. cuneifolia* and *Neophyllotocus* spp. may be exceptional. As such, studying the mechanism of pollinator attraction in *I. cuneifolia* may provide insight on how this strategy has arisen. In other plant families and geographic regions, beetle-pollinated plants often have brightly coloured, upward facing bowl-like flowers (Dafni et al. 1990; Goldblatt et al. 1998; Bernhardt 2000). *Isotropis cuneifolia* fits this pattern with its unusual upward facing flowers with particularly large standard petals. Given that this orientation of the standard petals could increase beetle visitation while making bee foraging relatively inefficient, this floral trait could potentially represent an adaptation to beetle pollination.

#### *Apis mellifera and the pollination of Western Australian pea plants*

In this study, the feral honey bee *A. mellifera* was likely to be a pollinator of most species of pea plant, representing over 10% of total floral visitations for any given pea plant species. Indeed, for several species *A. mellifera* comprised over 50% of total visiting insects that contacted the reproductive structures. *Apis mellifera* exhibited similar foraging behaviour to native bees in terms of nectar and pollen foraging. However, the orientation of *A. mellifera* on the keel during foraging was very variable (Table 2), raising the possibility that less pollen may be deposited on the insect body during some visitations. The effectiveness of *A. mellifera* at pollinating Australian pea plants was tested by Gross (2001), who found that 14.5% of visits by *A. mellifera* to flowers of *Dillwynia juniperia* led to fruit set, a similar level to that resulting from visits by *Trichocolletes* bees. While further experiments are needed, *A. mellifera* may currently represent an important pollinator for several species of pea plant in the SWAFR. However, while *A. mellifera* is likely to be a pollinator of these pea plants, it may potentially compete with native pollinators, leading to reduced population sizes of some native bee species

(e.g. Paini and Roberts 2005). Given the high diversity of pea plants and bee species in the SWAFR, understanding the effects of *A. mellifera* on native bees and the pea plants they pollinate would be important from a conservation perspective.

## **Appendix**

**Appendix S1, Table S1-S8:** Examples of generalist and specialist interactions among pea plants towards their potential pollinators.

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**Appendix, S1** Protocol for nectar analysis adopted for measuring the proportions of sugars in pea plants species of PH and PC regions.

### **Methodology**

The content of each microcapillary tube was extracted with 25  $\mu$ l ribitol solution (0.20 mg/ml) and transferred to a GC vial (2 ml). For each of the extracts, the solvent was evaporated to dryness with a stream of nitrogen. Methoxyamine-HCl (20  $\mu$ l of 20 mg/ml solution in pyridine; Sigma-Aldrich, St Louis, MI, USA) was added and the sealed vials were heated for 2 hours in a heating block at 37 °C. At the same temperature, the extracts were treated with *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA, 35  $\mu$ l; Sigma-Aldrich, St Louis, MI, USA) in the same sealed vials for 1 hour before GC-MS analysis (Lisec et al. 2006). GC-MS analysis was performed on an Agilent system (Agilent, Palo Alto, Ca, USA), consisting of a 5973 mass selective detector connected to an 6890 GC equipped with a BPX5 column [(5 % phenyl polysilphenylene-siloxane), 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m film thickness, SGE Australia], using helium as the carrier gas. An Agilent 7683 autoinjector was used to make 3  $\mu$ l injections in split mode (1 to 10). Tentative identification of trimethylsilylated sugars was based on the comparison of retention index and mass spectra with data from a mass spectral library (NIST-11). All tentative identifications were confirmed by co-injections with synthetic standards. Calculations of the ratios between glucose, fructose and sucrose were achieved by comparison of peak areas of the total ion chromatograms (TIC) of nectar samples. The response factors for respective carbohydrate sampled were measured and the calculated peak areas were adjusted accordingly.

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**Appendix, Table S1** List of the plant species studied per family collected in the field sites, subsequently vouchered at the Herbarium of Western Australia, Perth and relative list of field sites

Family name	Study species	Voucher	Collecting location	Field sites	Coordinates
Fabaceae	<i>Hovea chorizemifolia</i>	DS 1	31°29' 01.2" S; 115° 57' 55.0" E	Canning Rd, Lesmurdie	32°01'45.6"S, 116°06'01.3"E; 32°01'41.9"S, 116°05'41.7"E
Fabaceae	<i>Hovea pungens</i>	DS 7	31°56' 28.6" S; 116° 02' 52.7" E	Canning Rd, Lesmurdie; The Zig Zag, Kalamunda	32°01'45.6"S, 116°06'01.3"E; 31°56'35.3"S, 116°02'46.5"E
Fabaceae	<i>Daviesia rhombifolia</i>	DS 3	31°29' 01.2" S; 115° 57' 55.0" E	Canning Rd, Lesmurdie	32°01'45.6"S, 116°06'01.3"E; 32°01'41.9"S, 116°05'41.7"E
Fabaceae	<i>Bossiaea aquifolium</i> subsp. <i>aquifolium</i>	DS 5	31°29' 01.2" S; 115° 57' 55.0" E	Canning Rd, Lesmurdie	32°01'45.6"S, 116°06'01.3"E; 32°01'41.9"S, 116°05'41.7"E
Fabaceae	<i>Daviesia decurrens</i> subsp. <i>decurrens</i>	DS 6	31°29' 01.2" S; 115° 57' 55.0" E	Canning Rd, Lesmurdie	32°01'45.6"S, 116°06'01.3"E; 32°01'41.9"S, 116°05'41.7"E
Fabaceae	<i>Daviesia horrida</i>	DS 8	31°56' 28.6" S; 116° 02' 52.7" E	Canning Rd, Lesmurdie; The Zig Zag, Kalamunda	32°01'45.6"S, 116°06'01.3"E; 31°56'35.3"S, 116°02'46.5"E
Fabaceae	<i>Hardenbergia comptoniana</i>	DS12	31°50' 00.6" S; 115° 51' 13.8" E	Koondoola bushland; May Dr, Kings Park	31°50'12.7"S 115°52'02.8"E; 31°57'24.3"S 115°50'03.4"E
Fabaceae	<i>Jacksonia sternbergiana</i>	DS14	31°50' 00.6" S; 115° 51' 13.8" E	Koondoola bushland; May Dr, Kings Park	31°50'12.7"S 115°52'02.8"E; 31°57'24.3"S 115°50'03.4"E
Fabaceae	<i>Isotropis cuneifolia</i> subsp. <i>cuneifolia</i>	DS15	31°50' 00.6" S; 115° 51' 13.8" E	Koondoola bushland; May Dr, Kings Park	31°50'12.7"S 115°52'02.8"E; 31°57'24.3"S 115°50'03.4"E
Fabaceae	<i>Bossiaea eriocarpa</i>	DS16	31°50' 00.6" S; 115° 51' 13.8" E	Koondoola bushland; May Dr, Kings Park	31°50'12.7"S 115°52'02.8"E; 31°57'24.3"S 115°50'03.4"E
Fabaceae	<i>Daviesia divaricata</i> subsp. <i>divaricata</i>	DS17	31°50' 00.6" S; 115° 51' 13.8" E	Koondoola bushland; May Dr, Kings Park	31°50'12.7"S 115°52'02.8"E; 31°57'24.3"S 115°50'03.4"E
Fabaceae	<i>Mirbelia dilatata</i>	DS19	32°47' 38.9" S; 116° 00' 37.93" E	Waroona – in proximity Nanga Rd	32°47'38.9"S 116°00'37.93"E
Fabaceae	<i>Bossiaea linophylla</i>	DS20	33°57' 07.8" S; 115° 02' 28.6" E;	East Kevill Rd, Margaret River; Hillview Rd, Augusta	33°57'07.8"S 115°02'28.6"E; 34°18'51.3"S 115°07'30.7"E
Fabaceae	<i>Bossiaea disticha</i>	DS21	34°05' 46.5" S; 115° 02' 51.6" E;	Boranup forest; Conto Campground, Augusta	34°05'46.5"S 115°02'51.6"E; 34°04'44.4"S 115°01'09.3"E
Fabaceae	<i>Viminaria juncea</i>	DS22	33°57' 07.8" S; 115° 02' 28.6" E;	East Kevill Rd, Margaret River; Hillview Rd, Augusta	33°57'07.8"S 115°02'28.6"E 34°18'51.3"S 115°07'30.7"E

**Appendix, Table S2** Insects collected while foraging on pea plants. The identification is provided at genus, subgenus or species level, when possible. F: female; M: male

Species	Date	Region	Site	Genus subgenus	Sex
<i>Bossiaea aquifolium</i>	11/8/2016	PH	Lesmurdie - Pomeroy Rd	<i>Apis mellifera</i>	-
<i>Bossiaea disticha</i>	14/11/2015	MA	Boranup forest	<i>Apis mellifera</i>	-
<i>Bossiaea disticha</i>	17/11/2015	MA	Hillview Rd	<i>Apis mellifera</i>	-
<i>Bossiaea disticha</i>	17/11/2016	MA	Boranup forest	<i>Apis mellifera</i>	-
<i>Bossiaea disticha</i>	17/11/2016	MA	Boranup forest	<i>Apis mellifera</i>	-
<i>Bossiaea eriocarpa</i>	13/09/2016	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Bossiaea eriocarpa</i>	3/10/2017	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Bossiaea linophylla</i>	8/11/2016	MA	Kevil Rd East	<i>Apis mellifera</i>	-
<i>Bossiaea linophylla</i>	11/11/2016	MA	Kevil Rd East	<i>Apis mellifera</i>	-
<i>Daviesia decurrens</i>	7/8/2017	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Daviesia decurrens</i>	8/8/2017	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Daviesia decurrens</i>	18/08/2017	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	27/09/2015	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	27/09/2015	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	27/09/2015	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	27/09/2015	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	24/08/2016	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia divaricata</i>	24/08/2016	PC	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia horrida</i>	2/8/2016	PH	Koondoola Bushland	<i>Apis mellifera</i>	-
<i>Daviesia rhombifolia</i>	11/8/2017	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Daviesia rhombifolia</i>	25/08/2017	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Hovea pungens</i>	3/8/2016	PH	Lesmurdie - Canning Rd	<i>Apis mellifera</i>	-
<i>Viminaria juncea</i>	16/11/2015	MA	Hillview Rd	<i>Apis mellifera</i>	-
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Apis mellifera</i>	-
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Apis mellifera</i>	-
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Apis mellifera</i>	-

Species	Date	Region	Site	Genus subgenus	Sex
<i>Bossiaea linophylla</i>	8/11/2016	MA	Kevil Rd East	<i>Colymbomorpha</i>	-
<i>Viminaria juncea</i>	16/11/2015	MA	Hillview Rd	<i>Colymbomorpha</i>	-
<i>Jacksonia sternbergiana</i>	5/10/2016	PC	Koondoola Bushland	<i>Euhesma</i> sp. 1	F
<i>Jacksonia sternbergiana</i>	5/10/2016	PC	Koondoola Bushland	<i>Euhesma</i> sp. 1	F
<i>Bossiaea disticha</i>	13/11/2015	MA	Yatch club	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea disticha</i>	17/11/2016	MA	Boranup forest	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea disticha</i>	17/11/2016	MA	Boranup forest	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea disticha</i>	17/11/2016	MA	Boranup forest	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea disticha</i>	18/11/2016	MA	Boranup forest	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	13/11/2015	MA	Yatch club	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	8/11/2016	MA	Kevil Rd East	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	8/11/2016	MA	Kevil Rd East	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	8/11/2016	MA	Kevil Rd East	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	13/11/2016	MA	Kevil Rd East	<i>Exoneura Exoneura</i> sp.	F
<i>Bossiaea linophylla</i>	13/11/2016	MA	Kevil Rd East	<i>Exoneura Exoneura</i> sp.	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Viminaria juncea</i>	16/11/2015	MA	Hillview Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Exoneura Exoneura</i> sp.	F
<i>Isotropis cuneifolia</i>	8/9/2015	PC	Kings Park	<i>Lasioglossum</i> sp.	F
<i>Bossiaea disticha</i>	18/11/2016	MA	Boranup forest	<i>Lasioglossum Chilalictus</i> sp.	F
<i>Bossiaea linophylla</i>	11/11/2016	MA	Kevil Rd East	<i>Lasioglossum Chilalictus</i> sp.	F
<i>Hardenbergia comptoniana</i>	11/11/2016	MA	Kevil Rd East	<i>Lasioglossum Chilalictus</i> sp.	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Leioproctus Cladocerapis</i> sp.	M

Species	Date	Region	Site	Genus subgenus	Sex
<i>Daviesia divaricata</i>	13/09/2016	PC	Koondoola Bushland	<i>Leioproctus Euryglossidia</i> sp. 1	F
<i>Jacksonia sternbergiana</i>	5/10/2016	PC	Koondoola Bushland	<i>Leioproctus Euryglossidia</i> sp. 1	F
<i>Jacksonia sternbergiana</i>	11/10/2016	PC	Kings Park	<i>Leioproctus Euryglossidia</i> sp. 1	F
<i>Jacksonia sternbergiana</i>	11/10/2016	PC	Kings Park	<i>Leioproctus Euryglossidia</i> sp. 2	F
<i>Bossiaea aquifolium</i>	5/9/2016	PH	Lesmurdie -Canning Rd	<i>Leioproctus Leioproctus</i> WAM code F177	F
<i>Bossiaea aquifolium</i>	14/08/2016	PH	Lesmurdie - Canning Rd	<i>Leioproctus Leioproctus</i> sp. 1	F
<i>Bossiaea eriocarpa</i>	13/09/2016	PC	Koondoola Bushland	<i>Leioproctus Leioproctus</i> WAM code F177	F
<i>Daviesia decurrens</i>	19/07/2016	PH	Lesmurdie - Canning Rd	<i>Leioproctus Leioproctus</i> sp. 1	F
<i>Daviesia decurrens</i>	12/7/2017	PH	Lesmurdie - Canning Rd	<i>Leioproctus Leioproctus</i> sp. 1	F
<i>Daviesia decurrens</i>	12/7/2017	PH	Lesmurdie - Canning Rd	<i>Leioproctus Leioproctus</i> sp. 1	M
<i>Daviesia divaricata</i>	21/09/2015	PC	Koondoola Bushland	<i>Leioproctus Leioproctus</i> sp. 1	M
<i>Daviesia divaricata</i>	22/09/2015	PC	Koondoola Bushland	<i>Leioproctus Leioproctus</i> sp. 1	F
<i>Hardenbergia comptoniana</i>	14/10/2016	PC	Kings Park	<i>Leioproctus Leioproctus</i> sp. 1	F
<i>Hovea pungens</i>	14/08/2016	PH	Lesmurdie - Canning Rd	<i>Leioproctus Leioproctus</i> sp. 1	M
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Lipotriches australica</i>	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Lipotriches australica</i>	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Megachile Eutricharaea</i> sp. 1	F
<i>Bossiaea eriocarpa</i>	13/08/2016	PC	Koondoola Bushland	<i>Megachile Hackeriapis</i> sp. 1	F
<i>Isotropis cuneifolia</i>	7/9/2015	PC	Kings Park	<i>Neophyllotocus</i> sp. 1	
<i>Bossiaea disticha</i>	17/11/2015	MA	Hillview Rd	Syrphidae	
<i>Hardenbergia comptoniana</i>	14/10/2016	PC	Kings Park	Syrphidae	
<i>Bossiaea eriocarpa</i>	29/09/2016	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia decurrens</i>	26/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	M
<i>Daviesia decurrens</i>	26/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	F
<i>Daviesia decurrens</i>	11/8/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia decurrens</i>	11/8/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia decurrens</i>	13/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	M
<i>Daviesia decurrens</i>	13/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	M

Species	Date	Region	Site	Genus subgenus	Sex
<i>Daviesia decurrens</i>	20/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	F
<i>Daviesia decurrens</i>	18/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	M
<i>Daviesia decurrens</i>	18/07/2016	PH	Lesmurdie - Canning Rd	<i>Trichocolletes capillosus</i>	M
<i>Daviesia divaricata</i>	6/9/2016	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia divaricata</i>	12/9/2016	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia divaricata</i>	11/9/2016	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia divaricata</i>	13/09/2016	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia divaricata</i>	5/9/2017	PC	Koondoola Bushland	<i>Trichocolletes gelasinus</i>	F
<i>Daviesia divaricata</i>	13/09/2017	PC	Kings Park	<i>Trichocolletes gelasinus</i>	M
<i>Daviesia divaricata</i>	22/09/2015	PC	Koondoola Bushland	<i>Trichocolletes platyprosopis</i>	M
<i>Daviesia divaricata</i>	22/09/2015	PC	Koondoola Bushland	<i>Trichocolletes dives</i>	F
<i>Daviesia divaricata</i>	26/09/2015	PC	Koondoola Bushland	<i>Trichocolletes platyprosopis</i>	F
<i>Daviesia divaricata</i>	26/09/2015	PC	Koondoola Bushland	<i>Trichocolletes platyprosopis</i>	M
<i>Daviesia horrida</i>	2/8/2016	PH	Koondoola Bushland	<i>Trichocolletes leocogenys</i>	M
<i>Daviesia horrida</i>	2/8/2016	PH	Koondoola Bushland	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia horrida</i>	23/08/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia rhombifolia</i>	11/8/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia rhombifolia</i>	18/08/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia rhombifolia</i>	18/08/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Daviesia rhombifolia</i>	24/08/2017	PH	Lesmurdie - Canning Rd	<i>Trichocolletes leocogenys</i>	F
<i>Hardenbergia comptoniana</i>	3/10/2016	PC	Kings Park	<i>Trichocolletes gelasinus</i>	F
<i>Hardenbergia comptoniana</i>	11/10/2016	PC	Kings Park	<i>Trichocolletes gelasinus</i>	F
<i>Hardenbergia comptoniana</i>	14/10/2016	PC	Kings Park	<i>Trichocolletes gelasinus</i>	F
<i>Jacksonia sternbergiana</i>	11/10/2016	PC	Kings Park	<i>Trichocolletes gelasinus</i>	F
<i>Jacksonia sternbergiana</i>	12/9/2017	PC	Kings Park	<i>Trichocolletes gelasinus</i>	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Trichocolletes</i> sp.	F
<i>Mirbelia dilatata</i>	23/11/2015	W	East side of Nanga Rd	<i>Trichocolletes marginatus</i>	M
<i>Viminaria juncea</i>	20/11/2015	MA	Hillview Rd	<i>Trichocolletes</i> sp.	F

**Appendix, Table S3** Genera of potential pollinators that contacted the reproductive structures of any given species of pea plants. ‘Mean’ is the average of insects landing per Trial (SD = standard deviation); ‘n’ is number of total insect landing per genus; ‘Total N’ is the total number of visits from native potential pollinators while ‘Total NI’ is the number of visits from potential pollinators including native species and the introduced honey bees *Apis mellifera*.

PH	Trials	Native bees				Beetles		Introduced bees		Total N	Total NI
		<i>Trichocolletes</i>		<i>Leioproctus</i>		<i>Neophyllotocus</i>		<i>Apis</i>			
		Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n		
<i>Hovea chorizemifolia</i>	32	-	-	0.3 ± 0.52	10	-	-	0.4 ± 0.55	12	10	22
<i>Hovea pungens</i>	32	-	-	0.8 ± 1.07	25	-	-	2.8 ± 1.39	88	25	113
<i>Daviesia decurrens</i>	32	2.3 ± 1.54	74	0.0	0	-	-	0.8 ± 0.95	24	74	98
<i>Daviesia rhombifolia</i>	32	1.3 ± 1.17	43	0.3 ± 0.47	10	-	-	0.6 ± 0.75	20	53	73
<i>Daviesia horrida</i>	32	2.3 ± 1.38	75	0.0	0	-	-	2.5 ± 1.17	81	75	156
<i>Bossiaea aquifolium</i>	32	-	-	1.3 ± 1.63	40	1.5 ± 1.25	49	2.7 ± 1.23	85	90	175

PC	Trials	Native bees										Beetles		Introduced bees		Total N	Total NI
		<i>Trichocolletes</i>		<i>Euhesma</i>		<i>Lassioglossum</i>		<i>Leioproctus</i>		<i>Megachile</i>		<i>Neophyllotocus</i>		<i>Apis</i>			
		Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n	Mean (± SD)	n		
<i>Bossiaea eriocarpa</i>	18	0.7 ± 0.046	12	-	-	-	-	0.1 ± 0.32	2	-	-	0.6 ± 0.85	10	10.1 ± 4.18	182	24	206
<i>Hardenbergia comptoniana</i>	19	2.5 ± 2.14	48	-	-	0.5 ± 0.69	10	1.3 ± 0.88	25	-	-	-	-	0.9 ± 0.84	18	83	101
<i>Daviesia divaricata</i>	19	3.8 ± 1.34	72	-	-	-	-	3.6 ± 1.29	69	-	-	-	-	1.8 ± 0.89	35	141	176
<i>Isotropis cuneifolia</i>	16	-	-	-	-	0.1 ± 0.34	2	-	-	-	-	3.1 ± 1.78	49	0.6 ± 0.71	10	51	61
<i>Jacksonia sternbergiana</i>	16	1.6 ± 1.54	25	2.8 ± 2.50	45	-	-	2.0 ± 1.78	32	0.2 ± 0.44	3	-	-	1.5 ± 1.36	24	105	129

MA	Trials	Native bees						Beetles		Introduced bees		Total N	Total NI
		<i>Trichocolletes</i>		<i>Exoneura</i>		<i>Lassioglossum</i>		<i>Colymbomorpha</i>		<i>Apis</i>			
		Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n		
<i>Bossiaea linophylla</i>	17	0.6 $\pm$ 0.49	10	3.4 $\pm$ 1.63	58	0.2 $\pm$ 0.44	4	4.2 $\pm$ 3.04	71	1.5 $\pm$ 1.09	26	<b>143</b>	<b>169</b>
<i>Bossiaea disticha</i>	17	0.2 $\pm$ 0.57	4	4.2 $\pm$ 3.13	72.0	0.4 $\pm$ 0.61	6.0	-	-	2.1 $\pm$ 1.93	36	<b>82</b>	<b>118</b>
<i>Viminaria juncea</i>	16	0.3 $\pm$ 0.47	4	4.3 $\pm$ 2.24	68.0	0.0	0.0	9.0 $\pm$ 2.28	144.0	2.1 $\pm$ 40	34	<b>216</b>	<b>250</b>

W	Trials	Native bees						Introduced bees		Total N	Total NI		
		<i>Trichocolletes</i>		<i>Exoneura</i>		<i>Megachile</i>		<i>Lipotriches</i>				<i>Apis m</i>	
		Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n	Mean ( $\pm$ SD)	n			Mean ( $\pm$ SD)	n
<i>Mirbelia dilatata</i>	18	2.5 $\pm$ 1.04	45	0.9 $\pm$ 0.50	16	0.7 $\pm$ 0-48	12	0.2 $\pm$ 0.42	4	3.6 $\pm$ 1.09	65	<b>77</b>	<b>142</b>

**Appendix, Table S4** Pollen occurrence (%) on the body of bees caught foraging on pea plants. The pollen has been identified to the lowest taxonomic level possible. X denotes traces of pollen grains

Insects	Site	Caught on	FAMILY									FABOIDEAE							
			<i>Araliaceae</i>	<i>Asteraceae</i>	<i>Casuarinaceae</i>	<i>Chenopodiaceae</i>	<i>Cyperaceae</i>	<i>Ericaceae</i>	<i>Faboideae</i>	<i>Faboideae</i>	<i>Myrtaceae</i>	<i>Proteaceae</i>	<i>Bossiaea</i>	<i>Daviesia</i>	<i>Hardenbergia</i>	<i>Hovea</i>	<i>Isotrojis</i>	<i>Jacksonia</i>	<i>Mirtella</i>
<i>Apis mellifera</i>	PH	<i>Bossiaea aquifolium</i>									1			97.5					
<i>Apis mellifera</i>	MA	<i>Bossiaea disticha</i>									7			93					
<i>Apis mellifera</i>	MA	<i>Bossiaea disticha</i>									0.5			99.5					
<i>Apis mellifera</i>	PC	<i>Bossiaea eriocarpa</i>		X							X			100		X			
<i>Apis mellifera</i>	PC	<i>Bossiaea eriocarpa</i>			0.5		X				X			99					
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>									1			98					
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>									1			98					
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>									1			96.5					
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>		0.5							4			2	92				
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>												95					
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>												98					
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>			8						1			90					
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>		1							1			45.5					
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>		X	0.5						X			98.5					
<i>Apis mellifera</i>	PH	<i>Daviesia rhombifolia</i>												80					
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>		1							X	X							99
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>		1							X								98
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>		1							X	X							99
<i>Exeneura exeneura</i>	MA	<i>Bossiaea lynophylla</i>									2	0.5		97.5					
<i>Exeneura exeneura</i>	MA	<i>Viminaria juncea</i>																	100
<i>Leioproctus (Euryglossidia)</i>	PC	<i>Jacksonia sternebergiana</i>	0.5			0.5													99
<i>Leioproctus leioproctus</i>	PC	<i>Daviesia divaricata</i>									0.5	3	1.5		46				1

Insects	Site	Caught on	FAMILY									FABOIDEAE							
			<i>Araliaceae</i>	<i>Asteraceae</i>	<i>Casuarinaceae</i>	<i>Chenopodiaceae</i>	<i>Cyperaceae</i>	<i>Ericaceae</i>	<i>Faboideae</i>	<i>Faboideae</i>	<i>Myrtaceae</i>	<i>Proteaceae</i>	<i>Bossiaea</i>	<i>Daviesia</i>	<i>Hardenbergia</i>	<i>Hovea</i>	<i>Isatropis</i>	<i>Jacksonia</i>	<i>Mirbelia</i>
<i>Leioproctus leioproctus</i>	PH	<i>Bossiaea aquifolium</i>										100							
<i>Leioproctus leioproctus</i>	PC	<i>Bossiaea eriocarpa</i>		1	2.5					X		96.5							
<i>Lipotriches australiaca</i>	W	<i>Mirbelia dilatata</i>								100									
<i>Megachile (Hackeriapis)</i>	PC	<i>Bossiaea eriocarpa</i>				20				6	3	65							
<i>Trichocolletes capillosus</i>	PH	<i>Daviesia decurrens</i>								X			100						
<i>Trichocolletes gelasinus</i>	PC	<i>Bossiaea eriocarpa</i>		X	X							99					1		
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>											81	18.5					
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>										1	99						
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>		X	1								98	1					
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>											99				1		
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>											98.5						
<i>Trichocolletes gelasinus</i>	PC	<i>Jacksonia sternbergiana</i>		1							X			14			84		
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia decurrens</i>								X			100						
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia horrida</i>								2			97.5		0.5				
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia horrida</i>								X			100						
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia rhombifolia</i>								-			100						
<i>Trichocolletes sp.</i>	PC	<i>Daviesia divaricata</i>								X			100						
<i>Trichocolletes sp.</i>	W	<i>Mirbelia dilatata</i>						1			72							27	

Insects	Site	Caught on	OTHER GENERA											
			<i>Acacia</i>	<i>Banksia</i>	<i>Burchardia</i>	<i>Canastylis</i>	<i>Drosera</i>	<i>Gladiolus</i>	<i>Grevillea</i>	<i>Drakis</i>	<i>Petrophile</i>	<i>Sauerbaea</i>	<i>Stirlingia</i>	<i>Xanthorrhoea</i>
<i>Apis mellifera</i>	PH	<i>Bossiaea aquifolium</i>	X	1.5										
<i>Apis mellifera</i>	MA	<i>Bossiaea disticha</i>												
<i>Apis mellifera</i>	MA	<i>Bossiaea disticha</i>												
<i>Apis mellifera</i>	PC	<i>Bossiaea eriocarpa</i>	X										X	
<i>Apis mellifera</i>	PC	<i>Bossiaea eriocarpa</i>		0.5										
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>		1										
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>		1										
<i>Apis mellifera</i>	PH	<i>Daviesia decurrens</i>	1	1.5										
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>	0.5											
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>			2.5									
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>	0.5											
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>								52.5				
<i>Apis mellifera</i>	PC	<i>Daviesia divaricata</i>		1						X				
<i>Apis mellifera</i>	PH	<i>Daviesia rhombifolia</i>												
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>												
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>												
<i>Apis mellifera</i>	MA	<i>Viminaria juncea</i>												
<i>Exeneura exeneura</i>	MA	<i>Bossiaea lynophylla</i>												
<i>Exeneura exeneura</i>	MA	<i>Viminaria juncea</i>												
<i>Leioproctus (Euryglossidia)</i>	PC	<i>Jacksonia sternebergiana</i>												
<i>Leioproctus leioproctus</i>	PC	<i>Daviesia divaricata</i>					3					25		20
<i>Leioproctus leioproctus</i>	PH	<i>Bossiaea aquifolium</i>												

Insects	Site	Caught on	OTHER GENERA											
			<i>Acacia</i>	<i>Banksia</i>	<i>Burchardia</i>	<i>Conostylis</i>	<i>Drusera</i>	<i>Glabialis</i>	<i>Grevillea</i>	<i>Dualis</i>	<i>Petrophile</i>	<i>Sowerbaea</i>	<i>Stirlingia</i>	<i>Xanthorrhoea</i>
<i>Leioproctus leioproctus</i>	PC	<i>Bossiaea eriocarpa</i>										X		
<i>Lipotriches australiaca</i>	W	<i>Mirbelia dilatata</i>												
<i>Megachile (Hackeriapis)</i>	PC	<i>Bossiaea eriocarpa</i>		6										
<i>Trichocolletes capillosus</i>	PH	<i>Daviesia decurrens</i>						X						
<i>Trichocolletes gelasinus</i>	PC	<i>Bossiaea eriocarpa</i>												
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>			X						0.5			
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>												
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>	X	X										
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>												
<i>Trichocolletes gelasinus</i>	PC	<i>Daviesia divaricata</i>				0.5					1			
<i>Trichocolletes gelasinus</i>	PC	<i>Jacksonia sternbergiana</i>												
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia decurrens</i>												
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia horrida</i>												
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia horrida</i>												
<i>Trichocolletes leucogenys</i>	PH	<i>Daviesia rhombifolia</i>												
<i>Trichocolletes sp.</i>	PC	<i>Daviesia divaricata</i>												
<i>Trichocolletes sp.</i>	W	<i>Mirbelia dilatata</i>												

**Appendix, Table S5** Behavioural categories displayed by different potential pollinators: foraging on nectar and evidence of contacting the reproductive structures. Numbers are the total number of individuals exhibiting each behaviour.

Potential pollinator taxa	<i>Bossiaea aquifolium</i>	<i>Bossiaea disticha</i>	<i>Bossiaea eriocarpa</i>	<i>Bossiaea linophylla</i>	<i>Daviesia decurrens</i>	<i>Daviesia divaricata</i>	<i>Daviesia horrida</i>	<i>Daviesia rhombifolia</i>	<i>Hardenbergia comptoniana</i>	<i>Hovea pungens</i>	<i>Isotropis cuneifolia</i>	<i>Jacksonia sternbergiana</i>	<i>Mirbelia dilatata</i>	<i>Viminaria juncea</i>	Total	
<b><i>Apis mellifera</i></b>																
N insect landing	85	36	182	26	24	35	81	20	18	88	10	24	65	34	607	
N insect foraging nectar	67	32	170	18	24	35	75	20	16	30	4	21	65	34	482	
N insect contacted reproductive structures	67	18	120	15	10	18	68	17	10	4	1	10	18	24	315	
<b><i>Colymbomorpha</i></b>																
N insect landing				21										240	261	
N insect foraging nectar				15										97	112	
N insect contacted reproductive structures				18										138	156	
<b><i>Euhesma</i></b>																
N insect landing												45			45	
N insect foraging nectar												35			35	
N insect contacted reproductive structures												22			22	
<b><i>Exoneura</i></b>																
N insect landing		72		58										16	68	142
N insect foraging nectar		72		58										16	68	142
N insect contacted reproductive structures		48		44										2	24	70
<b><i>Lasioglossum</i></b>																
N insect landing		6							10		2					12
N insect foraging nectar		6							10		2					12
N insect contacted reproductive structures		4							10							10
<b><i>Leioproctus</i></b>																
N insect landing	40		2			69			25	25		32				153
N insect foraging nectar	35		2			69			19	25		26				116
N insect contacted reproductive structures	33		2			61			15	23		19				97
<b><i>Neophyllotocus</i></b>																
N insect landing	49		10								49					59
N insect foraging nectar	27		4								26					30
N insect contacted reproductive structures	32		6								34					40
<b><i>Megachile</i></b>																
N insect landing												3	12			15
N insect foraging nectar												3	12			15
N insect contacted reproductive structures												3	12			15
<b><i>Trichocolletes</i></b>																
N insect landing	1	4	12	10	74	72	75	43	48			25	45	4		408
N insect foraging nectar	1	4	12	10	64	67	64	31	48			25	45	4		370
N insect contacted reproductive structures	1	4	7	10	23	54	45	25	20			16	28	2		230

**Table S6** Average stamen lengths of pea plant species. Based on clear disjunction in average stamen length between species, pea plants could be classified into two broad groups: short and long stamens species.

	Pea plant species	Stamen length (mm) $\pm$ SD
Short stamens	<i>Hovea chorizemifolia</i>	0.73 $\pm$ 0.37
	<i>Hardenbergia comptoniana</i>	1.29 $\pm$ 0.26
	<i>Daviesia rhombifolia</i>	1.42 $\pm$ 0.14
	<i>Daviesia horrida</i>	1.5 $\pm$ 0.22
	<i>Daviesia divaricata</i>	1.5 $\pm$ 0.25
	<i>Daviesia decurrens</i>	1.98 $\pm$ 0.28
	<i>Hovea pungens</i>	2.08 $\pm$ 0.44
Long stamens	<i>Bossiaea eriocarpa</i>	4.83 $\pm$ 0.67
	<i>Jacksonia sternbergiana</i>	5.44 $\pm$ 0.67
	<i>Isotropis cuneifolia</i>	6.9 $\pm$ 0.57
	<i>Bossiaea aquifolium</i>	8.38 $\pm$ 0.34

**Appendix, Table S7** Stamen lengths ( $\pm$ SD) of pea plants from the PH and PC regions and body length of the visiting bees. For each bee the total length of the body is reported ( $\pm$  SD), except for the specimens represented by one individual. Names in parentheses are subgenera. F: female; M: male

Pea species	Stamen length (mm)	( $\pm$ ) SD	Bee taxa	Bee body length (mm)	( $\pm$ ) SD	Sex
<i>Bossiaea aquifolium</i>	8.38	0.34	<i>Apis mellifera</i>	11.01	0.63	-
<i>Bossiaea aquifolium</i>	8.38	0.34	<i>Leioproctus (Leioproctus)</i>	9.82	1.94	F
<i>Bossiaea eriocarpa</i>	4.83	0.67	<i>Apis mellifera</i>	11.01	0.63	-
<i>Bossiaea eriocarpa</i>	4.83	0.67	<i>Leioproctus (Leioproctus)</i>	9.82	1.94	F
<i>Bossiaea eriocarpa</i>	4.83	0.67	<i>Megachile (Hackeriapis)</i>	8.98	-	F
<i>Bossiaea eriocarpa</i>	4.83	0.67	<i>Trichocolletes gelasinus</i>	14.88	1.70	F
<i>Daviesia decurrens</i>	1.98	0.44	<i>Apis mellifera</i>	11.01	0.63	-
<i>Daviesia decurrens</i>	1.98	0.28	<i>Leioproctus (Leioproctus)</i>	9.82	1.94	F
<i>Daviesia decurrens</i>	1.98	0.28	<i>Trichocolletes capillosus</i>	13.44	0.21	F
<i>Daviesia decurrens</i>	1.98	0.28	<i>Trichocolletes leucogenys</i>	12.61	0.94	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Leioproctus (Euryglossidia)</i>	9.55	1.28	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Leioproctus (Leioproctus)</i>	9.82	1.94	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Trichocolletes dives</i>	17.84	-	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Trichocolletes gelasinus</i>	14.88	1.70	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Trichocolletes platyprosopis</i>	13.14	-	F
<i>Daviesia horrida</i>	1.50	0.22	<i>Apis mellifera</i>	11.01	0.63	-
<i>Daviesia horrida</i>	1.50	0.22	<i>Trichocolletes leucogenys</i>	12.61	0.94	F
<i>Daviesia rhombifolia</i>	1.42	0.14	<i>Apis mellifera</i>	11.01	0.63	-
<i>Daviesia rhombifolia</i>	1.42	0.14	<i>Trichocolletes leucogenys</i>	12.61	0.94	F
<i>Hardenbergia comptoniana</i>	1.29	0.26	<i>Lassioglossum</i>	7.69	0.77	F

Pea species	Stamen length (mm)	(±) SD	Bee taxa	Bee body length (mm)	(±) SD	Sex
<i>Hardenbergia comptoniana</i>	1.29	0.26	<i>Leioproctus (Leioproctus)</i>	9.82	1.94	F
<i>Hardenbergia comptoniana</i>	1.29	0.26	<i>Trichocolletes gelasinus</i>	14.88	1.70	F
<i>Hovea pungens</i>	2.08	0.44	<i>Apis mellifera</i>	11.01	0.63	-
<i>Isotropis cuneifolia</i>	6.90	0.57	<i>Lassioglossum</i>	7.69	0.77	F
<i>Jacksonia sternbergiana</i>	5.44	0.57	<i>Euhesma</i>	5.82	0.01	F
<i>Jacksonia sternbergiana</i>	5.44	0.67	<i>Leioproctus (Euryglossidia)</i>	9.55	1.28	F
<i>Jacksonia sternbergiana</i>	5.44	0.67	<i>Trichocolletes gelasinus</i>	14.88	1.70	F
<i>Daviesia divaricata</i>	1.50	0.25	<i>Leioproctus (Leioproctus)</i>	7.98	0.36	M
<i>Daviesia divaricata</i>	1.50	0.25	<i>Trichocolletes platyprosopis</i>	12.64	0.35	M
<i>Daviesia rhombifolia</i>	1.42	0.14	<i>Trichocolletes capillosus</i>	11.62	0.76	M
<i>Daviesia decurrens</i>	1.98	0.28	<i>Trichocolletes capillosus</i>	11.62	0.76	M
<i>Daviesia horrida</i>	1.5	0.22	<i>Trichocolletes leucogenys</i>	12.88	-	M
<i>Hovea pungens</i>	2.08	0.44	<i>Leioproctus (Leioproctus)</i>	7.98	0.36	M
<i>Daviesia decurrens</i>	1.98	0.28	<i>Leioproctus (Leioproctus)</i>	7.98	0.36	M
<i>Hardenbergia comptoniana</i>	1.29	0.26	<i>Trichocolletes gelasinus</i>	12.98	-	M

**Appendix, Table S8** Nectar volume of pea plant species, mean and standard deviation

Pea plant species	Nectar volume $\mu\text{l}$ (Mean $\pm$ SD)
<i>Bossiaea aquifolium</i>	0.14 $\mu\text{l} \pm 0.10$
<i>Bossiaea eriocarpa</i>	0.28 $\mu\text{l} \pm 0.27$
<i>Daviesia decurrens</i>	0.15 $\mu\text{l} \pm 0.06$
<i>Daviesia divaricata</i>	0.25 $\mu\text{l} \pm 0.10$
<i>Daviesia horrida</i>	0.09 $\mu\text{l} \pm 0.04$
<i>Daviesia rhombifolia</i>	0.09 $\mu\text{l} \pm 0.04$
<i>Hardenbergia comptoniana</i>	0.16 $\mu\text{l} \pm 0.06$
<i>Hovea chorizemifolia</i>	0.14 $\mu\text{l} \pm 0.07$
<i>Hovea pungens</i>	0.21 $\mu\text{l} \pm 0.16$
<i>Isotropis cuneifolia</i>	0.15 $\mu\text{l} \pm 0.07$
<i>Jacksonia sternbergiana</i>	0.36 $\mu\text{l} \pm 0.25$

## Chapter 3

This Chapter will be send to *Botanical Journal of the Linnean Society*.

### **A general pea flower image? Ecological factors affecting reproductive success in an orchid that exhibits imperfect floral mimicry**

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## Chapter 3

### A general pea flower image? Ecological factors affecting reproductive success in an orchid that exhibits imperfect floral mimicry

#### ABSTRACT

Co-occurring rewarding plants can enhance the reproductive success of plants that are pollinated by mimicry of rewarding flowers. However, it is not always possible to readily discern between the effects of model and non model plants that can act as magnet to pollinators. Here we tested for mimicry of co-occurring pea plants (Fabaceae) in *Diuris magnifica* (Orchidaceae) and if other ecological factors, such as number of pea plant flowers, habitat remnant size and frequency of conspecifics influence reproductive success of *D. magnifica*.

*D. magnifica* was pollinated by *Trichocolletes* bees (Colletidae) which displayed similar behaviours when attempting to feed at the labellum of the orchid and the keel of several co-occurring pea plants. Quantification of floral spectral reflectance suggested that the pea plants *Bossiaea eriocarpa*, *Daviesia divaricata*, *Jacksonia sternbergiana* were likely to represent model plants, while the pea plant *Hardenbergia comptoniana* was likely a non model pea plant. Orchid reproductive success was not affected by the number of flowers of the model plants, but there was evidence that pollination rate is enhanced by the presence of flowering *H. comptoniana*. Pollination success of the orchid decreased with higher density of conspecifics, but was not related to *Trichocolletes* occurrence, possibly due to the likely contribution of sub-optimal pollinators (*Apis* and beetles). Lastly, there was evidence for higher fruit set for *D. magnifica* in larger habitat remnants.

*D. magnifica* may reveal new ecological insights, especially on the definition of model and magnet plants, the role of the foraging behaviour and habitat size in food mimicry systems.

**Key words:** *Diuris magnifica*, Fabaceae, Colletidae, plant fitness, mimicry, plant frequency, pollinator behaviour, food deception

## INTRODUCTION

Rather than providing a reward to pollinators, it has been estimated that one third of orchid species attract their pollinators through deception (van der Pijl & Dodson, 1966; Dressler, 1981; Ackerman, 1986; Renner, 2006). A diversity of deceptive strategies are used, including mimicry of floral rewards (Ackerman, 1986), females of the pollinator species (Coleman, 1928; Schiestl *et al.*, 1999, 2003), brood and shelter sites (Jones, 1960; Martos *et al.*, 2015), and alarm pheromones (Brodmann *et al.*, 2009). Among deceptive orchids, the most common pollination strategy is food deception, where the rewardless orchid displays floral signals typically associated with rewarding plants (Jersáková, Johnson, Kindlmann, 2006). Pollination by food deception ranges between generalised food deception, where the orchid uses floral signals that are attractive to pollinators but without closely resembling any specific model species (Jersáková *et al.*, 2006), and floral mimicry, where the orchid closely mimics one or more species of model food plants (Brown & Kodric-Brown, 1979; Roy & Widmer, 1999; Johnson, 1994; 2000). Aside from morphological and colour similarity to lure pollinators, in mimicry systems it is expected that pollinators will exhibit the same foraging behaviour on model and mimic (e.g. Scaccabarozzi *et al.*, 2018; De Jager & Anderson, 2019), and that mimicry will be more effective with higher ratio of model to mimic flowers (Anderson & Johnson, 2006) through reduced opportunity for pollinator learning (Bierzychudek, 1981; Dafni & Ivri, 1981; Ruxton, Sherratt, Speed, 2004).

While there is experimental evidence that mimic fitness is greater when they are scarce relative to the model (Johnson, 1994; Anderson & Johnson, 2006), or occur in populations at low density (Ackerman, Meléndez Ackerman, Salguero Faria, 1997, Ferdy *et al.*, 1999; Smithson & Gigord, 2001, Pellegrino *et al.*, 2005), other factors can also influence reproductive success in food deceptive systems. In particular, the presence of rewarding plant species can increase the pollination success of non-rewarding or less rewarding co-flowering plant species in the floral community (i.e. the magnet effect species; Thomson, 1978; Feinsinger *et al.*, 1986, Feinsinger, 1987; Lavery, 1992; Johnson, Alexandersson, Linder, 2003) through increasing the local abundance of pollinators (cf. Kunin, 1993). Several studies highlight that the vicinity to other rewarding plant species influences the frequency of pollination or foraging

behaviour (Thomson, 1978; Root, 1973; Holt & Lawton, 1994; Callaway, 1995; Hambačk, Agren, Ericson, 2000). For example, the pollination success of the orchid *Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W. Chase is enhanced by the co-presence of the nectar producing plants *Geum rivale* L. (Rosaceae) and *Allium schoenoprasum* L. (Alliaceae) (Johnson *et al.*, 2003). Alternatively, other studies have found evidence for competition, where neighbouring plants that provide a greater reward draw pollinators away from plants (Free, 1968; Waser, 1983; Lammi & Kuitunen, 1995) with no or meagre reward. In *Dactylorhiza sambucina* (L.) Sođ, an orchid pollinated by generalised food deception, fitness was reduced by the abundance of the rewarding *Muscari neglectum* Ten. (Asparageaceae) which displays dissimilar floral colour (Internicola *et al.*, 2006). As such, deceptive orchids can be subject to both facilitation and competition from co-occurring plants, and the effect of magnet plants on the pollination success on non-rewarding plants is far from generalizable (Peter & Johnson, 2008).

Habitat fragmentation, as one of the leading cause of biodiversity decline (Pimm & Raven, 2000; Goddard, Andrew, Benton., 2010), can cause to reduced abundance of pollinators in small and / or isolated habitat remnants (Cunningham, 2000), leading to reduced plant reproductive success via pollination limitation (Nayak & Davidar, 2010; Pauw & Bond, 2011). The impact of habitat fragmentation may often be most severe on plants with specialised pollination systems (Newman *et al.*, 2013), as they are vulnerable to loss of just a single pollinator species. For example, in a guild of orchids pollinated by oil-collecting bees, there has been a gradual extinction of species from urban remnants following decline of the pollinator species, with more clonal species showing greater persistence (Pauw & Bond, 2011; Pauw & Hawkins, 2011). Nonetheless, the ability of some species to persist in the face of pollinator decline (Murren, 2002) highlights that the full effects of habitat fragmentation on many orchid populations are yet to be witnessed (e.g. Phillips *et al.*, 2015), and that more studies are needed to test the potential effect of habitat fragmentation on plant-pollination interactions, especially on the long-term (Xiao *et al.* 2016).

In particular, there has been relatively little research on the response of orchids pollinated by food mimicry to habitat fragmentation, but given that these systems are often specialised on one or few pollinator species (Newman, Anderson, Johnson, 2012; Johnson & Schiestl, 2016), they are expected to be highly vulnerable.

Based on pronounced similarity in colour and morphology to co-occurring pea plants, several species in the primarily Australian orchid genus *Diuris* Sm. are predicted to be pollinated by floral mimicry of a guild (Beardsell *et al.*, 1986; Dafni & Bernhardt, 1990). The mimicry system tends to be specialised on faboideae legumes (Edens-Meier & Bernhardt, 2014) which are primarily visited by *Trichocolletes* bees (Rayment, 1929, 1935; Houston, 2018). While this hypothesis has subsequently received support in the form of pollinator sharing between *Diuris* and pea plants, and similar patterns of UV reflection (Beardsell *et al.*, 1986; Indsto *et al.*, 2006), mimicry in *Diuris* has only recently been tested in detail. In *Diuris brumalis*, it was shown that not only do models and mimic show overlap in floral colour and greater morphological similarity than the remainder of the plant community, but the bee pollinator exhibits the same stereotyped foraging behaviours on both model and mimic (Scaccabarozzi *et al.*, 2018). In *D. brumalis*, fitness increased with frequency of model plants, though this is likely to be through a facilitation effect as the bee pollinator feeds almost exclusively on *Daviesia* Sm. pea-plants and was almost absent from sites where *Daviesia* were rare or absent (Scaccabarozzi *et al.*, 2018).

#### *Aims and hypotheses*

Here we investigate the pollination of *Diuris magnifica* D.L.Jones, a species hypothesised to be a putative pea flower mimic, on the basis of similarity in flower shape and colour of co-occurring pea plants. Based on morphology, *D. magnifica* is believed to be closely allied to *D. brumalis* (Brown *et al.*, 2013), but it grows in areas with several co-flowering pea plant species, and much of its remaining habitat is now in habitat remnants within an urban environment. Firstly, we investigated the possibility of floral mimicry testing the following predictions: (i) *D. magnifica* shares a pollinator with co-occurring pea plants (ii) colour overlaps between putative models and mimic, but not with the remainder of the floral community; (iii) the flowering phenology of the proposed mimic overlaps with the models; (iv) the pollinator exhibits with the mimic the deceived behaviour normally only associated with the model. Subsequently, we tested whether the reproductive success of *D. magnifica* (i) increases with the frequency of model species (ii) increases with the frequency of non-model food plants (iii) decreases at higher orchid density and (iv) decreases in small habitat remnants.

## MATERIALS AND METHODS

### *Study species*

*Diuris magnifica* is endemic to the Swan Coastal Plain in Western Australia, with its main distribution centered on what is now the Perth metropolitan area. Flowering occurs from late winter to early spring, with between three and nine yellow, orange and purple flowers produced per inflorescence (Brown *et al.*, 2013). The primary habitat of *D. magnifica* is mixed L.f., *Allocasuarina* L.A.S.Johnson and *Eucalyptus* L'Her. woodland (Brown *et al.*, 2013), where it co-occurs with a range of co-flowering Faboideae species (Fig. 1): *Daviesia divaricata* Benth., *Bossiaea eriocarpa* Benth. *Hardenbergia comptoniana* (Andrews) Benth., *Jacksonia sternbergiana* Benth. and *Isotropis cuneifolia* (Sm.) Benth. ex Heynh. (Marshall, 1995). These pea plants produce floral nectar, while *D. magnifica* does not [Newmann *et al.*, 2013; Appendix, S1]. As for the related *D. brumalis*, a vector is required for pollination of *D. magnifica* (Scaccabarozzi *et al.*, 2018), the flowers are self-compatible [Supplementary data, Appendix S1] and pollen deposition is the primary limitation to reproduction in *D. magnifica*, as most or all flowers on a scape produce fruit when hand-pollinated (Newman *et al.*, 2013).



**Fig. 1** A, *Diuris magnifica* (Orchidaceae) and co-flowering pea plants (Faboideae): B, *Daviesia divaricata*; C, *Bossiaea eriocarpa*; D, *Isotropis cuneifolia*; E, *Jacksonia sternbergiana*; F, *Hardenbergia comptoniana*.

### *Study sites*

We surveyed populations of *D. magnifica* for reproductive success during 2015 and 2017, while in 2015 and 2016 we carried out pollinator observations. The orchid populations were distributed across 10 bushland remnants within the metropolitan area of the city of Perth [Supplementary data, Table S1, A-B]. *D. magnifica* was the only *Diuris* species observed in flower in the study sites / period. All the study species were vouchered and identified at the Herbarium of Western Australia in Perth [Supplementary data, Table S2].

### *Observation of pollinators on Diuris magnifica*

To identify the pollinators of *D. magnifica* and quantify their behaviour, observation periods of insects visiting orchid flowers were performed at two sites in Koondoola bushland (S 31°50'06.8" E 115°51'83.4") and Kings Park (S 31°57'25.9" E 115°49'89.9") between 26 August to 28 September 2015 and 24 August to 13 September 2016. These sites were selected as they are two of the largest woodland remnants within the study region, have relatively intact vegetation communities, and *D. magnifica* was common. We conducted 248, 15-minutes observation periods (for a total of 3720 minutes observation), recording the insect behavior with EOS M video camera (Canon, Tokyo, Japan) for a subsequent examination in slow motion. Observations were conducted between 9.00 to 17.30, with temperatures ranging between 14 °C to 30 °C, as measured with a Smartsensor AR827 set 20 cm above the ground. Observations were made using arrays of picked orchid flowers (two inflorescences per vial, each with 4–6 flowers, three vials per trial, 10-20 cm apart; Scaccabarozzi *et al.*, 2018) placed 1–2 meters from flowering individuals of *D. divaricata*, *B. eriocarpa*, *J. sternbergiana* and *I. cuneifolia*.

For each insect visiting a flower of *Diuris* and pea plants, the behaviour was recorded for eight categories following Scaccabarozzi *et al.*, 2018 [Supplementary data, Table S3]. Due to the very rapid approaches of pollinators, behaviour was only recorded for the first flower visited, as tracking accurately the subsequent visit, was often impossible.

Insects pollinating *D. magnifica* and pea plant flowers, especially those bearing the visible white pollinaria of *D. magnifica*, were collected for identification. All collected

specimens were submitted as vouchers to the Western Australian Museum as voucher specimens [Supplementary data, Table S4]. To confirm the food plants of the floral visitors of *D. magnifica* and pea plants, pollen identification from the bodies of bees caught was previously done as part of a study of the pollination of communities of Faboideae.

#### *Pollinators of pea plants and behavioural comparison*

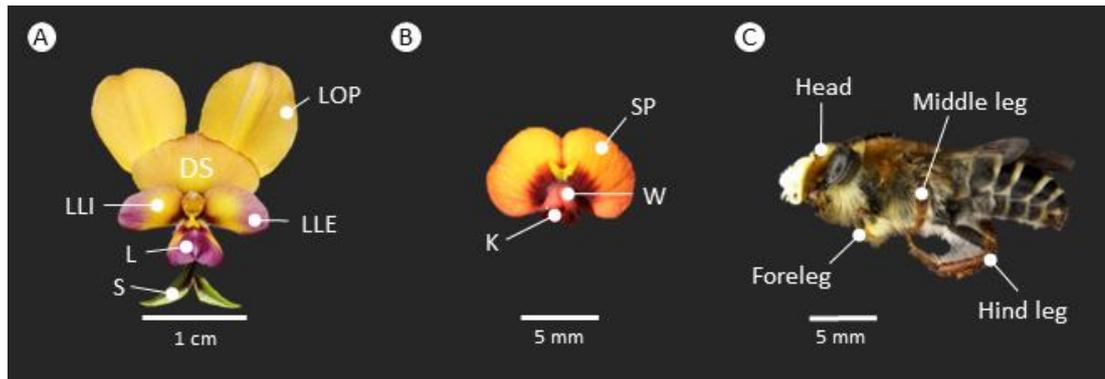
Observations of floral visitors at pea plants at the Koondola bushland and Kings Park sites were previously undertaken as part of a study of the pollination of communities of Faboideae (Scaccabarozzi *et al.*, in review). Based on the previous observations, we calculated the *Trichocolletes* frequency (number of visits per hour) on the co-flowering pea plants. This dataset enables us to test if *D. magnifica* shares pollinators with co-occurring Faboideae species and make comparisons of the behavior of bees when visiting *D. magnifica* and Faboideae. These observations were made between the 26<sup>th</sup> of August and the 28<sup>th</sup> of September 2015 and between the 24<sup>th</sup> August and 14<sup>th</sup> of October 2016 from 9.00 and 17.30. During 20-minute observation periods pollinator visits were recorded with the same video camera as described above. Two of the eight behavioural categories recorded, landing and manipulation, were selected for a formal comparison of pollinator behaviour on the orchid and pea plants. We used a Generalised Linear Model (GLM) assuming a Bernoulli distribution of the response variable (which is, presence-absence of the behaviour studied) in R Studio Version 1.0.44. Pea plant species was treated as a fixed effect. We tested the difference between *D. magnifica* and co-occurring pea plants (*B. eriocarpa*, *D. divaricata*, *H. comptoniana*, *J. sternbergiana*) in the proportion of (1) bees landing on the flower (2) among landing insects, bees manipulating the tri-lobed labellum / keel, the latter category including attempts to forage nectar and pollen. Because of the multiple comparisons involving *D. magnifica* and different pea plant species (four comparisons in total), the threshold for the significance was considered to be 0.0125 through a Bonferroni correction. *Isotropis cuneifolia* was not included in the analysis because it was not visited by *Trichocolletes*.

### *Pollen analysis*

As a complementary approach to resolving the food plants of the floral visitors, pollen was identified from the bodies of insects caught during pollinating *D. magnifica* and pea plants. Pollen observed on the tibiae or abdomen of pollinators during identification was removed by washing the insect with distilled water, acetolysed following the methods of Erdtmann (1960), and mounted on glass microscope slides. All pollen samples were identified under high magnification (Olympus-BX 51 microscope with Olympus-DP71 camera, Olympus, Tokyo, Japan) by comparison with acetolysed mounted pollen samples from herbarium specimens of *Bossiaea eriocarpa*, *Daviesia divaricata*, *Hardenbergia comptoniana*, *Jacksonia sternbergiana* and other commonly co-flowering plant taxa.

### *Spectral reflectance*

To test if bees are likely to be able to discern the floral color of *D. magnifica* flowers from the co-flowering pea plants species (*B. eriocarpa*, *D. divaricata*, *H. comptoniana*, *I. cuneifolia*, *J. sternbergiana*), we measured and analyzed floral spectral reflectance. Spectral reflectance was analysed using the colour hexagon model, based on the sensitivities of photoreceptors of the bee *Apis mellifera* (Chittka, 1992; Chittka & Kevan, 2005). Additionally, spectral reflectance was also measured for other common yellow-flowered rewarding species occurring at all sites, i.e. *Hibbertia hypericoides* (DC.) Benth. (Dilleniaceae), *Acacia pulchella* R.Br. (Fabaceae), *Conostylis aculeata* R.Br. (Haemodoraceae) and the pink flowered *Hypocalymma robustum* Schauer (Myrtaceae). Two flowers per plant from six randomly chosen individuals of each species were selected for measuring spectral reflectance using a spectrometer (Jaz, DH-2000 UV-VIS-NIR Light source) with an integration time of 50 milliseconds. In *D. magnifica*, spectral reflectance measurements were undertaken from the outer lateral petals (LOP), the center of the dorsal sepal (DS), the labellum (L), and the internal (LLI) and external (LLE) parts of the lateral labellum lobe. In pea plants measurements were taken from the standard (SP) and wing (W) petals (Fig. 2, A-B). For the other co-occurring species measurements were taken from the corolla, or stamens in the case of *A. pulchella*. Distances between colour loci were quantified using Euclidean distance in the colour hexagon model.



**Fig. 2** A, Floral morphology of *Diuris magnifica* (Orchidaceae) based on the terminology from Hoffman and Brown (2011): LOP (lateral outer petal), DS (dorsal sepal), LLE (external labellum lateral lobe), LLI (internal labellum lateral lobe), L (labellum), S (sepal); B, a pea-like flower (Faboideae) morphology; C, Male of *Trichocolletes gelasinus* carrying orchid pollinaria on the head with three pair legs used for foraging nectar (foreleg and middle leg) and storing pollen (hind leg) on pea plants.

#### *Flowering phenology of study species*

To test if the flowering period of *D. magnifica* overlaps with the flowering period of the proposed models, flowering was quantified across the study period for *D. magnifica* and the co-occurring pea plants. For each species, weekly counts of open flowers were undertaken in 30 x 30 m quadrats at three sites (two in Koondola and one in Kings Park) from 28 June to 18 October 2017. For pea plants, due to the high number of flowers, we scored the total number of flowers per quadrat as binned categories from 1 (100 flowers) to 25 (2500 flowers) increasing in 100 flower increments. However, in the case of *D. magnifica* and *I. cuneifolia*, due to the small number of flowers per inflorescence, the total number of flowers on each plant was quantified.

#### *Reproductive success of D. magnifica in relation to the abundance of pea plant models*

In 2015 and 2017 the proportion of *D. magnifica* flowers with pollinaria removal and the proportion of fruit formation was quantified at 15 sites (populations) in a single 30 x 30 meter quadrat. We focused on these large remnants in an attempt to minimize the effect of habitat fragmentation when attempting to understand the role of food plants on fitness of *D. magnifica*. At the peak flowering period for *D. magnifica* we recorded: (i) the estimated number of flowers for each pea plant species; (ii) the number of *D. magnifica* plants and

flowers. For the pea plants, variable (iii) was estimated by averaging the number of flowers per stem for ten stems and then multiplying by the number of stems (Scaccabarozzi *et al.*, 2018). In both years, at the end of the flowering period of *D. magnifica* we collected data on the number of flowers in the population with pollinaria removal and the number of produced fruits.

We analysed the relationship of proportion of pollinaria removal and proportion of fruit set with the following independent variables: i) number of flowers of putative model pea plants, ii) number of flowers of the non-model food plant *H. comptoniana*, iii) number of orchid plants per quadrat. *Isotropis cuneifolia* was excluded from the quantification of co-flowering plants because it is not visited by the primary pollinator of *D. magnifica*. A test using a Pearson correlation coefficient confirmed that these variables were not collinear and were therefore included in the same model. Data was analysed using GLMM (Generalized Linear Mixed Effect Model) in R Studio Version 1.0.44 through lme4 and nlme packages. The model was a two-way nested GLMM that included identity of the habitat remnant and population as random effects. The response variables (proportion of pollinaria removal and proportion of fruit set) were assumed to be binomially distributed.

In the case of a binomial model we have that the average of the response variable is equal to  $e^{(\text{intercept} + BX)} / 1 + e^{(\text{intercept} + BX)}$ . Therefore the relation if significant, is shaped as an exponential, unless we provided a transformation in the independent variable but this is not the case. Year was originally included as covariate but, due to a lack of significant effect and increasing the AIC, it was removed from the final model. However, the repeat surveys across sites (in 2015 and 2017) were accounted for by having site as a random effect.

Pearson type residuals were extracted from the model and were tested as a response variable in a GAM (Generalized Additive Model) to check for any non-linear patterns. When testing the effect of the number of *H. comptoniana* flowers on residuals from the GLMM, for fruit set there was 34.2% of the deviance of the residuals from the model was explained by non-linear patterns of the number of *H. comptoniana* flowers. As such, we repeated the analysis using a GAMM (General Additive Mixed Effect Model) in R Studio version 1.0.44 by using gamm4 package. The GAMM approach provides the benefit when residuals from the linear model show clear non-linear

pattern, as seen here (Zuur, 2012). For the GAMM analysis, we considered the same covariates (with the addition of a smooth term only for the covariate number of *H. comptoniana* flowers) and random effects. The use of the GAMM rather than GLMM lead to a decrease in the AIC value of more than 11 points (from 128.8 to 117.42) suggesting a better fitting model.

*Pollinator occurrence, habitat remnant size, and orchid reproductive success*

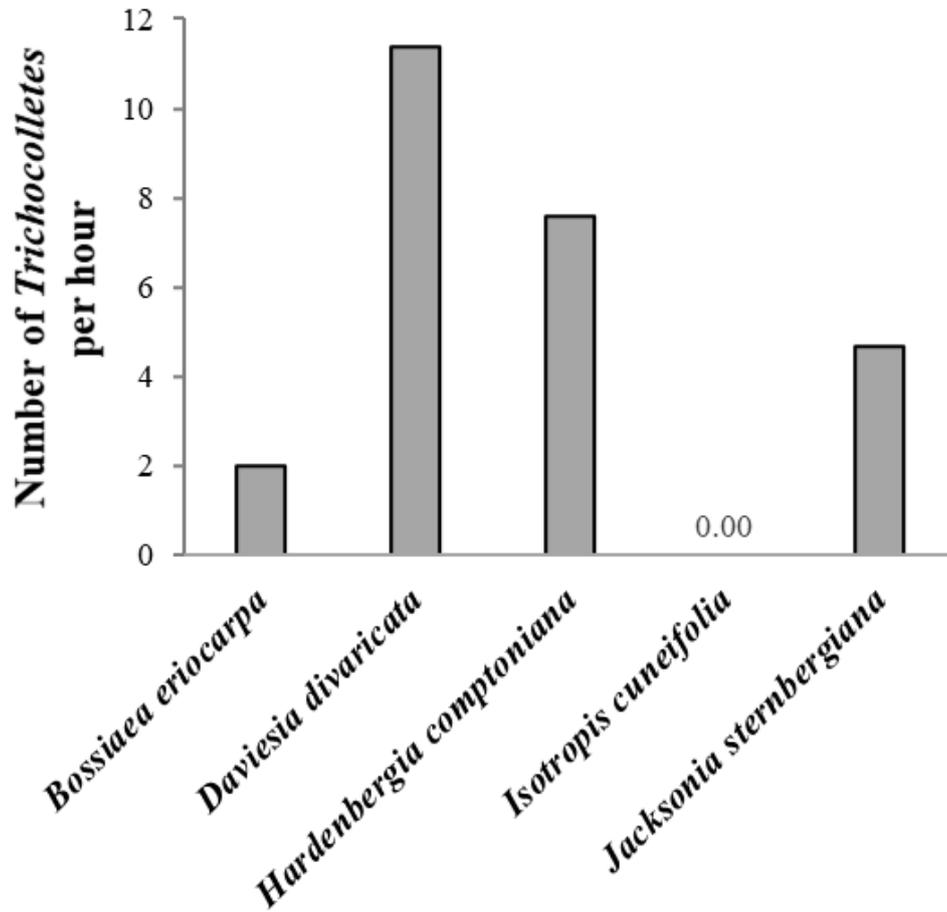
To test if the habitat remnant size and the presence of *Trichocolletes* affected the reproductive success of *D. magnifica*, in 2017 we quantified plant reproductive success (pollen removal, fruit set) for additional five sites (over the 15 ones used in previous analysis on orchid reproductive success), with one in each of five small habitat remnants [Supplementary data, Table S1 B]. We carried out two observation transects for all 20 sites from the 5<sup>th</sup> of September to 15<sup>th</sup> of September 2017, recording the occurrence of *Trichocolletes* along a transect centered on the quadrats used to quantify reproductive success of *D. magnifica*. These transects were 100 m in length, and were positioned so that the quadrat where orchid fitness was measured included the central part of the transect. Each transect took 40 minutes to complete, with an average of approximately three minutes of observations per flowering plant in the understory. Transects were repeated a week after the initial survey, following the same route. For the analysis, *Trichocolletes* occurrence was expressed as presence/absence, to reflect that the survey may not have provided accurate quantification of their abundance. Sizes of habitat remnants were taken from those reported in Bush Forever (2000). For both the analysis of pollinaria removal and fruit set, to avoid collinearity separate GLMMs were undertaken for the variables *Trichocolletes* occurrence and remnant size. For both the analyses, bushland remnant was treated as a random effect to take into account the multiple sites within the larger remnants.

## RESULTS

### *Pollinators of Diuris magnifica*

In total 248 insects were observed visiting experimental arrays of *D. magnifica*. Of the total visits, 98 were by *Trichocolletes* spp. (Colletidae, Hymenoptera), 65 by the introduced honey bee *Apis mellifera* (Apidae; Hymenoptera), 19 by *Neophyllotocus* sp. (Scarabeidae; Coleoptera), 11 by Syrphidae (Diptera), 47 by *Pollaninus* sp. (Zygaenidae; Lepidoptera), 7 by *Lassioglossum* sp. (Halictidae; Hymenoptera) and 1 by *Leioproctus* sp. (Colletidae; Hymenoptera). Only *Trichocolletes* spp., *Apis mellifera* and *Neophyllotocus* sp. were observed carrying pollinaria of *D. magnifica*. In each case, pollinaria was attached to the frontal region of the head (Fig. 2, C). Twenty-five *Trichocolletes* were observed to remove the pollinaria, with two individuals observed carrying and depositing pollinia. Alternatively, *A. mellifera* and *Neophyllotocus* sp. were observed three times to extract and deposit orchid pollinia on the stigma of the same flower.

During observations of floral visitors, fifteen *Trichocolletes* individuals were observed carrying pollinaria of *D. magnifica*, nine while visiting the orchid, and six while foraging on either *D. divaricata*, *J. sternbergiana* and *H. comptoniana*. No other insect species were observed carrying the pollen of *D. magnifica* when foraging on other plant species. A total of 15 insects (nine *Trichocolletes gelasinus*, two *A. mellifera*, two *Neophyllotocus* sp., one *Pollaninus*, one Syrphidae) were caught for identification during arrays of orchid flower experiments and 34 during observations of pea plants [Supplementary data, Table S4]. The individuals of *Trichocolletes* spp. caught on *D. magnifica* and on pea plants included both females (4) and males (7). Only one *Trichocolletes platyprosopis* was identified in 2015, whereas ten *T. gelasinus* were identified both in 2016 and 2017 [Supplementary data, Table S4]. It has previously been showed by Scaccabarozzi *et al.* (2018) that in this area *Trichocolletes* forage on the pea plants. *Trichocolletes* frequency (number of visits per hour) on pea plants revealed the pollinators visited primary *D. Divaricata* (~11), secondly *H. comptoniana* (~7), thirdly *J. sternbergiana* (~4) and lastly *B. eriocarpa* (~2). No *Trichocolletes* were observed on *I. cuneifolia* (Fig. 3).



**Fig. 3** Number of *Trichocolletes* insects per hour on the co-flowering pea plants with *Diuris magnifica*.

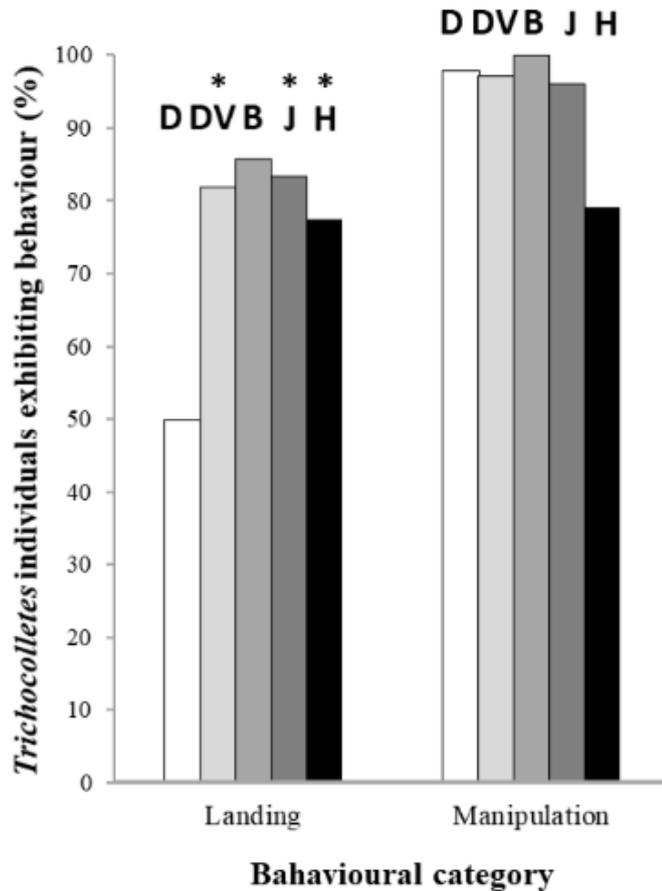
#### *Description of pollinator behaviour*

Male and females of *Trichocolletes* spp. visited individual flowers of *D. magnifica* for 1–2 seconds. Visits included apparent patrolling behaviour by males, when they inspected multiple flowers without landing. Of the *Trichocolletes* visiting *D. magnifica*, 50 % landed on the flowers, with the body aligned along the centre of the labellum, with the head facing towards the column. Of the *Trichocolletes* that alighted (n = 98), 98% attempted to manipulate the labellum, with repeated movements of fore-middle legs as observed on pea plants, facing with the head at the base of the corolla when foraging nectar (Fig. 4). Due to the quick visits by *Trichocolletes*, we only recorded the behaviour of *Trichocolletes* that landed for more than one second. Of the insects attempting to manipulate the labellum (n = 48), 52 % removed the pollinaria, 8.3% deposited the pollen on the stigma and 20.8% visited another orchid flower.



**Fig. 4** Foraging activity of *Trichocolletes gelasinus* on different pea plant species: A, *Daviesia divaricata*; B, *Hardenbergia comptoniana*; C, *Jacksonia sternbergiana*. The arrays indicate the use of fore, middle and hind legs for manipulating the wing petals when foraging on pea plants.

Significantly, more visitors landed on the pea plant species *D. divaricata* ( $n=88$ ;  $\beta = 1.5 \pm 0.34$  SE;  $p < 0.001$ ), *J. sternbergiana* ( $n=30$ ;  $\beta = 1.61 \pm 0.53$  SE;  $p = 0.002$ ) and *H. comptoniana* ( $n=62$ ;  $\beta = 1.23 \pm 0.37$  SE;  $p < 0.001$ ) compared with *D. magnifica* but not on *B. eriocarpa* ( $n=14$ ;  $\beta = 1.79 \pm 0.79$  SE;  $p = 0.023$ ; Fig 5). There was no significant difference in the frequency with which *Trichocolletes* attempted to manipulate the keel when foraging on *D. magnifica* and *B. eriocarpa* ( $n=12$ ;  $\beta = 14.7 \pm 1.82$  SE;  $p = 0.994$ ), *H. comptoniana* ( $n=38$ ;  $\beta = -2.54 \pm 1.07$  SE;  $p = 0.018$ ), *D. divaricata* ( $n=70$ ;  $\beta = -0.32 \pm 1.24$  SE;  $p = 0.799$ ) and *J. sternbergiana* ( $n=24$ ;  $\beta = -0.69 \pm 1.44$  SE;  $p = 0.629$ ; Fig. 5).

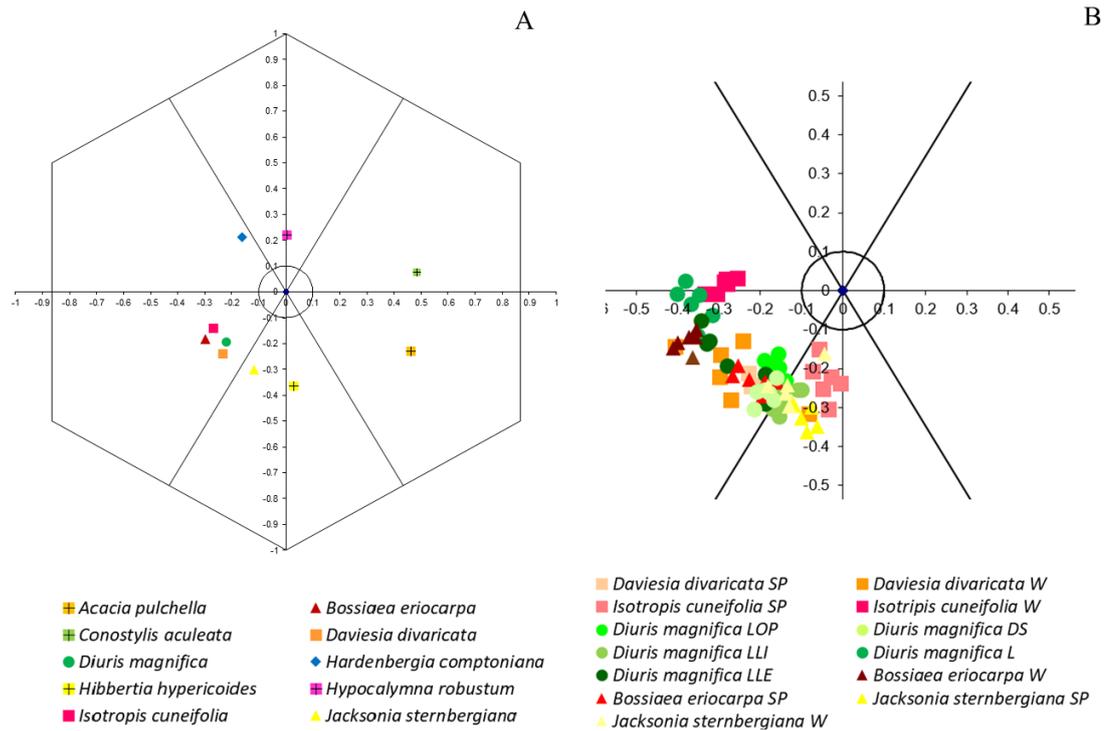


**Fig. 5** Behavioural categories (Landing and Manipulation) comparison between *Diuris magnifica* (D) and the co-flowering pea plants visited by *Trichocolletes*: *Daviesia divaricata* (DV), *Bossiaea eriocarpha* (B), *Jacksonia sternbergiana* (J), *Hardenbergia comptoniana* (H). Landing: alight on the orchid or pea plant flower; Manipulation: attempt to manipulate the flower during the foraging behaviour for either nectar or pollen. \*: indicates a significant difference between the pea plant species and the orchid of a given behavioural category.

#### *Spectral reflectance*

Based on the hexagon bee vision model (Chittka, 1992; Chittka & Kevan, 2005), the average colour loci of the spectral reflectance of *D. magnifica*, *B. eriocarpha*, *D. divaricata* and *I. cuneifolia* was in the UV-region. The average colour loci of *H. comptoniana* corresponded to the UV-blue region (Fig. 6, A). *J. sternbergiana* and *H. hypericoides* average colour loci were positioned in the UV-green, *A. pulchella* and *C. aculeata* were situated in the green region, while *Hypocalymna robustum* was in the blue region. Distances of the mean colour loci measured on flower parts between *D. magnifica* and *B. eriocarpha*,

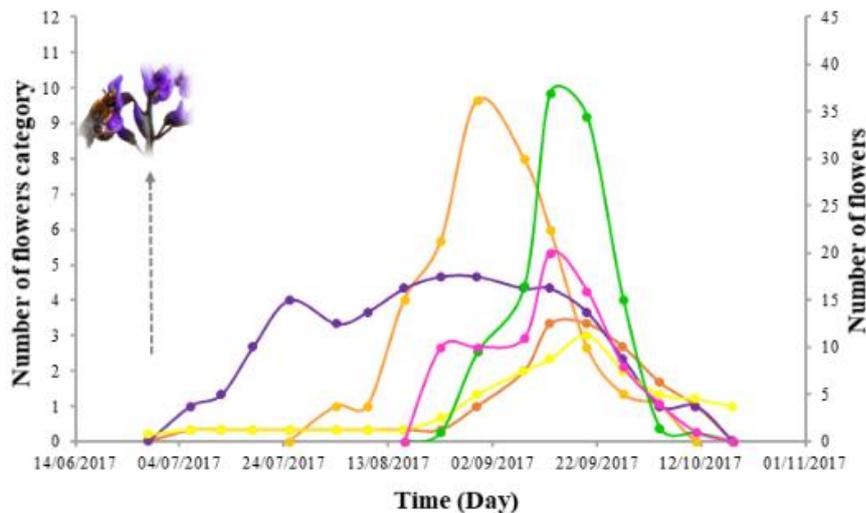
*D. divaricata*, *I. cuneifolia* and *J. sternbergiana* were 0.07, 0.03, 0.10 and 0.16 respectively [Supplementary data, Table S4]. Single colour loci from flower parts of individuals of *B. eriocarpa*, *D. divaricata*, *J. sternbergiana* were distributed in the coordinates range y:[-0.36; -0.10] x: [-0.06;-0.41], overlapping the distribution of *D. magnifica* single colour loci, extending across the positions y:[0.02; -0.32] x: [-0.10;-0.40] (Fig.6, B). In *I. cuneifolia* the colour loci from individuals didn't overlap with the colour loci of *D. magnifica* for any individual plants (Fig. 6, B).



**Fig. 6** A) Mean values of colour loci were calculated for floral parts of *Diuris magnifica*, *Bossiaea eriocarpa*, *Daviesia divaricata*, *Hardenbergia comptoniana*, *Isotropis cuneifolia*, *Jacksonia sternbergiana* and additionally other yellow-flowered species present in all the sites, *Acacia pulchella* (Fabaceae), *Conostylis aculeata* (Haemodoraceae), *Hibbertia hypericoides* (Dilleniaceae), and a co-occurring pink species, *Hypocalymna robustum* (Myrtaceae) to test model similarity based on floral colour. B) Distribution of colour loci most similar to the colour of *D. magnifica*. Measurements of spectral reflectance were taken for *D. magnifica*: LOP = lateral outer petal; DS = dorsal sepal; LLE = external labellum lateral lobe; ; LLI = internal labellum lateral lobe; L = labellum; for pea plant species (Faboideae): SP = standard petal; W = wing petals. The calculations were made using the Hexagon colour model of bee vision (Chittka, 1992).

### Compared flowering phenology of target species

*Diuris magnifica* showed overlap in flowering period with all the co-occurring pea plants (Fig. 7). Species visited by *Trichocolletes* spp., showed flowering peaks in the following order: *H. comptoniana* (three weeks before *D. magnifica* peak), *D. divaricata* (two weeks before *D. magnifica* peak), *B. eriocarpa* (concurrently with *D. magnifica* peak), *J. sternbergiana* (one week later than the *D. magnifica* peak).



**Fig. 7** Flowering phenology of *Diuris magnifica* and co-occurring Faboideae species at three sites the costal bushland remnants. Phenology data was collected in a single 30 x 30 metre quadrat per site. Due to the high number of flowers for *Bossiaea eriocarpa* (red line), *Daviesia divaricata* (orange line), *Hardenbergia comptoniana* (violet line) and *Jacksonia sternbergiana* (yellow line), we estimated the total number of flowers and assigned categories (primary y axis): (1) 1-100, (2) 101-200, (3) 301-400, (4) 401-500... to 1100. For *D. magnifica* (green line) and *Isotropis cuneifolia* (purple line) the number of flowers per quadrat (secondary y axis) was directly scored. *Trichocolletes* were observed to appear in correspondence of the flowering start of *H. comptoniana*.

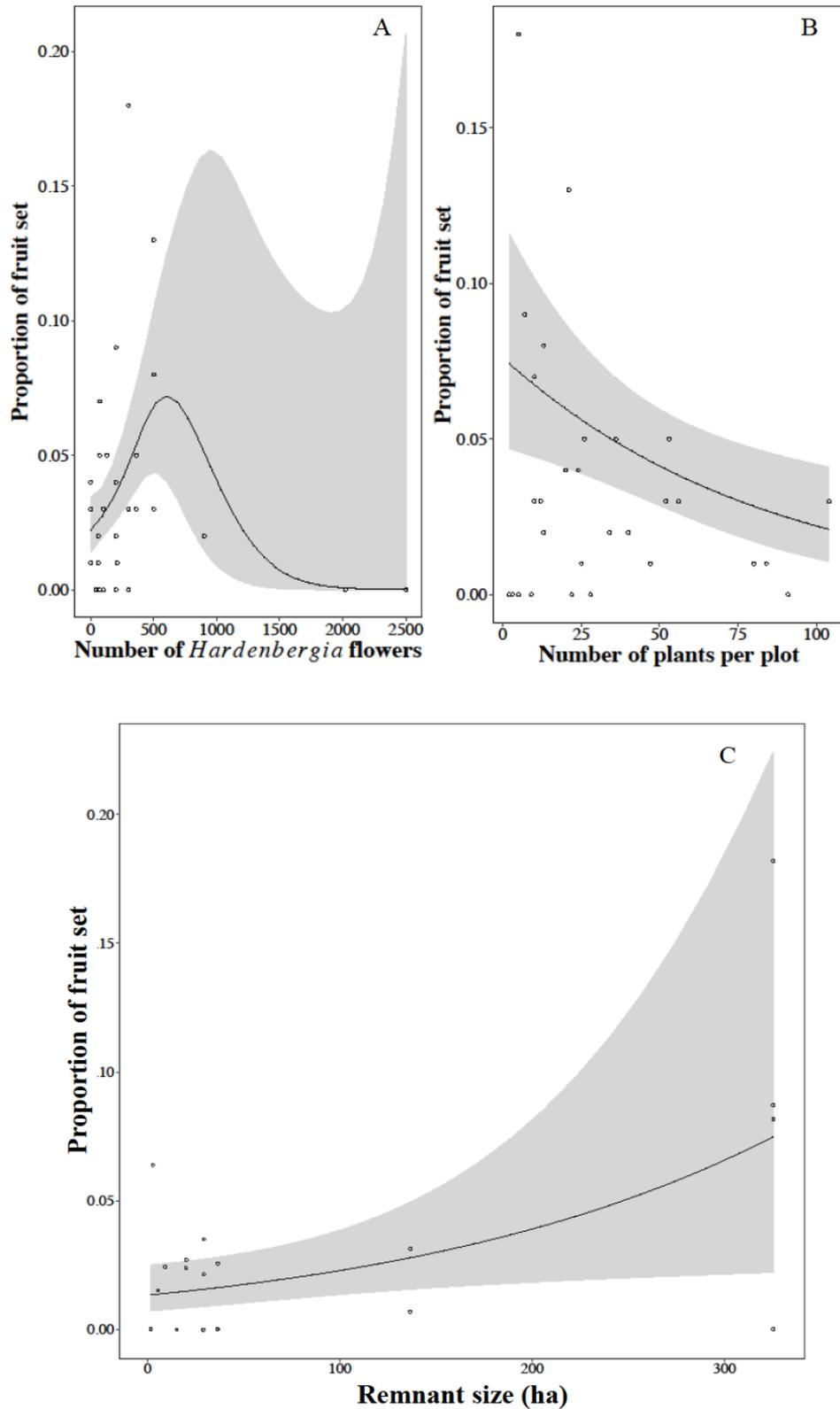
### Orchid fitness in relation to abundance of models and non-models

Reproductive success of *D. magnifica* was generally low across both 2015 (Mean SE fruit set =  $2.87 \pm 0.18$ ) and 2017 (Mean SE fruit =  $2.00 \pm 0.15$ ). The proportion of *D. magnifica* with pollinaria removal did not show a significant relationship with the number of flowers

of yellow-red pea plants (*B. eriocarpa*, *D. divaricata*, *J. sternbergiana*), which were identified as putative models for *D. magnifica* (male fitness:  $\beta = 0,017 \pm 0,078$  SE;  $p = 0,83$ ). The proportion of flowers with pollinaria removal showed no significant relationship with the number of flowers of the non-model *H. comptoniana* (pollinaria removal:  $\beta = 0.025 \pm 0.081$  SE;  $p = 0.756$ ). The output from the GAMM showed a significant non-linear trend for the reproductive success as a function of the number of non-model *H. comptoniana* flowers (smoother term = 2.326;  $p = 0.004$ ; Fig. 8, A). The best fitting model was a non-linear curve, though the decrease at high values of model flowers was likely driven by two outlying points. The orchid reproductive success increased until approximately 700 *H. comptoniana* flowers, though the very large confidence intervals above 700 *Hardenbergia comptoniana* flowers. Finally, the proportion of pollinaria removal showed no relationship with the number of individuals per patch ( $\beta = -0.148 \pm 0.106$  SE;  $p = 0.162$ ) while the proportion of fruit set show a significant negative relation with the number of orchids per quadrat ( $\beta = -0.366 \pm 0.128$  SE;  $p = 0.004$ ; Fig. 8 B).

#### *Orchid fitness in relation to pollinator occurrence and patch size*

The proportion of pollinaria removed did not show any significant relationship with either the presence of *Trichocolletes* or size of the habitat remnant. Neither pollinaria removed ( $\beta = 0.136 \pm 0.3$  SE;  $p = 0.649$ ) or fruit set ( $\beta = -0.265 \pm 0.7$  SE;  $p = 0.705$ ) exhibited a significant relationship with the presence of *Trichocolletes*. The proportion of fruit set showed a significant positive relationship with bushland remnant size ( $\beta = 0.684 \pm 0.296$  SE;  $p = 0.021$ ; Fig. 8, C), while there was no significant relationship for the proportion of pollinaria removed ( $\beta = 0.306 \pm 0.163$  SE;  $p = 0.061$ ).



**Fig. 8** Female reproductive success (fruit set on number of flowers per square) over 2016 and 2017 in function of A, number of flowers of *Hardenbergia comptoniana* (non-model) and B, number of orchid plants per plot (square). C, Proportion of fruit set in function of bushland remnant size in 2017.

## DISCUSSION

### *Pollination and behavioural evidence for mimicry in Diuris magnifica*

We present preliminary evidence of pollination by mimicry in *D. magnifica* by testing the fundamental criteria of floral mimicry such as sharing of pollinators, similar pollinator behaviour on model and mimic, overlap of flowering period and colour similarity (Roy & Widmer, 1999; Johnson & Schiestl, 2016). Based on pollinator visitation data and observations of wild bees carrying orchid pollen, *D. magnifica* appears to be primarily pollinated by the colletid bee *T. gelasinus*. This bee foraged on the sympatric yellow-red pea plants, *B. eriocarpa*, *D. divaricata*, *J. sternbergiana*, as well as the violet coloured *H. comptoniana*. Male and female *Trichocolletes* attempting to forage on *D. magnifica* exhibited the same keel-parting behaviour (Fig. 4) as seen by bees of this genus when foraging both nectar and pollen on pea plants, where they attempt manipulation of the orchid labellum using the fore and middle legs (Fig. 2, C). As previously seen in *D. brumalis* (Scaccabarozzi *et al.*, 2018), this behaviour is a distinctive aspect of mimicry towards pea plants, where the bees exhibit a behaviour with the orchid typically associated with pea plants. ‘Patrolling’ behaviour of *Trichocolletes* males (see Houston, 2018) was also observed occasionally in proximity to flowers of *D. magnifica*. Given that in *Trichocolletes* this mate searching behaviour (see (Haas, 1960; Barrows, 1976; Paxton, 2005) is usually seen around pea plants, patrolling provides further behavioural evidence of effective mimicry of pea plants by *D. magnifica*.

### *Evidence for mimicry based on floral traits*

As already found in *Diuris brumalis* (Scaccabarozzi *et al.*, 2018), a species morphologically very similar to *D. magnifica*, the color of *D. magnifica* floral parts overlapped with the colour loci of three yellow-red pea plants that we identified as putative models, suggesting that the flowers of those pea plant species may not be consistently distinguishable by pollinators from *D. magnifica* based on colour alone. Experiments suggests that bees are unable to distinguish colour distances less than 0.04 hexagon units, but colour distances between 0.04 and 0.11 can be distinguished with differential conditioning (Dyer, 2006; Dyer *et al.*, 2012). While some individual orchids do overlap with the colour of these pea plants, the average colour loci differences between *D. magnifica* and *D. divaricata* (0.03), *B. eriocarpa* (0.07) and *J.*

*sternbergiana* (0.16), suggest that it will depend on the colour of individual plants as to how well the pollinators distinguish between them. While not directly addressed here, it is likely that the plants will exhibit some difference in colour pattern, which would likely enhance the ability of pollinators to distinguish between models and mimics.

#### *A potential role for secondary pollinators?*

While never observed carrying pollen of *D. magnifica* while feeding on co-occurring plants, a few observations of *A. mellifera* and *Neophyllotocus* beetles removing and depositing pollinia on the same flower suggests that other visitors may occasionally contribute to the pollination of *D. magnifica*. The introduced *A. mellifera* forage on an exceptionally wide range of plant species (Paton, 1993) and frequently visit all of these species of Faboideae (Scaccabarozzi *et al.*, in review), so it is not surprising that they also visit *D. magnifica*. *Neophyllotocus* visit several species of brightly coloured understory plants in the study area, on which they both use as a food source and a site to congregate and mate (Keighery, 1975; Schatral, 1996). For both of these species it remains to be confirmed if they are effective pollinators of *D. magnifica*. Firstly, while the landing position of *Trichocolletes* was strictly aligned with the labellum as occurs when foraging on pea flowers, these other visitors more rarely moved into the correct position for pollen removal and deposition. Secondly, they were only seen removing and depositing pollinia on the same flower meaning that they may contribute towards fruit set via a level of self-pollination. A small number of visits were observed by two other species of native bee, but given the rarity of these visits, it seems likely that *Trichocolletes* are the primary native pollinators.

#### *Orchid fitness and co-occurring pea plants*

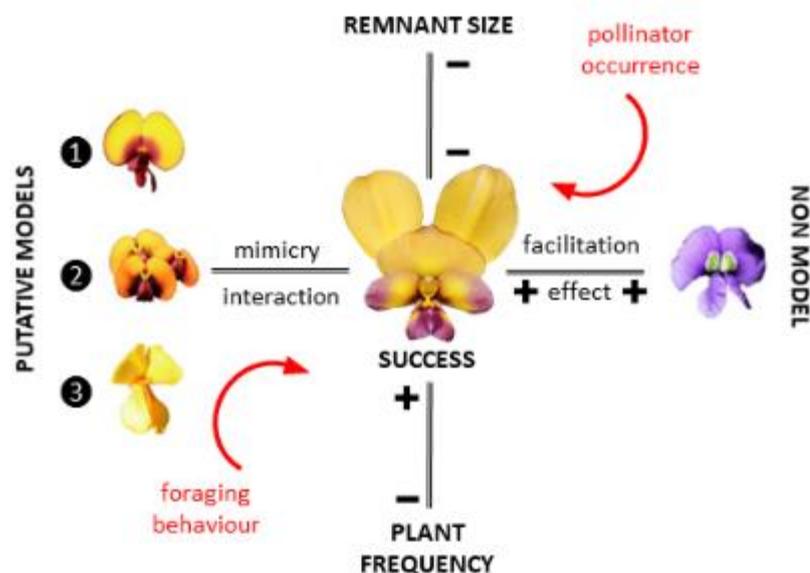
In mimicry systems it is expected that the fitness of the mimic should increase relative to the local abundance of the model (Anderson & Johnson, 2006). In *D. magnifica* fruit set declined with higher density of conspecifics, suggesting either pollinator learning or a limited number of pollinators relative to the number of orchids. However, we found that reproductive success of *D. magnifica* was not dependent on the total flower abundance of occurring yellow-red pea plants species, the putative models for this orchid. While this may in part be due to low levels of reproduction of the orchid (< 3% fruit set) increasing chance effects in the data, there are several possible ecological explanations why this expectation

was not fulfilled. Firstly, pea plants may vary in their importance as model species, but the relatively modest number of study sites compared with the diversity of species of pea plants did not allow for a test of the effect of single pea species on orchid fitness (as seen in Peter & Johnson 2008; Juillet *et al.*, 2007; Jersáková *et al.*, 2016). Secondly, in multiple ecological factors could be important and interact with each other (e.g. putative model and non-model pea plants, habitat fragmentation, pollinator availability and habitat suitability, role of secondary pollinators), making it difficult to tease out trends. Thirdly, foraging behaviour towards pea plants and orchids may vary between sites depending on the relative abundance of pea plants species (Fig. 9) that vary in their similarity to the orchid. For example, floral constancy (Waser, 1985; Chittka, Tomson, Waser, 1999; Gegear & Terence, 2004), a selective foraging behaviour whereby pollinators may optimize their foraging activity on a given and abundant pea species at each site, could lead to changes in the effectiveness of orchid pollination systems depending on which species pollinators typically forage on. In order to unravel the fitness dependence of *D. magnifica* on yellow- red plants, it would be of interest to investigate with experimental arrays of orchid flowers the preference of pollinators between each of the putative models and the orchid.

		Pea plant flower composition per site (%)														
Sites 2015		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B				3	6					13		14				
DD		10	19						13				13	11		43
J		90	81	36	39				68	9	2	55	26	77	67	
H				61	55	100		100	19	78	98	32	61	12	33	57
Sites 2017		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B		18	6	38	46					3						
DD		55	22					50	67		43			27		33
J		27	56	50	19					10	21	33	40	27	60	17
H			17	13	35	100	100	50	33	86	36	67	60	45	40	50

**Fig. 9** Pea plant flower proportion per species on all the total number of flowers of pea plants at each of the 15 sites. B, *Bossiaea eriocarpa*; DD, *Daviesia divaricata*, J, *Jacksonia sternbergiana*, H, *Hardenbergia comptoniana*.

While the GLMM analysis found no significant relationships with orchid fitness, the GAMM analysis found evidence for a relationship between fruit set of *D. magnifica* and the number of flowers of the non-model pea plant *H. comptoniana*. Fruit set initially increased with increasing number of flower of *H. comptoniana* as expected under a magnet effect (Fig. 10), where the local aggregation of pollinators on food plants benefit the pollination success of other nearby plants (Thomson, 1978; Laverty, 1992). At large numbers of flowers the relationship decreased, suggestive of a competition effect (i.e. Lammi & Kuitunen, 1995; Internicola *et al.*, 2006), whereby co-occurring rewarding species may affect negatively the success of the orchid. However, in *D. magnifica* the decrease in fitness with large numbers of food flowers appears to be driven by two outliers where there was one exceptionally large and prominently flowering individual of *H. comptoniana*. However, as cautionary note, it must be stressed that the decrease in fitness of *D. magnifica* at higher numbers of rewarding flowers appears to be driven by two outliers sites, where there was one exceptionally large and prominently flowering individual of *H. comptoniana*. Further work is needed to test for a decreasing trend, preferably including experimental manipulation of the availability of *H. comptoniana*. Lastly, as expected in deceptive systems (Pellegrino, 2005), we found that the *D. magnifica* fruit set declined with higher density of conspecifics (Fig. 10).



**Fig. 10** A summary of the ecological interactions potentially driving the reproductive success in *Diuris magnifica*. The interactions are based on the imperfect mimicry towards model yellow-red pea plants (1, *Bossiaea eriocarpa*; 2, *Daviesia divaricata*;

3, *Jacksonia sternbergiana*) and on the reproductive facilitation led by *Hardenbergia comptoniana*, a non-model pea plant. As expected, when the orchid is more frequent a decline in the reproductive success has been observed, while with smaller bushland remnants comparing with bigger ones, the fruit set was lower. All these ecological interactions may be indirectly influenced by the pollinator occurrence and their foraging behaviour.

#### *Fitness and habitat fragmentation*

Given that *Trichocolletes* appear to be the primary pollinator of *D. magnifica*, it was expected that reproductive success of *D. magnifica* would be greater at sites where *Trichocolletes* were present. Interestingly, we detected no significant difference in either pollen removal or fruit set for the orchid. However, it should be noted that levels of fruit set were low across all populations (less than 3% in any given year). While *Trichocolletes* may have remained undetected at some sites in our survey, it is possible that sub-optimal pollinators such as *A. mellifera* and *Neophyllotocus* beetles may be contributing to the reproductive success of the orchid sufficiently to obscure any difference in reproductive success between sites due to the presence or absence of *Trichocolletes*. It would be of interest to investigate the fitness of seed originating from pollination events from *Trichocolletes* versus other pollinators, and if seeds from sites without *Trichocolletes* tended to be of lower fitness, potentially from more pollen transfer within clumps of *D. magnifica*.

As predicted, the size of bushland remnants was positively related to fruit set of *D. magnifica* (Fig. 10), in accordance with previous research where habitat fragmentation causes lower fruit set through pollen limitation (Cunningham, 2000). However, it should be noted that the sites with high fruit set were mostly in Kings Park, the largest of the remnants. Lower fruit set in small remnants could be because habitat is less suitable for pollinators, or the remnants are too small to support viable populations of *Trichocolletes*, a remnant dependent genus. The proportion of flowers with pollen removal did not exhibit a significant relationship with any of the tested variables, though it is expected that pollen removal will be more effected by suboptimal pollinators removing but not transferring pollinia.

*Imperfect mimicry by exploitation of a 'general pea flower image'*

As found in the related species *D. brumalis* (Scaccabarozzi *et al.*, 2018), *D. magnifica* may receive an advantage from a broad mimicry system that includes multiple yellow-red pea plant species, rather than precisely mimic any one species. In fact, the orchid may benefit from the pollinator having a 'general search image' (Johnson & Schiestl, 2016) that encompasses all of the co-occurring pea plant species of similar colouration. This would represent a form of imperfect mimicry deriving from the imitation of multiple models (Sherratt, 2002; Gilbert, 2005). Interestingly, in *D. magnifica* flowering commenced well after the first of the model species and the emergence of the pollinator, which was first seen in around the first week of July (D. Scaccabarozzi pers. obs), four weeks prior the start of flowering in *D. magnifica*. This means that the pollinators could be already familiar with a 'pea flower image' when *D. magnifica* starts to flower, rather than the orchid being reliant on the exploitation of perceptual biases (Schaefer & Ruxton, 2009).

However, the benefit by *H. comptoniana* (non-model) on the fitness of *D. magnifica* arises some questions on the broadness and accuracy of the mimicry system.

Even though the pigmentation patterns were dissimilar and the colour reflectance didn't match with the orchid, we wonder if they have a similar smell and in this case the bees are attracted by scent rather than visual cues. Further investigations based on scent, including the lateral curved sepals that might retain scent glands, would be interesting to define the role of *Hardenbergia* in the mimicry species.

In generalized food deception, the plants lack floral traits that confer a similarity to a specific model plant (Dafni, 1984; Nilsson, 1992) and as consequence of general nature of signals they tend to have a wide group of pollinators (Nilsson, 1983, Cozzolino *et al.*, 2001; 2005) instead of specialised pollinators (Newman *et al.*, 2012). While an usual generalized food deception is not likely to occur, the impact on the fitness is due to the species most abundant across the sites (*H. comptoniana*). So, it would be of interest to test if *Trichocolletes* are dependent to the presence of the species most abundant rather than the model and non-model pea plants. In this sense, the use of resources by pollinators at spatial scale may be crucial for maintaining the orchid success.

### *Conclusions*

Based on similarity of floral traits and pollinator behaviour, *D. magnifica* is likely to be engaged in the generalised mimicry of multiple co-flowering pea plants, without closely resembling in any one species. However, it remains to be tested if there a specific model plant that is more important for supporting populations of the pollinator or increasing fitness for the orchid. We observed that the orchid fitness is affected by ecological interactions aside from floral mimicry. In particular, there was evidence that the abundance of the rewarding species *H. comptoniana* affected orchid reproductive success. From a conservation perspective, pollination success was not significantly different in the absence of *Trichocolletes*, but *A. mellifera* and beetles may come into play in maintaining the orchid's reproduction, especially in an urban fragmented habitat where *D. magnifica* occurs. However, whether these species are capable of maintaining populations of the orchid, or if their visits mostly lead to self-pollination, could be important for deciding the fate of *D. magnifica* in small habitat remnants.

Due to the peculiarity of the study system based on model and non-model species in a fragmented habitat, *D. magnifica* may reveal new ecological insights, especially on the definition of model / non-model species, the impact of the foraging behaviour and habitat size in mimicry systems.

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## SUPPLEMENTARY DATA

Supplementary data consist of the following. Appendix S1: floral biology of *Diuris magnifica* and co-occurring Faboideae; Table S1: A,B: Sites and reproductive data of *D. magnifica*; Table S2: plant species vouchered at the WA Herbarium; Table S3: observations of floral visitors to *D. magnifica* and co-occurring Faboideae; Table S4: insects caught on *D. magnifica*; Table S5: Means and standard deviation of colour loci of *D. magnifica* and co-occurring plants.

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## **SUPPLEMENTARY DATA, Appendix S1.** Observations of the floral biology of *Diuris magnifica*

### **Introduction**

Before undertaking a study of floral mimicry and its consequences for plant reproductive success, it is necessary to address general questions on the floral biology of the species. *Diuris magnifica* has no visible nectar while the co-occurring pea plants (Fabaceae) produces a nectar reward (Scaccabarozzi et al., 2019, in review). However, it has not been tested if *D. magnifica* produces fruit by autogamy and if it is self-compatible.

### **Methods**

#### *Nectar production*

To test for nectar production in *D. magnifica*, nectar content in flowers of each species was measured in August 2016. One inflorescence per individual was bagged for 10 randomly selected individuals at three sites (Shanton Park, Trigg bushland and Koondoola bushland). Inflorescences were bagged in the afternoon, with nectar collection predicted the following day during the warmest hours (from 11.00 to 14.00) to ensure maximum nectar production (Corbet, 1995; Corbet, 2003) using a 2 µl microcapillary tube (Drummond Microcaps, Broomall; Pa., USA).

#### *Testing for autogamy and self-compatibility in Diuris magnifica*

To test for autogamy, in August 2016, inflorescences with newly-opened flowers and no observed pollinia deposited on stigma were covered with a fine, insect proof nylon bag until floral senescence (ca. four weeks). To test for self-compatibility, one flower on each inflorescence was manually pollinated with pollinia from a different flower on the same inflorescence before the pollinated flower was covered with a fine, insect proof nylon bag until senescence or fruit formation (ca. four weeks). Six individuals were randomly selected at each of the three largest populations (Shanton Park, Trigg bushland and Koondoola bushland) for each test, with one inflorescence selected per individual for a total of eighteen inflorescences tested.

## Results

### *Nectar content*

No nectar was produced by any part of studied *D. magnifica* flowers, and any nectar was therefore collected.

### *Testing for autogamy and self-compatibility in Diuris brumalis*

None of the bagged flowers produced fruits, demonstrating that *D. magnifica* requires a vector to achieve pollination. Experimental hand pollination revealed that *D. brumalis* is able to produce seed capsules through self-pollination, with 80% (n=18) of flowers forming a capsule.

## Conclusions

As expected, *D. magnifica* is nectarless. *Diuris magnificia* was shown to require a vector for pollination, and to be self-compatible.

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**SUPPLEMENTARY DATA, Table S1, A.** List of the 15 populations sites of *Diuris magnifica* surveyed in 2015 above. All the values are related to plants surveyed within a 30 x 30 meter quadrat. DM: *D. magnifica*. Model pea plants: *Bossiaea eriocarpa*, *Daviesia divaricata*, *Jacksonia sternbergiana*. Non-model pea plants: *Hardenbergia comptoniana*. All co-occurring pea plants: model and non-model pea plants. **B.** Below, list of the 20 populations sites of *Diuris magnifica* surveyed in 2017. All the values are related to plants surveyed within 30 x 30 meter quadrat. DM: *D. magnifica*. The extra sites (number 16-20) in 2017 consist with small bushland remnants.

Population number	Site	Latitude, longitude	N plants of DM	N flowers DM	N flowers DM without pollinaria	N pollinated flowers DM	Proportion of pollinaria removal	Proportion of fruit set	N total flowers Model pea plants	N total flowers non Model pea plants	N total flowers all co-occurring pea plants
1	Koondoola bushland A	31°50'06.8" °S, 115°51'83.4" °E	56	241	13	7	0.28	0.08	315	0	315
2	Koondoola bushland B	31°50'12.6" °S, 115°51'94.2" °E	24	101	14	4	0.23	0.01	444	0	444
3	Trigg bushland A	31°52'07.9" °S, 115°45'63.2" °E	47	119	7	1	0.14	0.07	135	210	345
4	Trigg bushland B	31°52'20.1" °S, 115°45'64.7" °E	53	127	20	6	0.04	0.01	300	360	660
5	Shanton Park A	31°57'50.8" °S, 115°47'90.8" °E	28	91	7	0	0.06	0.07	0	70	70
6	Shanton Park B	31°57'32.6" °S, 115°47'52.7" °E	84	219	17	2	0.24	0.05	0	0	0
7	Shanton Park C	31°57'37.0" °S, 115°48'00.8" °E	36	98	9	5	0.07	0.10	0	130	130
8	Shanton Park D	31°57'40.5" °S, 115°48'01.2" °E	40	113	7	2	0.00	0.05	250	60	310
9	Wireless Reserve A	31°01'71.7" °S, 115°49'83.7" °E	21	62	14	8	0.00	0.00	140	500	640
10	Wireless Reserve B	32°01'88.5" °S, 115°49'75.0" °E	91	259	20	0	0.00	0.00	35	2020	2055
11	Wireless Reserve C	32°01'97.8" °S, 115°49'88.7" °E	10	30	6	2	0.05	0.01	150	70	220
12	Kings Park A	31°57'20.9" °S, 115°50'08.2" °E	12	34	4	1	0.01	0.02	230	360	590
13	Kings Park B	31°57'38.2" °S, 115°50'13.2" °E	25	74	9	1	0.00	0.01	450	60	510
14	Kings Park C	31°57'33.8" °S, 115°49'55.8" °E	26	79	12	4	0.06	0.00	145	70	215
15	Kings Park D	31°57'29.06" °S, 115°49'48.8" °E	22	65	4	0	0.05	0.00	30	40	70

Population number	Site	Latitude, longitude	N plants of DM	N flowers DM	N flowers DM without pollinaria	N pollinated flowers DM	Proportion of pollinaria removal	Proportion of fruit set	N total flowers Model pea plants	N total flowers non Model pea plants	N total flowers all co-occurring pea plants	<i>Trichocolletes</i> occurrence	Remnant size (ha)
1	Koondoola bushland A	31°50'06.8" °S, 115°51'83.4" °E	80	286	32	2	0.11	0.01	1100	0	1100	1	136.48
2	Koondoola bushland B	31°50'12.6" °S, 115°51'94.2" °E	10	32	3	1	0.09	0.03	1500	300	1800	1	136.48
3	Trigg bushland A	31°52'07.9" °S, 115°45'63.2" °E	52	147	11	4	0.07	0.03	700	100	800	0	19.69
4	Trigg bushland B	31°52'20.1" °S, 115°45'64.7" °E	13	42	5	1	0.12	0.02	1700	900	2600	0	19.69
5	Shanton Park A	31°57'50.8" °S, 115°47'90.8" °E	3	5	0	0	0.00	0.00	0	200	200	0	28.94
6	Shanton Park B	31°57'32.6" °S, 115°47'52.7" °E	9	27	1	0	0.04	0.00	0	100	100	0	28.94
7	Shanton Park C	31°57'37.0" °S, 115°48'00.8" °E	20	57	9	2	0.16	0.04	200	200	400	0	28.94
8	Shanton Park D	31°57'40.5" °S, 115°48'01.2" °E	34	93	5	2	0.05	0.02	400	200	600	0	28.94
9	Wireless Reserve A	31°01'71.7" °S, 115°49'83.7" °E	5	17	1	0	0.06	0.00	400	2500	2900	1	36.47
10	Wireless Reserve B	32°01'88.5" °S, 115°49'75.0" °E	104	312	13	8	0.04	0.03	900	500	1400	1	36.47
11	Wireless Reserve C	32°01'97.8" °S, 115°49'88.7" °E	2	8	0	0	0.00	0.00	100	200	300	0	36.47
12	Kings Park A	31°57'20.9" °S, 115°50'08.2" °E	3	4	0	0	0.00	0.00	200	300	500	1	325.09
13	Kings Park B	31°57'38.2" °S, 115°50'13.2" °E	13	49	5	4	0.10	0.08	600	500	1100	1	325.09
14	Kings Park C	31°57'33.8" °S, 115°49'55.8" °E	7	23	2	2	0.09	0.09	300	200	500	1	325.09
15	Kings Park D	31°57'29.06" °S, 115°49'48.8" °E	5	22	2	4	0.09	0.18	300	300	600	1	325.09
16	Apple Bossom Polyantha reserve	31°57'50.06" °S, 115°49'82.8" °E	104	315	22	0	0.07	0.00	400	0	400	1	1.5
17	Shephards Reserve	31°57'38.20" °S, 115°50'13.2" °E	26	82	4	0	0.05	0.00	600	200	800	0	15.3
18	Paloma Park	31°57'25.90" °S, 115°49'89.9" °E	54	199	8	3	0.04	0.02	800	100	900	1	5.05
19	Alfreton Reserve	31°57'56.00" °S, 115°49'92.2" °E	13	47	2	3	0.04	0.06	50	200	250	0	2.38
20	Brekler Reserve	31°57'50.80" °S, 115°47'90.8" °E	48	124	3	3	0.02	0.02	0	200	600	0	8.89

**SUPPLEMENTARY DATA, Table S2.** List of the plant species per family collected in the field sites, subsequently vouchered at the Herbarium of Western Australia, Perth.

Number	Family name	Specimens collected in field and vouchered at Herbarium	Voucher	Latitude; Longitude
1	Fabaceae	<i>Hardenbergia comptoniana</i>	DS 12	31°50' 12.7" S; 115° 52' 02.8" E
2	Orchidaceae	<i>Diuris magnifica</i>	DS 13	31°50' 00.6" S; 115° 51' 13.8" E
3	Fabaceae	<i>Jacksonia sternbergiana</i>	DS 14	31°50' 12.7" S; 115° 52' 02.8" E
4	Orchidaceae	<i>Isotropis cuneifolia</i> subsp. <i>cuneifolia</i>	DS 15	31°57' 24.3" S; 115° 50' 03.4" E
5	Fabaceae	<i>Bossiaea eriocarpa</i>	DS 16	31°50' 12.7" S; 115° 52' 02.8" E
6	Fabaceae	<i>Daviesia divaricata</i> subsp. <i>divaricata</i>	DS 17	31°50' 00.6" S; 115° 51' 13.8" E
8	Fabaceae	<i>Diuris magnifica</i>	DS 18	31°56' 06.8" S; 115° 51' 83.4" E

**SUPPLEMENTARY DATA, Table S3.** Observations of floral visitors to *Diuris magnifica* and co-flowering pea plants species (*Bossiaea eriocarpa*, *Daviesia divaricata*, *Hardenbergia comptoniana*, *Isotropis cuneifolia*, *Jacksonia sternbergiana*). Eight behavioural categories as in Scaccabarozzi et al. (2018) were distinguished to reflect the pollination process. For Category (II), the types of behaviour for insects approaching the flower were: zig-zag flight = moving side to side in flight as they approach the flowering plant; direct flight = flying in a straight line as they approach the flower; aligned = body of visitor aligned along the midpoint of the labellum/keel during attempts to forage; patrolling = appearing to inspect multiple flowers around the plant; approach but choose another flower = the bee approaches a flower closely (<5cm) but then chooses to alight on a different flower. In addition, it was recorded if males were observed patrolling for females around the flower. Highlighted are the insect species observed depositing pollen on orchid stigma.

<i>Diuris magnifica</i>		<i>Apis mellifera</i>	<i>Lassioglossum</i>	<i>Leioproctus</i>	<i>Neophyllotocus</i>	<i>Pollaninus sp.</i>	<i>Syrphidae</i>	<i>Trichocolletes gelasinus</i>
(I)	N insects approaching the flower	65	7	1	19	47	11	98
(II)	Behaviour when approaching the flower	zig-zag flight	direct flight	direct flight	direct flight	zig-zag flight	zig-zag flight	direct flight -aligned or patrolling
(III)	N insects carrying orchid pollen on arrival	0	0	0	0	0	0	1
(IV)	N insects landing on the flower	26	5	1	19	29	8	49
(V)	Visiting time $\geq$ 1s	3	2	3	2	3	4	1
(VI)	N insects attempting labellum manipulation	19	5	1	15	6	2	48
(VII a)	N insects removing pollen	2	0	0	2	0	0	25
(VII b)	N insect depositing pollen	2	0	0	1	0	0	4
(VIII)	N insects visiting another orchid flower	8	2	0	2	0	0	10

<b><i>Bossiaea eriocarpa</i></b>						
	<b>Behaviour categories</b>	<i>Apis mellifera</i>	<i>Neophyllotocus</i>	<i>Leioproctus</i>	<i>Pollanisis sp.</i>	<i>Trichocolletes gelasinus</i>
(I)	N insects approaching the flower	182	10	4	20	14
(II)	Behaviour typology approaching the flower	zig-zag flight	direct flight	direct flight	zig-zag flight	direct flight -aligned or patrolling
(III)	N insects landing on the flower	182	10	2	20	12
(IV)	Visiting time $\geq$ 1s	4	38	4	3	2
(V)	N insects attempting keel manipulation	172	10	2	14	12
(VI)	N insects foraging nectar	170	4	2	14	12
(VII)	N insect collecting pollen	120	10	2	0	7
(VIII)	N insects visiting to another flower of pea plant	4	2	1	2	2

<b><i>Daviesia divaricata</i></b>					
	<b>Behaviour categories</b>	<i>Apis mellifera</i>	Syrphidae	<i>Leioproctus</i>	<i>Trichocolletes gelasinus</i>
(I)	N insects approaching the flower	35	48	69	81
(II)	Behaviour typology approaching the flower	zig-zag flight	zig-zag flight	direct flight	direct flight -aligned or patrolling
(III)	N insects landing on the flower	35	44	69	72
(IV)	Visiting time $\geq$ 1s	3	3	2	1.5
(V)	N insects attempting keel manipulation	35	25	69	72
(VI)	N insects foraging nectar	35	32	69	67
(VII)	N insect collecting pollen	18	0	61	54
(VIII)	N insects visiting to another flower of pea plant	6	2	2.5	22

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***Hardenbergia comptoniana***

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	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Lassioglossum</i>	<i>Leioproctus</i>	<i>Trichocolletes gelasinus</i>
(I)	N insects approaching the flower	35	48	69	62
(II)	Behaviour typology approaching the flower	zig-zag flight	direct flight	direct flight	direct flight -aligned or patrolling
(III)	N insects landing on the flower	35	44	69	48
(IV)	Visiting time $\geq$ 1s	3	3	2	1.5
(V)	N insects attempting keel manipulation	35	25	69	48
(VI)	N insects foraging nectar	35	32	69	48
(VII)	N insect collecting pollen	18	0	61	20
(VIII)	N insects visiting to another flower of pea plant	6	2	2.5	3

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***Isotropis cuneifolia***

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	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Lassioglossum</i>	<i>Neophyllotocus</i>
(I)	N insects approaching the flower	35	5	49
(II)	Behaviour typology approaching the flower	zig-zag flight	direct flight	direct flight
(III)	N insects landing on the flower	10	5	49
(IV)	Visiting time $\geq$ 1s	3	1	240
(V)	N insects attempting keel manipulation	8	5	45
(VI)	N insects foraging nectar	4	5	31
(VII)	N insect collecting pollen	1	0	43
(VIII)	N insects visiting to another flower of pea plant	3	1	2

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***Jacksonia sternbergiana***

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	<b>Bahaviour categories</b>	<i>Apis mellifera</i>	<i>Euhesma</i>	<i>Leioproctus</i>	<i>Megachile</i>	<i>Trichocolletes gelasinus</i>
(I)	N insects approaching the flower	24	45	32	3	34
(II)	Behaviour typology approaching the flower	zig-zag flight	zig-zag flight	direct flight	direct flight	direct flight -aligned or patrolling
(III)	N insects landing on the flower	24	45	32	3	25
(IV)	Visiting time $\geq$ 1s	3	6.5	5	1.5	1.5
(V)	N insects attempting keel manipulation	21	34	26	3	25
(VI)	N insects foraging nectar	21	35	26	3	25
(VII)	N insect collecting pollen	10	22	19	3	16
(VIII)	N insects visiting to another flower of pea plant	4	10	6	5	3

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**SUPPLEMENTARY DATA, Table S4.** List of insects caught on *Diuris magnifica* using arrays of orchid flowers above and co-occurring pea plants below. All the insects were sexed and identified at the Western Australia Museum, where possible at species level. Sex: F: female; M: male; W: worker. Pollen: o: carrying orchid pollinaria; d: depositing orchid pollinaria coming from orchids in natural habitat; p: pea plant pollen carried on legs and abdomen. \*: insect removing *Diuris* pollinaria and depositing on the stigma of the same flower.

Insects caught on <i>Diuris magnifica</i>					
Code	Date	Site	Taxon	Sex	Pollen
D01/15	9/7/2015	Shanton Park	<i>Apis mellifera</i>	F	o *
D02/15	9/7/2015	Shanton Park	Scarabaeidae: <i>Neophyllotocus</i> sp.	-	o *
D03/15	9/7/2015	Shanton Park	Scarabaeidae: <i>Neophyllotocus</i> sp.	-	-
D04/15	9/9/2015	Koondoola bushland	Zygaenidae, <i>Pollanisus</i> sp.	-	-
D05/15	9/21/2015	Koondoola bushland	Syrphidae: Syrphidae	-	-
D06/15	9/26/2015	Koondoola bushland	<i>Apis mellifera</i>	F	o *
DM01/16	8/24/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	M	o
DM02/16	8/25/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	F	o
DM03/16	8/26/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	F	o
DM04/16	8/29/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	M	o
DM05/16	8/29/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	M	o
DM06/16	8/29/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	M	o
DM07/16	8/29/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	F	d
DM08/16	9/13/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	F	o
DM09/16	8/24/2016	Koondoola bushland	<i>Trichocolletes gelasinus</i>	M	d

Insects caught on pea plants						
Code	Date	Site	Caught on	Taxon	Sex	Pollen
P03/15	9/8/2015	Shanton Park	<i>Isotropis cuneifolia</i>	Halictidae - <i>Lasioglossum</i>	F	-

P04/15	9/21/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Leioproctus Leioproctus sp.</i>	M	-
P05/15	9/22/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes platyprosopis</i>	M	0
P06/15	9/22/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes dives</i>	F	-
P07/15	9/22/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Leioproctus Leioproctus sp.</i>	F	-
P08/15	9/26/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes platyprosopis</i>	F	p
P09/15	9/26/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes platyprosopis</i>	M	-
P10/15	9/27/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
P11/15	9/27/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
P12/15	9/27/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
P13/15	9/27/2015	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
DDI01/16	8/24/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
DDI02/16	8/24/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Apis mellifera</i>	F	P
DDI03/16	9/6/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	F	-
DDI04/16	9/12/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	F	P
DDI05/16	9/11/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	F	P
DDI06/16	9/13/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Leioproctus</i>	F	P
DDI07/16	9/13/2016	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	F	P
BE01/16	8/13/2016	Koondoola Bushland	<i>Bossiaea eriocarpa</i>	<i>Megachile (Hackeriapis)</i>	F	P
BE02/16	9/13/2016	Koondoola Bushland	<i>Bossiaea eriocarpa</i>	<i>Leioproctus (Leioproctus)-WAM code F177</i>	F	P
BE03/16	9/13/2016	Koondoola Bushland	<i>Bossiaea eriocarpa</i>	<i>Apis mellifera</i>	F	P
BE04/16	9/29/2016	Koondoola Bushland	<i>Bossiaea eriocarpa</i>	<i>Trichocolletes gelasinus</i>	F	p
HC01/16	10/3/2016	Kings Park	<i>Hardenbergia comptoniana</i>	<i>Trichocolletes gelasinus</i>	F	-
HC02/16	10/11/2016	Kings Park	<i>Hardenbergia comptoniana</i>	<i>Trichocolletes gelasinus</i>	F	-
JS01/16	10/5/2016	Koondoola Bushland	<i>Jacksonia sternbergiana</i>	<i>Euhesma sp. 1</i>	F	-
JS02/16	10/5/2016	Koondoola Bushland	<i>Jacksonia sternbergiana</i>	<i>Leioproctus (Euryglossidia) sp.1</i>	F	-
JS03/16	10/5/2016	Koondoola Bushland	<i>Jacksonia sternbergiana</i>	<i>Euhesma sp.1</i>	F	P
JS04/16	10/11/2016	Kings Park	<i>Jacksonia sternbergiana</i>	<i>Trichocolletes gelasinus</i>	F	P

JS05/16	10/11/2016	Kings Park	<i>Jacksonia sternbergiana</i>	<i>Leioproctus (Euryglossidia) sp.1</i>	F	P
JS06/16	10/11/2016	Kings Park	<i>Jacksonia sternbergiana</i>	<i>Leioproctus (Euryglossidia) sp.2</i>	F	P
JS01/17	9/12/2017	Kings Park	<i>Jacksonia sternbergiana</i>	<i>Trichocolletes gelasinus</i>	F	P
DDI01/17	9/05/2017	Koondoola Bushland	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	F	P
DDI02/17	9/13/2017	Kings Park	<i>Daviesia divaricata</i>	<i>Trichocolletes gelasinus</i>	M	-
BE01/17	10/3/2017	Koondoola Bushland	<i>Bossiaea eriocarpa</i>	<i>Apis mellifera</i>	F	p

**SUPPLEMENTARY DATA, Table S5.** Means (averages across flower parts) and standard deviation of colour loci coordinates for each plant species measured: *Diuris magnifica*, pea plants (Faboideae) species (*Bossiaea eriocarpa*, *Daviesia divaricata*, *Hardenbergia comptoniana*, *Isotropis cuneifolia*, *Jacksonia sternbergiana*), other yellow flowered species present at sites, *Acacia pulchella*, *Conostylis aculeata* and *Hibbertia hypericoides*, lastly a pink co-flowering species, *Hypocalymna robustum*. Means are based on colour measurements for six individuals. Colour loci were calculated using the Hexagon colour model of bee vision (Chittka, 1992).

<i>Species</i>	Mean (x)	Mean (y)	SD (x)	SD (y)
<i>Acacia pulchella</i>	0.46	-0.23	0.01	0.02
<i>Bossiaea eriocarpa</i>	-0.30	-0.18	0.09	0.06
<i>Conostylis aculeata</i>	0.48	0.08	0.52	0.23
<i>Daviesia divaricata</i>	-0.23	-0.24	0.08	0.06
<i>Diuris magnifica</i>	-0.22	-0.19	0.09	0.10
<i>Hardenbergia comptoniana</i>	-0.16	0.21	0.06	0.03
<i>Hibbertia hypericoides</i>	0.03	-0.36	0.08	0.03
<i>Hypocalymna robustum</i>	0.01	0.22	0.16	0.15
<i>Isotropis cuneifolia</i>	-0.27	-0.14	0.13	0.13
<i>Jacksonia sternbergiana</i>	-0.12	-0.30	0.04	0.05

## Chapter 4

This Chapter will be send to *Methods in Ecology and Evolution* Journal.

### **Rotating arrays of orchid flowers: a simple and effective methodology for studying pollination in food deceptive plants**

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## Chapter 4

### Rotating arrays of orchid flowers: a simple and effective methodology for studying pollination in deceptive orchids

#### Abstract

1. Pollination in deceptive plants is often difficult to assess and requires several hours of field observations. Here we describe a simple and novel approach to increase the effectiveness of pollination studies of food deceptive orchids where mimics are presented in rotating arrays near model plants.
2. Using a mimic orchid, *Diuris brumalis* (Orchidaceae), and putative model plants belonging to the genus *Daviesia* (Fabaceae), rotating arrays of orchid flowers were set and moved in proximity to model plants, resulting in effective and rapid attraction of the pollinators of *D. brumalis*. We validated the methodology recording the pollinaria removal as an indicator of reproductive success, i.e. comparing pollinaria removal in orchids in their natural habitat with pollinaria removal using rotating arrays of orchid flowers.
3. The proposed method greatly enhances the pollinator attractiveness in the model-mimic system that otherwise can have false negative outcomes due to the low frequency of visitation.
4. The methodology has universal applications to other food deceptive pollination syndromes and also has relevance for examining behavioral patterns and developing ecological assessments of pollinator capability. The methodology may be extended to all plants with very low pollinator visitation rates.

**Key words:** bait orchids, floral mimicry, food deceptive plants, pollinator attraction, pollinator behavior, rotating arrays of orchid flowers, visitation rate

## Introduction

Lack of nectar in deceptive orchids leads to low visitation rates of pollinators (Gill, 1989; Neiland & Wilcock, 1998; Tremblay, Ackerman, Zimmerman, & Calvo, 2005), so making the pollination strategies often difficult to assess. This is particularly frustrating after the first days of flowering as the pollinators quickly learn the fraud flowers and avoid the orchids (Gumbert, 2000; Internicola, Juillet, Smithson, & Gigord, 2006).

For sexually deceptive orchids, Stoutamire (1974) and Peakall (1990) developed a method of floral presentation via baiting stations that has been deployed and revisited in other orchids showing sexual mimicry strategies (Gaskett, Winnick, & Herberstein, 2008; Phillips et al., 2013; Whitehead & Peakall, 2013).

In food mimicry orchids a similar 'baiting station' approach, based on a bifurcated stick presenting two inflorescences, has been developed with the aim to compare the insect visitation rate between the mimic orchid and its rewarding model plants (i.e. pollinator choice experiments) (Johnson & Midgley, 1997; Johnson, 2000; Johnson, Peter, Nilsson, & Ågren, 2003).

During a study of the food deceptive orchid *Diuris brumalis* we needed a methodology to consistently attract pollinators which were extremely difficult to observe due to their low visitation rate and fleeting floral visitation times (often  $\leq 1$  sec.; Scaccabarozzi et al., 2018). Indeed, in *Diuris brumalis* there was evidence of visitation by a potential pollinator species (i.e., *Trichocolletes* spp., Hymenoptera: Colletidae) but, despite several hours of visual observations, the removal of pollen had never been assessed, resulting in failure of pollinator confirmation.

Based on the mimicry pollination syndrome of multiple pea species in the genus *Daviesia* (Faboideae) (Fig. 1; Scaccabarozzi et al., 2018), we developed and validated a methodology using artificial arrays of the orchid *D. brumalis* to enhance attractiveness for insects and therefore, increase the number of pollinator observations to determine behavioral patterns. As *D. brumalis* grows in vegetative clusters of up to 50 plants (Dixon, Buirchell, & Collins, 1989), we have adapted the sexual deceptive baiting method and modified the original approach to create artificial arrays or clumps of flowers (Scaccabarozzi et al., 2018). By rotating these arrays position we were able to attract a larger number of effective pollinators (pollinaria removal) for *D. brumalis*. Rotation appears to provide a refreshed landscape of floral attractions to diminish the

potential for floral avoidance due to the pollinators ‘learnt’ behavior when blooms are stationary.



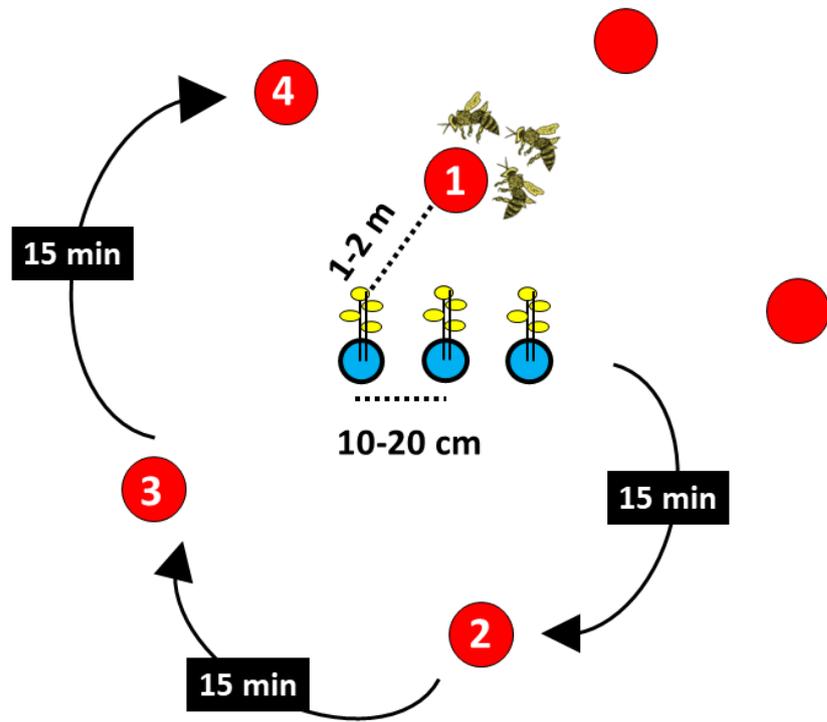
**Fig. 1.** (a),(c): The rewardless *Diuris brumalis* (Orchidaceae); (b),(d): *Daviesia decurrens* (Faboideae), one of the model species involved in the orchid floral mimicry.

## Description of the methodology

Arrays of orchid flowers were established at three study sites (in orchid-rich habitats in southwest Australia, 30 km east of Perth) with observations over six sunny days during the flowering period of orchids (12<sup>th</sup>, 18<sup>th</sup>, 25<sup>th</sup> July, 2<sup>nd</sup>, 9<sup>th</sup>, 15<sup>th</sup> August 2016) when pollinators were expected to be active. Experiments were conducted for two days per site, between 10.30 am to 3.30 pm when temperatures were higher than 17°C (around the optimum for pollinator activity, Scaccabarozzi et al., 2018), detected by an electronic thermometer, Smartsensor AR827, set 20 cm above the ground. Arrays of orchid flowers were placed to replicate the colony-forming pattern of *D. brumalis* and comprised multiple inflorescences that had been cut and placed in three glass vials with water on the bottom (two inflorescences per vial, with 4-6 flowers each; Fig. 2). All the flowers in the arrays had pollinaria at the beginning of the experiment. Vials were spaced 10–20 cm apart and positioned to create a conspicuous floral display, with vials placed 1–2 meters from flowering individuals of the model food pea-plant (*Daviesia* species). Each 15 minutes (including a minute for moving the arrays of orchid flowers), the vials were moved in proximity to another model plant. A total of four model plants randomly chosen per site were used for the experiment that was repeated hourly for 20 replicas (15 min each) per day, rotating the orchid arrays among the same four selected model plants (Fig. 3). Three experimental artificial arrays were employed.



**Fig. 2** Arrays of orchid flowers of *D. brumalis*, presenting two stems per each of the three vials.



**Fig. 3.** Representation of arrays of rotating orchid flowers used to establish pollinator effectiveness in *Diuris brumalis*. The rotation is repeated hourly in different locations with 15 minutes of observations on four pea plants. Filled red circles: model plants (*Daviesia*); arrays of flowers in the open blue circles (vials) with water, containing two inflorescences of the mimic orchid *Diuris brumalis*.

## Validation of the methodology and discussion

To test the effectiveness of artificial arrays, and to validate concurrence with natural pollinator activity, at the end of each day we scored pollinaria removal from intact clumps of flowering orchids and from arrays of orchid flowers rotating around the model plants. Experimental observations were conducted on the same day, selecting daily the natural orchid clump to score pollinaria removal.

Floral abundance was standardised by selecting natural orchid clumps comprising at least the same number of flowers displayed by arrays of orchid inflorescences.

A pairwise comparison was performed by using the *G*-test GenAIEx 6.5 (Peakall & Smouse, 2006, 2012), based on the total pollinaria removed in the three experimental artificial arrays and in a natural orchid clump in the same site. The test showed a significant difference between pollinaria removed ( $n=9$ ) in natural orchid clumps ( $N_{total\ flowers}=200$ ) and removal of pollinaria removed ( $n=31$ ) in rotating arrays of orchid flowers ( $N_{total\ flowers}=180$ ), with a higher and consistent outcome with the latter (16.731,  $p$ -value < 0.001).

The effectiveness of using arrays of orchid flowers may be due to the periodical moving of bait orchids to various model plants. This results in reducing the ‘learning behaviour’ of visiting insects when static displays are present (Dyer, 1996), and the consequent avoidance of non-rewarding plants previously visited (Goulson, 1997).

The current method provides a practical and quicker approach than traditional observational methods. This method can be applied to the study of other food deceptive orchid pollination, from generalised deception to specialised Batesian floral mimicry. The methodology also allows the behavior of the insect approaching to be observed in a quick and effective manner, ensuring sufficient replicas for data analysis. Furthermore, this methodology may also be applied to pollination studies on other plants with low insect visitation rates. In this case, arrays of flowers can be placed in proximity to magnet species (Thomson, 1978) that increase the local abundance of pollinators (cf. Kunin, 1993), or in sites characterised by an abundance of potential pollinators (as close to nest sites), so favoring more visits on the study plant.

A final recommendation is provided for storing the inflorescences and re-using for more days: picked inflorescences can be maintained fresh up to 3-4 days in plastic or glass sample vials stored in a refrigerator at 4°C, with some water in the bottom, and covering the inflorescence with a plastic bag.

## **Conclusions**

The proposed methodology enhances the visitation rate of pollinators and shortens the time required for pollinator observations, particularly for time-consuming studies of food deceptive orchids. Further, this method can be used for any pollination study involving plants with low visitation rates.

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## **Authors' contributions**

Daniela Scaccabarozzi conceived the ideas, designed methodology and collected the data; Daniela Scaccabarozzi and Andrea Galimberti analysed the data. Daniela Scaccabarozzi, Salvatore Cozzolino, Andrea Galimberti and Kingsley Dixon led the writing of the manuscript.

All authors contributed critically to the drafts and gave final approval for publication.

## **Data accessibility**

No data were used in this article.

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## Conclusions

We have presented a comprehensive evaluation of the guild mimicry for the *Diuris*-pea plants system. In addition to meeting the criteria for sharing pollinators and flowering times, pollinators exhibited pea plants-specific foraging behaviour on *Diuris*, providing strong evidence that the mimic had successfully deceived the pollinator. This evidence was further supported by data on morphology and colour, showing that not only *Diuris* and pea plants are very similar, but also that, based on bee vision model, the colour of *Diuris* and the proposed model species will not be readily distinguishable to pollinators. Reproductive success of *D. brumalis* was greater in the presence of *Daviesia*, though evidence suggests that this is likely through some combination of both learning and greater pollinator abundance at sites where the model is present. Alternatively, the reproductive success of *D. magnifica* was independent by the abundance of putative model species, while was influenced by the presence of a non-model species, *Hardenbergia comptoniana*. The diversity of species of *Diuris* with pea-like floral traits (*Diuris corymbosa* complex) suggests that this may be an effective system for understanding diversification in lineages that use floral Batesian mimicry.

The pea plant species that we investigated in the SWAFR were mostly bee-pollinated, although some species also attracted a large number of beetles that fed and mated on the flowers. If these beetles are proven to be effective pollinators, this would represent an unusual finding among the pea plants. Only two species of pea plants were visited by a broad spectrum of potential pollinators, while genera such as *Daviesia* and *Hovea* appear to be predominantly pollinated by just a single genus of bee. These findings suggest a level of specialisation and a potential role of pollinators in contributing to the diversity of the pea plants in the SWAFR. Experimental approaches are now needed to understand the basis of pollinator attraction in these specialised systems. While differences in nectar composition are unlikely to explain differences in visitation, the role of colour, odour, and foraging efficiency need to be investigated. In addition to native pollinators, the introduced bee *A. mellifera* visited all of the pea plants that we studied, sometimes in high frequency, and accessed both nectar and pollen. As such, *A. mellifera* could potentially both contribute to pollination and exclude the native pollinator species. Given the high diversity of pea plants and bee

species in the SWAFR, understanding the effects of *A. mellifera* on native bees and the pea plants they pollinate would be important from a conservation perspective.

The work provides new insights in the pollination ecology of species of Australian Fabaceae. Revealing fundamental plant-pollinator dynamics, through understanding mimicry interactions, the work offers the bases for advanced ecological studies in the field of restoration ecology in SWAFR, with particular attention to pollinator niches and pollinator functional groups.

## Publication List

### PhD candidate Daniela Scaccabarozzi

#### Indexed Publications ISI-WoS

- 1 D Scaccabarozzi, KW Dixon, S Tomlinson, Lynne Milne, Björn Bohman, RD. Phillips, Salvatore Cozzolino. *Pea plants in the southwestern Australia biodiversity hotspot: pronounced differences in potential pollinators between co-occurring species*. 2019. (Submitted to Plant Ecology Journal, in review).
- 2 D Scaccabarozzi, S Cozzolino, L Guzzetti, A Galimberti, L Milne, KW Dixon and RD Phillips. *Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid*. 2018. *Annals of botany* 122, 1061-107.
- 3 D Galimberti A, De Mattia FF, Bruni I, Scaccabarozzi D, Sandionigi A, Barbuto M, Casiraghi M, Labra M. *DNA barcoding approach to characterize pollen collected by honeybees*. 2014. *Ploce One* 8, 1-13.
- 4 Bruni I, Galimberti A, Caridi L, Scaccabarozzi D, De Mattia F, Casiraghi M, Labra, A *DNA Barcoding Approach to Identify Plant Species in Multiflower Honey*. 2014. *Food Chemistry* 170, 308-315.
- 5 Phillips RD, Scaccabarozzi D, Retter BA, Hayes C, Brown GR, Dixon KW and Peakall R. *Pollination of sexually deceptive trap-flowers by fungus gnats in Pterostylis (Orchidaceae)*. 2013. *Annals of Botany* 113, 629–641.
- 6 Brusa G, Castiglioni L, Scaccabarozzi D, Camozzini G and Cerabolini B. *La vegetazione delle pozze d'alpeggio: valutazioni ecologiche orientate alla definizione di criteri naturalistici nella progettazione*. 2011 *Studi trentini di Scienze Naturali* 88, 77-88.

## Accomplishments

- April 2019 Western Australia Semifinal - FameLab 2019 Science Communication Competition
- April 2019 - Research Rumble Student Poster Display, Poster competition across Faculties at Curtin University, Winner
- April 2019 - Semifinalist at FameLab of Western Australia
- November 2018 - Paper selected as Cover page of the special issue 6 Ecology on the Journal *Annals of Botany*, Vol 122;
- July 2018 - Royal Society Symposium of Western Australia – Poster awarded;
- May 2018 - Friends of Kings Park Scholarship - Conference scholarship award;
- August 2016 - Department of Environment and Agriculture Curtin University Postgraduate Research Scholarship (CIPRS) and Curtin Strategic International Research Scholarship (CSIRS) Scholarship award;
- February 2016 - July 2016 Australian Government - Endeavour Programme - Fellowship award;
- August 2016 - Australian Orchids Foundation - Grant for project support.

## Presentations on the PhD research

- **IOCC – International Orchic Conservation Conference, Kew, UK, 28 May-1 June 2019**, Oral presentation of mimicry pollination system in *Diuris brumalis*
- **Royal Society of Western Australia, Perth, 28-29 July 2018**, Masquerading as pea plants: mimicry in an Australian orchid, Poster presentation;
- **Scientific seminars Plant Biodiversity, Food Quality and Human Health – University of Milano-Bicocca, Milan, Italy** Strategies of 'food mimicry' in Australian orchids, 26 Jan 2018, Presentation;
- **Kings Park Festival, Perth, Western Australia - Science Seminars**, Deception, reward and native bees – unravelling a novel pollination syndrome in southwest Australia, 7 Sept 2017, Presentation;
- **Esa Conference, Fremantle, Western Australia** *Pterostylis* (Orchidaceae) pollination by sexual deception, 28 Nov-2 Dec 2016, Presentation