

Introduced honeybees (*Apis mellifera*) in orchid pollination: surrogate pollinators or pollen wasters?

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Abstract

Biological invasion is one of the leading threats to global biodiversity. Invasive species can change the structure and dynamics of landscapes, communities, and ecosystems, and even alter mutualistic relationships across species such as pollination. Orchids are one of the most threatened plant families globally and known to have established specialised pollination mechanism to reproduce, yet the impact of invasive bees on orchid reproduction has not been comprehensively assessed. We conduct a literature survey to document global patterns of the impact of invasive honeybees on orchids' pollination. We then present a study case from Australian orchids, testing the extent to which introduced honeybees can successfully pollinate orchids across different degrees of habitat alteration, using *Diuris brumalis* and *D. magnifica* (Orchidaceae). Globally, *Apis mellifera* is the principal alien bee potentially involved in orchid pollination. We show that pollinator efficiency and fruit set in *D. brumalis* is higher in wild habitats in which both native bees and invasive honeybees are present, relative to altered habitat with introduced honeybees only. Pollen removal and fruit set of *D. magnifica* rise with native bees' abundance whilst pollinator efficiency decreases with honeybee abundance and increases with habitat size. Complementarily to our findings, our literature survey suggests that the presence of introduced honeybees adversely impacts orchid pollination, likely via inefficient pollen transfer. Given the worldwide occurrence of introduced honeybees, we warn that some orchids may be negatively impacted by these alien pollinators, especially in altered and highly fragmented habitats where natural pollination networks are compromised.

Introduction

Biological invasions are one of the leading threats to global biodiversity (Bellard et al. 2016), impacting the structure and dynamics of landscapes, communities, and ecosystems (Traveset and Richardson 2014). The cascade effect of alien species can adversely affect mutualistic relationships among plant and animals, including pollination (Traveset and Richardson 2014). Particularly, invasive bees can change the original plant-pollinator network and even harm both partners (Agüero et al. 2020). By competing with native pollinators for floral resources and nesting sites (Goulson 2003; Agüero et al. 2018; Thomson and Page 2020), invasive bees can impact pollinator fitness and population dynamics (Thomson, 2004; Paini & Roberts, 2005; Hudewenz and Klein 2015). Through altering pollen flow, alien pollinators are expected to compromise plant reproductive success (Dohzono and Yokoyama 2010), limit pollen availability to native pollinators (Do Carmo et al. 2004; Traveset and Richardson 2014; Dohzono and Yokoyama 2010) and increase heterospecific pollen deposition (Traveset and Richardson 2014; Marrero et al. 2016).

European honeybees (*Apis mellifera*) have become principal floral visitors of plant species of ecosystems around the world (Herrera 2020), but their effect on plant reproductive success is complex to detect (Page and Williams 2023) and to assess (Agüero et al. 2020). Honeybees are generalist pollinators and frequent plant visitors but may not necessarily benefit plant reproduction of all species (Ollerton et al. 2012), especially when they competitively replace native pollinators and become ineffective surrogates (Page et al. 2021). Conversely, in cases where native pollinators are rare or locally extinct, honeybees often boost pollination (Lomov et al. 2010; Hanna et al. 2013) or can even recover plant fitness from reproductive collapse in fragmented habitat (Dick 2001). However, most studies have documented how honeybees impact native bee communities through floral resource competition, whilst their effect on plant reproduction remains poorly documented (Agüero et al. 2020; Page and Williams 2023).

Orchids present highly specialised pollination mechanisms, and therefore the impact of invasive honeybees on the fitness of these plants might be important. Beyond their renowned diversity of pollination systems, orchids can attract pollinators with nonrewarding flowers via various modes of deception (Van der Pijl and Dodson 1966; Dressler 1981; Dafni 1984; Schiestl 2005; Jersáková et al. 2006). About 46% of all orchid species globally are thought to lack reward (Shrestha et al. 2020; Ackerman et al. 2023), typically resulting in lower insect visitation rates compared to rewarding ones (Scopece et al. 2010; Brundrett 2019), deserving careful consideration for their conservation biology. Being that orchids offer pollen in discrete pollinia, instead of unpacked pollen dust as occurs in other flowering plants, is even more important to maximise the pollen transfer and deposition among flowers during pollinator visits (Johnson and Edwards 2000). A measure of the effectiveness of pollen transfer is pollination efficiency (PE) that is typically measured as the ratio of pollinated flowers on flowers with pollinia removed (Johnson et al. 2004; Tremblay et al. 2005). During transfer by pollinators, pollen losses in orchids are expected to be high when mediated by generalist pollinators and pollinator types overlap (Cozzolino et al. 2005; Scopece et al. 2010). For these reasons, pollinator efficiency in orchids might be hampered by exotic and generalist honeybees that manage to collect the pollinia but are not morphologically configured to successfully deposit the pollinia and guarantee reproduction of the plant. Whilst in most cases pollinia removal and fruit set are similar across populations (Schemske 1980; Ackerman and Montalvo 1990; Proctor and Harder 1995; Li et al. 2011), in some orchid species these trends can diverge. For example, the food deceptive Australian orchid species *Diuris brumalis* and *D. magnifica* show diverse raise of male and female pollination success in relation to model plants' abundance, with the first according to an exponential growth and the second to a logarithmic growth (Scaccabarozzi et al. 2018; Scaccabarozzi et al. 2020). However, studies on evaluating the effect of introduced honeybees on orchid pollination success are very scarce.

Here, we test whether orchid pollination success varies in response to the co-occurrence of introduced and native bees. To do that, our study focuses on two orchids' species of genus *Diuris* (Orchidaceae) with analogous pollination strategies (food deception) but occupying different habitats that are subject to different human alteration degrees. Both species are pollinated by native bees of the genus *Trichocolletes* and are occasionally visited by the introduced *Apis mellifera* that potentially acts as a sub-optimal pollinator (Scaccabarozzi et al. 2018, 2020). In fact, whilst *Apis mellifera* is ubiquitous in all study sites, the occurrence of native bees (*Trichocolletes*) is often discontinuous across the sites. Given that pollination success for *D. brumalis* varies according to habitat type (forest vs disturbed woodland; Scaccabarozzi et al. 2018), and for *D. magnifica* varies according to habitat size (Banksia woodland; Scaccabarozzi et al. 2020), our hypothesis focussed on the occurrence of native pollinators for *D. brumalis* and native pollinator abundance for *D. magnifica*. We expected that pollinia removal i) differed significantly in *D. brumalis* relative to the occurrence of native pollinators; and ii) increased in *D. magnifica* relative to native pollinators abundance. We also tested whether honeybees' interaction with native pollinators augmented the orchid fruit set by confirming that fruit set was higher iii) in *D. brumalis* in sites where native pollinator co-occurred with honeybees and iv) in *D. magnifica* increased relative to native pollinator abundance. Lastly, we predicted that PE v) was higher in *D. brumalis* in sites with native pollinators across habitat types (forest vs disturbed

woodland); vi) increased in *D. magnifica* with habitat size and vii) decreased with higher density of introduced honeybees. To contextualise and complement our study case, we provide an overview of incidence of honeybees in orchid pollination, with a focus on introduced honeybee potentially pollinating native and alien orchid species.

Materials and methods

2.1 Literature survey: incidence of honeybees in pollination of orchids

We searched the global literature to identify and summarise studies in which native and introduced honeybees have been reported as potential pollinators in orchid species. In Google Scholar and Web of Science Core Collection we searched the the following key words: 'Apis', 'pollinat', 'visitor' and 'honeybee' and 'orchid'. The first search was conducted on 1 July 2022 and repeated on 1 March 2023 any paper that mentioned an orchid-honeybee interaction was included. In addition, we included a book, co-authors' photos, and personal observations in which invasive honeybees were reported as a substitute potential pollinator of Australian orchid species. During the survey, the introduced honeybee was recorded as a visitor (V, when only observed landing on a flower); successful pollinator (SP, when successfully pollinating the flowers at least once) or potential pollinators (PP, when removing pollinia at least once).

2.2 Study species

Diuris (Orchidaceae) comprises approx. 120 species distributed principally in Australia, with centres of diversity in south-western and south-eastern Australia (Backhouse et al. 2019). *Diuris* are terrestrial geophytes, producing a solitary scape per plant yearly (Jones, 2006); most species within the genus seem capable of clonal reproduction through vegetative propagation of tubers (Dixon et al. 1989). We selected two species, *Diuris brumalis* and *D. magnifica*, with known pollination strategy and published reproductive data (Scaccabarozzi et al. 2018, 2020). *Apis* was observed to act as a potential pollinator of both species (Fig. 2, a-e).

Endemic to southwestern Australia, *Diuris brumalis*, is very common in Perth city area, and produces yellow brown nectar less flowers during July and August, with between three and 15 flowers per inflorescence (Hoffman and Brown 2011). *Diuris magnifica* is endemic to the Swan Coastal Plain in Western Australia, with its main distribution centred on the Perth metropolitan area (Fig. 1; Hoffman and Brown 2011). Flowering occurs from late winter to early spring, with between three and nine yellow-purple flowers per inflorescence (Hoffman and Brown 2011). Given the species were visited by introduced honeybees and occupied two different habitats, subject to anthropogenic alteration, they were chosen as model species to test for our hypothesis.

2.3 Study sites

We studied 14 populations of *D. brumalis* in the Darling Range, near Perth in Western Australia (Fig. 1). The populations were selected across two different habitat types: Jarrah Forest (hereafter referred to as 'forest') dominated by *Eucalyptus marginata* with *Corymbia calophylla* and open Jarrah Forest with *Eucalyptus marginata* and *Allocasuarina fraseriana* highly subject to fragmentation because of urbanization (hereafter referred to as 'disturbed woodland'). Populations of *D. magnifica* were distributed across 15 sites in bushland remnants within the metropolitan area of the city of Perth (Fig. 1). Habitat was uniform across populations and characterised by *Banksia* woodland, an ecological community adjacent to the Swan Coastal Plain of Perth with a tree layer of *Banksia* with scattered *Eucalyptus* or *Allocasuarina* species and a diverse understorey including sclerophyllous shrubs, graminoids and forbs. Both the orchid species co-flowered with a range of Faboideae that represent a conspicuous component of the understorey vegetation.

2.4 Orchid pollination success

Pollination success data come from previously published studies (Scaccabarozzi et al. 2018; Scaccabarozzi et al. 2020, for *D. brumalis* and *D. magnifica* respectively). Additional data was included to increase the sample size for *D. magnifica* adding two populations. For *D. brumalis* the proportion of flowers with pollinia removal and the proportion of pollinated flowers at the end of the flowering period (i.e., the number of flowers found with at least one pollen massula on the stigma) was quantified in 2016 and in 2017, using a 30 × 30 m quadrat centred on each population. As per *D. brumalis*, at the end of flowering period in 2017, the proportion of *D. magnifica* flowers with pollinia removal and the proportion of pollinated flowers was recorded.

2.5 Observational transects on pollinator occurrence

We carried out observation along transects of 100 m length for 10 sites (populations) in September 2016 and 14 sites in September 2017 during *D. brumalis* flowering. We recorded the occurrence of native pollinator, *Trichocolletes* spp. (Colletidae) bees, the introduced honeybee by observing all the flowering species of the understory vegetation along the transect (Fig. 2a-e) and habitat type. Transects were centred on the same quadrats used to quantify pollination success of *D. brumalis* (see next paragraph). Observations along transect lasted 40 min, spending approximately 3 min per flowering plant. Transects were repeated one week after the initial survey, following the same route. For *D. magnifica* we carried out two observation transects for all the bushland reserves, from 5 to 15 September 2017, by recording the frequency (number of insects) of native pollinator, *Trichocolletes* spp. bees, and the introduced honeybee per 3 min of observation per flowering plant. Beetles were included too since they have been observed to act as sub-optimal pollinator in 2015 by extracting the pollinia and depositing it on the stigma of same orchid flower on two occasions (Scaccabarozzi et al. 2020). Sizes of bushland reserves were obtained from Scaccabarozzi et al. (2020). To quantify the effectiveness of pollen transfer, we calculated pollination efficiency (PE) for each population of both species as a ratio of pollinated flowers on flowers with pollinia removed (Johnson et al. 2004; Tremblay et al. 2005). PE was expressed as a ratio of F_p/F_r where F_p is the number of pollinated flowers and F_r is the number of flowers found with one or both pollinia removed. The value of PE potentially ranges between 0 and 1, with 1 representing the maximum and 0 the lowest efficiency.

2.6 Statistical analysis

We analysed the relationship between the proportion of pollinia removed, proportion of fruits, and pollination efficiency with the following independent variables via generalised linear mixed models: co-occurrence of honeybees and native bees, lack of co-occurrence (for *D. brumalis*), and abundance of honeybees and abundance of native bees (for *D. magnifica*). Year was included in each model as a fixed factor, while population was included as a random effect to account for repeated measures over time.

We also evaluated the effect of pollinator occurrence and year on the proportion of pollinia removed, on the proportion of fruit set and on the overall pollination efficiency in *D. brumalis*. To do so, we employed generalized linear regression models with binomial or quasi-binomial distributions of the response variables, depending on the overdispersion parameter. We firstly evaluated the role of the factor sampling site on the response variables to avoid possible data dependency. Regression models were evaluated for collinearity among covariates using the VIF criterion ($VIF < 3$). All the models were subjected to a backward regression approach to remove non-significant variables through the AICc criterion ($\Delta AICc > 3$). For *D. magnifica* we wanted to assess the effect of habitat size on orchid pollination success (pollinia removed and fruit set) and pollination efficiency. To do so, we tested the effects of number of plants, native and honeybee abundance, beetle abundance, and remnant size on the same response variables analysed for *D. brumalis*. The statistical analyses followed the same workflow described above. Furthermore, the relationship between remnant size and native bee abundance was evaluated through a negative binomial GLM to account for the overdispersion of the residuals occurring in the Poisson model. All the analyses were carried out in R ver 4.2.0 (R Core Team 2022) exploiting the following packages “ggplot2” (Wickham 2016), “plyr” (Wickham 2011), “MuMIn” (Barton 2009), “mass” (Venables and Ripley 2002).

Results

3.1 Incidence of honeybees in pollination of orchids

A total of 82 publications were included in the literature survey, covering 65 different orchid species overall (see Table, Fig. S1) that were potentially pollinated by native or introduced honeybees. These included all continents where orchids occur, except Africa. Asia represented the 35% of total cases, followed by Europe (34%), Oceania (18%) and America (14%) (Fig. S2). Honeybee pollination (or potential pollination) was recorded most often for the Orchidoideae, followed by Epidendroideae subfamily and Cypripedioideae. *Cymbidium* was the most frequently reported genus, with 14 species documented as pollinated (or potentially pollinated) by *Apis cerana* (Fig. S2). The introduced honeybee was observed to act as a: visitor (V, when only observed landing on a flower) for five orchid species; a successful pollinator (SP, when successfully depositing a pollinia at least once) in seven cases or potential pollinators (PP, when removing pollinia at least once) in 15 cases. *Apis mellifera* was the main alien bee involved in orchid pollination, occurring across 25 documented cases whilst the Africanized honeybee (hybrid) has been recorded twice as an alien species. In a few cases, *A. mellifera* was accompanied by other introduced bee genera such as *Bombus*, *Centris* and *Euglossa*.

3.2 Orchid pollination in relation to occurrence of native and alien honeybees

We found an overall effect of sampling year on pollinia removal and fruit set. In detail, the pollinia removal was higher in 2017 ($\chi^2 = 7.4677$, $p = 0.006$), whilst the fruit set was higher in 2016 ($\chi^2 = 4.6356$, $p = 0.03$). For *D. brumalis*, the occurrence of honeybees alone and the co-occurrence of honeybees and the native bees did not impact the pollinia removal (Fig. 3a) ($\chi^2 = 2.8637$, $p = 0.091$), but significantly impacted the fruit set ($\chi^2 = 5.4698$, $p = 0.019$) with lowest values where native bees lacked (Fig. 3b). Pollination efficiency was significantly lower where native bees were missing (disturbed woodland) relative to sites in which occurred with honeybees (forest) ($\chi^2 = 6.1869$, $p = 0.012$) (Fig. 3c).

With respect to *D. magnifica*, the abundance of native bees was associated with a significant increase in both pollinia removal ($\chi^2 = 19.572$, $p < 0.001$) and fruit set ($\chi^2 = 5.1371$, $p = 0.023$) (Fig. 4a, b; Table S1). In particular, the abundance of honeybees led to a decrease of pollination efficiency ($\chi^2 = 7.2195$, $p = 0.007$) (Fig. 4c; Table S1), whilst abundance of native bees did not affect pollination efficiency (Table S1). Overall pollination efficiency resulted lower in 2017 ($\chi^2 = 4.1719$, $p = 0.04$) (Fig. 4c; Table S1).

3.3 Effect of habitat type and size on pollination success and efficiency

In *D. brumalis* populations, as honeybees alone were found in disturbed woodland only, while honeybees and native bees occurred together in forest habitat, habitat type reflected the same effect on pollinia removal, fruit set and pollination efficiency than pollinator occurrence (Fig. 2a, b, c). Consequently, disturbed woodland had lower fruit set than forest. In *D. magnifica* populations, habitat remnant size was correlated with of pollination efficiency ($\chi^2 = 6.7399$, $p = 0.009$) with a positive logarithmic trend (Fig. 5).

Discussion

Our study combined an analysis of experimental data on *Diuris* reproductive success with a literature survey that addresses the role of introduced honeybees in orchid pollination. We also examined whether alien honeybees adversely affect pollination success or have the capacity to support orchid pollination in altered landscapes.

4.1 Introduced honeybees likely impacting orchid pollination globally

The role of introduced honeybees, as a pollinator (or potential) for orchid species remains unresolved since most studies on interactions between introduced and native bees have focused on other plant families. In our literature survey, *Apis mellifera* was the principal potential alien bee involved in orchid pollination (Table 1). Pollination by honeybees (native and introduced) is not really common in orchid species, resulting in only 65 documented cases, in

marked contrast with the prevalence of specialized pollination by the other corbiculate Apidae (orchid bees, bumblebees and stingless bees) which are known to be the primary pollinators of numerous orchid species (van der Pijl and Dodson 1966; van der Cingel 1995, 2001). This is unexpected given the widespread distribution of honeybees in Eurasia and Africa (Michener 2007), but in line with the relatively specialised pollination strategies of most orchids that often involve specific foragers rather than super-generalist pollinators as honeybees (Valido et al. 2019). Even though honeybees are the most frequently observed native pollinator of Mediterranean orchids, due to their omnipresence, but none of these species specializes on this pollinator (Joffard et al. 2019). Pollination by introduced honeybee is especially widespread among *Cymbidium* and *Cypripedium* species, in Asia and America respectively, and quite frequent among the North-Centre American *Cyrtopodium* (Table 1). In these cases, the introduced honeybees have a large body, comparable or even larger in size to *Apis* (i.e. *Bombus*, *Trichocolletes*, *Megachile*) (De Luca and Vallejo-Marin 2013; Scaccabarozzi et al. 2020; Table 1). Therefore, a prerogative of an alien surrogate pollinator seems to be the level of morphological fit between the alien bee and the newly acquired flower.

Table 1

Literature survey presenting the incidence of *Apis mellifera* as a native and alien species in orchid pollination across continents, according to the following categories: V: visitor; PP: potential pollinator; SP: successful pollinator. Personal observations and photos are included to support some evidence focusing on Australian orchid species.

Continent	Country	Subfamily	Plant species	Native or alien plant species	Native bee or other native pollinators	Introduced bee species	Pollination by introduced species	Literature source
Europe	Israel	Orchidoideae	<i>Anacamptis collina</i>	native	<i>Apis mellifera</i>	-	-	Dafni and Ivry 1979
Europe	Israel	Orchidoideae	<i>Anacamptis fragrans</i>	native	<i>Vespula vulgaris</i> , <i>Xylocopa iris</i> , <i>Zygaena grasilini</i> (Lepidoptera), <i>Apis mellifera</i>	-	-	Dafni and Ivry 1979
Europe	Austria and South of Italy	Orchidoideae	<i>Anacamptis morio</i>	native	<i>Bombus</i> sp., <i>Apis mellifera</i> , <i>Andreana</i> sp., <i>Eucera</i> sp. and other bees	-	-	Vöth 1987; Cozzolino et al. 2005
Europe	Greece	Orchidoideae	<i>Anacamptis papilionacea</i>	native	<i>Apis mellifera</i> , <i>Nomada</i> sp., <i>Osmia</i> sp., <i>Eucera</i> sp.	-	-	Vöth 1989
Europe	Austria	Orchidoideae	<i>Anacamptis pyramidalis</i>	native	<i>Apis mellifera</i> , <i>Zygaena purpuralis</i>	-	-	Vöth 1999
America	Puerto Rico	Epidendroideae	<i>Arundina graminifolia</i>	native	<i>Megachile yaeyamaensi</i> , <i>Thyreus takaonis</i>	Africanized honeybee (hybrid)	PP	Sugiura 2014; Ackerman 2021
Asia	Japan, South Korea	Epidendroideae	<i>Bletilla striata</i>	native	likely <i>Tetralonia nipponensis</i>	<i>Apis mellifera</i>	SP	Sugiura 1995; Ogawa and Takashi, 2020; Chung and Chung 2005
America	Chile, Argentina Andes	Orchidoideae	<i>Brachystele unilateralis</i>	native	<i>Bombus dahlbomii</i>	<i>Apis mellifera</i> , <i>Bombus terrestris</i> , <i>Bombus ruderatus</i>	SP	Sanguinetti and Singer 2014
Asia	China	Epidendroideae	<i>Bulbophyllum ambrosia</i>	native	<i>Apis cerana</i>	-	-	Chen and Gao 2011
Oceania	Western Australia	Orchidoideae	<i>Caladenia flava</i>	native	<i>Neophyllotocus</i> , native bee	<i>Apis mellifera</i>	V	Adams and Lawson 1993; Fig S1 and Daniela Scaccabarozzi personal observation
Oceania	Western Australia	Orchidoideae	<i>Caladenia xantha</i>	native	n.a.	<i>Apis mellifera</i>	V	Figure S1
Asia	Japan	Epidendroideae	<i>Calanthe discolor</i>	native	<i>Apis cerana japonica</i> , <i>Eucera nipponensis</i> , <i>Osmia cornifrons</i>	-	-	Suetsugu and Fukushima 2014
Asia	China	Epidendroideae	<i>Changnienia amoena</i>	native	<i>Bombus tritasciatus</i> , likely <i>Apis cerana</i>	-	-	Sun et al. 2003
America	Chile, Argentina Andes	Orchidoideae	<i>Chloraea virescens</i>	native	<i>Bombus dahlbomii</i>	<i>Apis mellifera</i> , <i>Bombus terrestris</i> , <i>Bombus ruderatus</i>	SP	Sanguinetti and Singer 2015
Asia	Nepal	Epidendroideae	<i>Coelogyne flaccida</i>	native	<i>Apis cerana</i>	-	-	Subedi et al. 2011
Asia	Nepal	Epidendroideae	<i>Coelogyne nitida</i>	native	<i>Apis cerana</i>	-	-	Subedi et al. 2011
Asia	India	Epidendroideae	<i>Cymbidium aloifolium</i>	native	<i>Apis cerana indica</i>	-	-	Adit et al. 2022

Continent	Country	Subfamily	Plant species	Native or alien plant species	Native bee or other native pollinators	Introduced bee species	Pollination by introduced species	Literature source
Asia	Japan	Epidendroideae	<i>Cymbidium dayanum</i>	native	<i>Apis cerana japonica</i>	-	-	Matsuda and Sugiura 2019
Asia	Nepal, Sikkim, Bhutan, and North Thailand	Epidendroideae	<i>Cymbidium devonianum</i>	native	<i>Apis cerana japonica</i>	-	-	Sugahara 2006
Asia	Borneo, Malesia	Epidendroideae	<i>Cymbidium finlaysonianum</i>	native	<i>Apis cerana</i>	-	-	Chan et al. 1994; Ong 2010
Asia	Japan	Epidendroideae	<i>Cymbidium floribundum</i>	native	<i>Apis cerana japonica</i>	-	-	Sugahara et al. 2013; Sugahara et al. 2010; Sasagawa et al. 2005
Asia	China	Epidendroideae	<i>Cymbidium goeringii</i>	native	<i>Apis cerana</i>	-	-	Yu et al. 2008
Asia	Japan	Epidendroideae	<i>Cymbidium goeringii</i>	native	<i>Apis cerana japonica</i>	-	-	Tsuji and Kato 2010
Asia	Japan	Epidendroideae	<i>Cymbidium kanran</i>	native	<i>Apis cerana japonica</i>	-	-	Tsuji and Kato 2010
Asia	China	Epidendroideae	<i>Cymbidium lancifolium</i>	native	<i>Apis cerana</i>	-	-	Cheng et al. 2007
Asia	Japan	Epidendroideae	<i>Cymbidium lancifolium</i>	native	likely <i>Apis cerana cerana</i>	-	-	Suetsugu 2015
Asia	Japan	Epidendroideae	<i>Cymbidium macrorhizon</i>	native	<i>Apis cerana cerana</i>	-	-	Suetsugu 2015
Asia	India	Epidendroideae	<i>Cymbidium pendulum</i>	native	<i>Apis cerana</i>	<i>Apis mellifera</i>	SP	Attri and Kant 2011; Jagdeep et al. 2012; Verma et al. 2012
Asia	Japan	Epidendroideae	<i>Cymbidium pumilum</i>	alien	<i>Apis cerana japonica</i>	western honeybee not attracted	-	Sasaki et al. 1991
Asia	China	Epidendroideae	<i>Cymbidium qubeiense</i>	native	<i>Apis cerana cerana</i>	-	-	Hu et al. 2018
Asia	Myanmar	Epidendroideae	<i>Cymbidium suavissimum</i>	native	<i>Apis cerana japonica</i>	-	-	Sugahara 2006
America	USA	Cypripedioideae	<i>Cypripedium candidum</i>	native	likely <i>Andrena</i> sp., <i>Odontomyia pubescens</i> (Diptera)	<i>Apis mellifera</i>	PP	Pearn 2013; Grantham et al. 2018
America	USA	Cypripedioideae	<i>Cypripedium parviflorum</i>	native	likely <i>Andrena</i> sp., <i>Odontomyia pubescens</i> (Diptera), <i>Lasioglossum zonulum</i>	<i>Apis mellifera</i>	PP	Pearn 2013; Grantham et al. 2019
America	USA, Canada	Cypripedioideae	<i>Cypripedium reginae</i>	native	likely <i>Anthophora</i> ; <i>Megachile</i> spp.	<i>Apis mellifera</i>	PP	Edens Meier et al. 2011
America	Mexico	Epidendroideae	<i>Cyrtopodium macrobulbon</i>	native	likely <i>Centris</i> or <i>Xylocopa</i>	<i>Apis mellifera</i>	PP	Miranda-Molina et al. 2021
Asia	China	Epidendroideae	<i>Cyrtopodium polyphyllum</i>	alien	<i>Centris tarsata</i> ; <i>Centris labrosa</i>	<i>Apis mellifera</i> , <i>Centris nitida</i> , <i>Centris errans</i>	PP	Liu and Pemberton 2010; Pansarin et al. 2008

Continent	Country	Subfamily	Plant species	Native or alien plant species	Native bee or other native pollinators	Introduced bee species	Pollination by introduced species	Literature source
America	Florida, USA	Epidendroideae	<i>Cyrtopodium punctatum</i>	native	<i>Xylocopa</i> sp.	<i>Apis mellifera</i> , <i>Euglossa viridissima</i> , <i>Centris errans</i>	V	Ackerman 1955; Pemberton and Liu 2008; Dutra et al. 2009
Europe	Poland	Orchidoideae	<i>Dactylorhiza majalis</i>	native	<i>Apis mellifera</i>	-	-	Ostrowiecka et al. 2019
Europe	France	Orchidoideae	<i>Dactylorhiza majalis</i>	native	<i>Bombus</i> sp., <i>Apis mellifera</i>	-	-	Berger 2003
Europe	Sweden	Orchidoideae	<i>Dactylorhiza sambucina</i>	native	<i>Bombus</i> sp., <i>Osmia bicolor</i> , <i>Apis mellifera</i>	-	-	Nilsson 1980
Europe	Poland and England	Orchidoideae	<i>Dactylorhiza</i> spp. (<i>D. incarnata</i> , <i>D. fuchsii</i> and <i>D. majalis</i>)	native	<i>Apis mellifera</i>	-	-	Wroblewska et al. 2019; Ostrowiecka et al. 2019; Dafni and Woodell 1986
America	Puerto Rico	Epidendroideae	<i>Dendrobium crumenatum</i>	alien	<i>Apis cerana</i>	Africanized honeybee (hybrid)	PP	Leong and Wee 2013; Meurgey 2017; Ackerman 2017
Oceania	Eastern Australia	Epidendroideae	<i>Dendrobium kingianum</i>	native	n.a.	<i>Apis mellifera</i>	PP	Figure S1
Oceania	Australia	Epidendroideae	<i>Dendrobium speciosum</i> var. <i>hillii</i>	native	likely <i>Trigona</i> sp., <i>Homalictus</i> sp., <i>Lassioglossum</i> , <i>Hylaeus</i>	<i>Apis mellifera</i>	V	Slater and Calder 1988
Oceania	Western Australia	Orchidoideae	<i>Diuris brumalis</i>	native	<i>Tichocolletes capillosus</i> , <i>Trichocolletes leucogenys</i>	<i>Apis mellifera</i>	PP	Scaccabarozzi et al. 2018
Oceania	Eastern Australia	Orchidoideae	<i>Diuris maculata</i>	native	<i>Trichocolletes venustus</i>	<i>Apis mellifera</i>	SP	Beardsell et al. 1986; Indsto et al. 2006
Oceania	Western Australia	Orchidoideae	<i>Diuris magnifica</i>	native	<i>Tichocolletes gelasinus</i> , <i>T. dives</i>	<i>Apis mellifera</i>	SP	Scaccabarozzi et al. 2019
Oceania	Australia	Orchidoideae	<i>Diuris sulphurea</i>	native	<i>Paracolletes</i> sp., <i>Amegilla</i> sp., <i>Lipotriches</i> sp.	<i>Apis mellifera</i>	PP	Rayment 1932; Fig. S1
Europe	Sweden	Epidendroideae	<i>Epipactis palustris</i>	native	<i>Apis mellifera</i> , <i>Andrena</i> sp., <i>Lassioglossum</i> sp., <i>Bombus</i> and other insects	-	-	Nilsson 1978
Europe	Poland and Czech	Epidendroideae	<i>Epipogium aphyllum</i>	native	<i>Apis mellifera</i>	-	-	Jakubska-Busse et al. 2014
Europe	Czech Republic & Poland	Epidendroideae	<i>Epipogium aphyllum</i>	native	<i>Apis mellifera</i> , <i>Bombus</i> sp.	-	-	Jakubska-Busse et al. 2014
Asia	China	Epidendroideae	<i>Epipogium roseum</i>	native	<i>Apis cerana cerana</i>	-	-	Zhou et al. 2012
Asia	China	Epidendroideae	<i>Eria coronaria</i>	native	<i>Apis cerana</i>	-	-	Shangguan et al. 2008
Oceania	Western Australia	Orchidoideae	<i>Eriochilus dilatatus</i>	native	Halictidae	<i>Apis mellifera</i>	PP	Bundrett 2014; Daniela Scaccabarozzi personal observation

Continent	Country	Subfamily	Plant species	Native or alien plant species	Native bee or other native pollinators	Introduced bee species	Pollination by introduced species	Literature source
Asia	China	Orchidoideae	<i>Goodyera foliosa</i>	native	<i>Apis cerana</i>	-	-	Zha et al. 2016; Liu et al. 2020
Europe	Germany	Orchidoideae	<i>Goodyera repens</i>	native	<i>Bombus</i> sp., <i>Lassioglossum</i> sp., <i>Apis mellifera</i>	-	-	Vöth 1999; Classens and Kleynen 2013
Europe	France	Orchidoideae	<i>Gymnadenia conopsea</i>	native	<i>Apis mellifera</i> , <i>Colias phicomone</i> (Lepidoptera), <i>Erebia albertanus</i> (Lepidoptera)	-	-	Berger 2009
Europe	Austria	Orchidoideae	<i>Himantoglossum adriaticum</i>	native	<i>Apis mellifera</i> , <i>Colletes similis</i>	-	-	Vöth 1999
Europe	Hungary	Orchidoideae	<i>Himantoglossum adriaticum</i>	native	<i>Apis mellifera</i> (Apiary)	-	-	Biro et al. 2014
Europe	Sweden	Orchidoideae	<i>Neottia ovata</i>	native	<i>Apis mellifera</i> and a broad range of insects	-	-	Nilsson 1981
Europe	Greek	Orchidoideae	<i>Orchis boryi</i>	native	<i>Apis mellifera</i>	-	-	Gumbert and Kunze 2001
Europe	Greece	Orchidoideae	<i>Orchis italica</i>	native	<i>Apis mellifera</i> , <i>Chelostoma transversum</i> , <i>Anthidium septemdentatum</i>	-	-	Vöth 1998
Europe	Austria	Orchidoideae	<i>Orchis militaris</i>	native	<i>Apis mellifera</i> , <i>Andrena</i> sp., <i>Halictus</i> sp., <i>Osmia</i> sp., <i>Tropinota hirta</i> (Coleoptera)	-	-	Vöth 1999
Europe	France	Orchidoideae	<i>Orchis purpurea</i>	native	<i>Apis mellifera</i>	-	-	Berger 2004
Oceania	Australia	Orchidoideae	<i>Prasophyllum elatum</i>	native	native bee	<i>Apis mellifera</i>	V	Figure S1
Oceania	Australia	Orchidoideae	<i>Prasophyllum</i> sp.	native	native bees and wasps	<i>Apis mellifera</i>	PP	Photo and personal observation by Rudie Kuiter Fig. S1
Oceania	Eastern Australia	Orchidoideae	<i>Spiranthes australis</i>	native	<i>Amegilla asserta</i> (likely primary pollinator);	<i>Apis mellifera</i>	PP	Ren personal observation; Kuiter 2023
Asia	Japan	Orchidoideae	<i>Spiranthes australis</i>	native	<i>Megachile nipponica</i> ; <i>M. japonica</i> ; <i>Halictidae</i> sp.	<i>Apis mellifera</i>	SP	Suetsugu and Abe 2021; Iwata et al. 2012
Europe	Ireland	Orchidoideae	<i>Spiranthes romanzoffiana</i>	native	<i>Apis mellifera</i>	-	-	Duffy and Stout 2008
Oceania	Australia	Orchidoideae	<i>Spiranthes sinensis</i>	native	guild of native bees	<i>Apis mellifera</i>	PP	Coleman 1933
Asia	China	Orchidoideae	<i>Spiranthes sinensis</i>	native	<i>Apis cerana</i> , <i>Bombus</i> sp., <i>Ceratina</i> , Halictidae	-	-	Tao et al. 2018
Europe	Greek	Orchidoideae	<i>Spiranthes spiralis</i>	native	<i>Apis mellifera</i>	-	-	Petanidou et al. 2013
Europe	NA	Orchidoideae	<i>Spiranthes spiralis</i>	native	<i>Apis mellifera</i>	-	-	Reinhard et al. 1991
America	USA	Orchidoideae	<i>Spiranthes vernalis</i>	native	native bee	<i>Apis mellifera</i>	PP	Catling 1983
Europe	Austria	Orchidoideae	<i>Traunsteinera globosa</i>	native	<i>Bombus</i> sp., <i>Lassioglossum</i> sp., <i>Apis mellifera</i> and other insects	-	-	Vöth 1994

Our literature search shows that in most of cases the introduced honeybee was ineffective in replacing native pollinators. There were 25 recorded cases where introduced honeybees were observed as a visitor or a potential pollinator but in only seven cases they were recorded as a pollinator. However, none of these cases except our study case has investigated the impact of introduced honeybees on the orchid pollination efficiency or the frequency of their pollination events.

4.2 Orchid pollination success and efficiency in relation to occurrence of native and alien honeybees

In our empirical study, western honeybees occurred in all study sites for both target species (*D. brumalis* and *D. magnifica*) whilst occurrence of native bees (*Trichocolletes* spp.) was erratic across sites. In *D. brumalis*, honeybees predominantly occurred along with native bees in the genus *Trichocolletes* (Scaccabarozzi et al. 2018), but in the absence of native bees, orchid fruit set had the lowest values (Fig. 3b). Notably, there was no difference on orchid pollinia removal between sites where honeybees occurred alone and sites where it co-occurred with native bees (Fig. 3a), indicating that honeybees led to comparable level of pollinia removal to native bees. Thus, honeybees are capable of successfully removing pollinia from flowers of *D. brumalis* (Fig. 3, a, b, c, d), but since fruit set and pollination efficiency were lowest when honeybees occurred alone, we hypothesise that they deplete pollen supplies available to native pollinators (Ackerman 2021) and fail to be effective at pollen deposition. This highlights the value of native pollinator specificity in orchid pollen deposition. According to the *lock* and *key* hypothesis food deceptive species showed higher levels of correlation between pollinarium and stigmatic cavity lengths comparing to sexual deceptive species (Lussu et al. 2019), to avoid heterospecific pollen deposition of sympatric species, so that pollinator specificity is very crucial in food deceptive species.

In *D. magnifica* both male and fruit set exponentially raised with native bee abundance (*Trichocolletes gelasinus*; Fig. 4a, b) and they were not impacted by the abundance of *Apis mellifera* along study sites. The output was similar among pollinia removal and fruit set and conforms with our expectations that optimal pollinator frequency would enhance the orchid reproductive success.

Interestingly, in *D. magnifica*, the increasing of honeybees abundance inversely influenced the orchid pollination efficiency likely because they withdraw pollinia without successfully depositing them on the next flower (Fig. 2, b, c, e; Fig. 4c; Argüero 2020; Ackerman 2021) as per in *D. brumalis*. However, the abundance of native bees did not influence the pollination success and PE for this species. This could be explained by the discontinuous occurrence of native bees across the bushland remnants, especially in smaller bushland reserves. It is also plausible that other factors might interfere with the ability of native pollinators to fulfil their pollination service, i.e., presence of suboptimal pollinators such as beetles, that were observed to remove pollinia and deposit it on the same flowers on few occasions (Scaccabarozzi et al. 2020) and competition between honeybees and wild bees for access to floral resources (Agüero et al. 2020; Page and Williams 2023). In addition, plant success often relies more on bee assemblage and diversity than abundance per se (Klein et al. 2003). However, the significant impact of honeybees' abundance on *D. magnifica* pollination efficiency provided compelling evidence for the detrimental effect of honeybees' abundance on orchid reproduction. Honeybee is well known for its modest efficiency in pollination service (Hung et al. 2018; Page et al. 2022) and in some cases is even possible to observe a shift from a mutualistic relationship between the plant and the pollinator to an antagonistic one where costs (i.e., associated with nectar replenishment or damage to flowers) exceed the benefits for the plant pollination (Aizen et al. 2014).

Findings here suggest an accurate and considered management of beekeeping activity that can influence the abundance of alien bees relate to native ones so reducing antagonistic costs for the plants. However, we intend to cautiously consider this hypothesis because our study sites did not include orchid populations with native bees only. To test for the effect of native bees and introduced honeybees on orchid pollination and if this latter is influenced by resource overlap between native and introduced bees more conclusively, would be necessary to i) isolate the effects of native bee occurrence from honeybee occurrence (this may not be feasible since honeybees are often ubiquitous); ii) test if a lack of native bees is primarily caused by habitat change or competition with honeybees, and iii) investigate honeybee abundance in intact and altered habitat respectively.

4.3 Effect of habitat type and size on pollination success and efficiency

Habitat type influenced the orchid reproductive success in *D. brumalis*. but habitat type (wild vs disturbed) also influenced the co-occurrence of honeybees and native bees (Fig. 3, b, c), since only honeybees occurred in disturbed woodland. *Diuris brumalis* pollination success was highest with the occurrence of native bees and lowest with honeybees only.

We were not able to determine the causes of lack of native pollinators in some of the study sites, but we hypothesize that anthropogenic habitat alteration (disturbance linked to urban development) might have led to their decline (Potts et al. 2010; Scheper et al. 2014). Given that *Trichocolletes* native bees are ground-nesting bees (Houston et al. 2023), habitat change might interfere with nesting and foraging sites (Goulson et al. 2005; Biesmeijer et al. 2006; Baude et al. 2016), eventually leading to their local loss. Species that employ Batesian floral mimicry as *D. brumalis* have specialised pollination and rely on few pollinator types (Scaccabarozzi et al. 2018). These outcomes point the attention on the conservation of bee fauna in land use and management especially for crucial pollinators of rare species and highly specific pollinators. For *D. magnifica*, larger bushland reserves led to an increase of pollination efficiency (Fig. 5). Specifically, the growth was sharp in the first half of the predicted trend, where values from 0 to 0.5 PE were linked to habitats within a range of 1–60 ha. This means that even relatively small bush fragments can sustain an effective pollination service. However, only bigger bushland reserves (over 100 ha) showed PE > 0.5, suggesting that the continuous habitat provided a more optimal pollination service. This trend might be explained by the expectation that larger habitat sizes sustain a higher biodiversity of native bees (number and richness) (Blaauw & Isaacs, 2014).

Conclusion

We empirically show that *Diuris* pollination was impacted by the interplay between exotic bees and native bees. In *D. brumalis* the pollination efficiency (PE) was higher in the wild habitat where native and alien honeybees co-occurred and was lower in the altered habitats with only introduced honeybees. Pollination efficiency was also positively impacted by habitat type and size respectively for *D. brumalis* and *D. magnifica*. Because many members of the orchid family are at high risk of extinction, resolving their pollination status in areas occupied by introduced honeybees is vital for their conservation through effective land management. Our study provides evidence that biological invasion by honeybees can impact orchid pollination efficiency and that this effect is exacerbated by habitat disturbance. Given that European honeybees have occupied all continents except Antarctica and are the primary alien bees involved in orchid pollination, our literature survey highlights the importance on conducting studies on the interaction of native and alien pollinator species globally. Our findings recommend an accurate and considered management of beekeeping activity such as introduction of honeybees to new areas, carefully determining the minimum distance of hives to orchid populations and monitoring the number of honeybees relative to native bees in the sites where hives are located. This knowledge is required for ensuring the survival of many orchid species, especially where the habitat is altered and highly fragmented and the effect of honeybees on orchid reproductive success is most severe.

Declarations

Supplementary material

Supplementary material associated with this article includes Figure S1, Figure S2, Table S1 and Datafile S1.

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Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

Daniela Scaccabarozzi, Zong-Xin Ren, Salvatore Cozzolino contributed to the study conception and design. Material preparation, data collection, graphics and analysis were performed by Daniela Scaccabarozzi, Lorenzo Guzzetti, Emiliano Pioltelli, Andrea Aromatisi, Zong-Xin Ren, Mark Brundrett, Mario Vallejo-Marin and Giovanni Polverino. The first draft of the manuscript was written by Daniela Scaccabarozzi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

References

1. Ackerman JD, Phillips RD, Tremblay RL, Karremans A, Reiter N, Peter CI, Bogarín D, Pérez-Escobar OA, Liu H (2023) Beyond the various contrivances by which orchids are pollinated: global patterns in orchid pollination biology. *Bot J Linn Soc* 202:295–324. <https://doi.org/10.1093/botlinnean/boac082>
2. Ackerman JD (2017) Orchidées invasives: accélération de la colonisation et de la propagation. *L'Orchidophile* 213:167–173
3. Ackerman JD (2021) Island invasions by introduced honey bees: what can be expected for Puerto Rico and the Caribbean? *Front Ecol Evol* 8:556744. <https://doi.org/10.3389/fevo.2020.556744>
4. Ackerman JD, Montalvo AM (1990) Short-and long-term limitations to fruit production in a tropical orchid. *Ecol* 71:263–272. <https://doi.org/10.2307/1940265>
5. Agüero JI, Rollin O, Torretta JP, Aizen MA, Requiere F, Garibaldi LA (2018) Impactos de la abeja melífera sobre plantas y abejas silvestres en hábitats naturales. *Ecosistemas* 27:60–69. <https://doi.org/10.7818/ECOS.1365>
6. Agüero JI, Pérez-Méndez N, Torretta JP, Garibaldi LA (2020) Impact of invasive bees on plant-pollinator interactions and reproductive success of plant species in mixed *Nothofagus Antarctica* forests. *Neotrop Entomol* 49:557–567. <https://doi.org/10.1007/s13744-020-00787-6>
7. Adams PB, Lawson SD (1993) Pollination in Australian Orchids: a Critical-Assessment of the Literature 1882–1992. *Aust J Bot* 41:553–575. <https://doi.org/10.1071/BT9930553>
8. Adit A, Koul M, Choudhary AK, Tandon R (2022) Interaction between *Cymbidium aloifolium* and *Apis cerana*: Incidence of an outlier in modular pollination network of oil flowers. *Ecol Evol* 12:e8697. <https://doi.org/10.1002/ece3.8697>
9. Aizen MA, Morales CL, Vázquez DP, Garibaldi LA, Sáez A, Harder LD (2014) When mutualism goes bad: density-dependent impacts of introduced bees on plant reproduction. *New Phytol* e8697. <https://doi.org/10.1002/ece3.8697>
10. Attri LK, Kant R (2011) Orchid Pollination: an observation on pollination-pollinator interaction in *Cymbidium pendulum* (Sw.) Roxb. *Curr Bot* 2:05–08

11. Backhouse G, Bates RJ, Brown AP, Copeland LM (2019) A Checklist of the Orchids of Australia Including its Island Territories. Self-published, Melbourne. ISBN 978-0-9946489-3-8
12. Beardsell DV, Clements MA, Hutchinson JF, Williams EG (1986) Pollination of *Diuris maculata* R Br (Orchidaceae) by Floral Mimicry of the Native Legumes *Daviesia* spp and *Pultenaea scabra* R Br. Aust J Bot 34:165–173. <https://doi.org/10.1071/BT9860165>
13. Bellard C, Cassey P, Blackburn TM (2016) Alien Species as a Driver of Recent Extinctions. Biol Lett 12:20150623. <https://doi.org/10.1098/rsbl.2015.0623>
14. Berger L (2003) Observations sur le comportement de quelques pollinisateurs d'orchidées (2e partie) – L'Orchidophile 159:277–290
15. Berger L (2004) Observations sur le comportement de quelques pollinisateurs d'orchidées (3e partie). – L'Orchidophile 160:19–35
16. Berger L (2009) La pollinisation du genre *Nigritella* (L.C.M. Richard). – J Europäischer Orchideen 41:411–432
17. Bíró E, Bodis J, Nagy T, Toekoelyi J, Molnar VA (2015) Honeybee (*Apis mellifera*) mediated increased reproductive success of a rare deceptive orchid. Appl Ecol Environ Res 13:181–192
18. Blaauw BR, Isaacs R (2014) Larger patches of diverse floral resources increase insect pollinator density, diversity, and their pollination of native wildflowers. Basic Appl Ecol 15:701–711. <https://doi.org/10.1016/j.baae.2014.10.001>
19. Brundrett M (2014) Identification and ecology of southwest Australian orchids. Western Australian Naturalists' Club Inc, Perth
20. Burton K (2009) Mu-MIn: Multi-model inference. R package, Version 0.12.2/r18. <https://mumin.r-forge.r-project.org/>
21. Catling P (1983) Pollination of northeastern North American *Spiranthe* (Orchidaceae). Can J Bot 61:1080–1093. <http://dx.doi.org/10.1139/b83-116>
22. Claessens J, Kleynen J (2013) Honigbienen (*Apis mellifera*) als Bestäuber von *Goodyera repens*. – J Europäischer Orchideen 45:133–138
23. Diller C, Castañeda-Zárate M, Johnson SD (2022) Why honeybees are poor pollinators of a mass-flowering plant: experimental support for the low pollen quality hypothesis. Am J Bot 109:1305–1312. <https://doi.org/10.1002/ajb2.16036>
24. Chen L, Gao J (2011) Reproductive ecology of *Bulbophyllum ambrosia* (Orchidaceae). Acta Pharmacol Sin 35:1202–1208. <https://www.plant-ecology.com/EN/10.3724/SP.J.1258.2011.01202>
25. Chan CL, Lamb A, Shim PS, Wood JJ (1994) Orchids of Borneo, volume 1: introduction and a selection of species. Sabah Society Kota Kinabalu, Kew, Royal Botanic Gardens, Kew, London
26. Chung MY, Chung MG (2005) Pollination biology and breeding systems in the terrestrial orchid *Bletilla striata*. Plant Syst Evol 252:1–9. <https://doi.org/10.1007/s00606-004-0256-6>
27. Coleman E (1933) Further notes on the pollination of *Spiranthes sinensis* (Pers.) Ames. Vic Naturalist 50:61–64
28. Cozzolino S, Schiestl FP, Müller A, De Castro O, Nardella AM, Widmer A (2005) Evidence for pollinator sharing in Mediterranean nectar-mimic orchids: absence of pre-mating barriers? Proc Royal Soc B 272:1271–1278. <https://doi.org/10.1098/rspb.2005.3069>
29. Dafni A (1984) Mimicry and deception in pollination. Ann Rev Ecol Evol Syst 15:259–278. <https://doi.org/10.1146/annurev.ev.es.15.110184.001355>
30. Dafni A, Yariv I (1979) Pollination ecology of, and hybridization between *Orchis coriophora* L. and *O. collina* Sol. Ex Russ. (Orchidaceae) in Israel. New Phytol 83:181–187
31. Dafni A, Woodell SRJ (1986) Stigmatic Exudate and the Pollination of *Dactylorhiza fuchsii* (Druce) Soo. Flora 178:343–350. [https://doi.org/10.1016/S0367-2530\(17\)31521-9](https://doi.org/10.1016/S0367-2530(17)31521-9)
32. De Luca PA, Vallejo-Marín M (2013) What's the 'buzz' about? The ecology and evolutionary significance of buzz-pollination. Curr Opin Plant Biol 16:429–435. <https://doi.org/10.1016/j.pbi.2013.05.002>
33. Dick WC (2001) Genetic rescue of remnant tropical trees by an alien pollinator. Proc R Soc B: Biol Sci 268:2391–2396. <https://doi.org/10.1098/rspb.2001.1781>
34. Dixon KW, Buirchell BJ, Collins MT (1989) Orchids of Western Australia: Cultivation and Natural History, 2nd edn. Western Australian Native Orchid Study and Conservation Group, Victoria Park, Perth
35. Do Carmo RM, Franceschinelli EV, da Silveira FA (2004) Introduced honeybees (*Apis mellifera*) reduce pollination success without affecting the floral resource taken by native pollinators. Biotropica 36:371–376. <https://doi.org/10.1111/j.1744-7429.2004.tb00329.x>
36. Dodson CH (1966) Ethology of some bees of the tribe Euglossini (Hymenoptera: Apidae). J Kans Entomol Soc 39:607–629. <https://www.jstor.org/stable/25083566>
37. Dohzono I, Yokoyama J (2010) Impacts of alien bees on native plant-pollinator relationships: a review with special emphasis on plant reproduction. Appl Entomol Zool 45:37–47. <https://doi.org/10.1303/aez.2010.37>
38. Dressler RL (1981) The orchids: natural history and classification. Harvard University Press, Cambridge
39. Duffy KJ, Stout JC (2008) The effects of plant density and nectar reward on bee visitation to the endangered orchid *Spiranthes romanzoffiana*. Acta Oecol 34:131–138. <https://doi.org/10.1016/j.actao.2008.04.007>
40. Dutra D, Kane ME, Adams CR, Richardson L (2009) Reproductive biology of *Cyrtopodium punctatum* in situ: implications for conservation of an endangered Florida orchid. Plant Species Biol 24:92–103. <https://doi.org/10.1111/j.1442-1984.2009.00242.x>
41. Edens-Meier R, Arduser M, Westhus E, Bernhardt P (2011) Pollination ecology of *Cypripedium reginae* Walter (Orchidaceae): size matters. Telopea 13:327–340
42. Goulson D (2003) Effects of introduced bees on native ecosystems. Ann Rev Ecol Evol Syst 34:1–26. <https://doi.org/10.1146/annurev.ecolsys.34.011802.132355>

43. Government of Western Australia (2000) Bush forever, policies, principles and processes, vol 1. Western Australian Planning Commission, Perth
44. Grantham MA, Ford BA, Worley AC (2019) Pollination and fruit set in two rewardless slipper orchids and their hybrids (*Cypripedium*, Orchidaceae): large yellow flowers outperform small white flowers in the northern tall grass prairie. *Plant Biol* 21:997–1007. <https://doi.org/10.1111/plb.13026>
45. Gumbert A, Kunze J (2001) Colour similarity to rewarding model plants affects pollination in a food deceptive orchid, *Orchis boryi*. *Biol J Linn* 72:419–433. <https://doi.org/10.1111/j.1095-8312.2001.tb01328.x>
46. Hanna C, Foote D, Kremen C (2013) Invasive species management restores a plant–pollinator mutualism in Hawaii. *J Appl Ecol* 50:147–155. <https://doi.org/10.1111/1365-2664.12027>
47. Henneresse T, Tyteca D (2016) Insect Visitors and Potential Pollinators of *Orchis militaris* (Orchidaceae) in Southern Belgium. *J Insect Sci* 16:104. <https://doi.org/10.1093/jisesa/iew088>
48. Herrera CM (2020) Gradual replacement of wild bees by honeybees in flowers of the Mediterranean Basin over the last 50 years. *Proc Royal Soc B* 287:20192657. <https://doi.org/10.1098/rspb.2019.2657>
49. Hoffman N, Brown A (2011) Orchids of south-west Australia, 3rd edn. University of Western Australia, Perth
50. Houston TF, Dods K, Milne LA, Scaccabarozzi D (2023) New insights into the unusual nesting biology of the bee *Trichocolletes orientalis* (Hymenoptera: Colletidae, Neopasiphaeinae), particularly its larval ‘oil bath’. *Apidologie* 54:11. <https://doi.org/10.1007/s13592-022-00981-y>
51. Hu S, Xin R, Guo H, Wang X, Zhang Z, Cheng J (2018) Accuracy detection of predicting pollinator from pollination syndromes: taking *Cymbidium qiubeiense* as an example. *J Beijing for Univ* 40:101–110
52. Hudewenz A, Klein AM (2015) Red mason bees cannot compete with honeybees for floral resources in a cage experiment. *Ecol Evol* 5:5049–5056. <https://doi.org/10.1002/ece3.1762>
53. Hung KLJ, Kingston JM, Albrecht M, Holway DA, Kohn JR (2018) The worldwide importance of honeybees as pollinators in natural habitats. *Proc R Soc B: Biol Sci* 285:20172140
54. Indsto JO, Weston PH, Clements MA, Dyer AG, Batley M, Whelan RJ (2006) Pollination of *Diuris maculata* (Orchidaceae) by male *Trichocolletes venustus* bees. *Aust J Bot* 54:669–679. <https://doi.org/10.1071/BT05146>
55. Iwata T, Nagasaki O, Ishii HS, Ushimaru A (2012) Inflorescence architecture affects pollinator behaviour and mating success in *Spiranthes sinensis* (Orchidaceae). *New Phytol* 193:196–203. <https://doi.org/10.1111/j.1469-8137.2011.03892.x>
56. Jakubská-Busse A, Jasicka-Misiak I, Poliwoda A, Święczkowska E, Kafarski P (2014) The chemical composition of the floral extract of *Epipogium aphyllum* Sw. (Orchidaceae): a clue for their pollination biology. *Arch Biol Sci* 66:989–998. 10.2298/ABS1403989B
57. Jersáková J, Johnson SD, Kindlmann P (2006) Mechanisms and evolution of deceptive pollination in orchids. *Biol Rev* 81:219–235. <https://doi.org/10.1017/S1464793105006986>
58. Jin C, Shiyong L, Rong H, Xinlian W, Yibo L (2007) Food-deceptive pollination in *Cymbidium lancifolium* (Orchidaceae) in Guangxi, China. *Biodiv Sci* 15:608–617. <https://www.biodiversity-science.net/EN/10.1360/biodiv.070091>
59. Joffard N, Massol F, Grenié M, Montgelard C, Schatz B (2019) Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids. *J Ecol*. <https://doi.org/10.1111/1365-2745.13013>
60. Johnson SD, Edwards TJ (2000) The structure and function of orchid pollinaria. *Plant Syst Evol* 222:243–269. <https://doi.org/10.1007/BF00984105>
61. Johnson SD, Peter CI, Ågren J (2004) The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proc R Soc B: Biol Sci* 271:803–809. <https://doi.org/10.1098/rspb.2003.2659>
62. Klein AM, Steffan–Dewenter I, Tschamtkke T (2003) Fruit set of highland coffee increases with the diversity of pollinating bees. *Proc R Soc B: Biol Sci* 270:955–961. <https://doi.org/10.1098/rspb.2002.2306>
63. Kuitert HR (2023) Orchid pollinators of Victoria. 5 Eds, Aquatic Photographics, Victoria, Australia
64. Leong TM, Yeow CW (2013) Observations of pollination in the pigeon orchid, *Dendrobium crumenatum* Swartz (Orchidaceae) in Singapore. *Nat Singap* 6:91–96
65. Li P, Huang BQ, Pemberton RW, Luo YB, Cheng J (2011) Floral display influences male and female reproductive success of the deceptive orchid *Phaius delavayi*. *Plant Syst Evol* 296:21–27. <https://www.jstor.org/stable/43558601>
66. Liu H, Pemberton R (2010) Pollination of an invasive orchid, *Cyrtopodium polyphyllum* (Orchidaceae), by an invasive oil-collecting bee, *Centris nitida*, in southern Florida. *Botany* 88:290–295. 10.1139/B10-017
67. Liu N, Xiao H, Chen X, Chi Y, Luo H, Xiong D, Yang B (2020) Nectar secretion characteristics and their effects on insect pollination of *Goodyera foliosa*, a nectar rewarding orchid. *J Trop Subtrop Bot* 28:265–270. <https://doi.org/10.1007/s00606-011-0473-8>
68. Lomov B, Keith DA, Hochuli DF (2010) Pollination and plant reproductive success in restored urban landscapes dominated by a pervasive exotic pollinator. *Landsc Urban Plan* 96:232–239. <https://doi.org/10.1016/j.landurbplan.2010.03.009>
69. Lussu M, De Agostini A, Cogoni A, Marignani M, Cortis P (2019) Does size really matter? A comparative study on floral traits in orchids with two different pollination strategies. *Plant Biol J* 21:961–966. <https://doi.org/10.1111/plb.12993>
70. Matsuda Y, Sugiura N (2019) Specialized pollination by honeybees in *Cymbidium dayanum*, a fall-winter flowering orchid. *Plant Species Biol* 34:19–26. <https://doi.org/10.1111/1442-1984.12231>
71. Marrero HJ, Medan D, Zarlavsky GE, Torretta JP (2016) Agricultural land management negatively affects pollination service in Pampean agro-ecosystems. *Agric Ecosyst Environ* 218:28–32. <https://doi.org/10.1016/j.agee.2015.10.024>

72. Meurgey F (2016) Bee species and their associated flowers in the French West Indies (Guadeloupe, Les Saintes, La Désirade, Marie Galante, St Barthelemy and Martinique) (Hymenoptera: Anthophila: Apoidea). *Ann Soc Entomol Fr* 52:209–232. <https://doi.org/10.1080/00379271.2016.1244490>
73. Miranda-Molina YM, Gonzalez EJ, Marquez-Guzman J, Meave JA, Perez-Garcia EA (2021) Pollination success in three tropical dry forest orchid species from Mexico: insights from floral display, visitation rates, and flower micromorphology. *Bot Sci* 99:771–790. <https://doi.org/10.17129/botsci.2785>
74. Nilsson LA (1978) Pollination ecology of *Epipactis palustris* (Orchidaceae). *Bot Notiser* 131:255–368
75. Nilsson LA (1980) The pollination ecology of *Dactylorhiza sambucina* (Orchidaceae). *Bot Notiser* 133:367–385
76. Ogawa Y, Miyake T (2020) How do rewardless *Bletilla striata* flowers attract pollinators to achieve pollination? *Plant Syst Evol* 306:78. <https://doi.org/10.1007/s00606-020-01709-0>
77. Ollerton J, Price V, Armbruster WS, Memmott J, Watts S, Waser NM, Totland Ø, Goulson D, Alarco R, Stout J, Tarrant S (2012) Overplaying the role of honeybees as pollinators: a comment on Aebi and Neumann. *Trends Ecol Evol* 27:141–142. <https://doi.org/10.1016/j.tree.2011.12.001>
78. Ong PT (2010) Notes on bee pollination in three peninsular Malaysian orchids. *Malay Orchid Rev* 44:73–75
79. Ostrowiecka B, Talalaj I, Brzosko E, Jermakowicz E, Mirski P, Kostro-Ambroziak A, Mielczarek L, Lason A, Kupryjanowicz J, Kotowicz J, Wroblewska A (2019) Pollinators and visitors of the generalized food-deceptive orchid *Dactylorhiza majalis* in North-Eastern Poland. *Biologia* 74:1247–1257. <https://doi.org/10.2478/s11756-019-00285-0>
80. Page ML, Nicholson CC, Brennan RM, Britzman AT, Greer J, Hemberger J, Kahl H, Müller U, Peng Y, Rosenberger NM, Stuligross C, Wang L, Yang LH, Williams NM (2021) A meta-analysis of single visit pollination effectiveness comparing honeybees and other floral visitors. *Am J Bot* 108:2196–2207. <https://doi.org/10.1002/ajb2.1764>
81. Page ML, Williams NM (2022) Honey bee introductions displace native bees and decrease pollination of a native wildflower. *Ecology* 104:e3939. <https://doi.org/10.1002/ecy.3939>
82. Paini DR, Roberts JD (2005) Commercial honey bees (*Apis mellifera*) reduce the fecundity of an Australian native bee (*Hylaeus alcyoneus*). *Biol Conserv* 123:103–112. <https://doi.org/10.1016/j.biocon.2004.11.001>
83. Pansarin LM, Pansarin ER, Sazima M (2008) Reproductive biology of *Cyrtopodium polyphyllum* (Orchidaceae): a Cyrtopodiinae pollinated by deceit. *Plant Biol* 10:650–659. <https://doi.org/10.1111/j.1438-8677.2008.00060.x>
84. Peam MA (2013) Pollination and comparative reproductive success of lady's slipper orchids *Cypripedium candidum*, *C. parviflorum*, and their hybrids in southern Manitoba. Dissertation, University of Manitoba
85. Pemberton RW, Liu H (2008) Potential of invasive and native solitary specialist bee pollinators to help restore the rare cowhorn orchid (*Cyrtopodium punctatum*) in Florida. *Biol Conserv* 141:1758–1764. <https://doi.org/10.1016/j.biocon.2008.04.016>
86. Petanidou T, Duffy KJ, Karatza A, Kantsa A (2013) Reduced fecundity in large populations of a Mediterranean orchid - Evidence for pollinator limitation. *Basic Appl Ecol* 14:36–43. <https://doi.org/10.1016/j.baae.2012.11.007>
87. R Core Team (2022) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org>
88. Rayment T (1932) Two orchids and a bee. *Vic Naturalist* 49:140–140
89. Reinhard HR, Götz P, Peter R, Wildermuth H (1991) Die Orchideen der Schweiz und angrenzender Gebiete. – Fotorotar, Egg 348 pp
90. Sanguinetti A, Singer RB (2014) Invasive bees promote high reproductive success in Andean orchids. *Biol Conserv* 175:10–20. <https://doi.org/10.1016/j.biocon.2014.04.011>
91. Sasagawa H, Kadowaki T, Matsuyama S (2005) Honeybee communication system and pollination tactics of an oriental orchid that secretes honeybee semiochemicals. *Zool Sci* 22:1513–1513
92. Sasaki M, Ono M, Asada S, Yoshida T (1991) Oriental orchid (*Cymbidium pumilum*) attracts drones of the Japanese honeybee (*Apis cerana japonica*) as pollinators *Experientia* 47:1229–1231. <https://doi.org/10.1007/BF01918392>
93. Scaccabarozzi D, Cozzolino S, Guzzetti L, Galimberti A, Milne L, Dixon KW, Phillips RD (2018) Masquerading as pea plants: behavioural and morphological evidence for mimicry of multiple models in an Australian orchid. *Ann Bot* 122:1061–1073. <https://doi.org/10.1093/aob/mcy166>
94. Scaccabarozzi D, Guzzetti L, Phillips RD, Milne L, Tommasi N, Cozzolino S, Dixon KW (2020) Ecological factors driving pollination success in an orchid that mimics a range of Fabaceae. *Bot J Linn Soc* 194:253–269. <https://doi.org/10.1093/botlinnean/boaa039>
95. Scaccabarozzi D, Galimberti A, Dixon KW, Cozzolino S (2020) Rotating arrays of orchid flowers: a simple and effective method for studying pollination in food deceptive plants. *Diversity* 12:286. <https://doi.org/10.3390/d12080286>
96. Schiestl FP (2005) On the success of a swindle: pollination by deception in orchids. *Sci Nat* 92:255–264. <https://doi.org/10.1007/s00114-005-0636-y>
97. Schweiger O, Biesmeijer JC, Bommarco R, Hickler T, Hulme PE, Klotz S, Kühn I, Moora M, Nielsen A, Ohlemüller R, Petanidou T, Potts SG, Pyšek P, Stout JC, Sykes MT, Tscheulin T, Vilà M, Walthers GR, Westphal C, Winter M, Zobel M, Settele J (2010) Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. *Biol Rev* 85:777–795. <https://doi.org/10.1111/j.1469-185X.2010.00125>
98. Schemske DW (1980) Evolution of floral display in the orchid *Brassavola nodosa*. *Evol* 489–493. <https://doi.org/10.1111/j.1558-5646.1980.tb04838.x>
99. Scopece G, Cozzolino S, Johnson SD, Schiestl FP (2010) Pollination efficiency and the evolution of specialized deceptive pollination systems. *Am Nat* 175:98–105. <https://doi.org/10.1086/648555>
100. Shangguan F, Cheng J, Yuanxin X, Yibo L (2008) Deceptive pollination of an autumn flowering orchid *Eria coronaria* (Orchidaceae). *Biodiv Sci* 16:477–483. <https://doi.org/10.3724/SP.J.1003.2008.08096>

101. Shrestha M, Dyer AG, Dorin A, Ren ZX, Burd M (2020) Rewardlessness in orchids: how frequent and how rewardless? *Plant Biol* 22:555–561. <https://doi.org/10.1111/plb.13113>
102. Sugahara M (2006) *Cymbidium devonianum* and *Cymbidium suavisissimum* as well as *Cymbidium floribundum* attracts Japanese honeybees (*Apis cerana japonica*). *Zool Sci* 23:1225
103. Slater AT, Calder DM (1988) The Pollination Biology of *Dendrobium speciosum* Smith: a case of false advertising? *Aust J Bot* 36:145–158. <https://doi.org/10.1071/BT9880145>
104. Subedi A, Chaudhary RP, van Achterberg C, Heijerman T, Lens F, van Dooren TJM, Gravendeel B (2011) Pollination and protection against herbivory of Nepalese Coelogyninae (Orchidaceae). *Am J Bot* 98:1095–1103. <https://doi.org/10.3732/ajb.1000306>
105. Suetsugu K (2015) Autonomous self-pollination and insect visitors in partially and fully mycoheterotrophic species of *Cymbidium* (Orchidaceae). *J Plant Res* 128:115–125
106. Suetsugu K, Abe Y (2021) Unexpected contribution of the introduced honeybee *Apis mellifera* to high fruit set in *Spiranthes australis* (Orchidaceae). *Entomol News* 129:559–563. <https://doi.org/10.3157/021.129.0511>
107. Suetsugu K, Fukushima S (2014) Bee pollination of the endangered orchid *Calanthe discolor* through a generalized food-deceptive system. *Plant Syst Evol* 300:453–459. <https://doi.org/10.1007/s00606-013-0894-7>
108. Sugahara M, Izutsu K, Nishimura Y, Sakamoto F (2013) Oriental Orchid (*Cymbidium floribundum*) Attracts the Japanese Honeybee (*Apis cerana japonica*) with a Mixture of 3-hydroxyoctanoic Acid and 10-hydroxy-(E)-2-decenoic Acid. *Zool Sci* 30:99–104. <https://doi.org/10.2108/zsj.30.99>
109. Sugahara M, Minamoto T, Fuchikawa T, Michinomae M, Shimizu I (2010) *Apis cerana japonica* Discriminates between Floral Color Phases of the Oriental Orchid, *Cymbidium floribundum*. *Zool Sci* 27:901–906. <https://doi.org/10.2108/zsj.27.901>
110. Sugiura N (1995) The pollination ecology of *Bletilla striata* (Orchidaceae). *Ecol Res* 10:171–177. <https://doi.org/10.1007/BF02347939>
111. Sugiura N (2014) Pollination and floral ecology of *Arundina graminifolia* (Orchidaceae) at the northern border of the species' natural distribution. *J Plant Res* 127:131–139. <https://doi.org/10.1007/s10265-013-0587-x>
112. Sun HQ, Luo YB, Ge S (2003) A preliminary study on pollination biology of an endangered orchid, *Changnienia amoena*, in Shennongjia. *J Integr Plant Biol* 45:1019–1023. <https://www.jipb.net/EN/Y2003/V45/I9/1019>
113. Tao ZB, Ren ZX, Bernhardt P, Liang H, Li HD, Zhao YH, Wang H, Li DZ (2018) Does reproductive isolation reflect the segregation of color forms in *Spiranthes sinensis* (Pers.) Ames complex (Orchidaceae) in the Chinese Himalayas? *Ecol Evol* 8:5455–5469. <https://doi.org/10.1002/ece3.4067>
114. Thomson D (2004) Competitive interactions between the invasive European honeybee and native bumble bees. *Ecol* 85:458–470. <https://doi.org/10.1890/02-0626>
115. Thomson DM, Page ML (2020) The importance of competition between insect pollinators in the Anthropocene. *Curr Opin Insect Sci* 38:55–62. <https://doi.org/10.1016/j.cois.2019.11.001>
116. Traveset A, Richardson DM (2014) Mutualistic interactions and biological invasions. *Ann Rev Ecol Evol Syst* 45:89–113. <https://doi.org/10.1146/annurev-ecolsys-120213-091857>
117. Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN (2005) Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biol J Linn Soc* 84:1–54. <https://doi.org/10.1515/biocr-2015-0024>
118. Tsuji K, Kato M (2010) Odor-guided bee pollinators of two endangered winter/early spring blooming orchids, *Cymbidium kanran* and *Cymbidium goeringii*, in Japan. *Plant Species Biol* 25:249–253. <https://doi.org/10.1111/j.1442-1984.2010.00294.x>
119. Venables WN, Ripley BD (2022) *Modern Applied Statistics with S*, 4th edn. Springer, New York
120. Valido A, Rodríguez-Rodríguez MC, Jordano P (2019) Honeybees disrupt the structure and functionality of plant-pollinator networks. *Sci Rep* 9:1–11. <https://doi.org/10.1038/s41598-019-41271-5>
121. Van der Pijl L, Dodson H (1966) *Orchid flowers: Their pollination and evolution*, 1st edn. The Fairchild Tropical Garden and the University of Miami Press, Coral Gables
122. Verma J, Thakur K, Sembi JK, Attri LK, Kant R, Vij SP (2012) Pollination in *Cymbidium pendulum*. (Roxb) Sw (Orchidaceae) *Vegetos* 25:298–302
123. Vöth W (1987) Bestäubungsbiologische Beobachtungen an *Orchis militaris* L. *Die Orchidee* 38:77–84
124. Vöth W (1998) *Orchis italica* und die angetroffenen Insekten. *Die Orchidee* 49:219–221
125. Vöth W (1989) Werden *Ophrys aesculapii* RENZ von *Andrena muscaria* WARNCKE. (Andrenidae) bestäubt? *Mitt BI Arbeitskr Heim Orch Baden-Württ* 21:133–142
126. Vöth W (1994) Bestäuber und Besucher der Blüten von *Traunsteinera globosa* (L.) Rchb., Orchidaceae, in Niederösterreich. – *Linzer Biologische Beitrage* 26:133–148
127. Vöth W (1999) *Lebensgeschichte und Bestäuber der Orchideen am Beispiel von Niederösterreich*. Stapfia 65 Biologiezentrum. Oberösterreichisches Landesmuseum, Linz
128. Wilckman H (2011) The split-apply-combine strategy for data analysis. *J Stat Softw* 40:1–29. <https://www.jstatsoft.org/article/view/v040i01>
129. Wickham H (2016) *ggplot2: elegant graphics for data analysis*. Springer-Verlag, New York. ISBN 978-3-319-24277-4
130. Wilcove DS, Rothstein D, Dubow J, Phillips A, Losos E (1998) Quantifying threats to imperiled species in the United States. *Bioscience* 48:607–615. <https://doi.org/10.2307/1313420>

131. Wroblewska A, Szczepaniak L, Bajguz A, Jedrzejczyk I, Talalaj I, Ostrowiecka B, Brzosko E, Jermakowicz E, Mirski P (2019) Deceptive strategy in *Dactylorhiza* orchids: multidirectional evolution of floral chemistry. *Ann Bot* 123:1005–1016. <https://doi.org/10.1093/aob/mcz003>
132. Yu XH, Luo YB, Dong M (2008) Pollination biology of *Cymbidium goeringii* (Orchidaceae) in China. *J Syst Evol* 46:163–174. <https://doi.org/10.3724/SP.J.1002.2008.06203>
133. Zha ZB, Tang J, Liang YL, Ding H, Luo HL, Yang BY (2016) Breeding system and pollination biology of *Goodyera foliosa* (Orchidaceae). *Trop Subtrop Bot* 24:333–341
134. Zhou X, Lin H, Fan XL, Gao JY (2012) Autonomous self-pollination and insect visitation in a saprophytic orchid, *Epipogium roseum* (D. Don) Lindl. *Aust J Bot* 60:154–159. <https://doi.org/10.1071/BT11265>

Figures

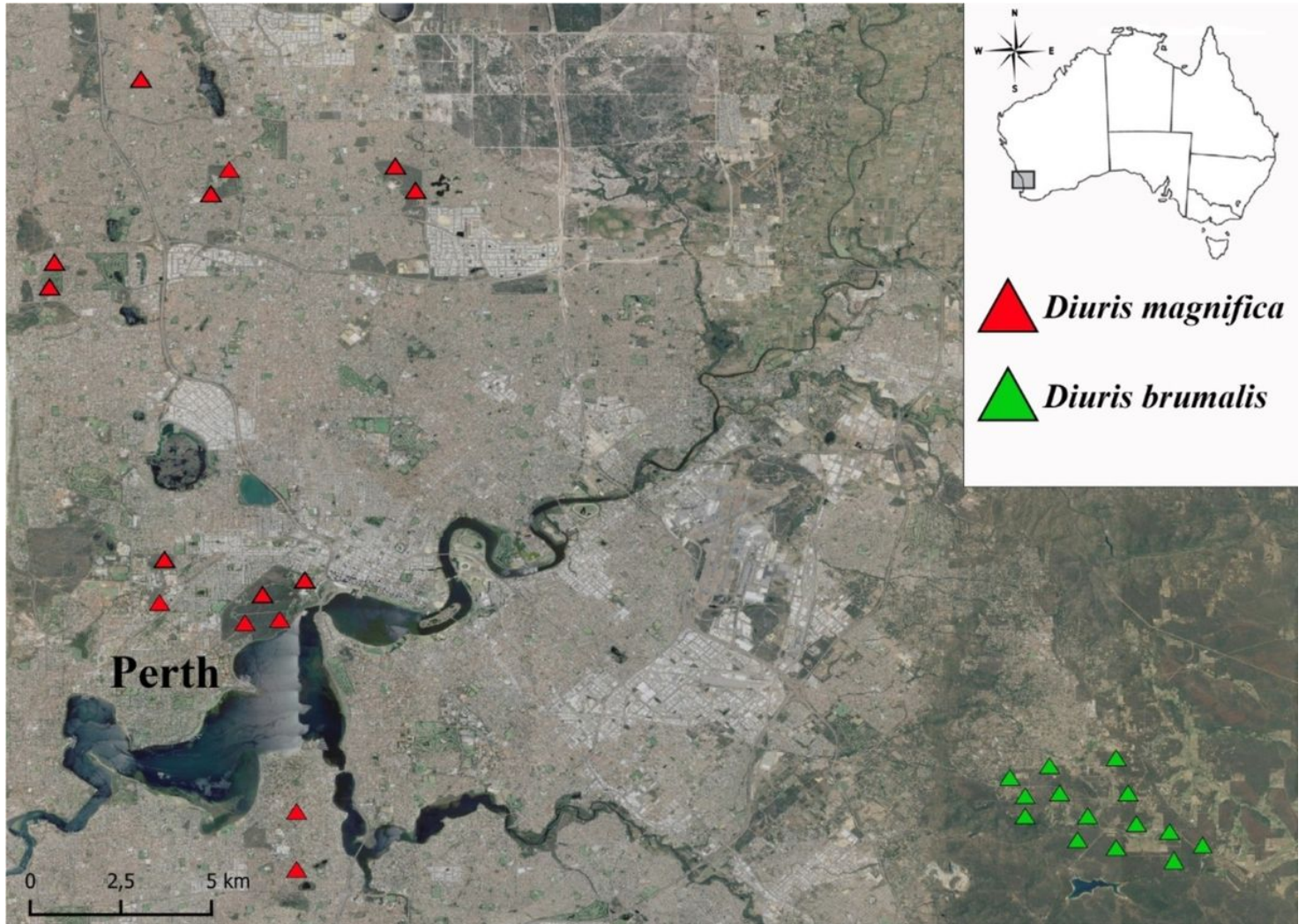


Figure 1

Distribution of field sites of *Diuris brumalis* and *D. magnifica* in Southwestern Australia.

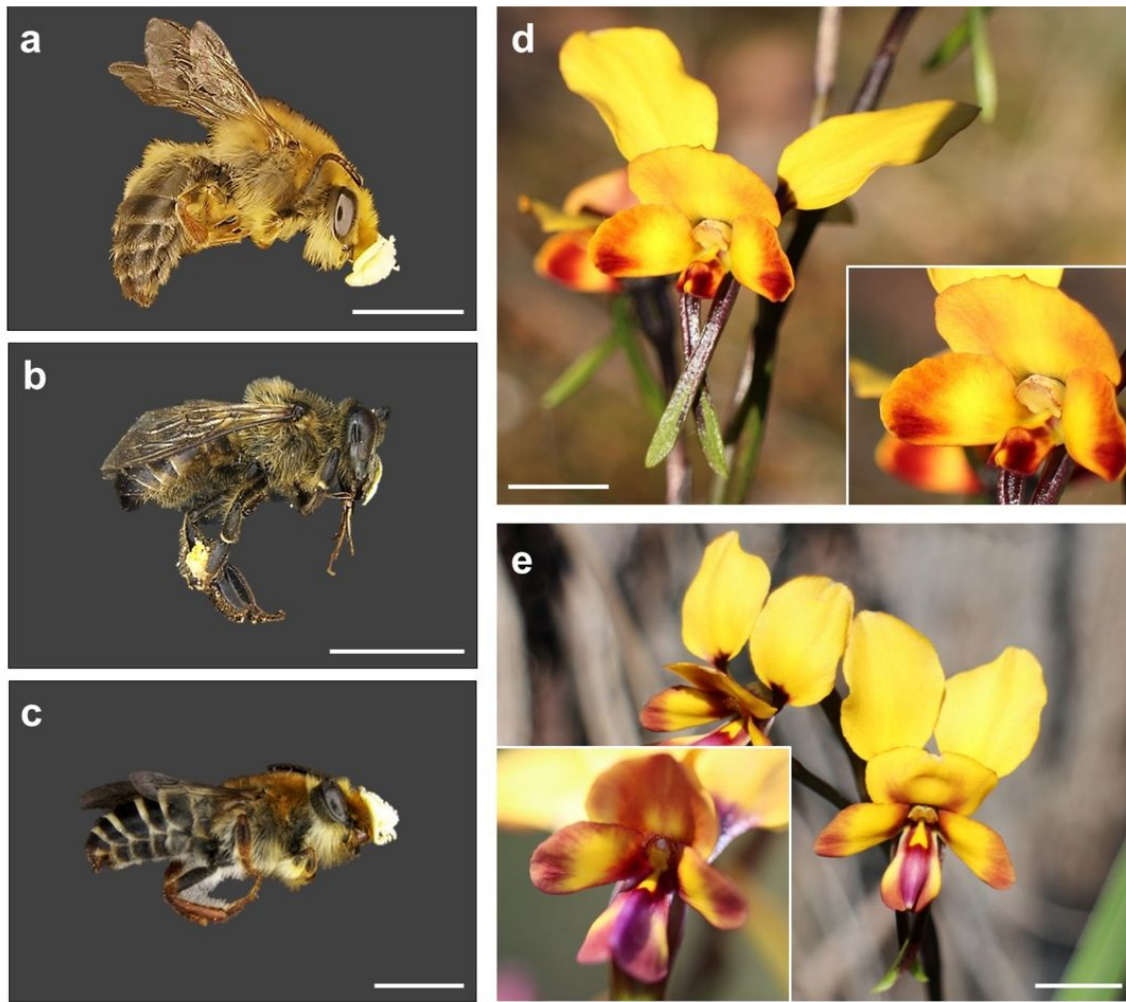


Figure 2

Orchid pollinia placement: on *Trichocolletes capillosus* (a), native pollinator for *Diuris brumalis*, on *Apis mellifera*, potential pollinator for *D. brumalis* and *D. magnifica* (b); and *Trichocolletes gelasinus* (c), *D. magnifica*-native pollinator; flower morphology and focus on flower of *D. brumalis* (d) and *D. magnifica* (e) showing the wide from the top column where the pollinia is placed and the labellum platform. Scale bar of 5 mm. Credit: Daniela Scaccabarozzi

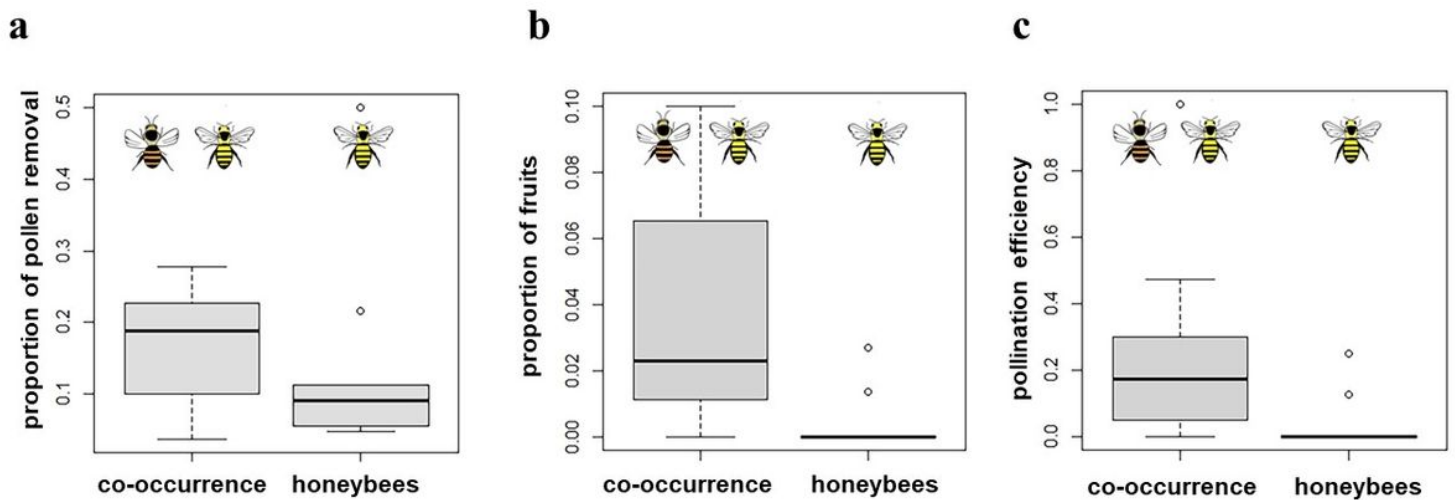


Figure 3

Effects of co-occurrence of honeybees and native bees (co-occurrence) vs. honeybees alone (honeybees) on pollinia removal (a), fruit set (b), and pollination efficiency (c) of *Diuris brumalis*.

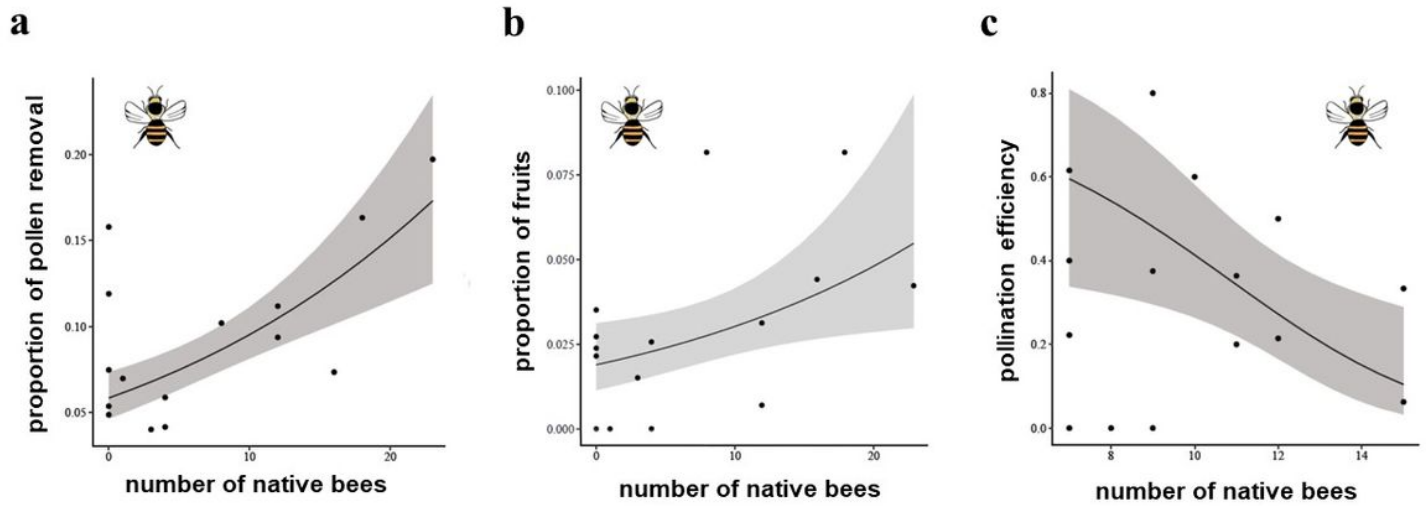


Figure 4
 Number of native bees along transects influences pollinia removal (a), and fruit set (b) of *Diuris magnifica*, while number of introduced honeybees influences pollination efficiency (c).

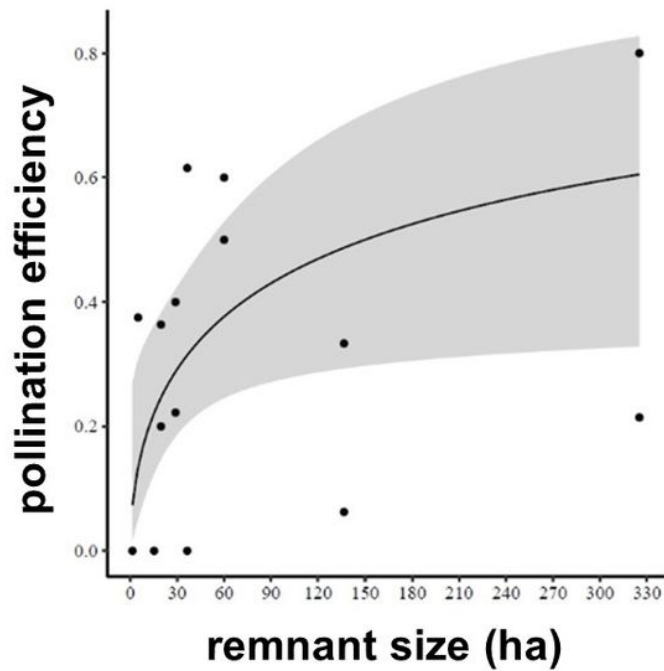


Figure 5
 Pollination efficiency of *Diuris magnifica* as a function of bushland reserve size (habitat size).

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