

Beyond the various contrivances by which orchids are pollinated: global patterns in orchid pollination biology

JAMES D. ACKERMAN^{1,*}, RYAN D. PHILLIPS^{2,3,4}, RAYMOND L. TREMBLAY⁵, ADAM KARREMANS^{6,7}, NOUSHKA REITER^{3,4}, CRAIG I. PETER⁸, DIEGO BOGARÍN⁶, OSCAR A. PÉREZ-ESCOBAR⁹ and HONG LIU¹⁰

¹Department of Biology, University of Puerto Rico, San Juan, Puerto Rico, USA

²Department of Ecology, Environment and Evolution, La Trobe University, Melbourne, Victoria, Australia

³Royal Botanic Gardens Victoria, Cranbourne, Victoria, Australia

⁴Ecology and Evolution, Research School of Biology, Australian National University, Canberra, ACT, Australia

⁵Intercampus PhD Program, University of Puerto Rico, San Juan, Puerto Rico, USA

⁶Jardín Botánico Lankester, Universidad de Costa Rica, Cartago, Costa Rica

⁷Evolutionary Ecology Group, Naturalis Biodiversity Center, Leiden, The Netherlands

⁸Department of Botany, Rhodes University, Grahamstown, South Africa

⁹Royal Botanic Gardens, Kew, Richmond, Surrey, UK

¹⁰Department of Earth and Environment, Florida International University, Miami, Florida, USA

Received 12 July 2022; revised 30 October 2022; accepted for publication 30 December 2022

Orchidaceae show remarkable diversity in pollination strategies, but how these strategies vary globally is not entirely clear. To identify regions and taxa that are data-rich and lend themselves to rigorous analyses or are data-poor and need attention, we introduce a global database of orchid reproductive biology. Our database contains > 2900 species representing all orchid subfamilies and 23 of 24 tribes. We tabulated information on habit, breeding systems, means of pollinator attraction and the identity of pollinators. Patterns of reproductive biology by habit, geography and taxonomy are presented graphically and analysed statistically. On the basis of our database, most orchid species sampled are pollinator dependent (76%) and self-compatible (88%). Pollinator attraction based on rewards occurs in 54% of the species, whereas 46% use some means of deceit. Orchids generally have highly specific pollinator interactions (median number of pollinator species = 1). Nonetheless, on average, specificity is lower for species offering rewards, occurring in multiple continental regions or Northern America (as defined by the Taxonomic Database Working Group Level 1 regions). Although our database reveals impressive knowledge gains, extensive gaps in basic observations of orchid reproductive biology exist, particularly in tropical regions and diverse lineages of fly-pollinated species. The database is expected to facilitate targeted studies, further elucidating the ecological and evolutionary drivers of orchid diversity.

ADDITIONAL KEYWORDS: biogeography – breeding systems – floral deception – Orchidaceae – pollinator diversity – pollinator rewards – reproductive biology – sexual deceit.

INTRODUCTION

Charles Darwin is credited for identifying Orchidaceae, with all their fantastic flowers, vegetative forms and extraordinary species richness as a model system for studying evolutionary processes (Darwin, 1862). Specifically, Darwin interpreted the various contrivances (pollination mechanisms) as means to

enhance the probability of outcrossing, a cornerstone of his theory on the origin of species through natural selection (Darwin, 1859). Unparalleled by any other plant family, orchids have evolved a plethora of strategies to attract pollinators, using rewards, such as nectar, lipids, fragrances, trichomes, pollen and resins, and various forms of deception (van der Pijl & Dodson, 1966; van der Cingel, 2001). Because of the unusual floral traits and often unconventional pollination attraction strategies, orchids have been

*Corresponding author. E-mail: ackerman.upr@gmail.com

at the forefront to understand floral adaptations to pollinators (Johnson & Schiestl, 2016). We also have compelling evidence that differences in pollinator attraction can lead to reproductive isolation (Hills 1972; Williams & Dodson, 1972; Schiestl & Schluter, 2009; Xu *et al.*, 2011; Peter & Johnson, 2014; Whitehead & Peakall, 2014), and therefore are likely to be important contributors to speciation. This highlights the importance of pollination biology for unravelling the origins of the enormous morphological and taxonomic diversity of orchids but also for understanding their macro-evolutionary dynamics (Pérez-Escobar *et al.*, 2017) (Fig. 1).

A hallmark of the orchid family is the high proportion of species that employ deceit to attract pollinators by exploiting the cognitive and sensory abilities of pollinators via chemical, visual or tactile stimuli, generally in combination (e.g. van der Pijl & Dodson, 1966; Kullenberg & Bergström, 1976; Ackerman, 1986; Nilsson, 1992; Jersáková, Johnson & Kindlmann, 2006; Renner, 2006; Jersáková, Johnson & Jürgens, 2009; Peter, 2011). Estimates of the proportion of deceptive orchid species vary from 25% (Dressler, 1993) to 33% (as 'nectarless' in van der Pijl & Dodson, 1966) and, more recently, 36% (Peter, 2011). Orchids exhibit two major forms of deceit. The first involves food deception, which may entail specific mimicry of a co-occurring rewarding model species (known as Batesian mimicry) (e.g. Dafni & Ivri, 1981; Kjellsson, Rasmussen & Dupuy, 1985; Peter & Johnson, 2008; Scaccabarozzi *et al.*, 2018) or generalized food deception where a deceptive species exploits innate preferences of insects (e.g. Ackerman, 1981; Jersáková *et al.*, 2006; Peter & Johnson, 2013). The second prevalent form of deceitful pollination is sexual deception, where male pollinators are enticed to visit flowers that provide visual, tactile and/or olfactory signals that are indicative of a female insect (Schiestl *et al.*, 1999, 2003; de Jager & Peakall, 2015; Bohman *et al.*, 2017). The floral signals can be so persuasive that insects attempt copulation and may even ejaculate (Blanco & Barboza, 2005; Gaskett, Winnick & Herberstein, 2008; Cohen *et al.*, 2021). A third means of deception is brood-site deception, typically involving mimicry of larval food such as mushrooms, dung, carrion or prey to attract female flies (Johnson & Schiestl, 2016). Although such means of deceit occur in numerous genera of flowering plants (Jürgens *et al.*, 2013), to date they have rarely been reported for orchids (e.g. Atwood, 1984; van der Niet, Hansen & Johnson, 2011; Martos *et al.*, 2015; Jiang *et al.*, 2020).

In addition to nectar, orchid flowers offer several other types of reward, including floral volatiles, trichomes, lipids, pollen, resins and sleep sites (Dodson *et al.*, 1969; Goss, 1977; Gregg, 1991; Singer

& Koehler, 2004; Pansarin & Pansarin, 2010; Davies & Stpiczyńska, 2012; Vereecken *et al.*, 2012), none of which provides nutrition for the forager. Trichomes, oils and pollen are foodstuffs for brood; resins, waxes and perhaps oils are for nest construction; floral volatiles are for attracting mates. Methods for detecting rewards are much more sophisticated than in the past, even enabling the discovery of nanogram levels of sugar (Reiter *et al.*, 2018). Such overlooked minuscule quantities of rewards are being reported with greater frequency (e.g. Gomiz, Torretta & Aliscioni, 2017; Davies & Stpiczyńska, 2019; Pansarin, 2021) and may significantly influence pollinator behaviours and, therefore, plant reproductive success (Reiter *et al.*, 2018).

Most orchids are self-compatible (Tremblay *et al.*, 2005), which sets the stage for the evolution of autogamy, especially when pollinator services do not exist, are infrequent or unpredictable (Ortiz-Barney & Ackerman, 1999). There is some evidence that orchids at high latitudes, on islands and at the periphery of their geographical or elevational range are more likely to be self-pollinating than at lower latitudes or in continental regions, which is congruent with the reproductive assurance hypothesis (e.g. Hagerup, 1952; Jain, 1976; Ackerman, 1985; Catling, 1990). The frequency of self-pollination may also be habit-dependent. Martín-Rodríguez *et al.* (2015) showed that autonomous self-pollination was higher in rupicolous and epiphytic species than terrestrial species of Neotropical Gesneriaceae. Is this true for orchids as well?

Van der Pijl & Dodson (1966) estimated that more than half of all orchid species are pollinated by bees (55%), whereas Peter (2011) found no such dominance by any one pollinator group (Table 1). Nevertheless, Peter (2011) noted that bees were the most reported pollinators, serving 40% of orchid species studied. According to van der Pijl & Dodson (1966) and Peter (2011), Diptera are the second most common taxonomic order of pollinators for orchids, and such records are likely to increase simply because fly-pollinated orchids are understudied, with the pollinators being challenging to observe and identify. Furthermore, several species-rich groups such as *Bulbophyllum* Thouars (2111 species; POWO, 2021), Malaxidinae (1255 species; Chase *et al.*, 2015) and most Pleurothallidinae (> 5500 species; Karremans & Vieira-Urbe, 2020) exhibit fly pollination syndromes (van der Pijl & Dodson, 1966). Similarly, records of moth pollination are also likely to increase, especially for orchids pollinated by settling moths (smaller, non-hovering Geometridae, Noctuidae etc.), a functional group segregated from the large, long-tongued hovering moths of Sphingidae (Vogel, 1954, 2006; Peter & Venter, 2017). Like Diptera, settling moths are often challenging to observe and identify.

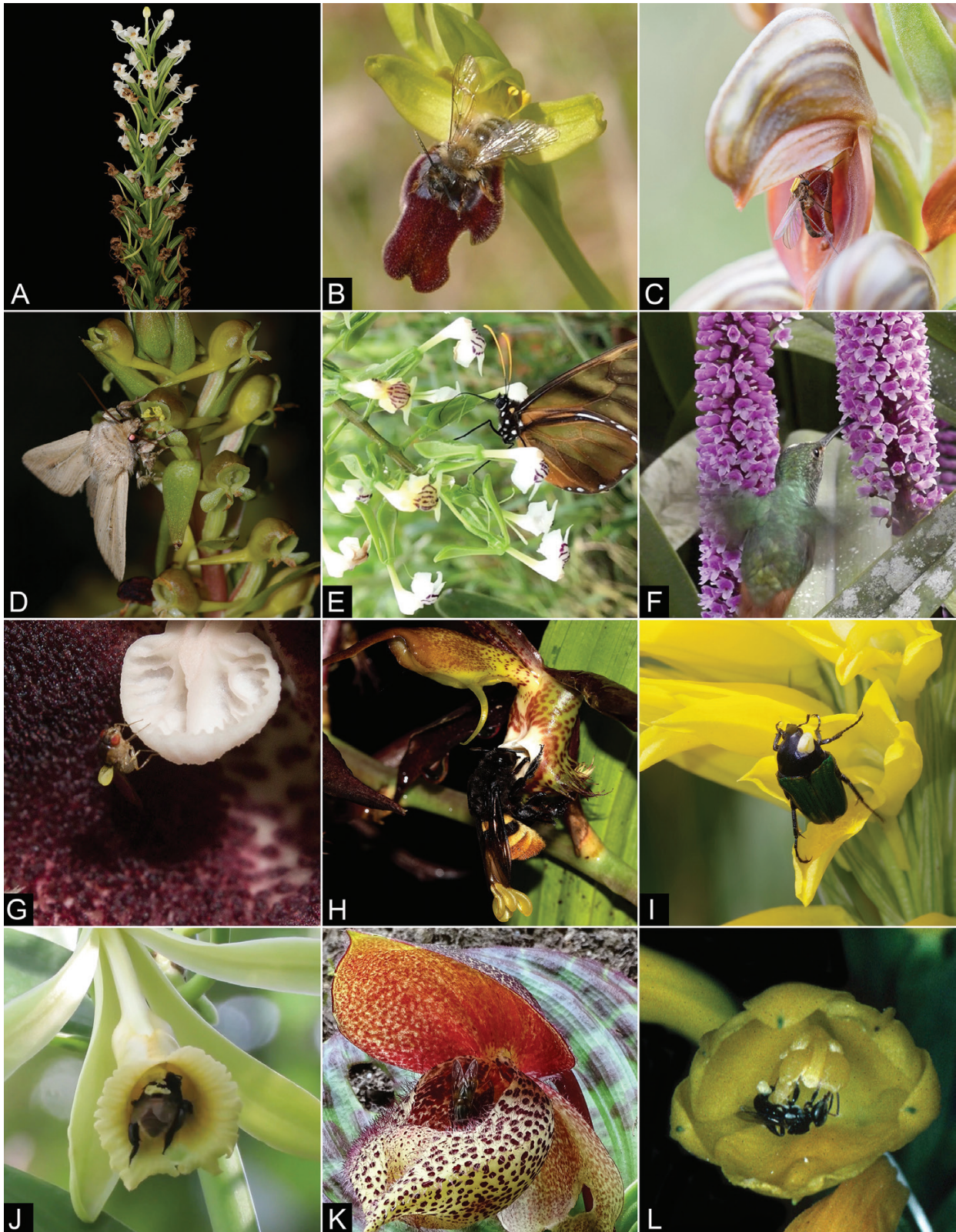


Figure 1. Examples of the diversity of orchid pollination strategies. A, *Habenaria monorrhiza* (Orchidoideae), a Neotropical terrestrial, autonomously self-pollinating. B, *Ophrys lojaconoi* (Orchidoideae), pollinated by *Andrena* sp. (Andrenidae) through sexual deception in Italy. C, *Pterostylis orbiculata* (Orchidoideae), pollinated by *Mycomya* sp. (Mycetophilidae)

Whether or not specialization or generalization predominates among pollination systems has been debated in the literature (Waser *et al.*, 1996; Johnson & Steiner, 2000), but part of this disagreement stems from variation in the prevalence of specialized systems among geographical regions and plant families (Johnson & Steiner, 2003; Ollerton *et al.*, 2009; Johnson & Wester, 2017). Orchids are recognized as a group with a high incidence of specialization regardless of how it is defined (Nilsson, 1992; Tremblay, 1992; Phillips *et al.*, 2020b). Indeed, the literature is replete with well-studied orchid species with one or few pollinator species (Tremblay, 1992; Ray & Gillett-Kaufman, 2022). This applies to species representative of a range of different pollinator attraction strategies (e.g. nectar reward: Johnson *et al.*, 2011; floral fragrance reward: Ackerman, 1983a; food deception: Peter & Johnson, 2013; sexual deception: Paulus & Gack, 1990). In many cases, specialization appears to arise from orchids using floral signals targeted at a particular pollinator species (e.g. Peter & Johnson, 2014). However, specialization is reinforced by the requirement of pollinators of a specific size to fill the gap between the labellum and the column. This means that of the range of animal species visiting an orchid flower, only a subset of them may be the appropriate

size and shape to remove and deposit pollen (e.g. Li *et al.*, 2008; Phillips *et al.*, 2020b).

With a nearly exponential increase in the number of orchid pollination studies since van der Pijl & Dodson (1966) (van der Cingel, 1995, 2001; Peter, 2011), we now have a much greater understanding of orchid biology, and the time to re-appraise the trends is overdue. Therefore, we combined data from as many published orchid pollination studies as possible to yield a global database providing information on 2962 orchid species. Specifically, we sought data on breeding systems, means of attracting pollinators and the number of pollinators per orchid species. From these data, we identify general patterns and knowledge gaps limiting our understanding of orchid biology at the global level. Specifically, we address the following trends in the database. (1) What are the frequencies of different means for attracting pollinators, and how are they distributed within the family? (2) What are the frequencies of different breeding systems? (3) What are the diversity and relative frequency of animal taxa that serve as pollinators? (4) Do orchids show high levels of specificity for pollinators? We then explore whether patterns are dependent on plant habit (terrestrial or epiphytic) and geography [latitudinal zones and Level 1 regions of the International Working Group on Taxonomic Databases (TDWG)].

Table 1. Distribution of major groups of orchid pollinators and the frequency of autogamy. Pollinator percentages (in italics) of present data add up to more than 100% because some species employ multiple pollinator classes. P&D = van der Pijl & Dodson (1966). Exclusively autonomous self-pollination plus agamospermy ('Selfers') percentage is calculated from the list by $\text{autogamy}/(\text{chasmogamy} + \text{autogamy-mixed pollination}) \times 100$

| Source | Bees | Wasps | Moths | Butterflies | Flies | Beetles | Birds | Others | Selfers |
|----------------|-------------|-------------|------------|-------------|-------------|-------------|------------|------------|-------------|
| P&D, data | 222 | 25 | 15 | 11 | 40 | 6 | 20 | 1 | — |
| | <i>68.3</i> | <i>7.7</i> | <i>4.6</i> | <i>3.4</i> | <i>12.3</i> | <i>12.3</i> | <i>6.2</i> | <i>0.3</i> | |
| P&D, estimates | 55 | 5 | 8 | 3 | 15 | — | 3 | — | 3 |
| Peter, 2011 | 520 | 88 | 55 | 20 | 101 | 15 | 52 | 7 | 396 |
| | <i>39.9</i> | <i>6.8</i> | <i>4.2</i> | <i>1.5</i> | <i>7.8</i> | <i>1.2</i> | <i>4.2</i> | <i>0.6</i> | <i>30.4</i> |
| Present data | 1006 | 224 | 141 | 62 | 383 | 72 | 60 | 15 | 468 |
| | <i>57.5</i> | <i>12.8</i> | <i>8.1</i> | <i>3.5</i> | <i>21.9</i> | <i>4.1</i> | <i>3.4</i> | <i>0.9</i> | <i>18.8</i> |

through sexual deception in Australia. D, *Satyrium parviflorum* (Orchidoideae), pollinated by *Vietteania* sp. (Noctuidae) through nectar reward in South Africa. E, *Epidendrum piliferum* (Epidendroideae), pollinated by *Dircenna klugii* (Nymphalidae) through food deception in Costa Rica. F, *Arpophyllum giganteum* (Epidendroideae), pollinated by *Amazilia tzacatl* (Trochilidae) through nectar reward in Costa Rica. G, *Dracula vinacea* (Epidendroideae), pollinated by *Zygothrica* sp. (Drosophilidae) through brood-site deception in Colombia. H, *Catasetum saccatum* (Epidendroideae), pollinated by *Eulaema* sp. (Apidae: Euglossini) through fragrance collection in Peru. I, *Eulophia ensata* (Epidendroideae), pollinated by *Leucocelis haemorrhoidalis* (Scarabaeidae) in South Africa. J, *Vanilla pompona* (Vanilloideae), a vine pollinated by *Eulaema cingulata* (Apidae: Euglossini) through food deception in Costa Rica. K, *Cyrtopodium lichiangense* (Cyrtopodioideae), pollinated by *Ferdinandea cuprea* (Syrphidae) through brood-site mimicry in China. L, *Newwiedia veratrifolia* (Apostasioideae), pollinated by *Trigona laeviceps* (Apidae: Meliponini) through pollen rewards in Malaysia. Photographs by A.P. Karremans (A, E, F), A. Perilli (B), T. Hayashi (C), C. Peter (D, I), N. Gutierrez (G), L.E. Yupanki (H), C. Watteyn (J), C.C. Zheng (K) and A. Kocyan (L). Published with permission from the photographers.

METHODS

LITERATURE SEARCH

We sought information on the reproductive biology of orchids, especially pollinator observations, means of attraction (including rewards) and breeding systems from literature published since Darwin (1877). Our cut-off publication date for incorporating information into the database for our analyses was 31 December 2020. We used several means to discover literature because many publications on orchid pollination are in the ‘orchid literature’ (e.g. orchid society journals), which are not often included in the more scholarly-oriented search engines. Particularly helpful have been *Bibliorchidea* (Jenny, 2007) and the compilations of van der Pijl & Dodson (1966); Adams & Lawson (1993); van der Cingel (1995, 2001); Pridgeon *et al.* (1999, 2001, 2003, 2005, 2009); Claessens & Kleynen (2011), Argue (2012a, 2012b) and online resources, notably Webofscience.com, Scopus.com, Scholar.google.com, Researchgate.com and Academia.net. We also used our networks of colleagues and scanned the literature cited within the publications accessed. The Pollination List, metadata and cited literature in the list are publicly available and in Zenodo (Ackerman *et al.*, 2022). In addition, we added a few unpublished pollination observations by the authors and their colleagues documented in photographs, which are in the Supporting Information (Fig. S1, evidence of pollinators and self-pollination). We did not necessarily include every reference we found for a given species if the information contained had already been included in the entry for a particular species.

ORCHID TAXONOMY AND HABIT

We followed the orchid classification of Chase *et al.* (2015), except where otherwise noted. Those exceptions usually arise when alternative treatments have had wide acceptance in the region where the taxa occur, or more recent phylogenetic studies resolve ambiguities. Species counts for genera are based on Chase *et al.* (2015), except where more recent counts have been published. Unless otherwise noted, our default treatment at the species level was *Plants of the World Online* (POWO, plantsoftheworldonline.org). Particularly problematic has been the genus *Ophrys* L. where the number of species ranges from ten (Bateman, Sramkó & Paun, 2018) to 353 (Delforge, 2016), the differences being rooted in both methodological and philosophical issues (Paulus, 2019; Cozzolino *et al.*, 2020; Bateman *et al.*, 2021). We have listed pollinators of *Ophrys* using the taxonomy adopted by the authors, as it is easier to work plant–pollinator data from narrower to broader concepts rather than the reverse, particularly when dealing with geographical patterns of variation.

We scored whether an orchid species was achlorophyllous, terrestrial, rupicolous or epiphytic or a climbing vine. Species that routinely grow in more than one category (e.g. some populations are rupicolous, others are epiphytic) were assigned to both categories. Such information was taken from the references cited for that species entry or from floras, monographs or other taxonomic literature and internet sources such as POWO (2021).

GEOGRAPHICAL REGION

We classified species by both the latitudinal zones in which they occur (temperate: > 35.00°; subtropical: 23.27–35.00°; tropical: < 23.27°) and by TDWG Level 1 of the World Geographical Scheme for Recording Plant Distributions (Fig. 2; Brummitt, 2001). If a species occurs in multiple Level 1 regions, then we list them all. We conducted our analyses using both geographical systems.

BREEDING SYSTEMS

We follow the recommendations by Neal & Anderson (2005) and define breeding systems as those traits that influence fertilization (e.g. compatibility/incompatibility mechanisms). Although breeding systems can involve a variety of floral traits such as dioecy and dichogamy, we only quantified the frequencies of self-incompatibility. We scored a species as self-incompatible (SI) if it was tested experimentally with hand pollinations that resulted in either 0% fruit set or < 5% seed set from self-pollination (all such studies included comparisons with cross-pollination). A species is scored as self-compatible (SC) if the fruit set and seed set exceed those criteria (Agnew, 1986). The genetic mechanisms for self-incompatibility are known to be variable but are rarely identified (Zhang *et al.*, 2021), so we did not record this.

A species was classified as exhibiting autonomous self-pollination or agamospermy (similar fruit set outcome, but rarely distinguished) on the basis of experimental evidence or unusually high fruit set with no evidence of pollinators. We scored species as chasmogamous if their flowers open and there is no evidence of autonomous selfing or if autonomous selfing occurs but pollinators are known (mixed pollination system). In the latter case, the columns for both autogamy and chasmogamy were marked as well as the mixed pollination column. Evidence for autonomous selfing was categorized based on the strength of evidence: we scored ‘1’ if autonomous selfing was demonstrated experimentally (pollinator exclusion conditions) or ‘2’ if autogamy is assumed because (1) flowers are cleistogamous, (2) pollinators are unknown and fruit set is exceptionally high, (3) if there are observations of pollen tubes coming

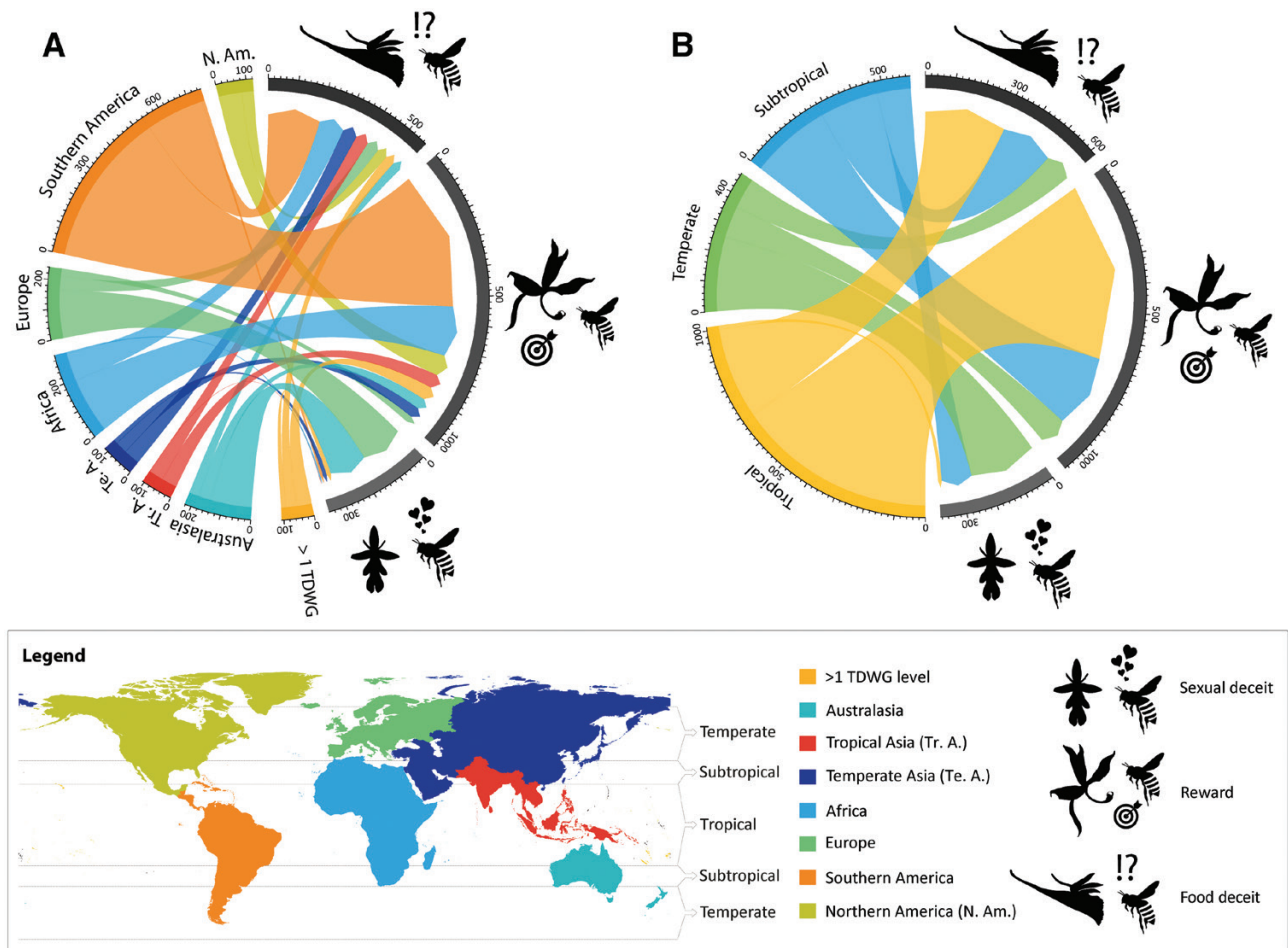


Figure 2. Frequency distribution of modes of pollinator attraction by TDWG Level 1 continental regions and latitudinal zones. Data are from the Pollination List Database. Only the three most common means of pollinator attraction are included. A, Frequency of modes of pollinator attraction in TDWG Level 1 regions. The Pacific and Antarctic regions are excluded because they have few records. N. Am. = Northern America; Te. A. = Temperate Asia; Tr. A. = Tropical Asia; >1 TDWG = more than one TDWG Level 1 region. B, Frequency of modes of pollinator attraction by latitudinal zones. Tropics: < 23.7°; subtropics: 23.7–35°; temperate: 35–66.3°. The scale at the margin of each circle represents the number of records.

directly from the anther, (4) lack of a rostellum, (5) supernumerary anthers, (6) pollinaria bending down to touch the stigma or (7) pollinia (or parts thereof) appear to fall onto the stigma.

We tested whether frequencies of autogamous and chasmogamous systems varied by habit and latitudinal zones using Pearson's chi-squared analyses. When considering growth habit, to reduce bias, we analysed only data from the tropics, as epiphytic species are generally rare or absent in subtropical and temperate regions. However, many rupicolous species also grow as epiphytes, so we combined the two growth habits for the analyses.

POLLINATOR ATTRACTION STRATEGIES

How pollinators are attracted to orchids may be divided into two broad categories: those that offer a reward and those that use deception. The rewarding species were

categorized based on the evidence for their reward status. Reward was scored as '1' when the quantity or composition of reward was ascertained. When evidence for the presence of a reward is inferred on the basis of chemical staining, human senses (visual, tactile, olfactory or taste), or pollinator behaviour (e.g. pollen packed in corbiculae, oil or fragrance collecting behaviours of pollinators), then it is scored a '2'. Deceitful species were classified as either exhibiting food, sexual or brood-site deception. Definitions and examples of these categories are given in Table 2. We used Pearson's chi-squared test to ascertain whether the frequencies of deceit and reward strategies differ among growth habits (epiphytic, rupicolous, terrestrial).

IDENTIFICATION OF POLLINATORS

Pollinators were included in the database for a given orchid species if they had been observed removing

Table 2. Definitions of pollinator attraction strategies used in the database. Reward strategies are listed first. References are intended as examples, not an exhaustive list

| Strategy | Definition | Example | Reference |
|----------------------|--|--|---|
| Nectar | Pollinators consume nectar while visiting the flower | <i>Platanthera bifolia</i> (L.) Rich. | Nilsson (1983) |
| Pollen | Pollen is collected, which is associated with pollination | <i>Neuwiedia borniensis</i> de Vogel | Inoue, Kato & Inoue (1995) |
| Trichomes | The consumption or collection of trichomes is associated with pollination | <i>Polystachya caracasana</i> Rchb.f. | Otero & Alomia (2016) |
| Fragrance | Collecting or ingesting behaviours of volatile compounds by male insects that are later used in courtship rituals | <i>Gongora fulva</i> Lindl. <i>Bulbophyllum patens</i> King ex Hook.f. | Dressler (1968a); Tan & Nishida (2000) |
| Lipids | Lipids are collected by female bees to construct or provision nest cells | <i>Gomesa bifolia</i> (Sims) M.W.Chase & N.H.Williams | Aliscioni <i>et al.</i> (2009); Torretta <i>et al.</i> (2011) |
| Sleep sites | Primary reason for the pollinator visiting the flower is as a site to sleep ('primary' allows for possibility that the odd individual may seek food or attempt to mate with a female that is there to sleep) | <i>Serapias cordigera</i> L. | Vereecken <i>et al.</i> (2012) |
| Resins | Resins collected by female bees and used for nest cell construction | <i>Heterotaxis superflua</i> (Rchb.f.) F.Barros | Krahl <i>et al.</i> (2019) |
| Brood-site deception | Primary pollen vectors are female insects searching for a suitable site to lay eggs ('primary' allows for the possibility that males may occasionally visit and try to mate with approaching females) | <i>Satyrium pumilum</i> Thunb. | van der Niet <i>et al.</i> (2011) |
| Food deception | Pollinators exhibit food seeking behaviour in the absence of a floral reward | <i>Diuris brumalis</i> D.L.Jones | Scaccabarozzi <i>et al.</i> (2018) |
| Sex deception | Attempted copulation or courtship behaviour exhibited with the flower by the pollinator species | <i>Drakaea glyptodon</i> Fitzg. | Peakall (1990) |

or depositing pollen or the vector bore identifiable pollinaria in a position on the body that is consistent with mechanics of pollinarium removal and deposition, a circumstance that applies primarily to euglossine bee-pollinated species (Dressler, 1976; Ackerman, 1983a). Those pollinator reports based on the identity of pollinarium loads are indicated as such in the list. Studies listing probable pollinators inferred based on a floral syndrome are excluded from the list. Observations of non-indigenous pollinators are quite rare but are included. Species-level taxonomy of pollinators follows that of the authors of such studies. Higher taxonomic levels are based on entries in the Catalogue of Life (www.catalogueoflife.org), except when noted otherwise. If the pollinator is not identified to species level, but the author indicates that only one species is involved, then it is scored as a single species. If no indication is given that there is but a single species or that there were multiple species of the taxon involved, then the taxa or taxon below the identified level is left blank.

To obtain an estimate of the frequencies of pollinator taxa by subfamilies and subtribes, we obtained the proportion of observed orchid taxa pollinated by each

group of pollinators and extrapolated to the total species known for each orchid subfamily and subtribe. We also tested for differences in the mean number of pollinator species per orchid species among growth habits, means of pollinator attraction, TDWG Level 1 geographical regions and latitudinal zones.

STATISTICAL APPROACH AND DATA VISUALIZATION

The frequency distributions for the number of pollinator species per orchid have long tails, so using traditional methods of calculating the mean and variance would be biased towards the outliers. Instead, we used 'Robust statistics' (Wilcox, 2017; Maronna *et al.*, 2019), which uses mathematical approaches to reduce the effect of outliers on the descriptive and inferential statistics. We used the Harrell–Davis estimator (hdpd), which calculates the mean location and the quantiles for descriptive statistics, and used the R function 'hdpd' of R package WRS (<https://dornsife.usc.edu/labs/rwilcox/software/>) described in Wilcox (2017: p. 123). The Harrell–Davis estimator reduces the effect of outliers on the central tendencies (median or mean) and dispersion parameters (quantiles and SD) using

a bootstrap method ($N = 10\,000$) where the data are trimmed (removing the outliers) by 20% posterior to the bootstrap (Efron & Tibshirani, 1993). A test of comparison of two independent groups was performed using Yuen's method (Yuen, 1974; Wilcox, 2017: p. 169). This method is similar to Welsh's mean comparison but adjusted to consider outliers. Whenever we report a statistical 'mean' we refer to the 'mean location'.

We performed contingency table analyses to test for factor independence using the R package gmodels (Warnes *et al.*, 2018) and the function CrossTables. Comparison among means of multiple groups was made using an ANOVA-type approach that considers outliers, as described by Wilcox (2017: p. 326, function 'fac2list'), and the *post hoc* test followed the step-down multiple comparison of Wilcox (2017: p. 349, function 'lincon'). Plots were made in R Studio with the packages circlize, ggplot2 and migest (Abel, 2013; Gu *et al.*, 2014; Wickham, 2016; R Core Team, 2020; RStudio Team, 2020).

RESULTS

TAXA WITH DATA AND HABIT DISTRIBUTION

We included data from 1211 publications with records of some aspect of pollination biology for 2921 orchid species representing 416 genera. This represents a sample of *c.* 56% of the genera and 10% of the species in the family. Only 83 of those references (0.8 per year) were published before van der Pijl & Dodson (1966), whereas 1128 were published between 1966 and 2020 (20.9 per year). All five subfamilies are represented in the list, and 23 of 24 tribes (including Apostasioideae and Cyrtipedioidae, neither of which is divided into tribes). Xerorchideae is the only tribe not represented. In addition, all subtribes are represented except for Diceratostelinae, Galeottiellinae and Manniellinae. In total, 51% of species in the database were terrestrial and 52% of the species grow as epiphytes. Only 7% of species were rupicolous, and vines (species of *Vanilla* Plum. ex Mill.) were represented by 0.6% of the species with data. Achlorophyllous species accounted for just 1% of the species (all terrestrials). The percentages add up to more than 100% because some species were placed in more than one category (mostly rupicolous and epiphytic).

GEOGRAPHICAL REPRESENTATION OF POLLINATION STUDIES

Approximate geographical locations are available for all the species in the list, and most (1727) are in tropical latitudes, followed by subtropical latitudes (702 species) and temperate latitudes (585 species). The TDWG Level 1 distribution of orchid species

records are shown in Table 3. Because Level 1 regions Pacific and Antarctica (Falkland Islands) only have few records (eight and one, respectively), these are omitted from analyses using TDWG regions. We found that sampling for orchid reproductive biology data is heavily biased towards Europe and Northern America north of Mexico. Australasia and Africa have 15 and 20% coverage of their orchid diversity, respectively, whereas orchid floras of Temperate Asia, Tropical Asia and Southern America, including Mexico are much under-represented (Table 3).

BREEDING SYSTEMS

Data on compatibility systems are available for 1076 species, with 12% of these being SI. Self-incompatible species in our list are mostly clustered in three tribes and subtribes of Epidendroideae: Epidendreae, Pleurothallidinae (SI/SC: 19/45 species); Malaxideae, Dendrobiinae (64/92) and Cymbidieae: Oncidiinae (25/29).

We have records for 2466 species that indicate whether they are pollinator dependent or not. Approximately 81% of species in the database received pollinator services, and 76% were entirely dependent on pollinators for reproduction. Mixed pollination systems occurred in 4.8% of species, whereas 19.0% were exclusively autonomously self-pollinated or agamospermic (Table 1). However, there was experimental evidence for only 44% of those species considered to be self-pollinated or agamospermic. The others were thought to be

Table 3. Orchid reproductive biology sampling effort by geographical region. Regions 6 (Pacific Islands) and 9 (Antarctica) are excluded because few records of orchid reproductive biology exist from those areas. Data for Mexico are moved to Region 8 due to weak affinities of its orchid flora to the north and overwhelmingly strong phylogenetic and biogeographic affinities to the south

| TDWG Level 1 region | Current estimates | Species with data | Percentage coverage |
|------------------------------------|---------------------|-------------------|---------------------|
| 1 Europe | 592 ^a | 303 | 51 |
| 2 Africa | 2855 ^a | 423 | 15 |
| 3 Temperate Asia | 2020 ^a | 178 | 9 |
| 4 Tropical Asia | 9419 ^a | 331 | 6 |
| 5 Australasia | 1582 ^a | 309 | 20 |
| 7 Northern America north of Mexico | 209 ^b | 153 | 73 |
| 8 Southern America plus Mexico | 14 700 ^a | 1236 | 8 |

^aEstimate from POWO (accessed 7 September 2021).

^bEstimate based on the list from North American Orchid Conservation Center minus the Hawaiian and non-indigenous species (<https://goorchids.northamericanorchidcenter.org> accessed 7 September 2021).

reproducing without pollinator services based on a variety of circumstantial evidence (see Methods). Most achlorophyllous (holomycotrophic) species in the database are autonomously self-pollinated ($N = 38$, 74%). The relative frequencies of the different breeding systems used by orchids vary by growth habit. For example, the frequency of autonomous self-pollination plus agamospermy for terrestrial orchid species was much higher than expected. In contrast, frequencies of pollinator dependent (chasmogamous) species were over-represented in epiphytic species (chi squared = 109.10, d.f. = 2, $P < 0.001$; Table 4). The relative frequencies of breeding systems also varied by latitudinal regions. Temperate orchid species were significantly less likely to be exclusively and autonomously self-pollinating (or agamospermic) than species in subtropical and tropical regions (chi-squared test = 56.42, d.f. = 4, $P < 0.001$; Table 5). The results were the same when we compared temperate orchids and the combination of subtropical and tropical species (chi-squared test = 18.13, d.f. = 2; $P < 0.001$).

POLLINATOR ATTRACTION STRATEGIES

We found records of 1112 orchid species (54%) in 43 subtribes that offer pollinator rewards, and about half of those (51%) produce nectar. Orchids that are pollinated by insect pollinators collecting floral fragrances, account for 24% of the rewarding species, whereas those that produce floral oils account for c. 15%. The remaining 10% comprises species that offer trichomes (food hairs, pseudopollen), resins, pollen or sleep sites (Supporting Information, Table S1).

Deception, including food, brood-site and sexual deception, was recorded in 951 (46%) of the species for which data on the means of pollinator attraction are available. Food deception was the most frequently recorded means of deception accounting for 60% of deceptive species, with instances of food deception distributed across 39 subtribes. Sexual deception accounted for 38% of the records for deceit and is present in 13 subtribes. We found only 11 reports of brood-site deception (1%). Orchidoideae, and Orchidinae, especially, are heavily represented in the data relative to their species richness, whereas

Table 4. Orchid pollination systems by growth habit for tropical regions. ‘Autogamous’ includes autonomously self-pollinating as well as agamospermous species. ‘Mixed’ refers to mixed pollination systems (species with both chasmogamous and autonomous self-pollinating pollination systems or facultatively self-pollinating). ‘Chasmogamous’ species are those that are pollinator dependent. Observed values refer to the number of species in the database, Expected values are based on Pearson’s chi squared = 109.10, d.f. = 2, $P < 0.001$

| Pollination system | | Epiphytic + Rupicolous | Terrestrial | Row total |
|--------------------|----------|------------------------|-------------|-----------|
| Autogamous | Observed | 191 | 116 | 307 |
| | Expected | 248.4 | 58.6 | |
| Mixed | Observed | 50 | 25 | 75 |
| | Expected | 60.7 | 14.3 | |
| Chasmogamous | Observed | 887 | 125 | 1012 |
| | Expected | 818.9 | 193.1 | |
| Column total | | 1128 | 266 | 1394 |

Table 5. Orchid pollination systems by latitudinal region. ‘Autogamous’ includes autonomously self-pollinating as well as agamospermous species. ‘Mixed’ refers to mixed pollination systems (species with both chasmogamous and autonomous self-pollinating systems or facultatively self-pollinating). ‘Chasmogamous’ species are those that are pollinator dependent. Latitude zones: temperate 35.0–66.3°; subtropical 23.7–35.0°; tropical < 23.7°. Pearson’s chi squared = 56.42, d.f. = 4, $P < 0.001$

| Pollination system | | Temperate | Subtropical | Tropical | Row total |
|--------------------|----------|-----------|-------------|----------|-----------|
| Autogamous | Observed | 77 | 39 | 313 | 429 |
| | Expected | 102.0 | 71.6 | 255.4 | |
| Mixed | Observed | 47 | 11 | 75 | 133 |
| | Expected | 31.6 | 22.2 | 79.2 | |
| Chasmogamous | Observed | 442 | 347 | 1029 | 1818 |
| | Expected | 432.3 | 303.3 | 1082.4 | |
| Column Total | | 566 | 397 | 1417 | 2380 |

Epidendroideae, particularly Pleurothallidinae and Dendrobiinae, are under-represented (Fig. 3).

In our database, the distribution of the three basic types of pollinator attraction (reward, food deceit, sexual deceit) are not distributed evenly among the TDWG Level 1 ('continental') regions. Food reward and deceit comprise most of the records for all regions except Europe and Australasia, where the greatest number of records involve species pollinated via sexual deceit (Fig. 2A). Types of pollinator attraction are also unevenly distributed by latitude. Orchids in tropical latitudes (< 23.7°) mostly offer rewards or employ food deceit. Those residing in subtropical latitudes (23.7–35.0°) have attraction strategies more equitably distributed among reward, food deceit and sexual deceit. Orchid floras at temperate latitudes (35.0–66.3°) are striking in the dominance of records of sexual deceit, despite the lack of sexual deception records in Northern America (Fig. 2B). The frequency of deceit and reward strategies are dependent on growth habit (epiphytic, rupicolous, terrestrial), with deceitful means of pollinator attraction dominating among terrestrial species and the provision of rewards dominating among epiphytic species (chi squared = 268.7, d.f. = 2, $P \ll 0.0001$).

POLLINATOR GROUPS

Data on the identity of pollinators are available for 1758 species of orchids, representing just 6% of the family. Hymenoptera are the most reported higher taxonomic group of pollinators and are involved in the pollination of members of all five subfamilies (Fig. 4). However, when using the percentages of known pollinators at the subtribe level to infer the expected pollinator services of unstudied species, we found that Hymenoptera potentially interacts with > 8600 orchid species and Diptera with > 8000 orchid species. Hymenoptera interact with more subfamilies (five) and subtribes (51) than Diptera (subfamilies three, subtribes 29). However, Diptera are involved in the pollination of the most species-rich subtribes such as Dendrobiinae (mostly *Bulbophyllum* Thouars), Malaxidinae and Pleurothallidinae (Fig. 5; Supporting Information, Fig. S2, heatmap of pollinator orders by orchid subtribes; Supporting Information, Fig. S3, heatmap of pollinator families by orchid subtribes; Supporting Information, Fig. S4, expected distribution of pollinator orders among subtribes).

Approximately 8% of orchids in our database are pollinated by Lepidoptera (settling and hovering moths combined; Table 1). The two groups were recorded with approximately equal frequency as butterflies. Much less reported as pollinators were Coleoptera (beetles) and Aves (birds; Apodiformes

and Passeriformes) (Table 1; Fig. 5). Beetles were pollinators of 4.1% of orchid species and were mostly represented by species in Scarabaeidae, Cerambycidae and Oedemeridae, but 16 other families also served as pollen vectors (Supporting Information, Fig. S4, heatmap of pollinator families by orchid subtribes). Just 3.5% of the pollinator data were recorded being pollinated by birds. Nectar feeding birds that pollinate orchids include passerines and hummingbirds, the latter of which accounts for 45 of the 60 records.

We have placed all unusual records of pollinators under the 'miscellaneous' category, which includes Thysanoptera (thrips), Orthoptera (crickets), Hemiptera (true bugs), Araneae (spiders), Squamata (lizards) and Rodentia (mice). Most of these appear to be incidental or at most minor pollinators, but others seem to serve as primary pollinators (e.g. Orthoptera: Micheneau *et al.*, 2010; Rodentia: Wang *et al.*, 2008). While such incidences are intriguing, they are clearly outliers in the orchid pollinator community.

PATTERNS IN NUMBERS OF POLLINATORS

The number of pollinator species recorded per orchid species is strongly skewed to the right, with orchids having just a single known pollinator outnumbering the next category (two pollinators) by a margin of approximately three to one (Fig. 6). The median number of pollinator species is one, but the mean is 3.03 due to the skewed nature of the data. Outliers include a few supergeneralist species, such as *Neottia ovata* (L.) Bluff & Fingerh. (five orders, 34 families, 162 pollinator species), *Epipactis palustris* (L.) Crantz (four orders, 41 families, 136 species) and *Anacamptis pyramidalis* (L.) Rich. (four orders, 14 families, 75 species).

The statistical dispersion of the number of pollinators per species differs among subfamilies (Supporting Information, Fig. S5). The subfamilies with the highest number of known pollinators per orchid species, Cypripedioideae, Vanilloideae, Apostasioideae (mean number of pollinators = 1.440, 1.339 and 1.173, respectively), are also those with the fewest number of records ($N = 39, 27, 3$, respectively) and the most variable highest probability density intervals. The two most species-rich subfamilies, which are the two most intensely sampled, Orchidoideae (mean location = 1.100, $N = 647$) and Epidendroideae (mean location = 1.098, $N = 655$), have virtually the same mean but the former subfamily is more variable.

The number of known pollinator species varies with growth habit (Fig. 7). Orchid species that are rupicolous and species with multiple habits exhibit the most interspecific variation in the number of pollinator species. On average, terrestrial species have

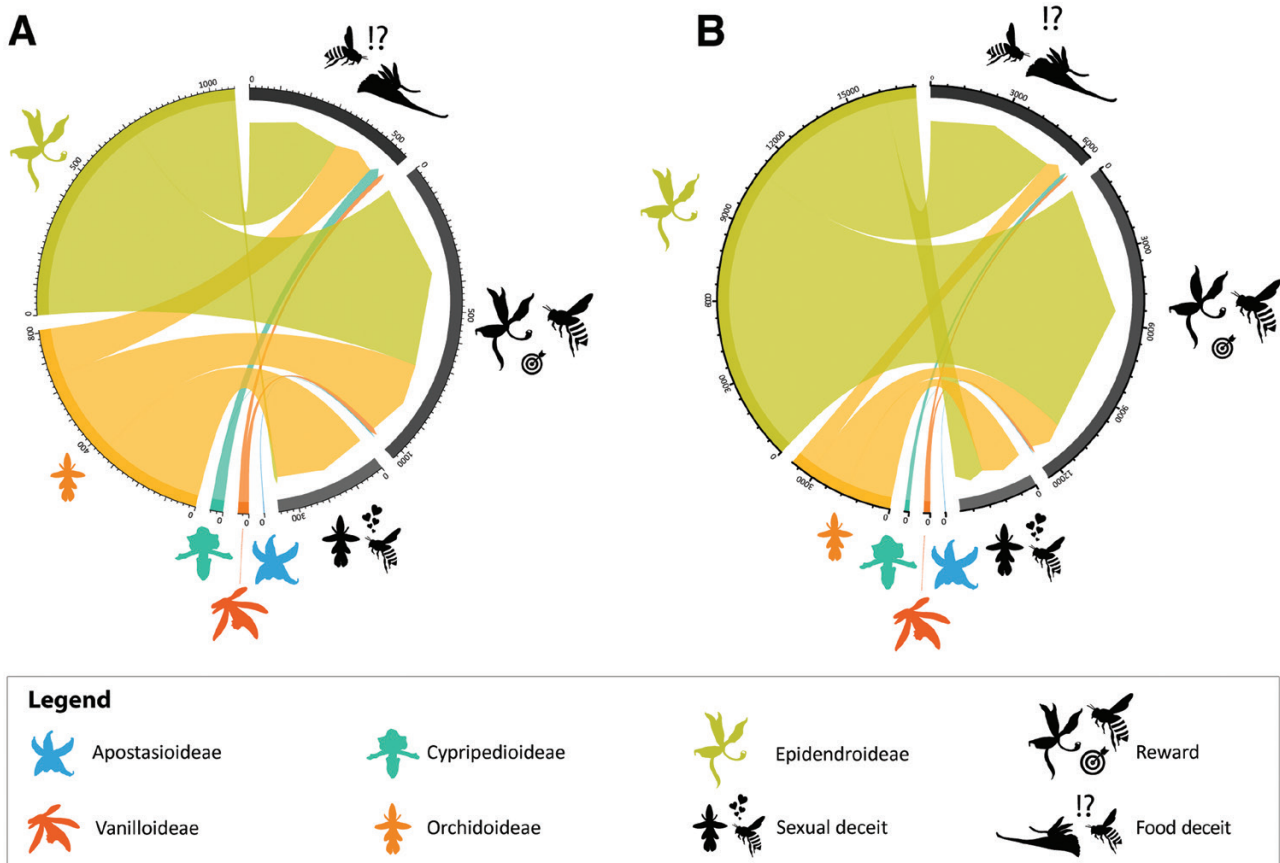


Figure 3. Observed and expected distribution of pollinator attraction strategies among subfamilies. A, Major orchid subfamilies and frequencies of their known pollinator attraction strategies. The scale at the margin of the circle represents the number of records. B, Expected frequencies between the major orchid subfamilies and pollinators, extrapolating the proportion of known strategies with the total known orchid species in each subfamily. The scale at the margin of the circle represents the total number of known species of the genera in each subfamily with pollination records.

the highest number of known pollinators (mean = 3.8; median = 1, $N = 827$), whereas epiphytic species are more specialized (mean = 1.9 median = 1, $N = 469$). Because of the highly skewed data resulting from some species having many pollinators, the mean is not a good representation of the central tendencies, and a more appropriate method is the Harrell–Davis estimator, which provides a mean for the number of pollinator species for both groups [terrestrial: mean = 1.7 (confidence interval, CI = 1.6–1.8), epiphytes: mean = 1.3 (CI = 1.2–1.4)]. The confidence intervals do not overlap and the difference between terrestrial and epiphytic species is significant (Yuen’s test, test-statistic = 4.89, d.f. = 710, $P < 0.001$).

The number of known pollinator species per orchid also varies geographically. Overall, the median number of pollinator species per orchid species is one, and that pattern persists in most regions of the world; however, the frequency distributions by geographical region are all skewed to the right (Fig. 6A). Our geographical

analysis indicates that the TDWG Level 1 regions differ significantly for the number of pollinator species per orchid species (test score = 15.12, $P < 0.0001$). Orchids of Australasia had the lowest mean number of pollinator species whereas Northern America had the most. In paired comparisons (adjusted p for multiple comparisons), both regions were significantly different in five paired comparisons (Supporting Information, Table S2). Because TDWG Level 1 ‘continents’ can include temperate to tropical regions, we also examined number of pollinator species by latitudinal zone and found that the frequency distributions were similarly skewed as those for TDWG regions (Fig. 6B).

The number of known pollinator species also varies by modes of pollinator attraction (Fig. 8). Unsurprisingly, chasmogamous species with sexual deceit strategies consistently have the fewest number of pollinator species (mean location = 1.0, CI = 1.0–1.0). In general, those that produce a nectar reward have significantly more pollinators (mean

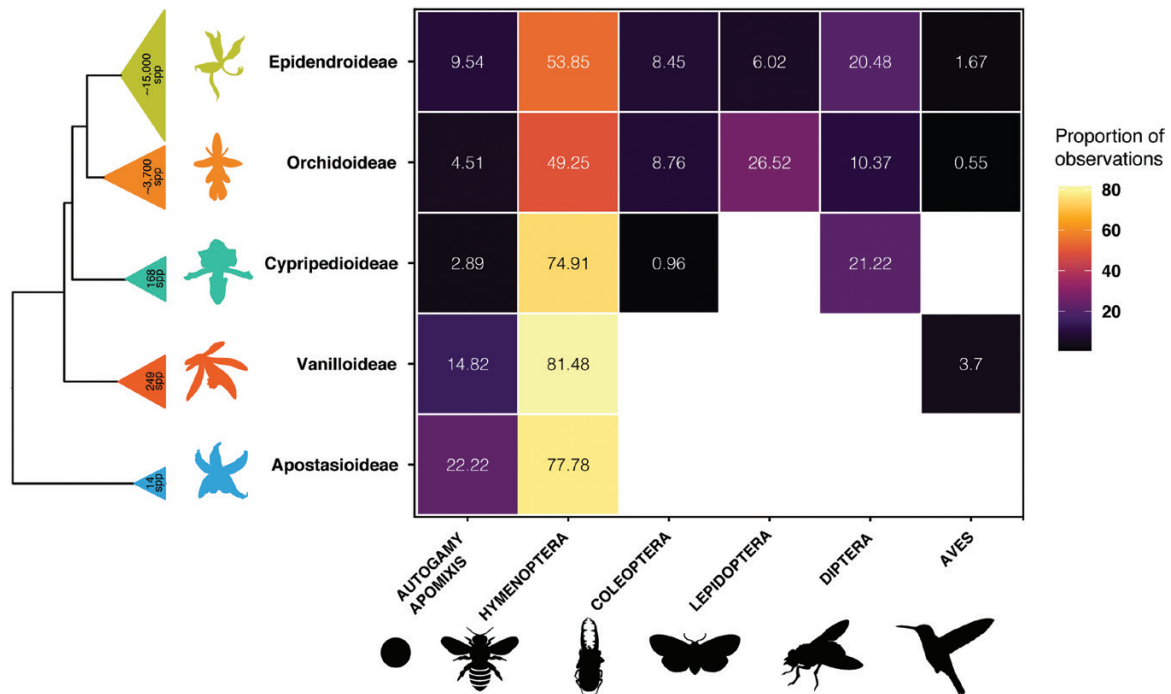


Figure 4. Percentage of orchid species using various pollinator groups in each subfamily. Minor pollinator groups are excluded (Araneae, Thysanoptera, Orthoptera, Hemiptera, Squamata, Rodentia). Percentages do not necessarily add to 100% since minor pollinator groups are excluded or some species are pollinated by multiple pollinator groups.

location = 2.04, CI = 2.0–2.6) than any other category of rewards (mean location = 1.0, CI = 1.0–1.1, Yuen's test = 6.14, d.f. = 271, $P < 0.0001$), including non-nectar forms of reward, which by their nature are relatively specialized rewards (e.g. volatile chemicals that attract orchid bees in the Neotropics and fruit flies in Tropical Asia). For orchid species with deceitful strategies, sexual deceit generally involves attracting one known pollinator species (mean location = 1, Fig. 8) with a confidence interval that is also one. In contrast, orchid species using food deceit tended to have more pollinator species, though this was more variable (mean location = 1.67, CI = 1.03–1.97). The difference in the number of pollinators between food-deceptive and sexually deceptive orchids is highly significant (Yuen's test, $P < 0.0001$) when data from all subfamilies are pooled and when data are separated by subfamily. In pairwise contrasts of the two subfamilies (Orchidoideae and Epidendroideae) that have both sex and food deceit modes of pollinator attraction reveals all combinations are significantly different, except the comparison of the two subfamilies by sex deceit (Table 6; Supporting Information, Table S3).

DISCUSSION

In the 55 years since the publication of the seminal work of van der Pijl & Dodson (1966) on orchid

pollination, the community of orchid biologists has expanded globally and the number of published studies in orchid reproductive biology has grown accordingly (Peter, 2011; Ray & Gillett-Kaufman, 2022). As a result, the coverage of orchid species for which we have reproductive data has increased five-fold since 1966, and with a few striking exceptions, many of the observed patterns described in van der Pijl & Dodson (1966) have stood the test of time. Nonetheless, we identify patterns that differ from expectations, gaps in our knowledge and hypotheses to be tested in the future.

REPRESENTATION OF ORCHID SPECIES IN THE DATABASE: HABIT AND GEOGRAPHY

In our survey, half of the species records with reproductive data are epiphytic. However, approximately 72% of orchid species are epiphytic (Gravendeel *et al.*, 2004), so these species are significantly under-represented in orchid pollination studies. This bias is unsurprising as it is often challenging to access and observe epiphytic plants. Furthermore, most orchid pollination biologists work in regions where orchid epiphytes are either rare or absent. Indeed, geographical biases concerning the number of studies undertaken are prevalent in the data. Temperate and subtropical latitudes are overly represented in

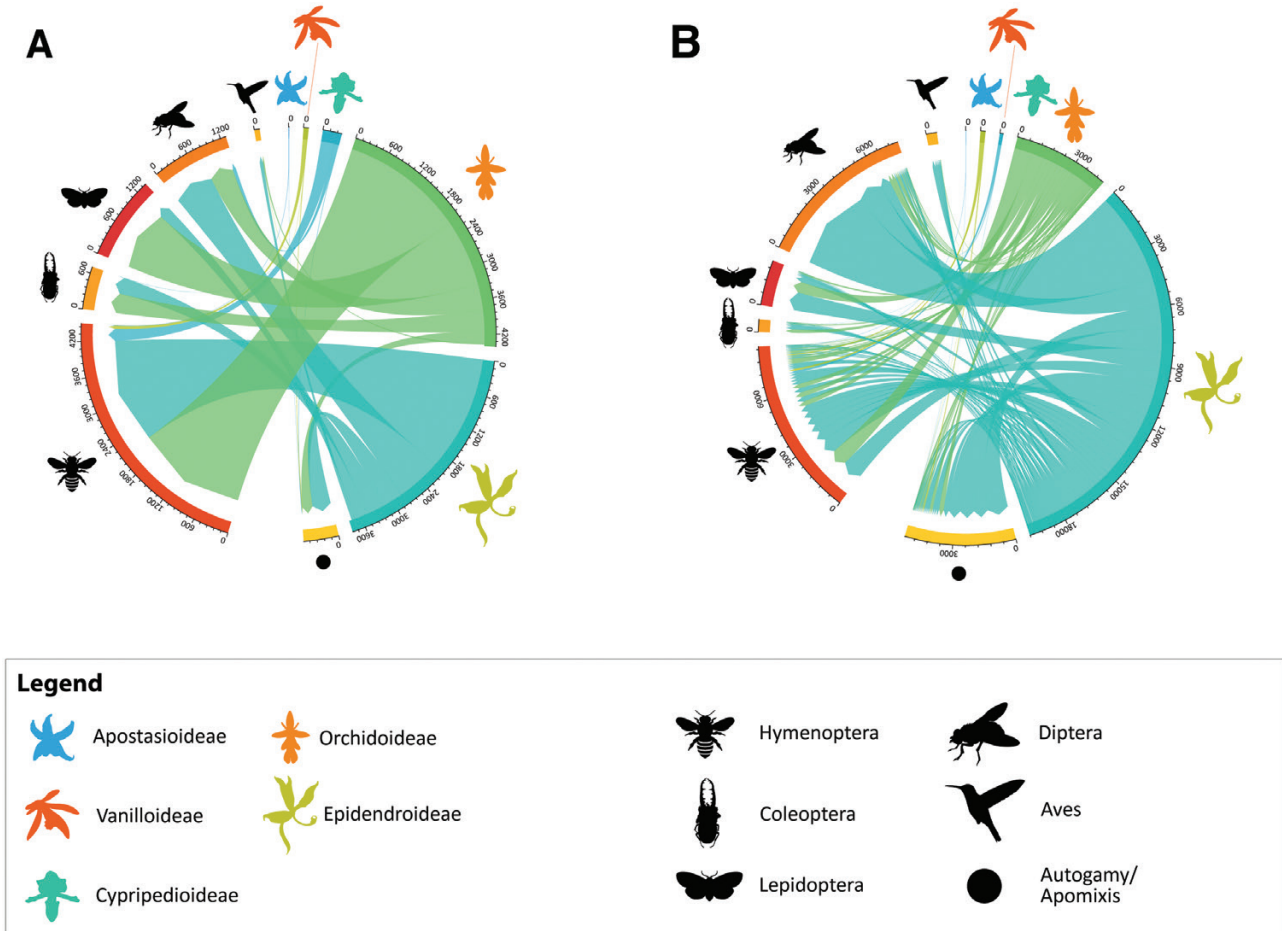


Figure 5. Observed and expected distributions of pollinator groups among subfamilies. Frequency of observed interactions of an orchid taxon is extrapolated to the total known species of the given taxon, which is provided on the scale at the margin of each circle. A, Observed frequencies of pollinator groups among subfamilies. B, Predicted frequencies of pollinator groups among subfamilies.

terms of the proportion of orchid species studied, largely because of the intense research activities in South Africa, southern Australia, Europe and Northern America north of Mexico (see also [Ray & Gillet-Kaufman, 2022](#)). Severely under-represented are tropical regions of Africa, Southern America, Temperate Asia and Tropical Asia ([Table 2](#)). Four decades ago, the orchid family had 19 616 described species ([Dressler, 1981](#)), which has increased by 56% to a current estimate of 30 543 ([POWO, 2021](#)). The increases are probably not proportionately distributed across the globe. The regions that are under-represented in our survey are those where biotas are most poorly sampled and where we can expect most new species to be discovered ([Raven et al., 2020](#); [Hughes et al., 2021](#)). Such biases would only exacerbate the under-representation of those tropical regions unless a concerted research effort on orchid reproductive biology is made.

BREEDING SYSTEMS

The predominance of SC species may be a characteristic of the orchid family. While analyses testing for features associated with the evolution of self-incompatibility in orchids are yet to be undertaken, one might expect that self-incompatibility is more likely to evolve when multiple flowers are open at a time on a plant when flowers have no mechanism for dichogamy (another aspect of breeding systems, but not quantified here) to enhance the probability of cross-pollination, and when pollen and seed dispersal is limited (e.g. [Furstenau & Cartwright, 2017](#)). Furthermore, if one considers that most orchids (especially epiphytes) occupy habitats or substrates that are ephemeral, depend on dispersal-driven metapopulation dynamics and are pollinator- and seed-limited ([Ackerman, Sabat & Zimmerman, 1996](#); [Tremblay et al., 2005](#); [Tremblay, Meléndez-Ackerman & Kapan, 2006](#); [Laube & Zotz, 2007](#); [Winkler, Hülber & Hietz, 2009](#); [Cruz-Fernández,](#)

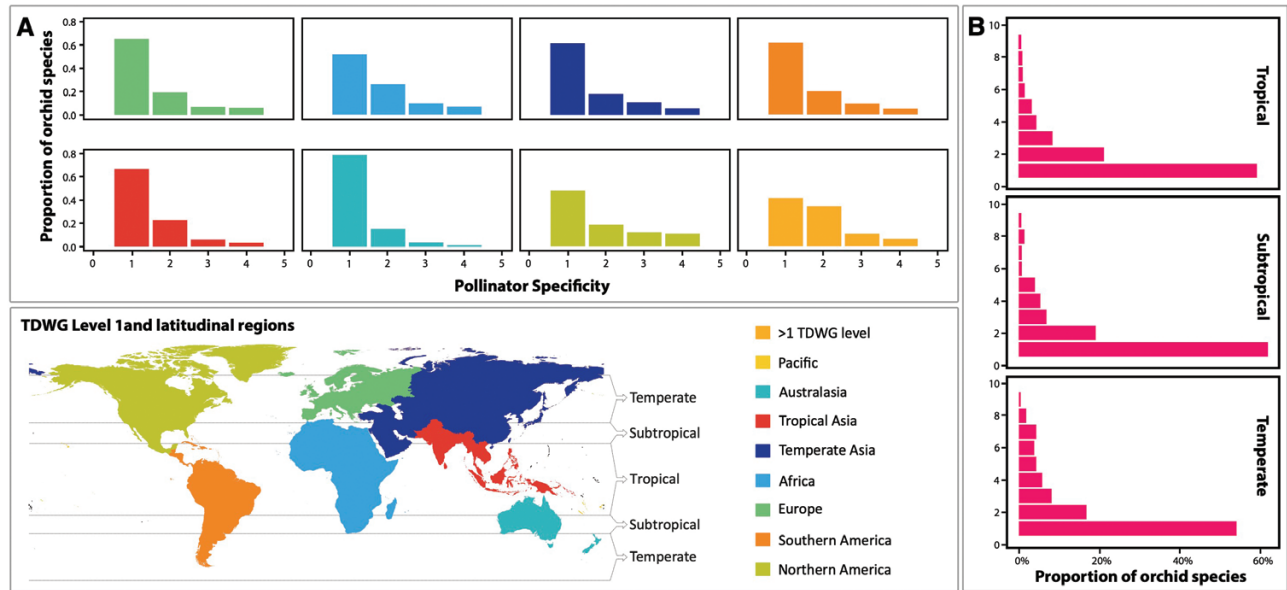


Figure 6. Frequencies of pollinator species per orchid by geographical regions. Specificities represent the number of pollinator species for each species. A, Specificity by TDWG Level 1 regions; specificities are cropped at four as beyond that the frequencies are very low and skewed (see Fig. 6). B, Specificities (N) by latitudinal zones; tropics: $< 23.7^\circ$, subtropics: $23.7\text{--}35^\circ$, temperate: $35\text{--}66.3^\circ$; y axis is the number of pollinator species cropped at nine.

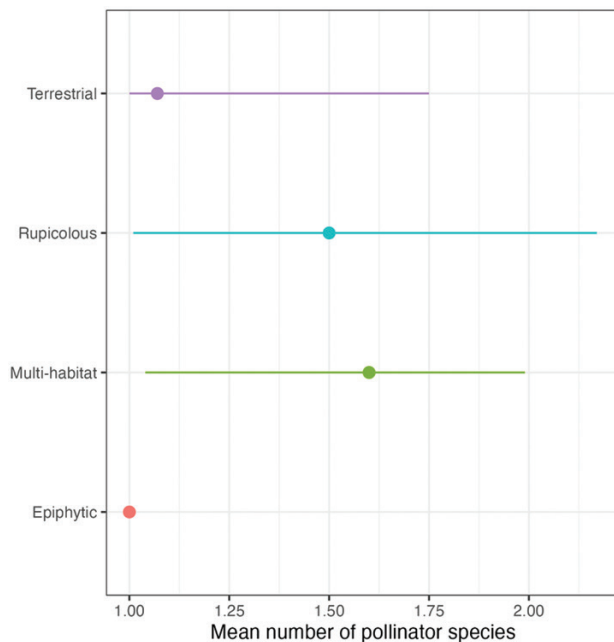


Figure 7. Mean location and highest density intervals for the number of pollinators per orchid species by growth habit. Sample sizes: terrestrial species $N = 819$; rupicolous $N = 16$; epiphytic $N = 465$ and multiple habits (multi-habit) $N = 68$.

Alquicira-Arteaga & Flores-Palacios, 2011; Acevedo *et al.*, 2015; Rasmussen & Rasmussen, 2018; Ackerman, 2019; Djordjevic & Tsiftsis, 2020), self-incompatibility

would seem to be a counter-productive strategy for species that are so dependent on their colonizing capabilities (Baker, 1955). Nonetheless, three subtribes of Epidendroideae (Dendrobiinae, Oncidiinae and Pleurothallidinae) with relatively high SI frequencies are primarily epiphytic, so the driver of SI in orchids is not yet apparent. On the other hand, widespread SC in the family may have led to breeding systems involving floral traits that enhance the probability of outcrossing in orchids, including deception and pollinarium reconfiguration (e.g. Jersáková *et al.*, 2006; Peter & Johnson, 2006). It was evident to Darwin (1862) and quite clear today that orchids have taken a multitude of pathways to enhance the probability of outcrossing.

Van der Pijl & Dodson (1966) estimated that about 3% of the species in the family are autogamous. In contrast, our database shows that 19% of the species for which we have data are exclusively autonomously selfing or agamospermic (and 81% are chasmogamous). The 573 species that we have reported represent a 64% increase over Catling's (1990) study and a 45% increase over Peter's (2011) review. We think that 3% is an underestimate, but we also suspect our numbers are biased in favour of detecting autogamy for at least three reasons. First, early efforts to record cases of self-pollination may have been inspired by attempts to discredit one of the cornerstones of Darwin's theory of natural selection, in which he states 'nature ... abhors perpetual self-fertilisation' (Darwin, 1876; Catling,

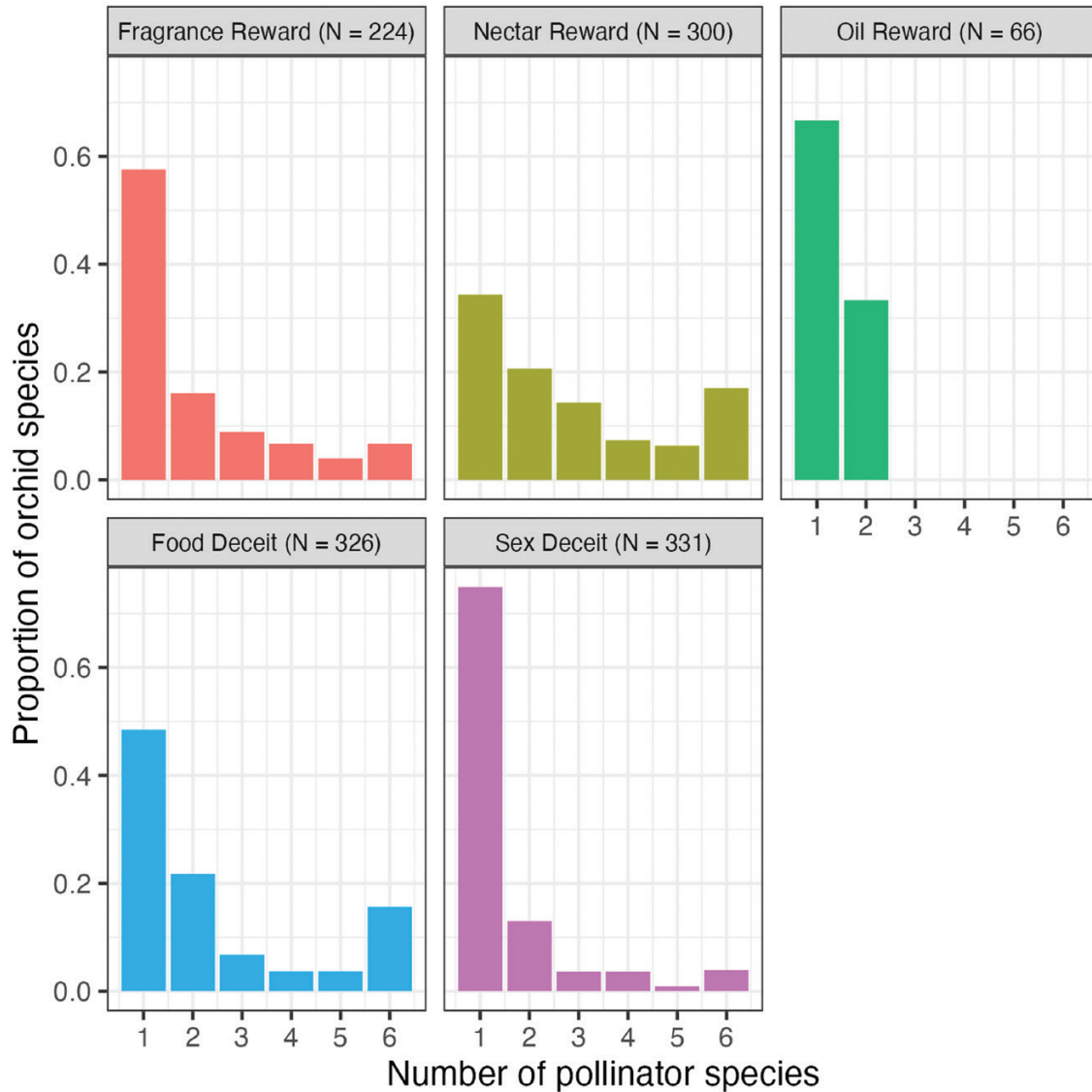


Figure 8. Frequency distributions for number of pollinator species among modes of pollinator attraction. Only those categories that have > 30 orchid species are included. Data for number of pollinators are cropped at four as beyond that number the frequencies are highly skewed to the right (see Fig. 6).

1990). Secondly, many of these cases come from high latitudes or from islands where self-pollination is more common than on continental areas of similar latitudes (e.g. Schlechter, 1914; Smith, 1928; Ackerman, 1985; Catling, 1990). Finally, but perhaps most importantly, 55% of our data on pollinator-free fruit and seed production is based on indirect evidence rather than experimentally derived conclusions. Nevertheless, our figure of 19% may be reasonable if one-third of flowering plants are indeed self-pollinating (Allard, 1975, cited by Allem, 2004).

Our data indicate that autonomous self-pollination in the orchid family is more common among terrestrial species than epiphytic and rupicolous

species. Neotropical Gesneriaceae show the opposite trend (Martén-Rodríguez *et al.*, 2015), suggesting that frequency patterns of breeding systems are not transferable across higher taxa with similar growth habits. The relatively low frequency of autonomous self-pollination among epiphytic orchids may be the result of high frequencies of SI in species-rich subtribes heavily populated with epiphytic species.

Autonomous self-pollination is advantageous when pollinator services are either absent or unpredictable, such as at high latitudes or elevations and on islands (Müller, 1883; Hagerup 1952; Ackerman, 1985; Jacquemyn *et al.*, 2005). Surprisingly, among terrestrial species in our database, the relative

Table 6. Number of pollinator species for varying modes of deceit for Epidendroideae and Orchidoideae. The confidence intervals for quantiles and median location are estimated using the Harrell–Davis method, which is a bootstrap process followed by trimming of 20% of the data at the lower (LCI) and upper (HCI) intervals of the distribution to remove outliers. Sample size is given as ‘*N*’. Only orchid species whose pollinators have been identified to species level are included. See [Supporting Information, Table S3](#) for tests of pairwise comparisons

| Subfamily | Deceit type | Median | LCI | HCI | <i>N</i> |
|----------------|---------------|--------|-------|------|----------|
| Epidendroideae | Sexual deceit | 1.11 | 1.000 | 1.65 | 7 |
| Epidendroideae | Food deceit | 1.03 | 1.001 | 1.50 | 194 |
| Orchidoideae | Food deceit | 2.26 | 1.980 | 3.46 | 95 |
| Orchidoideae | Sexual deceit | 1.00 | 1.000 | 1.00 | 326 |

frequency of autonomously self-pollinating species was high in tropical regions, perhaps indicating that pollinator services are irregular in the tropical forest understorey or at high elevations, so selection for reproductive assurance strategies may be more pervasive.

The high specialization of pollination systems in orchids makes them an interesting group to study the evolution of self-pollination. Relying on one or few pollinator species not only can leave populations vulnerable to local shortages of suitable pollinators, but also constrain expansions of geographical ranges (Pauw & Hawkins, 2011; Duffy & Johnson, 2017; Reiter *et al.*, 2017). Furthermore, orchids reliant on pollen vectors typically experience strong pollen limitation (mean of 37.1% pollination rate for rewarding species, 20.7% for rewardless species; Tremblay *et al.*, 2005). As such, orchids may evolve self-pollination to gain reproductive assurance when outcrossing fails to overcome the automatic selective advantage of self-pollination (e.g. Ortiz-Barney & Ackerman, 1999; CaraDonna & Ackerman, 2012). This could explain unusual situations where we see orchids with highly modified flowers exhibit self-pollination. For example, some members of the Australasian genus *Calochilus* R.Br. attract scoliid wasps via sexual deception (Fordham 1946; Jones & Gray, 1974; Bower & Branwhite, 1993) but are facultatively self-pollinating (Cady, 1972).

POLLINATOR ATTRACTION: REWARDS

A long-held notion is that approximately two-thirds of the orchid family use some type of reward to attract pollinators (van der Pijl & Dodson, 1966; Shrestha *et al.*, 2020). Such plants often have higher visitation rates and higher fruit production than rewardless orchids (Neiland & Wilcock, 1998; Tremblay *et*

al., 2005). Our data suggest that reward-offering strategies are less prevalent than initially thought (two-thirds of the species in the family; van der Pijl & Dodson, 1966). Among chasmogamous species, 54.2% (*N* = 1946) of orchids in our database use rewards to attract pollinators. However, these rewarding species are not evenly distributed geographically. Rewards are the dominant or co-dominant means of pollinator attraction in most TDWG Level 1 regions with orchids. Because these regions can span temperate to tropical latitudes, we also analysed the frequencies of reward-offering orchids by latitude. This approach revealed rewards as the most reported means of pollinator attraction in orchids of both tropical and subtropical regions (Figs 1, 2). Given those results, it is not surprising that orchids offering rewards are over-represented among epiphytes and under-represented among terrestrial species. Whether or not these results reflect differences in the abiotic and biotic environments, geological history or phylogenetic constraints remains to be assessed.

We recorded eight types of pollinator reward, and, as expected, nectar is the most common (Supporting Information, Table S1). In orchids, fragrance is the second most common reward, which is largely attributable to well-studied genera pollinated by male euglossine bees (Apidae) in the Neotropics (e.g. Dressler, 1968a) and *Bulbophyllum* spp. pollinated by fruit flies in Tephrididae in Tropical Asia (e.g. Tan, 2006; Ong, 2011). Numerous records of rewards (nectar, resins, oils) are based on non-traditional methods that sometimes detect minute quantities of these substances (e.g. Gomiz *et al.*, 2017; Reiter *et al.*, 2018, 2019a, b; Davies & Stpiczyńska, 2019; Pansarin, 2021). We have scored these minute quantities as rewards, and indeed the pollinators may perceive them as such. When pollinators are small (e.g. many Hymenoptera such as some braconid and ichneumonid wasps, *Trigona* and *Paratetrapedia* bees and many flies such as Mycetophilidae, Sciaridae, Drosophilidae and Tephrididae), minute rewards may be sufficient to maintain pollinator interest through significant gains relative to energetic costs, although not necessarily so (Ackerman & Mesler, 1979; Karremans *et al.*, 2015; Bogarín *et al.*, 2018). Alternatively, when pollinators are large and have high energy needs (e.g. bees of the genera *Eulaema* and *Xylocopa*, birds and hovering moths of Sphingidae), small rewards may be nothing more than teasers. They could be part of a deceitful strategy to entice pollinators to visit more than just one or few flowers (e.g. Watteyn *et al.*, 2021). This may also be the case where some plants in a population produce nectar whereas others do not [*Rodriguezia granadensis* (Lindl.) Rchb.f.: Ospina-Calderón *et al.*, 2015; *Caladenia nobilis* Hopper & A.P.Br.: Phillips *et al.*, 2020b]. The gain for orchids can be detectable, but

the benefit to the pollinator is more difficult to assess (Ackerman, Rodríguez-Robles & Meléndez, 1994). We do know that nectar-addition experiments in species with meagre or no rewards fail to increase pollinator visits and fruit production or result in an increase in geitonogamous pollinations with reduced viable seed production (Salguero-Farías & Ackerman, 1999; Smithson & Gigord, 2001; Johnson, Peter & Ågren, 2004; Jersáková & Johnson, 2006).

POLLINATOR ATTRACTION: DECEIT

Excluding autogamous species, a deceitful means of pollinator attraction is used by 46.1% of the species for which we have data. For both terrestrial and epiphytic species, the most common form of deceitful attraction is food deception, which was used by 28.5% of orchids in the database. Bees, wasps, flies, moths, butterflies and beetles are among the victims of this deceit. Sometimes this involves mimicry of a co-occurring rewarding plant (e.g. Kjellsson *et al.*, 1985; Johnson, 2000; Peter & Johnson, 2008), but in most species, it is generalized food deception. Here deceit operates by exploiting innate foraging preferences of their pollinators without mimicking a specific model (Heinrich, 1975; Ackerman, 1981, 1986; Steiner, 1998; Jersáková *et al.*, 2006). However, the boundaries of this dichotomy can become blurred for several reasons (Johnson *et al.*, 2003), including the following: (1) some orchid species crudely mimic a guild of morphologically similar species (e.g. Jersáková *et al.*, 2016; Scaccabarozzi *et al.*, 2018); (2) evidence is consistent with multiple hypotheses of pollinator attraction (Ackerman, 1983b) and (3) broad-scale convergent evolution of floral traits has occurred to attract the pollinator(s) in question (Johnson, Alexandersson & Linder, 2003; Jersáková *et al.*, 2009; Papadopoulos *et al.*, 2013).

Pollination by sexual deceit has been reported for 22 orchid genera, representing all TDWG Level 1 regions of the world except Northern America and Antarctica. While the first sexually deceptive systems studied in detail involved bees and wasps (Coleman, 1928; Ames & Ames, 1937; Kullenberg, 1961; Stoutamire, 1975; Paulus & Gack, 1990; Singer, 2002), there is now a range of animal groups known to be exploited via sexual deception including ants (Peakall, 1989), three families of fungus gnats (Blanco & Barboza, 2005; Phillips *et al.*, 2014; Reiter *et al.*, 2019c), tachinid flies (Martel *et al.*, 2016) and three families of beetles (Arakaki *et al.*, 2016; Joffard *et al.*, 2019, 2020; Cohen *et al.*, 2021). A single genus, *Ophrys* of the European/Mediterranean region, accounts for 55% of the species with pollinator records, a percentage that may be inflated because of taxonomic uncertainty (e.g. Chase *et al.*, 2015; Delforge, 2016; Bateman *et al.*, 2018; POWO, 2021). Most of those records involve bees in

the families Apidae, Andrenidae and Megachilidae. Other reports of sexual deception primarily come from 11 Australian genera, several of which are pollinated by male thynnine wasps (Thynnidae).

At present, most documented cases of sexual deception involve bees and wasps. However, the sleeping giant of sexual deception is the Neotropical genus *Lepanthes* Sw. We have pollinator data for only ten of the 1158 species of the genus, all of which are pollinated by fungus gnats via means of pseudocopulation. The floral feature critical to the mechanics of successful pollen transfer is the size, shape and hirsuteness of the minute mid lobe of the labellum ('appendix' of Carlyle A. Luer's terminology) (Blanco & Barboza, 2005; Karremans & Díaz-Morales, 2019; Vieira-Uribe & Moreno, 2019). This morphological feature occurs in most *Lepanthes* spp. (Luer & Thorerle, 2012), so we expect sexual deceit to be much more widespread in this genus than recognized in the literature. However, testing this prediction will probably take a long time as observations have been serendipitous and are generally challenging to accomplish. Although not as diverse as *Lepanthes*, a similar situation may exist in the species-rich Australasian terrestrial genus *Pterostylis* R.Br., in which recent work has suggested that sexual deception of fungus gnats may predominate in several major clades in the genus (Phillips *et al.*, 2014; Reiter *et al.*, 2019c). Given that there are unstudied species in the tropics with flowers that are similar to the sexual deception syndrome [i.e. reduced floral parts, dull-coloured, often insectiform flowers such as some species in *Andinia* (Luer) Luer, *Cottonia* Wight, *Telipogon* Kunth and *Trichoceros* Kunth], it seems likely that additional genera using sexual deception remain to be discovered.

POLLINATOR GROUPS USED BY ORCHIDS

The frequency of orchids pollinated by different pollinator groups, as observed or estimated by van der Pijl & Dodson (1966), came remarkably close to our own tally (Table 1). Hymenoptera remains the most common participant in orchid pollination, especially in the families of the order Apoidea (mostly Apidae, Colletidae, Halictidae and Megachilidae). Apoidea contain several functional groups (e.g. solitary vs. social, long-tongued vs. short-tongued, large vs. small body size, bees vs. wasps vs. sawflies) worthy of a more specific analysis.

The largest difference between our data and that of van der Pijl & Dodson (1966) is in their estimate for orchids pollinated by dipterans (15%), whereas our count reached 20%. We expect that number to climb as observations are published for species-rich tropical groups such as Pleurothallidinae (> 5500 species; current count: 149/156 species pollinated by Diptera),

Malaxidinae (1255 species; current count: 6/6 pollinated by Diptera) and *Bulbophyllum* (> 1800 species; 47/49 species pollinated by Diptera). When extrapolating the percentages of known pollinators with the total known orchid species by subtribe, we found that Hymenoptera and Diptera potentially interact with a similar number of orchid species. Whereas Hymenoptera pollinate a high diversity of orchid subfamilies and subtribes, Diptera are involved in the pollination of the most species-rich groups in the family. As for Hymenoptera, we have not created multiple categories of Diptera, which could be divided into a variety of functional groups such as long-proboscid flies (Bombyliidae, Nemestrinidae, Tabanidae), flower flies (Syrphidae), carrion flies (Calliphoridae), fruit flies (Drosophilidae, Tephritidae) and fungus gnats (Keroplastidae, Mycetophilidae, Sciaridae), each of which has their own behavioural and anatomical peculiarities reflected in suites of characteristics of the flowers they pollinate (e.g. van der Pijl & Dodson, 1966; Johnson, 2006; Ong, 2011). Indeed, the number of dipteran families (*c.* 50) that pollinate orchids is quite remarkable (Supporting Information, Fig. S2).

Lepidoptera pollinate *c.* 10% of the orchids that have been studied, 85% of which are nectar-producing species. The frequency of deception is about twice as high among butterfly-pollinated orchids (37% of 64 species) as those pollinated by moths (19% of 141 species). The diversity of orchids pollinated by Lepidoptera is greatest in the tropics, particularly for butterflies, and there are relatively few orchid species from temperate and subtropical latitudes that commonly use them as pollen vectors (e.g. *Gymnadenia* R.Br., *Platanthera* Rich. subgenus *Fimbriella* (Butzin) Efimov and some species of *Disa* P.J.Bergius and *Calanthe* R.Br.; Smith & Snow, 1976; Johnson & Bond, 1994; Chapurlat *et al.*, 2018; Luo *et al.*, 2020). Like Diptera, Lepidoptera are another group of orchid pollinators that may increase in frequency with further observation, particularly for the large Neotropical genus *Epidendrum* L. *s.l.* (1800 species; Karremans, 2021). Many members of this genus have nocturnally fragrant and green or white flowers, suggesting pollination by settling or hovering moths (Goss & Adams, 1976; Ackerman & Montalvo, 1990), or odourless to the human nose and colourful (e.g. yellow, orange, red, pink, purple and combinations of these colours), traits more typical of butterfly-pollinated flowers (e.g. Bierzychudek, 1981; Almeida & Figueiredo, 2003).

Coleopterans have rarely been considered important orchid pollinators and are typically regarded as an incidental component of the pollinator fauna in communities where generalist plant–pollinator interactions are common (e.g. Europe, North America; Nilsson, 1978; Steiner, 1998; Arakaki *et al.*, 2016). However, an increasing number of orchids are being

revealed to be specialized for beetle pollination (e.g. Peter & Johnson, 2006), even though some, on the basis of floral syndrome traits of morphology and colour, were predicted to be bee pollinated. Examples include the terrestrial *Cyanicula gemmata* (Lindl.) Hopper & A.P.Br. (Peakall, 1987) and the epiphytic *Myrmecophila thomsoniana* (Rchb.f.) Rolfe (Rose-Smyth, 2019). On the other hand, pollinator-mediated selection has produced two forms of *Eulophia parviflora* (Lindl.) A.V.Hall, one of which is specialized for beetle pollinators in an otherwise bee-pollinated clade (Peter & Johnson, 2014).

Most records of bird pollination of orchids are from tropical and subtropical regions. In the Western Hemisphere, primarily in the tropics, hummingbirds (Apodiformes: Trochilidae) are the only recorded avian pollinators of orchids, where they pollinate members of the two largest subfamilies, Epidendroideae and Orchidoideae, involving terrestrial, rupicolous and epiphytic species (e.g. Rodríguez-Robles, Meléndez & Ackerman, 1992; Singer & Sazima, 2000; Supporting Information, Figs S3, S5). In contrast, Passeriformes are pollinators of orchids in TDWG Level 1 regions of Africa (sunbirds: Nectariniidae; white-eyes: Zosteropidae) and probably Tropical Asia. Indeed, some of the most detailed studies of bird pollination in orchids are from the Africa region (e.g. Johnson, 1996; Micheneau *et al.*, 2008; Johnson & van der Niet, 2019). To date, there are no reports of bird pollination of orchids in Australia, New Zealand or New Guinea, despite the prevalence of bird pollination, particularly honeyeaters (Melephagidae), in other plant families (Armstrong, 1979; Phillips *et al.*, 2010). Similarly, reports of bird pollination of orchids in Asia are few (Liu *et al.*, 2013), although it is likely that sunbirds and other families may be more widely involved in pollination. Based on the floral syndrome exhibited by some *Dendrobium* Sw. in Tropical Asia and New Guinea (Slade, 1962), bird pollination is probably more widespread in this region than is currently documented. Only 16% of 62 orchid species pollinated by birds employ deception, and only three of those deceitful orchids (5%) are exclusively pollinated by them, which is much less than the overall frequency of deception pollination (46.1%) in the family. Compared with insects, birds appear to be less prone to exploitation by the deceitful antics of many orchids.

SPECIFICITY OF POLLINATION SYSTEMS

Orchids are thought to have a high incidence of specialized pollination strategies, with many species attracting just one or few pollinator species (Tremblay, 1992; Ray & Gillett-Kaufman, 2022). Our analysis confirmed that the median number of pollinator

species for orchid species is one. High specialization on pollinators would suggest higher risk of extinction (e.g. Waser *et al.*, 1996), particularly in anthropogenically modified landscapes (Pauw & Hawkins, 2011). The relationship among orchid pollinator specificity, diversification rate and extinction risk should be a fruitful avenue of inquiry, but this has rarely been explicitly addressed (e.g. Ackerman & Roubik, 2012; Givnish *et al.*, 2015).

Although orchids with few pollinator species occur in all subfamilies, there are some differences among clades. In the most species-rich subfamily, Epidendroideae, the early-branching tribe Neottieae exploit a broad range of pollinator taxa. In contrast, members of other clades tend to interact with a narrower taxonomic group of pollinators (Supporting Information, Figs S3, S6, Epidendroideae and pollinator families). Neottieae also include many of the most generalist species (e.g. species of *Epipactis* Zinn and *Neottia* Guett.). For the other subfamilies, the relationship between the number of pollinator families and orchid subtribes is not so obvious (Supporting Information, Fig. S7, Apostasioideae, Vanilloideae, Cypripedioideae and pollinator families; Supporting Information, Fig. S8, Orchidoideae and pollinator families).

Ollerton & Cranmer (2002) asked whether plant–pollinator interactions of tropical plants were more specialized than those of higher latitudes by using two data sets: one was taxon-specific (asclepiads) and the other based on community-level studies. They found no differences associated with latitude. Our data indicate that globally, orchids are characterized by a high frequency of species with one pollinator across all latitudes and all major geographical regions. However, regional variation is evident; our analysis highlights the more generalized nature of much of the Holarctic orchid flora (with the notable exception of *Ophrys*) relative to the tropics and higher latitudes of the Southern Hemisphere. Variation in sampling effort by observers may bias the results (Schatz *et al.*, 2021), but we have few data by which we can assess this. Biologists in Europe have been paying attention to orchid pollination since Sprengel (1793), but there have been numerous detailed studies of orchid pollination in other geographical regions (e.g. parts of TDWG Level 1 Africa), suggesting that this strong trend is likely to hold across those broad geographical categories. More geographically constrained comparisons, though, may differ. Johnson & Steiner (2003) showed that the European orchid flora is much more generalized (more pollinator species per orchid) than that of southern Africa, but our analysis comparing TDWG Level 1 regions, Europe and Africa, shows there are quite similar ($P = 0.91$; Supporting Information, Table S2).

NUMBER OF ORCHID POLLINATORS AND POLLINATOR REWARDS

Our analysis shows that, on average, nectar reward attraction strategies tend to be more generalized than any other means of pollinator attraction (Fig. 8). Indeed, in our database, many orchids with higher-than-average number of pollinators are nectar-producing terrestrial species from temperate regions of the Northern Hemisphere. Nonetheless, it is important to note that even for nectar-rewarding orchids, a high proportion of orchid species have just a single known pollinator species. This high specificity applies to species that are pollinated by a range of vectors, including long-tongued flies (Johnson, 2006), hawkmoths (Nilsson *et al.*, 1987), sunbirds (van der Niet, Cozien & Johnson, 2015), colletid bees (Reiter *et al.*, 2019a) and thynnine wasps (Reiter *et al.*, 2019b; Phillips *et al.*, 2020b), even when multiple species of the same pollinator functional group co-occur. These study systems highlight that even within a pollinator functional group, floral traits operate as filters such that only one or few species may be attracted to the flower or have the appropriate size and behaviour to achieve pollination.

Orchids in our database that produce oil as a reward have either one or two pollinator species (Fig. 8). Specificity is assisted by the fact that only two families (Hymenoptera: Apidae and Melittidae) have members that pollinate these orchids and are dependent on oils to provision or construct their nest cells. In some bee communities, further filtering of potential pollinators comes from the considerable variation in body size of oil bees (e.g. *Epicharis* vs. *Paratetrapedia*; Roubik, 1989), meaning that not all bee species are capable of pollinating a given orchid, and orchids generally have flower sizes that correspond to their primary pollinator (e.g. *Ornithocephalus* Hook. vs. *Trichocentrum* Poepp. & Endl.).

Fragrance rewards are also highly specific in the animals they attract, but like most other means of pollinator attraction in orchids, there is some variation (Fig. 8; Ackerman, 1983a). For example, orchids pollinated by fragrance-seeking male euglossine bees (Apidae) can be highly specific with filtering based primarily on the chemical composition of floral fragrances and secondarily by bee size (Dodson, 1962; Williams & Dodson, 1972; Williams & Whitten, 1983; Ackerman, 1983a), the combination of which can arise convergently in disparate lineages (Nunes *et al.*, 2017). On the other hand, in the fragrance rewarding orchids of lowland Panama, those species with longer flowering periods have a greater number of pollinator species (Ackerman & Roubik, 2012), making variation in the degree of specificity analogous to sampling effort. It would be interesting to test whether this pattern applies for orchids pollinated by other vectors and in

different regions, and whether longer flowering periods are the cause or the effect of variation in specificity.

SPECIALIZATION AND DECEIT

The high level of specificity in the two most common means of deception (sex and food) is similar to that of species offering more atypical rewards (oils and fragrances; Fig. 8). However, the filters leading to specialization on particular pollinators vary among the means of pollinator attraction. The basis for sexual deception is that females of many insects lure conspecific males using sex pheromones, a form of chemical signalling that orchids have exploited resulting in a high level of specialization (e.g. Schiestl *et al.*, 1999; Peakall *et al.*, 2010; Bohman *et al.*, 2014, 2017). Indeed, two-thirds of the species that employ sexual deceit have been reported to be pollinated by a single known pollinator species, with the mean number of pollinators for sexually deceptive species being 1.6. This trend of high specialization among orchids using sexual deceit is consistent worldwide. For example, based on the 11 Australian genera using sexual deceit, the mean number of pollinator species is 1.2 (e.g. Peakall *et al.*, 2010; Phillips *et al.*, 2017). In Southern America, six of seven species (in six genera) that utilize sexual deception, for which we have sufficient data, have just a single known pollinator species. This situation is slightly more complicated in the European genus *Ophrys*, depending on the taxonomic treatment used. If we combined more finely split species and considered them as local variants of more widespread taxa, then that would make the few remaining *Ophrys* spp. more generalist regarding the number of pollinator species. For example, Bateman *et al.* (2021) organized *Ophrys sphegodes* Mill. *s.l.* as a ‘macrospecies’ composed of nine ‘mesospecies’. When we use data from the Pollination List, this macrospecies has 19 pollinator species (distributed among four bee families). The ‘*O. sphegodes*’ mesospecies has eight pollinator species, whereas the other eight averaged 1.6 pollinator species. Recently, Schatz *et al.* (2021) discovered that in large populations of several *Ophrys* spp. previously thought to have a single pollinator species have a numerically dominant pollinator and multiple secondary pollinators. Thus, the number of pollinators for a given *Ophrys* sp. is not only dependent on species circumscriptions, but also on orchid population sizes.

Although filtering the pollinator pool for species using food deception is not as evident as for sex deception, many food-deceptive species still exhibit high specialization (median number of known pollinator species = 1). We suspect that species using Batesian mimicry will typically show greater

specialization than those using generalized food deception, as Batesian mimics resemble a model plant that often have just a small number of specific pollinators (Johnson & Schiestl, 2016). However, we did not distinguish between the two types of food deception in our database, partly because the two forms of deception appear to intergrade into each other, and partly because not all studies evaluate the criteria needed to separate these two strategies or distinguish mimicry from convergent evolution to exploit the same pollinator (Johnson & Schiestl, 2016). Although some of the most generalist orchids in our database are terrestrial species that use generalized food deception, there are still species using this strategy that are pollinated by only one or few pollinator species (e.g. Steiner, 1998; Phillips & Batley, 2020).

ADDRESSING GAPS IN POLLINATION KNOWLEDGE IN THE ORCHIDS

For macroecological or macro-evolutionary studies of plant reproduction and lineage diversification in general, it is critical to know which animal species are responsible for pollinating focal plant species (van der Niet, 2020). Pollination syndromes can be useful when they are validated as having a predictive capacity for the focal study group (Johnson & Wester, 2017) but are of more limited utility in poorly studied groups where functional groups and their associated floral traits are not fully resolved (van der Niet, 2020). This represents a problem for many studies of Orchidaceae, particularly in tropical regions that are highly diverse (e.g. Pérez-Escobar *et al.*, 2017) but have fewer active pollination biologists compared to temperate regions. Tropical groups such as *Epidendrum*, Pleurothallidinae, Malaxidinae, Dendrobiinae and Zygopetalinae have few observations relative to their species richness and should be targeted for study. As such, the challenge for orchid studies is to develop approaches to identify pollinators and resolve pollinator attraction strategies more rapidly. This kind of work can be particularly labour-intensive for orchids, as many species are rare and have low visitation rates (Tremblay *et al.*, 2005).

As most orchids are epiphytes and difficult to observe under most circumstances, creative means of identifying pollinators have been implemented. Dressler (1976) noted that for orchids pollinated by euglossine bees (Apidae), pollinarium placement on insect bodies can be specific and consistent within an orchid genus (Dressler, 1968b; Ackerman, 1983a). Furthermore, pollinarium morphology can vary among species of some genera to the extent that species may be recognized solely on based on their pollinaria (Dressler, 1976). Bees could be captured using chemical attractants or at nectar or pollen resources and examined for their pollinaria (e.g.

Ackerman, 1983b). Unusual pollinarium loads have even led to the discovery of new orchid species (Williams & Whitten, 1988). Nevertheless, identifying orchids based on pollinarium morphology has its limits (Dressler, 1977; Singer *et al.*, 2008). DNA barcoding has been used successfully to identify orchid pollinaria using Sanger sequencing technologies (Widmer *et al.*, 2000; Farrington *et al.*, 2009; Ramirez *et al.*, 2011; Waterman *et al.*, 2011; Pérez-Escobar *et al.*, 2017). However, to our knowledge no attempt to barcode pollinia using high-throughput sequencing has been conducted, an approach that could deliver much better representations of the nuclear and organellar genomes, from which many barcodes could be mined (Dodsworth, 2015).

In sexually deceptive orchids, rapid inroads have been made into identifying pollinators by using flowers as a bait (Stoutamire, 1975; Peakall, 1990). Here, picking and relocating a flower to a new position in the landscape can rapidly attract males (Peakall, 1990), which respond as if the flower is a newly emerged female. A modification of this technique is also effective for food-deceptive systems, in which scientists working with clonal species have increased pollinator visitation rates by picking an artificial clump and moving it to varying positions in the landscape (Scaccabarozzi *et al.*, 2018, 2020; Phillips *et al.*, 2020b). A similar approach has also been implemented by using groups of potted plants to increase the floral stimulus to pollinators compared with the scattered plants that occur in natural conditions (Reiter *et al.*, 2018, 2019c).

Recent years have seen the advent of using motion-activated game cameras to detect vertebrate pollinators, including orchids (Micheneau *et al.*, 2008; van der Niet *et al.*, 2015) and other herbaceous species (Kestel *et al.*, 2021). This approach is likely to be highly effective in bird-pollinated orchids, particularly as most bird species can be identified from images alone. In addition, recent work has shown that these cameras can also be used to capture footage of some insect species (Micheneau *et al.*, 2010; Danaher *et al.*, 2019; Houlihan *et al.*, 2019; Balducci, van der Niet & Johnson, 2020; Johnson *et al.*, 2020; Lombardi *et al.*, 2021), ideally using a camera with a short focal range. An exciting development is the use of Raspberry Pi computing systems as a cheap way of capturing footage of pollinators (Droissart *et al.*, 2021). With further development, it will be possible to use these as an affordable approach for motion-triggered video of small insect pollinators (Klemens, Tripepi & McFoy, 2021).

CONCLUSIONS

Our global compilation of studies of orchid reproduction will help address the deficit in natural history data needed for robust ecological and phylogenetic analyses

of pollinator-driven evolution (van der Niet, 2020). Further, it has enabled us to assess global trends in the pollination of orchids. We find that 76% of orchid species are pollinator dependent, and the majority rely on one species of pollinator. Most species are SC, although self-incompatibility is common in certain subtribes of Epidendroideae. Most orchids are chasmogamous, about one-fifth are autonomously and exclusively selfing or agamospermic, and only 5% have mixed pollination systems. Autonomous self-pollination is more frequently found in terrestrial orchids than in epiphytes. Excluding autogamous species, pollination by deceitful means occurs in nearly half of all orchid species studied, a significant increase from previous estimations. Food deception is the most common form of deceitful attraction, followed by sexual deception.

Based on current knowledge, the most common pollinator group in Orchidaceae is the Hymenoptera. However, Diptera are expected to found to be as important as Hymenoptera when highly diverse tropical orchid groups are studied further. Lepidoptera pollinate *c.* 10% of orchid species, and Coleoptera and birds follow closely. Our analysis confirms that many orchid species indeed have a highly specific means of pollination, with the median number of pollinator species per orchid being one. This trend holds among both geographical regions and growth forms. However, there are some super-generalists, particularly among the temperate terrestrial orchids of the Northern Hemisphere. On average, species offering nectar rewards were shown to be more generalist, whereas high specificity was found to be more common in both sexual- and food-deceptive orchids.

Despite containing > 2900 species, our database covers < 10% of the family, with orchids from tropical regions of Africa, Southern America and Asia, especially epiphytes, significantly under-represented in orchid pollination studies. The latter two regions also have the highest orchid species diversity but remain poorly sampled. Therefore, we encourage pollination biologists to focus on under-represented taxa and regions that would unequivocally identify the pollinators and why they visit the flowers, the floral stimuli to which pollinators respond and the fitness consequences of floral variation within and among populations. These data may not only inform syntheses for ecological and evolutionary studies but also for more practical applications in the refinement of local to global conservation strategies.

ACKNOWLEDGEMENTS

We thank our colleagues who had contributed their insights and literature to our efforts. These include

Peter Bernhardt, Emilia Brzosko, Sunil Charturvedi, Kerry Dressler, Gunter Gerlach, Kathrine Gregg, Steve Johnson, Eric Hágsater, Rudolf Jenny, Carlos Nunes, Poh Teck Ong, Thierry Pailler, Emerson Pansarin, Zong-Xin Ren, Rodrigo Singer, Kenji Suetsugu, Naoto Sugiura, Keng-Hong Tan and Ángel Vale.

Ackerman conceived the project, compiled most of the data, wrote most of the text and coordinated collaborator input. Phillips reviewed and added to the data from Australia, assisted in the direction of the manuscript, and wrote sections of the text. Tremblay did most of the statistical analyses, produced figures illustrating the statistics and wrote sections of the text. Karremans contributed data on Neotropical groups, created a figure and wrote sections of the text. Reiter contributed to the database, assisted in the direction of the manuscript and wrote sections of the text. Peter contributed to the database and improved the literature representation concerning African species. Bogarín added to the database, assisted in the direction of the paper and produced many of the final figures. Pérez-Escobar added to the database, assisted in the direction of the paper, explored phylogenetic analyses and produced some figures. Hong contributed to the database, improving the representation of records from China and Southeast Asia. All authors revised the manuscript.

FUNDING

O. A. Pérez-Escobar acknowledges support from the Swiss Orchid Foundation and the Lady Sainsbury Fellowship at the Royal Botanic Gardens, Kew.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

DATA AVAILABILITY

The data underlying this article are freely available in Zenodo; <https://zenodo.org/record/6350596#.Y12ZG-zMJ0J>; doi.org/10.5281/zenodo.7263689.

REFERENCES

- Abel GJ. 2013. *Migest: methods for the indirect estimation of bilateral migration*. Available at: <https://cran.r-project.org/web/packages/migest/index.html>
- Acevedo MA, Fletcher RJ Jr, Tremblay RL, Meléndez-Ackerman EJ. 2015. Spatial asymmetries in connectivity influence colonization-extinction dynamics. *Oecologia* **179**: 415–424.
- Ackerman JD. 1981. Pollination of *Calypso bulbosa* var. *occidentalis* (Orchidaceae): a food deception system. *Madroño* **28**: 101–110.
- Ackerman JD. 1983a. Specificity and mutual dependency of the orchid-euglossine bee interaction. *Biological Journal of the Linnean Society* **20**: 301–314.
- Ackerman JD. 1983b. Euglossine bee pollination of the orchid *Cochleanthes lipscombiae*: a food source mimic. *American Journal of Botany* **70**: 830–834.
- Ackerman JD. 1985. Pollination mechanisms of temperate and tropical orchids. In Tan K, ed. *Proceedings of the Eleventh World Orchid Conference*. Miami: Eleventh World Orchid Conference, 98–101.
- Ackerman JD. 1986. Mechanisms and evolution of food-deceptive pollination systems in orchids. *Lindleyana* **1**: 108–113.
- Ackerman JD. 2019. Orchids and the persistent instability principle. In Pridgeon AM, Arosemena AR, eds. *Proceedings of the 22nd World Orchid Conference, Vol. 1*. Guayaquil: Asociación Ecuatoriana de Orquideología, 42–51.
- Ackerman JD, Mesler MR. 1979. Pollination biology of *Listera cordata* (Orchidaceae). *American Journal of Botany* **66**: 820–824.
- Ackerman JD, Montalvo AM. 1990. Short and long-term limitations to fruit production in a tropical orchid. *Ecology* **71**: 263–272.
- Ackerman JD, Phillips RD, Tremblay RL, Karremans A, Reiter N, Peter CI, Bogarín D, Pérez-Escobar OA, Liu H. 2022. Beyond the various contrivances by which orchids are pollinated: global patterns in orchid pollination biology. *Zenodo*. <https://doi.org/10.5281/zenodo.7263689>. Online deposition date: 29 Oct 2022.
- Ackerman JD, Rodríguez-Robles JA, Meléndez EJ. 1994. A meager nectar offering by an epiphytic orchid is better than nothing. *Biotropica* **26**: 44–49.
- Ackerman JD, Roubik DW. 2012. Can extinction risk help explain plant-pollinator specificity among euglossine bee pollinated plants? *Oikos* **121**: 1821–1827.
- Ackerman JD, Sabat A, Zimmerman JK. 1996. Seedling establishment in an epiphytic orchid: an experimental study of seed limitation. *Oecologia* **106**: 192–198.
- Adams PB, Lawson SD. 1993. Pollination in Australian orchids: a critical assessment of the literature 1882–1992. *Australian Journal of Botany* **41**: 553–575.
- Agnew JD. 1986. Self compatibility/incompatibility in some orchids of the subfamily Vandoideae. *Plant Breeding* **97**: 183–186.
- Aliscioni SS, Torretta JP, Bello ME, Galati BG. 2009. Elaiophores in *Gomesa bifolia* (Sims) M.W. Chase & N.H. Williams (Oncidiinae: Cymbidieae: Orchidaceae): structure and oil secretion. *Annals of Botany* **104**: 1141–1149.
- Allard RW. 1975. The mating system and microevolution. *Genetics* **79** Suppl: 115–126.
- Allem AC. 2004. Optimization theory in plant evolution: an overview of long-term evolutionary prospects in the Angiosperms. *Botanical Review* **69**: 225–251.
- Almeida AM, Figueiredo RA. 2003. Ants visit nectaries of *Epidendrum denticulatum* (Orchidaceae) in a Brazilian rainforest: effects on herbivory and pollination. *Brazilian Journal of Biology* **63**: 551–558.
- Ames O, Ames B. 1937. Pollination of orchids through pseudocopulation. *Botanical Museum Leaflets, Harvard University* **5**: 1–29.

- Arakaki N, Yasuda K, Kanayama S, Jitsuno S, Oike M, Wakamura S. 2016.** Attraction of males of the cupreous polished chafer *Protaetia pryeri pryeri* (Coleoptera: Scarabaeidae) for pollination by an epiphytic orchid *Luisia teres* (Asparagales: Orchidaceae). *Applied Entomology and Zoology* **51**: 241–246.
- Argue GL. 2012a.** *The pollination biology of North American orchids, Vol. 1.* New York: Springer.
- Argue GL. 2012b.** *The pollination biology of North American orchids, Vol. 2.* New York: Springer.
- Armstrong JA. 1979.** Biotic pollination mechanisms in the Australian flora — a review. *New Zealand Journal of Botany* **17**: 467–508.
- Atwood JT. 1984.** Pollination of *Paphiopedilum rothschildianum*: brood-site imitation. *National Geographic Research* **1**: 247–254.
- Baker HG. 1955.** Self compatibility and establishment of long distance dispersal. *Evolution* **9**: 337–349.
- Balducci MG, van der Niet T, Johnson SD. 2020.** Diel scent and nectar rhythms of an African orchid in relation to bimodal activity patterns of hawkmoth pollinators. *Annals of Botany* **126**: 1155–1164.
- Bateman RM, Rudall PJ, Murphy ARM, Cowan RS, Devey DS, Pérez-Escobar OA. 2021.** Whole plastomes are not enough: phylogenomic and morphometric exploration at multiple demographic levels of the bee orchid clade *Ophrys* sect. *Sphegodes*. *Journal of Experimental Botany* **72**: 654–681.
- Bateman RM, Sramkó G, Paun O. 2018.** Integrating restriction site-associated DNA sequencing (RAD-seq) with morphological cladistic analysis clarifies evolutionary relationships among major species groups of bee orchids. *Annals of Botany* **121**: 85–105.
- Bierzuchudek P. 1981.** *Asclepias, Lantana, and Epidendrum*: a floral mimicry complex? *Biotropica* **13** Suppl: 54–58.
- Blanco MA, Barboza G. 2005.** Pseudocopulatory pollination in *Lepanthes* (Orchidaceae: Pleurothallidinae) by fungus gnats. *Annals of Botany* **95**: 763–772.
- Bogarín D, Fernández M, Borkent A, Heemskerk A, Pupulin F, Ramírez S, Smets E, Gravendeel B. 2018.** Pollination of *Trichosalpinx* (Orchidaceae: Pleurothallidinae) by biting midges (Diptera: Ceratopogonidae). *Botanical Journal of the Linnean Society* **186**: 510–543.
- Bohman B, Phillips RD, Flematti GR, Barrow RA, Peakall R. 2017.** The spider orchid *Caladenia crebra* produces sulfurous pheromone mimics to attract its male wasp pollinator. *Angewandte Chemie International Edition* **56**: 8455–8458.
- Bohman B, Phillips RD, Menz MHM, Berntsson BW, Flematti GR, Barrow RA, Dixon KW, Peakall R. 2014.** Discovery of pyrazines as pollinator sex pheromones and orchid semiochemicals: implications for the evolution of sexual deception. *New Phytologist* **203**: 939–952.
- Bower CC, Branwhite P. 1993.** Observations on the pollination of *Calochilus campestris* R. Br. *The Orchadian* **11**: 68–71.
- Brummitt RK. 2001.** *World geographical scheme for recording plant distributions, 2 edn.* Pittsburgh: Hunt Institute for Botanical Documentation.
- Cady L. 1972.** Notes on the pollination of *Calochilus campestris* R. Br. *The Orchadian* **4**: 52–53.
- CaraDonna PJ, Ackerman JD. 2012.** Reproductive assurance for a rewardless epiphytic orchid in Puerto Rico: *Pleurothallis ruscifolia* (Orchidaceae, Pterothallidinae). *Caribbean Journal of Science* **46**: 249–257.
- Catling PM. 1990.** Auto-pollination in the Orchidaceae. In Arditti J, ed. *Orchid biology, reviews and perspectives, Vol. 5.* Portland: Timber Press, 123–158.
- Chapurlat E, Anderson J, Ågren J, Friberg M, Sletvold N. 2018.** Diel pattern of floral scent emission matches the relative importance of diurnal and nocturnal pollinators in populations of *Gymnadenia conopsea*. *Annals of Botany* **121**: 711–721.
- Chase MW, Cameron KM, Freudenstein JV, Pridgeon AM, Salazar G, van den Berg C, Schuiteman A. 2015.** An updated classification of Orchidaceae. *Botanical Journal of the Linnean Society* **177**: 151–174.
- van der Cingel, NA. 1995.** *An atlas of orchid pollination: European orchids.* Rotterdam: AA Balkema.
- van der Cingel NA. 2001.** *An atlas of orchid pollination: America, Africa, Asia and Australia.* Rotterdam: AA Balkema.
- Claessens J, Kleynen J. 2011.** *The flower of the European orchid: form and function.* Netherlands: Claessens & Kleynen.
- Cohen C, Liltved WR, Coville JF, Shuttleworth A, Weissflog J, Svatoš A, Bytebier B, Johnson SD. 2021.** Sexual deception of a beetle pollinator through floral mimicry. *Current Biology* **31**: 1962–1969. e6.
- Coleman E. 1928.** Pollination of *Cryptostylis leptochila*. *Victorian Naturalist* **44**: 333–340.
- Cozzolino S, Scopece G, Roma L, Schlüter PM. 2020.** Different filtering strategies of genotyping-by-sequencing data provide complementary resolutions of species boundaries and relationships in a clade of sexually deceptive orchids. *Journal of Systematics and Evolution* **58**: 133–144.
- Cruz-Fernández QT, Alquicira-Arteaga ML, Flores-Palacios A. 2011.** Is orchid species richness and abundance related to the conservation status of oak forest? *Plant Ecology* **212**: 1091–1099.
- Dafni A, Ivri Y. 1981.** Floral mimicry between *Orchis israelitica* Baumann and Dafni (Orchidaceae) and *Bellevalia flexuosa* Boiss. (Liliaceae). *Oecologia* **49**: 229–232.
- Danaher MW, Ward C, Zettler LW, Covell CV. 2019.** Pollinia removal and suspected pollination of the endangered ghost orchid, *Dendrophylax lindenii* (Orchidaceae) by various hawk moths (Lepidoptera: Sphingidae): another mystery dispelled. *Florida Entomologist* **102**: 671–683.
- Darwin C. 1859.** *On the origin of species by means of natural selection or the preservation of favoured races in the struggle for life.* London: John Murray.
- Darwin C. 1862.** *The various contrivances by which British and foreign orchids are fertilised by insects.* London: John Murray.
- Darwin C. 1876.** *The effects of cross- and self-fertilisation in the vegetable kingdom.* London: John Murray.
- Darwin C. 1877.** *The various contrivances by which orchids are fertilised by insects, 2nd edn.* London: John Murray.

- Davies KL, Stpiczyńska M. 2012.** Comparative labellar anatomy of resin-secreting and putative resin-mimic species of *Maxillaria s.l.* (Orchidaceae: Maxillariinae). *Botanical Journal of the Linnean Society* **170**: 405–435.
- Davies KL, Stpiczyńska M. 2019.** Comparative anatomy of putative secretory floral structures in the *Camaridium cucullatum* complex and *Nitidobulbon* (Orchidaceae: Maxillariinae). *Botanical Journal of the Linnean Society* **190**: 165–191.
- De Jager ML, Peakall R. 2015.** Does morphology matter? An explicit assessment of floral morphology in sexual deception. *Functional Ecology* **30**: 537–546.
- Delforge P. 2016.** *Orchidées d'Europe, d'Afrique du Nord et de Proche-Orient, 4th edn.* Paris: Delachaux et Niestlé.
- Djordjevic V, Tsiftsis S. 2020.** The role of ecological factors in distribution and abundance of terrestrial orchids. In Mérillon J-M, Kodja H, eds. *Orchids phytochemistry, biology and horticulture*. Chamoni: Springer Nature, Switzerland, 1–71.
- Dodson CH. 1962.** The importance of pollination in the evolution of the orchids of tropical America. *American Orchid Society Bulletin* **31**: 525–534, 641–649, 731–735.
- Dodson CH, Dressler RL, Hills HG, Adams RM, Williams NH. 1969.** Biologically active compounds in orchid fragrances. *Science* **164**: 1243–1249.
- Dodsworth S. 2015.** Genome skimming for next-generation biodiversity analysis. *Trends in Plant Science* **20**: 525–527.
- Dressler RL. 1968a.** Observations on orchids and euglossine bees in Panama and Costa Rica. *Revista de Biología Tropical* **15**: 143–183.
- Dressler RL. 1968b.** Pollination by euglossine bees. *Evolution* **22**: 202–210.
- Dressler RL. 1976.** How to study orchid pollination without any orchids. In Senghas K, ed. *Proceedings 8th World Orchid Conference*. Frankfurt: Eighth World Orchid Conference, 534–537.
- Dressler RL. 1977.** El uso de los polinarios en la sistemática de las orquídeas. *Orquideología* **12**: 155–165.
- Dressler RL. 1981.** *The orchids: natural history and classification*. Cambridge: Harvard University Press.
- Dressler RL. 1993.** *Phylogeny and classification of the orchid family*. Portland: Dioscorides Press.
- Droissart V, Azandi L, Onguene ER, Savignac M, Smith TB, Deblauwe V. 2021.** PICT: A low-cost, modular, open-source camera trap system to study plant–insect interactions. *Methods in Ecology and Evolution* **12**: 1389–1396.
- Duffy KJ, Johnson SD. 2017.** Specialised mutualisms may constrain the geographical distribution of flowering plants. *Proceedings of the Royal Society of London B, Biological Sciences* **284**: 20171841.
- Efron B, Tibshirani R. 1993.** *An introduction to the bootstrap*. Boca Raton: Chapman & Hall/CRC.
- Farrington L, MacGillivray P, Faast R, Austin A. 2009.** Evaluating molecular tools for identification of *Caladenia* (Orchidaceae) species. *Australian Journal of Botany* **57**: 276.
- Fordham F. 1946.** Pollination of *Calochilus campestris*. *Victorian Naturalist* **62**: 199–201.
- Furstenau TN, Cartwright RA. 2017.** The impact of self-incompatibility systems on the prevention of biparental inbreeding. *PeerJ* **5**: e4085.
- Gaskett AC, Winnick CG, Herberstein ME. 2008.** Orchid sexual deceit provokes ejaculation. *American Naturalist* **171**: E206–E212.
- Givnish TJ, Spalink D, Ames M, Lyon SP, Hunter SJ, Zuluaga A, Iles WJD, Clements MA, Arroyo MTK, Leebens-Mack J, Endara L, Kriebel R, Neubig KM, Whitten WM, Williams NH, Cameron K. 2015.** Orchid phylogenomics and multiple drivers of their extraordinary diversification. *Proceedings of the Royal Society B: Biological Sciences* **282**: 20151553.
- Gomiz NE, Torretta JP, Aliscioni SS. 2017.** New evidence of floral elaiophores and characterization of the oil flowers in the subtribe Oncidiinae (Orchidaceae). *Plant Systematics and Evolution* **303**: 433–449.
- Goss GJ. 1977.** The reproductive biology of the epiphytic orchids of Florida 5 – *Epidendrum difforme* Jacquin. *American Orchid Society Bulletin* **46**: 630–636.
- Goss GJ, Adams RM. 1976.** The reproductive biology of the epiphytic orchids of Florida IV. – Sexually selective attraction of moths to the floral fragrance of *Epidendrum anceps* Jacquin. *American Orchid Society Bulletin* **45**: 997–1001.
- Gravendeel B, Smithson A, Slik FJW, Schuiteman A. 2004.** Epiphytism and pollinator specialisation: drivers for orchid diversity? *Philosophical Transactions of the Royal Society London B: Biological Sciences* **359**: 20041529.
- Gregg KB. 1991.** Defrauding the deceitful orchid: pollen collection by pollinators of *Cleistes divaricata* and *C. bifaria*. *Lindleyana* **6**: 214–220.
- Gu Z, Gu L, Eils R, Schlesner M, Brors B. 2014.** Circlize implements and enhances circular visualization in R. *Bioinformatics* **30**: 2811–2812.
- Hagerup O. 1952.** Bud autogamy in some northern orchids. *Phytomorphology* **2**: 51–60.
- Heinrich B. 1975.** Bee flowers: a hypothesis on flower variety and blooming times. *Evolution* **29**: 325–334.
- Hills HG, Williams NH, Dodson CH. 1972.** Floral fragrances and isolating mechanisms in the genus *Catasetum* (Orchidaceae). *Biotropica* **4**: 61–76.
- Houlihan PR, Stone M, Clem SE, Owen M, Emmel TC. 2019.** Pollination ecology of the ghost orchid (*Dendrophylax lindenii*): a first description with new hypotheses for Darwin's orchids. *Scientific Reports* **9**: 12850.
- Hughes AC, Orr MC, Ma K, Costello MJ, Waller J, Provoost P, Yang Q, Zhu C, Qiao H. 2021.** Sampling biases shape our view of the natural world. *Ecography* **44**: 12591–11269.
- Inoue K, Kato M, Inoue T. 1995.** Pollination ecology of *Dendrobium setifolium*, *Neuwiedia borneensis*, and *Lecanorchis multiflora* (Orchidaceae) in Sarawak. *Tropics* **5**: 95–100.
- Jacquemyn H, Micheneau C, Roberts DL, Paillet T. 2005.** Elevational gradients of species diversity, breeding system and floral traits of orchid species on Réunion Island. *Journal of Biogeography* **32**: 1751–1761.
- Jain SK. 1976.** The evolution of inbreeding in plants. *Annual Review of Ecology and Systematics* **7**: 469–495.

- Jenny R.** 2007. What is *Bibliorchidea*? *Lankesteriana* **7**: 169–174.
- Jersáková J, Johnson SD.** 2006. Lack of floral nectar reduces self-pollination in a fly-pollinated orchid. *Oecologia* **147**: 60–68.
- Jersáková J, Johnson SD, Jürgens A.** 2009. Deceptive behavior in plants. II. Food deception by plants: from generalized systems to specialised floral mimicry. In Baluska F, ed. *Plant-environment interactions*. Berlin: Springer-Verlag, 223–246.
- Jersáková J, Johnson SD, Kindlmann P.** 2006. Mechanisms and evolution of deceptive pollination in orchids. *Biological Review* **81**: 219–235.
- Jersáková J, Spaethe J, Streinzer M, Neumayer J, Paulus H, Dötterl S, Johnson SD.** 2016. Does *Traunsteinera globosa* (the globe orchid) dupe its pollinators through generalized food deception or mimicry? *Botanical Journal of the Linnean Society* **180**: 269–295.
- Jiang H, Kong J-J, Chen H-C, Xiang Z-Y, Zhang W-P, Han Z-D, Liao P-C, Lee Y-I.** 2020. *Cypripedium subtropicum* (Orchidaceae) employs aphid colony mimicry to attract hoverfly (Syrphidae) pollinators. *New Phytologist* **227**: 1213–1221.
- Joffard N, Arnal V, Buatois B, Schatz B, Montgelard C.** 2020. Floral scent evolution in the section *Pseudophrys*: pollinator-mediated selection or phylogenetic constraints? *Plant Biology* **22**: 881–889.
- Joffard N, Massol F, Grenié M, Montgelard C, Schatz B.** 2019. Data from: *Effect of pollination strategy, phylogeny and distribution on pollination niches of Euro-Mediterranean orchids, Dataset*. Available at: <https://doi.org/10.5061/dryad.58n11j4>, 30 June 2020.
- Johnson SD.** 1996. Bird pollination in South African species of *Satyrium* (Orchidaceae). *Plant Systematics and Evolution* **203**: 91–98.
- Johnson SD.** 2000. Batesian mimicry in the non-rewarding orchid *Disa pulchra*, and its consequences for pollinator behaviour. *Biological Journal of the Linnean Society* **71**: 119–132.
- Johnson SD.** 2006. Pollination by long-proboscid flies in the endangered African orchid *Disa scullyi*. *South African Journal of Botany* **72**: 24–27.
- Johnson SD, Alexandersson R, Linder HP.** 2003. Experimental and phylogenetic evidence for floral mimicry in a guild of fly-pollinated plants. *Biological Journal of the Linnean Society* **80**: 289–304.
- Johnson SD, Balducci MG, Bijl A, Castañeda-Zárata M, Cozien RJ, Ortman CR, Niet T.** 2020. From dusk till dawn: camera traps reveal the diel patterns of flower feeding by hawkmoths. *Ecological Entomology* **45**: 751–755.
- Johnson SD, Bond WJ.** 1994. Red flowers and butterfly pollination in the fynbos of South Africa. In Arianoutsou M, Groves RH, eds. *Plant-animal interactions in Mediterranean-type ecosystems*. Dordrecht: Kluwer Academic Publisher, 137–148.
- Johnson SD, van der Niet T.** 2019. Bird pollination in an African *Satyrium* (Orchidaceae) confirmed by camera traps and selective exclusion experiments. *Plant Systematics and Evolution* **305**: 477–484.
- Johnson SD, Peter CI, Ågren J.** 2004. The effects of nectar addition on pollen removal and geitonogamy in the non-rewarding orchid *Anacamptis morio*. *Proceedings of the Royal Society London B: Biological Sciences* **271**: 803–809.
- Johnson SD, Peter CI, Ellis AG, Boberg E, Botes C, van der Niet T.** 2011. Diverse pollination systems of the twin-spurred orchid genus *Satyrium* in African grasslands. *Plant Systematics and Evolution* **292**: 95–103.
- Johnson SD, Peter CI, Nilsson LA, Ågren J.** 2003. Pollination success in a deceptive orchid is enhanced by co-occurring reward magnet plants. *Ecology* **84**: 2919–2927.
- Johnson SD, Schiestl FP.** 2016. *Floral mimicry*. Oxford: Oxford University Press.
- Johnson SD, Steiner KE.** 2000. Generalization versus specialisation in plant pollinator systems. *Trends in Ecology and Evolution* **15**: 140–143.
- Johnson SD, Steiner KE.** 2003. Specialised pollination systems in southern Africa. *South African Journal of Science* **99**: 345–348.
- Johnson SD, Wester P.** 2017. Stefan Vogel's analysis of floral syndromes in the South African flora: an appraisal based on 60 years of pollination studies. *Flora* **232**: 200–206.
- Jones DL, Gray B.** 1974. The pollination of *Calochilus holtzei* F. Muell. *American Orchid Society Bulletin* **43**: 604–606.
- Jurgens A, Wee S-L, Shuttleworth A, Johnson SD.** 2013. Chemical mimicry of insect oviposition sites: a global analysis of convergence in angiosperms. *Ecology Letters* **16**: 1157–1167.
- Karremans AP.** 2021. With great biodiversity comes great responsibility: the underestimated diversity of *Epidendrum* (Orchidaceae). *Harvard Papers in Botany* **26**: 299–369.
- Karremans AP, Díaz-Morales M.** 2019. The Pleurothallidinae: extremely high speciation driven by pollinator adaptation. In Pridgeon AM, Arosemena AR, eds. *Proceedings of the 22nd World Orchid Conference, Vol. 1*. Guayaquil: Asociación Ecuatoriana de Orquideología, 363–388.
- Karremans AP, Pupulin F, Grimaldi D, Beentjes KK, Butot R, Fazzi GE, Kaspers K, Kruijzinga J, Roessingh P, Smets EF, Gravendeel B.** 2015. Pollination of *Specklinia* by nectar-feeding *Drosophila*: the first reported case of a deceptive syndrome employing aggregation pheromones in Orchidaceae. *Annals of Botany* **116**: 437–455.
- Karremans AP, Vieira-Urbe S.** 2020. *Pleurothallids: Neotropical jewels, Vol. I*. Quito: Imprenta Mariscal.
- Kestel JH, Phillips RD, Anthony J, Davis RA, Krauss SL.** 2021. Unexpectedly low paternal diversity is associated with infrequent pollinator visitation for a bird-pollinated plant. *Oecologia* **196**: 937–950.
- Kjellsson G, Rasmussen FN, Dupuy D.** 1985. Pollination of *Dendrobium infundibulum*, *Cymbidium insigne* (Orchidaceae) and *Rhododendron lyi* (Ericaceae) by *Bombus eximius* (Apidae) in Thailand: a possible case of floral mimicry. *Journal of Tropical Ecology* **1**: 289–302.
- Klemens JA, Tripepi M, McFoy SA.** 2021. A motion-detection based camera trap for small nocturnal mammals with low latency and high signal-to-noise ratio. *Methods in Ecology and Evolution* **12**: 1323–1328.
- Krahl AH, Holanda ASS, Krahl DRP, Martucci MEP, Gobbo-Neto L, Webber AC, Pansarin ER.** 2019. Study of the reproductive biology of an Amazonian *Heterotaxis*

- (Orchidaceae) demonstrates the collection of resin-like material by stingless bees. *Plant Systematics and Evolution* **305**: 281–291.
- Kullenberg B. 1961.** *Studies in Ophrys pollination*. Uppsala: Almquist and Wiksells.
- Kullenberg B, Bergström G. 1976.** The pollination of *Ophrys* orchids. *Botaniska Notiser* **129**: 11–20.
- Laube S, Zotz G. 2007.** A metapopulation approach to the analysis of long-term changes in the epiphyte vegetation on the host tree *Annona glabra*. *Journal of Vegetation Science* **18**: 613–624.
- Li P, Luo Y, Bernhardt P, Kou Y, Perner H. 2008.** Pollination of *Cypripedium plectrochilum* (Orchidaceae) by *Lasioglossum* spp. (Halictidae): the roles of generalist attractants versus restrictive floral architecture. *Plant Biology* **10**: 220–230.
- Liu ZJ, Chen LJ, Liu KW, Li LQ, Rao WH, Zhang YT, Tang GD, Huang LQ. 2013.** Adding perches for cross-pollination ensures the reproduction of a self-incompatible orchid. *PLoS ONE* **8**: e53695.
- Lombardi GC, Midgley JJ, Turner RC, Peter CI. 2021.** Pollination biology of *Erica aristata*: first confirmation of long-proboscid fly-pollination in the Ericaceae. *South African Journal of Botany* **142**: 403–408.
- Luer CA, Thorerle L. 2012.** Icones Pleurothallidarum XXXII. *Lepanthes* of Colombia (Orchidaceae). *Monographs in Systematic Botany from the Missouri Botanical Garden* **123**: 1–296.
- Luo H, Liang Y, Xiao H, Liu N, Chen Y, Wang W, Tang J, Xiong D, Yang B, Ren Z. 2020.** Deceptive pollination of *Calanthe* by skippers that commonly act as nectar thieves. *Entomological Science* **23**: 3–9.
- Maronna RA, Martin RD, Yohai VJ, Salibián-Barrera M. 2019.** *Robust statistics: theory and methods (with R)*. Hoboken: John Wiley & Sons.
- Martel C, Cairampoma L, Stauffer FW, Ayasse M. 2016.** *Telipogon peruvianus* (Orchidaceae) flowers elicit pre-mating behavior in *Eudejeania* (Tachnidae) males for pollination. *PLoS ONE* **11**: e0165896.
- Martén-Rodríguez S, Quesada M, Castro A-A, Lopezaraiza-Mikel M, Fenster CB. 2015.** A comparison of reproductive strategies between island and mainland Caribbean Gesneriaceae. *Journal of Ecology* **103**: 1190–1204.
- Martos F, Cariou M-L, Paillet T, Fournel J, Bytebier B, Johnson SD. 2015.** Chemical and morphological filters in a specialised floral mimicry system. *New Phytologist* **207**: 225–234.
- Micheneau C, Fournel J, Humeau L, Paillet T. 2008.** Orchid-bird interactions: a case study from *Angraecum* (Vandaeae, Angraecinae) and *Zosterops* (white-eyes, Zosteropidae) on Reunion Island. *Botany* **86**: 1143–1151.
- Micheneau C, Fournel J, Warren BH, Hugel S, Gauvin-Bialecki A, Paillet T, Strasberg D, Chase MW. 2010.** Orthoptera, a new order of pollinator. *Annals of Botany* **105**: 355–364.
- Müller H. 1883.** *The fertilization of flowers*. (transl. by Thompson DW). London: Macmillan and Co.
- Neal PR, Anderson GJ. 2005.** Are ‘mating systems’ ‘breeding systems’ of inconsistent and confusing terminology in plant reproductive biology? Or is it the other way around? *Plant Systematics and Evolution* **250**: 173–185.
- Neiland MRM, Wilcock CC. 1998.** Fruit set, nectar reward, and rarity in the Orchidaceae. *American Journal of Botany* **85**: 1657–1671.
- van der Niet T. 2020.** Paucity of natural history data impedes phylogenetic analyses of pollinator-driven evolution. *New Phytologist* **229**: 1201–1205.
- van der Niet T, Cozien RJ, Johnson SD. 2015.** Experimental evidence for specialised bird pollination in the endangered South African orchid *Satyrium rhodanthum* and analysis of associated floral traits. *Botanical Journal of the Linnean Society* **177**: 141–150.
- van der Niet T, Hansen DM, Johnson SD. 2011.** Carrion mimicry in a South African orchid: flowers attract a narrow subset of the fly assemblage on animal carcasses. *Annals of Botany* **107**: 981–992.
- Nilsson LA. 1978.** Pollination ecology of *Epipactis palustris* (Orchidaceae). *Botaniska Notiser* **131**: 355–368.
- Nilsson LA. 1983.** Processes of isolation and introgressive interplay between *Platanthera bifolia* (L.) Rich and *P. chlorantha* (Custer) Reichb. (Orchidaceae). *Botanical Journal of the Linnean Society* **87**: 325–350.
- Nilsson LA. 1992.** Orchid pollination biology. *Trends in Ecology and Evolution* **7**: 255–259.
- Nilsson LA, Jonsson L, Ralison L, Randrianjohany E. 1987.** Angraecoid orchids and hawkmoths in Central Madagascar: specialised pollination systems and generalist foragers. *Biotropica* **19**: 310–318.
- Nunes CEP, Gerlach G, Bandeira KDO, Gobbo-Neto L, Pansarin ER, Sazima M. 2017.** Two orchids, one scent? Floral volatiles of *Catasetum cernuum* and *Gongora bufonia* suggest convergent evolution to a unique pollination niche. *Flora* **232**: 207–216.
- Ollerton J, Alarcón R, Waser NM, Price MV, Watts S, Cranmer L, Hingston A, Peter CI, Rotenberry J. 2009.** A global test of the pollination syndrome hypothesis. *Annals of Botany* **103**: 1471–1480.
- Ollerton J, Cranmer L. 2002.** Latitudinal trends in plant-pollinator interactions: are tropical plants more specialised? *Oikos* **98**: 340–350.
- Ong PT. 2011.** The importance of *Bactrocera* fruit flies as pollinators of *Bulbophyllum* orchids. *Conservation Malaysia* **14**: 4–5.
- Ortiz-Barney E, Ackerman JD. 1999.** The cost of selfing in *Encyclia cochleata* (Orchidaceae). *Plant Systematics and Evolution* **219**: 55–64.
- Ospina-Calderón NH, Duque-Buitrago CA, Tremblay RL, Otero JT. 2015.** Pollination ecology of *Rodriguezia granadensis* (Orchidaceae). *Lankesteriana* **15**: 129–139.
- Otero JT, Alomía Y. 2016.** Visitantes florales de *Polystachya caracasana* Rchb.f. (Orchidaceae) en condiciones de cultivo. *Orquideología* **33**: 154–163.
- Pansarin E. 2021.** *Vanilla* flowers: much more than food-deception. *Botanical Journal of the Linnean Society* **198**: 57–73.
- Pansarin E, Pansarin L. 2010.** Reproductive biology of *Trichocentrum pumilum*: an orchid pollinated by oil-collecting bees. *Plant Biology* **13**: 576–581.

- Papadopoulos AST, Powell MP, Pupulin F, Warner J, Hawkins JA, Salamin N, Chittka L, Williams NH, Whitten WM, Loader D, Valente LM, Chase MW, Savolainen V. 2013.** Convergent evolution of floral signals underlies the success of Neotropical orchids. *Proceedings of the Royal Society B: Biological Sciences* **280**: 20130960.
- Paulus HF. 2019.** Speciation, pattern recognition and the maximization of pollination: general questions and answers given by the reproductive biology of the orchid genus *Ophrys*. *Journal of Comparative Physiology A* **205**: 285–300.
- Paulus HF, Gack C. 1990.** Pollination of *Ophrys* (Orchidaceae) in Cyprus. *Plant Systematics and Evolution* **169**: 177–207.
- Pauw A, Hawkins JA. 2011.** Reconstruction of historical pollination rates reveals linked declines of pollinators and plants. *Oikos* **120**: 344–349.
- Peakall R. 1987.** *Genetic systems of Australian terrestrial orchids*. PhD Thesis, University of Western Australia, Perth.
- Peakall R. 1989.** The unique pollination of *Leporella fimbriata* (Orchidaceae): pollination by pseudocopulating male ants (*Myrmecia urens*, Formicidae). *Plant Systematics and Evolution* **167**: 137–148.
- Peakall R. 1990.** Responses of male *Zaspilothynnus trilobatus* Turner wasps to females and the sexually deceptive orchid it pollinates. *Functional Ecology* **4**: 159–167.
- Peakall R, Ebert D, Poldy J, Barrow RA, Francke W, Bower CC, Schiestl FP. 2010.** Pollinator specificity, floral odour chemistry and the phylogeny of Australian sexually deceptive *Chiloglottis* orchids: implications for pollinator-driven speciation. *New Phytologist* **188**: 437–450.
- Pérez-Escobar OA, Chomicki G, Condamine FL, Karremans AP, Bogarin D, Matzke NJ, Silvestro D, Antonelli A. 2017.** Recent origin and rapid speciation of Neotropical orchids in the world's richest plant biodiversity hotspot. *New Phytologist* **215**: 891–905.
- Peter CI. 2011.** Modes of pollination and the occurrence of deception in the Orchidaceae. In Elliott J, Kurzweil HF, O'Bryne P, Tan KW, van der Schans AS, Wong SM, Yam TW, eds. *20th World Orchid Conference Proceedings*. Singapore: 20th World Orchid Conference, 268–272.
- Peter CI, Johnson SD. 2006.** Doing the twist: a test of Darwin's cross-pollination hypothesis for pollinarium reconfiguration. *Biology Letters* **2**: 65–68.
- Peter CI, Johnson SD. 2008.** Mimics and magnets: the importance of color and ecological facilitation in floral deception. *Ecology* **89**: 1583–1595.
- Peter CI, Johnson SD. 2013.** Generalized food deception: colour signals and efficient pollen transfer in bee-pollinated species of *Eulophia* (Orchidaceae). *Botanical Journal of the Linnean Society* **171**: 713–729.
- Peter CI, Johnson SD. 2014.** A pollinator shift explains floral divergence in an orchid species complex in South Africa. *Annals of Botany* **113**: 277–288.
- Peter CI, Venter N. 2017.** Generalist settling moth pollination in the endemic South African epiphyte, *Mystacidium pusillum* Harv. (Orchidaceae). *Flora* **232**: 16–21.
- Phillips RD, Batley M. 2020.** Evidence for a food-deceptive pollination system using *Hylaeus* bees in *Caladenia hildae* (Orchidaceae). *Australian Journal of Botany* **68**: 146–152.
- Phillips RD, Bohman B, Brown GR, Tomlinson S, Peakall R. 2020b.** A specialised pollination system using nectar-seeking wasps in *Caladenia nobilis* (Orchidaceae). *Plant Biology* **22**: 157–166.
- Phillips RD, Brown GR, Dixon KW, Hayes C, Linde CC, Peakall R. 2017.** Evolutionary relationships among pollinators and repeated pollinator sharing in sexually deceptive orchids. *Journal of Evolutionary Biology* **30**: 1674–1691.
- Phillips RD, Hopper SD, Dixon KW. 2010.** Pollination ecology and the potential impacts of the environmental change in the Southwest Australian Biodiversity Hotspot. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **365**: 517–528.
- Phillips RD, Reiter N, Peakall R. 2020a.** Orchid conservation: from theory to practice. *Annals of Botany* **126**: 345–362.
- Phillips RD, Scaccabarozzi D, Retter BA, Hayes C, Brown GR, Dixon KW, Peakall R. 2014.** Caught in the act: pollination of sexually deceptive trap-flowers by fungus gnats in *Pterostylis* (Orchidaceae). *Annals of Botany* **113**: 629–641.
- van der Pijl L, Dodson CH. 1966.** *Orchid flowers: their pollination and evolution*. Coral Gables: University of Miami Press.
- POWO. 2021.** *Plants of the world online*. Facilitated by the Royal Botanic Gardens, Kew. Published on the internet. Available at: <http://www.plantsoftheworldonline.org>. Accessed 08 September 2021.
- Pridgeon A, Cribb PJ, Chase MW, Rasmussen FN. 1999.** *Genera Orchidacearum, Vol. 1*. Oxford: Oxford University Press.
- Pridgeon A, Cribb PJ, Chase MW, Rasmussen FN. 2001.** *Genera Orchidacearum, Vol. 2*. Oxford: Oxford University Press.
- Pridgeon A, Cribb PJ, Chase MW, Rasmussen FN. 2003.** *Genera Orchidacearum, Vol. 3*. Oxford: Oxford University Press.
- Pridgeon A, Cribb PJ, Chase MW, Rasmussen FN. 2005.** *Genera Orchidacearum, Vol. 4*. Oxford: Oxford University Press.
- Pridgeon A, Cribb PJ, Chase MW, Rasmussen FN. 2009.** *Genera Orchidacearum, Vol. 5*. Oxford: Oxford University Press.
- R Core Team. 2020.** *R: a language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing. Available at: <https://www.R-project.org/>.
- Ramírez SR, Eltz T, Fujiwara MK, Gerlach G, Goldman-Huertas B, Tsutsui ND, Pierce NE. 2011.** Asynchronous diversification in a specialised plant-pollinator mutualism. *Science* **333**: 1742–1746.
- Rasmussen HN, Rasmussen FN. 2018.** The epiphytic habitat on a living host: reflections on the orchid-tree relationship. *Botanical Journal of the Linnean Society* **186**: 456–472.
- Raven PH, Gereau RE, Phillipson PB, Chatelain C, Jenkins CN, Ulloa Ulloa C. 2020.** The distribution of biodiversity richness in the tropics. *Science Advances* **6**: eabc6228.
- Ray HA, Gillett-Kaufman JL. 2022.** By land and by tree: pollinator taxa diversity of terrestrial and epiphytic orchids. *Journal of Pollination Ecology* **32**: 174–185.

- Reiter N, Bohman B, Batley M, Phillips RD. 2019a.** Pollination of an endangered *Caladenia* species (Orchidaceae) by nectar-foraging behavior of a widespread species of colletid bee. *Botanical Journal of the Linnean Society* **189**: 83–98.
- Reiter N, Bohman B, Flematti GR, Phillips RD. 2018.** Pollination by nectar-foraging thynnine wasps: evidence of a new specialised pollination system for Australian orchids. *Botanical Journal of the Linnean Society* **188**: 327–337.
- Reiter N, Bohman B, Freestone M, Brown GR, Phillips RD. 2019b.** Pollination by nectar-foraging thynnine wasps in the endangered *Caladenia arenaria* and *Caladenia concolor* (Orchidaceae). *Australian Journal of Botany* **67**: 490–500.
- Reiter N, Freestone M, Brown G, Peakall R. 2019c.** Pollination by sexual deception of fungus gnats (Keroplastidae and Mycetophilidae) in two clades of *Pterostylis* (Orchidaceae). *Botanical Journal of the Linnean Society* **190**: 101–116.
- Reiter N, Vleck K, O'Brien N, Gibson M, Pitts D, Brown GR, Bower CC, Phillips RD. 2017.** Pollinator rarity limits reintroduction sites in an endangered sexually deceptive orchid (*Caladenia hastata*): implications for plants with specialised pollination systems. *Botanical Journal of the Linnean Society* **184**: 122–136.
- Renner SS. 2006.** Rewardless flowers in the angiosperms and the role of insect cognition in their evolution. In Waser NM, Ollerton J, eds. *Plant-pollinator interactions: from specialisation to generalization*. Chicago: University of Chicago Press, 123–144.
- Rodriguez-Robles JA, Meléndez EJ, Ackerman JD. 1992.** The effects of display size, flowering phenology, and standing crop of nectar on the visitation frequency of *Comparettia falcata* (Orchidaceae). *American Journal of Botany* **79**: 1009–1017.
- Rose-Smyth MC. 2019.** Investigating the pollination biology of a long-lived island endemic epiphyte in the presence of an adventive alien pollinator. In Pridgeon AM, Arosemena AA, eds. *Proceedings of the 22nd World Orchid Conference, Vol. 1*. Guayaquil: Asociación Ecuatoriana de Orquideología, 80–91.
- Roubik DW. 1989.** *Ecology and natural history of tropical bees*. Cambridge: Cambridge University Press.
- RStudio Team. 2020.** *RStudio: integrated development for R*. Boston: RStudio, PBC. Available at: <http://www.rstudio.com/>.
- Salguero-Farías JA, Ackerman JD. 1999.** A nectar reward: is more better? *Biotropica* **31**: 303–311.
- 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. *Annals of Botany* **122**: 1061–1073.
- Scaccabarozzi D, Galimberti A, Dixon KW, Cozzolino S. 2020.** Rotating arrays of orchid flowers: a simple and effective methods for studying pollination in food deceptive plants. *Diversity* **12**: 286.
- Schatz B, Genoud D, Escudí P, Geniez P, Wunsch KG, Joffard N. 2021.** Is *Ophrys* pollination more opportunistic than previously thought? Insights from different field methods of pollinator observation. *Botany Letters* **168**: 333–347.
- Schiestl FP, Ayasse M, Paulus HF, Löfstedt C, Hansson BS, Ibarra F, Francke W. 1999.** Orchid pollination by sexual swindle. *Nature* **399**: 421–421.
- Schiestl FP, Peakall R, Mant JG, Ibarra F, Schulz C, Franke S, Francke W. 2003.** The chemistry of sexual deception in an orchid-wasp pollination system. *Science* **302**: 437–438.
- Schiestl FP, Schlüter PM. 2009.** Floral isolation, specialised pollination, and pollinator behavior in orchids. *Annual Review of Entomology* **54**: 425–446.
- Schlechter R. 1914.** Die Orchidaceen von Deutsch-Neu-Guinea. *Repertorium specierum novarum regni vegetabilis. Beihefte, Bd. I*. Berlin: Gebrüder Borntraeger. (1982; translation by Katz R and Simmons JD; Melbourne: Australian Orchid Foundation).
- Shrestha M, Dyer AG, Dorin A, Ren Z-X, Burd M. 2020.** Rewardlessness in orchids: how frequent and how rewardless? *Plant Biology* **22**: 555–561.
- Singer RB. 2002.** The pollination mechanism in *Trigonidium obtusum* Lindl (Orchidaceae: Maxillariinae): sexual mimicry and trap flowers. *Annals of Botany* **89**: 157–163.
- Singer RB, Gravendeel B, Cross H, Ramírez SR. 2008.** The use of orchid pollinia or pollinaria for taxonomic identification. *Selbyana* **29**: 6–19.
- Singer RB, Koehler S. 2004.** Pollinarium morphology and floral rewards in Brazilian Maxillariinae (Orchidaceae). *Annals of Botany* **93**: 39–51.
- Singer RB, Sazima M. 2000.** The pollination of *Stenorrhynchos lanceolatus* (Aublet) L. C. Rich. (Orchidaceae: Spiranthinae) by hummingbirds in southeastern Brazil. *Plant Systematics and Evolution* **223**: 221–227.
- Slade GM. 1962.** Some attractive Dendrobiums from New Guinea. *American Orchid Society Bulletin* **31**: 993–996.
- Smith GR, Snow GE. 1976.** Pollination ecology of *Platanthera (Habenaria) ciliaris* and *P. blephariglottis* (Orchidaceae). *Botanical Gazette* **137**: 133–140.
- Smith JJ. 1928.** Zelfbevruchting bij Orchideen. *Natuurkundig Tijdschrift Nederlandsch-Indie* **88**: 122–140.
- Smithson A, Gigord LDB. 2001.** Are there fitness advantages in being a rewardless orchid? Reward supplementation experiments with *Barlia robertiana*. *Proceedings of the Royal Society London B: Biological Sciences* **268**: 1435–1441.
- Sprengel CK. 1793.** *Das entdeckte Geheimnis der Natur im Bau und der Befruchtung der Blumen*. Berlin: Friedrich Vieweg dem aeltern.
- Steiner KE. 1998.** The evolution of beetle pollination in a South African orchid. *American Journal of Botany* **85**: 1180–1193.
- Stoutamire WP. 1975.** Pseudocopulation in Australian terrestrial orchids. *American Orchid Society Bulletin* **44**: 226–232.
- Tan KH. 2006.** Fruit fly pests as pollinators of wild orchids. In: Fruit flies of economic importance: from basic to applied knowledge. *Proceedings of the 7th International Symposium on Fruit Flies of economic importance*. Salvador, Brazil, 195–206.

- Tan KH, Nishida R. 2000.** Mutual reproductive benefits between a wild orchid, *Bulbophyllum patens*, and *B. actrocera* fruit flies via a floral synomone. *Journal of Chemical Ecology* **26**: 533–546.
- Torretta JP, Gomiz NE, Aliscioni SS, Bello ME. 2011.** Biología reproductiva de *Gomesa bifolia* (Orchidaceae, Cymbidieae, Oncidiinae). *Darwiniana* **49**: 16–24.
- Tremblay RL. 1992.** Trends in the pollination ecology of the Orchidaceae: evolution and systematics. *Canadian Journal of Botany* **70**: 642–650.
- Tremblay RL, Ackerman JD, Zimmerman JK, Calvo RN. 2005.** Variation in sexual reproduction in orchids and its evolutionary consequences: a spasmodic journey to diversification. *Biological Journal of the Linnean Society* **84**: 1–54.
- Tremblay RL, Meléndez-Ackerman E, Kapan D. 2006.** Do epiphytic orchids behave as metapopulations? Evidence from colonization, extinction rates and asynchronous population dynamics. *Biological Conservation* **129**: 70–81.
- Vereecken NJ, Wilson CA, Hötling S, Schulz S, Banketov SA, Mardulyn P. 2012.** Pre-adaptations and the evolution of pollination by sexual deception: Cope's rule of specialisation revisited. *Proceedings of the Royal Society B: Biological Sciences* **279**: 4786–4794.
- Vieira-Urbe S, Moreno JS. 2019.** Three new *Lepanthes* (Orchidaceae: Pleurothallidinae) from the Alto de Ventanas Ecoregion in Antioquia, Colombia. *Lankesteriana* **19**: 63–75.
- Vogel S. 1954.** *Blütenbiologische Typen als Elemente der Sipplgliederung*. Jena: Fischer.
- Vogel S. 2006.** Floral syndromes: empiricism versus typology. *Botanische Jahrbücher für Systematik* **127**: 5–11.
- Wang Y, Zhang Y, Ma XK, Dong L. 2008.** The unique mouse pollination in an orchid species. *Nature Precedings* 1–6. <https://doi.org/10.1038/npre.2008.1824.1>.
- Warnes GR, Bolker B, Lumley T, Johnson RC. 2018.** *gmodels: various R programming tools for model fitting*. R package version 2.18.1. Available at: <https://CRAN.R-project.org/package=gmodels>
- Waser NM, Chittka L, Price MV, Williams NM, Ollerton J. 1996.** Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043–1060.
- Waterman RJ, Bidartondo MI, Stofberg J, Combs JK, Savolainen V, Barraclough TG, Pauw A. 2011.** The effect of above- and belowground mutualisms on orchid speciation and coexistence. *American Naturalist* **177**: E54–E68.
- Watteyn C, Scaccabarozzi D, Muys B, van der Schueren N, van Meerbeek K, Guizar Amador MF, Ackerman JD, Cedeño Fonseca M, Chinchilla IF, Reubens B, Pillco Huaracaya R, Cozzolino S, Karremans AP. 2021.** Trick or treat? Pollinator attraction in *Vanilla pompona* (Orchidaceae). *Biotropica* **54**: 268–274.
- Whitehead MR, Peakall R. 2014.** Pollinator specificity drives strong prepollination reproductive isolation in sympatric sexually deceptive orchids. *Evolution* **68**: 1561–1575.
- Wickham, H. 2016.** *ggplot2: Elegant graphics for data analysis*. New York: Springer-Verlag.
- Widmer A, Cozzolino S, Pellegrino G, Soliva M, Dafni A. 2000.** Molecular analysis of orchid pollinaria and pollinaria-remains found on insects. *Molecular Ecology* **9**: 1911–1914.
- Wilcox R. 2017.** *Introduction to robust estimation and hypothesis testing, 4th edn*. New York: Academic Press.
- Williams NH, Dodson CH. 1972.** Selective attraction of male euglossine bees to orchid floral fragrances and its importance in long distance pollen flow. *Evolution* **26**: 84–85.
- Williams NH, Whitten WM. 1983.** Orchid floral fragrances and male euglossine bees: methods and advances in the last sequidecade. *Biological Bulletin* **164**: 355–395.
- Williams NH, Whitten WM. 1988.** *Stanhopea panamensis*, a new species from central Panama (Orchidaceae). *Lindleyana* **3**: 9–11.
- Winkler M, Hülber K, Hietz P. 2009.** Population dynamics of epiphytic orchids in a metapopulation context. *Annals of Botany* **104**: 995–1004.
- Xu S, Schlüter PM, Scopece G, Breitkopf H, Gross K, Cozzolino S, Schiestl FP. 2011.** Floral isolation is the main reproductive barrier among closely related sexually deceptive orchids. *Evolution* **65**: 2606–2620.
- Yuen KK. 1974.** The two-sample trimmed t for unequal population variances. *Biometrika* **61**: 165–170.
- Zhang X, Jia Y, Liu Y, Chen D, Luo Y, Niu S. 2021.** Challenges and perspectives in the study of self-incompatibility in orchids. *International Journal of Molecular Sciences* **22**: 12901.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article on the publisher's website.

Table S1. The number of orchid species that offer varying types of floral reward. Percentage is based on all reward types.

Table S2. Mean number of pollinator species per orchid species among TDWG Level 1 regions. Analysis involves a bootstrap method considering outliers, function 'btrim' (Wilcox, 2017: p. 331). Analyses of paired comparisons based on step-down multiple comparisons approach using 'lincon' function (Wilcox, 2017: p. 349). Adjusted *P* value accounts for multiple comparisons.

Table S3. Pairwise comparisons of the number of pollinator species for subfamilies Orchidoideae and Epidendroideae and modes of deception (sex and food). The confidence intervals and median location are estimated

using the Harrell–Davis method, which is a bootstrap process followed by trimming of 20% of the data at the lower (LCI) and upper (HCI) intervals of the distribution to remove outliers. Only orchid species whose pollinators have been identified to species level are included. Tests were adjusted for multiple comparisons. See [Table 6](#) for mean locations.

Figure S1. Photographic evidence of unpublished pollination observations. A, *Epidendrum horichii* Hágsater pollinated by *Dircenna klugii* (Nymphalidae) in Costa Rica. B, *Epidendrum triangulabium* Ames & C.Schweinf. with cleistogamous flowers showing the swelling of the ovary. C, *Lepanthes minutissima* Endrés ex Luer with cleistogamous flowers *in situ* in Costa Rica. D, *Lepanthes pygmaea* Luer being pollinated by a fungus gnat (Sciaridae) in Costa Rica. E, *Masdevallia coriacea* Lindl. pollinated by Calliphoridae in Colombia. F, *Masdevallia coriacea* pollinated by Sarcophagidae in Colombia. G, *Octomeria costaricensis* Schltr.: a cleistogamous flower showing a swelling ovary. H, *Ornithocephalus lankesteri* Ames: an autogamous flower showing the pollinarium stuck to its stigma. I, *Pleurothallis homalantha* Schlechter being pollinated by a sciarid fly at Bosque de Paz Reserve in Costa Rica. J, *Pleurothallis tridentata* Klotzsch pollinated by a drosophilid fly. K, *Prosthechea spondiada* (Rchb.f.) W.E.Higgins: an autogamous species showing its fruits. L, *Xylobium elongatum* (Lindl. & Paxton) Hemsl. with its pollinator, a *Trigona* sp. (Apidae) bee at Bosque de Paz Reserve in Costa Rica. Photographs by A. P. Karremans (A, B, C, G, H), G. Barboza (D), K. Gil (E, F, I, K, L) and D. Pfarr (J).

Figure S2. Heatmap for frequency of major pollinator groups with orchid subtribes.

Figure S3. Observed and expected distribution of pollinator attraction strategies and pollinator orders among orchid subtribes. Expected frequencies are calculated by extrapolating the proportion of known strategies or orders with the total known orchid species in each subtribe. A, Observed frequencies of pollinator orders among orchid subtribes. B, Expected frequencies of pollinator orders among pollinator subtribes. C, Observed frequencies of modes of pollinator attraction among orchid subtribes. D, Expected frequencies of modes of pollinator attraction among orchid subtribes.

Figure S4. Heatmap for frequency of pollinator families versus orchid subfamilies and subtribes.

Figure S5. Mean location and highest density intervals for the number of pollinator species among subfamilies of Orchidaceae. Sample sizes: Apostasioideae, $N = 3$; Cyripedioideae, $N = 39$; Epidendroideae, $N = 663$; Orchidoideae, $N = 665$; Vanilloideae, $N = 27$.

Figure S6. Frequency associations between subfamily Epidendroideae and pollinator families.

Figure S7. Frequency associations between subfamilies Apostasioideae, Vanilloideae and Cyripedioideae and pollinator families.

Figure S8. Frequency associations between subfamily Orchidoideae and pollinator families.