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3 **Orchid Conservation – from theory to practice**

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1   ABSTRACT

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3   *Background*: Given the exceptional diversity of orchids (26,000+ species), improving

4   strategies for the conservation of orchids will benefit a vast number of taxa.

5   Furthermore, with rapidly increasing numbers of endangered orchids, and low success

6   rates in orchid conservation translocation programs worldwide, it is evident that our

7   progress in understanding the biology of orchids is not yet translating into widespread

8   effective conservation.

9   *Scope*: We highlight unusual aspects of the reproductive biology of orchids that can

10 have important consequences for conservation programs such as specialisation of

11 pollination systems, low fruit set but high seed production, and the potential for long-

12 distance seed dispersal. Further, we discuss the importance of their reliance on

13 mycorrhizal fungi for germination, including quantifying the incidence of specialised

14 versus generalised mycorrhizal associations in orchids. In light of leading

15 conservation theory and the biology of orchids, we provide recommendations for

16 improving population management and translocation programs.

17 *Conclusions*: Major gains in orchid conservation can be achieved by incorporating

18 knowledge of ecological interactions, for both generalist and specialist species. For

19 example, habitat management can be tailored to maintain pollinator populations, and

20 conservation translocation sites selected based on confirmed availability of

21 pollinators. Similarly, use of efficacious mycorrhizal fungi in propagation will

22 increase the value of *ex-situ* collections, and likely increase the success of

23 conservation translocations. Given the low genetic differentiation between

24 populations of many orchids, experimental genetic mixing is an option to increase

25 fitness of small populations, although caution is needed where cytotypes or floral

1 ecotypes are present. Combining demographic data and field experiments will provide

2 knowledge to enhance management and translocation success. Finally, high per-fruit

3 fecundity means that orchids offer powerful but overlooked opportunities to propagate

4 plants for experiments aimed at improving conservation outcomes. Given the

5 uncertainty of future environmental change, experimental approaches also offer

6 powerful ways to build more resilient populations.

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8 **Key-words:** orchid, conservation, genetics, mycorrhiza, pollination, conservation

9 translocations, reintroduction, restoration, demography

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1   INTRODUCTION

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3   The Orchidaceae is one of the most species rich of all angiosperm families, with in

4   excess of 26,000 named species (WCSPF, 2017). The distribution of the family spans

5   all continents except Antarctica, and includes most major island groups (Dressler,

6   1981). Orchids reach their highest diversity in the epiphytic communities of the

7   tropics, particularly at middle elevations, where they make up a large component of

8   plant species richness (Gentry and Dodson, 1987; Ibish *et al*., 1996; Vasquez *et al*.,

9   2003). While approximately 70 % of orchid species are epiphytic, there are also

10   diverse terrestrial communities in some tropical and temperate regions (Dressler,

11   1981).

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13   In some countries, such as Ecuador, China and Australia, orchids feature prominently

14   among lists of threatened plant species (e.g. León-Yánez *et al*., 2011; Qin *et al.*, 2017;

15   Australian Government, 2019). However, given that in biodiverse tropical countries

16   many orchid species are poorly known, the numbers of endangered orchid species are

17   likely to continue to grow on both national and international lists (Joppa *et al*.,

18   2011a,b). Like many other plant groups, orchids face unprecedented levels of threat

19   from habitat destruction and fragmentation, over-collecting, climate change, and a

20   range of other human induced issues (Diamond, 1989; Dixon *et al*., 2003; Thomas *et*

21   *al*., 2004; Swarts and Dixon, 2009; Reiter *et al*., 2016; Hinsley *et al*., 2018). However,

22   some unusual aspects of orchid biology suggest that many species may present unique

23   conservation challenges. Further, many species of terrestrial and epiphytic orchids

24   naturally occur in small isolated populations, in part as a result of specialised habitat

25   preferences (Dressler, 1981; Tremblay *et al*., 2005).

2 *Aims and approach*

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4 Despite considerable advances in our knowledge of orchid biology (see reviews of

5 Swarts and Dixon, 2009; McCormick and Jacquemyn, 2014; Rasmussen *et al*., 2015;

6 Bohman *et al*., 2016; Johnson and Schiestl, 2016; Fay, 2018; McCormick *et al*.,

7 2018), and some important conservation success stories (e.g. Figure 1; Willems, 2001;

8 Schrautzer *et al*., 2011; Reiter *et al*., 2018a), there are clear signs in the literature that

9 orchid conservation is not being as effective as required to avert the extinction of a

10 large number of species. Many threatened orchid species have already undergone

11 large population declines (Cribb *et al*., 2003) and, based on resources such as the

12 IUCN red list (IUCN, 2018) and government recovery plans (e.g. Australian

13 Government, 2019), most species that are listed as endangered experience threats

14 beyond the destruction of habitat (Wraith and Pickering, 2019). Further, in a global

15 review of 74 published conservation translocations, an action commonly applied for

16 threatened orchids, Reiter *et al*., (2016) found that only 25 % of studies observed any

17 fruit set, and just 2.8 % of studies observed recruitment. Given this lack of success, it

18 is clear that there is an urgent need for a critical appraisal of the current approaches to

19 orchid conservation.

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21 Practical techniques for orchid conservation such as propagation, seed storage, and

22 genetic analysis have been comprehensively reviewed elsewhere (Swarts and Dixon,

23 2017). Therefore, here we consider how biological knowledge of a species, and its

24 interactions, might be used to improve conservation outcomes. For example, no

25 matter how good the propagation technique or the *ex situ* collection, failure to

consider the availability of effective pollinators in a conservation translocation

program could mean that such efforts are merely a 'gardening exercise' with little

prospect of achieving a self-sustaining population. Our focus in this review is on

topics that are likely to be more prevalent in orchids than other plants, and on

conservation issues that are likely to be broadly applicable across geographic regions

and taxonomic groups within the Orchidaceae. Nonetheless, many of the issues we

raise also have some general applicability beyond orchids. We do not cover the

adverse effects of the trade in wild collected orchids (e.g. removal of wild orchids for

horticulture, food or medicine), as this has recently been comprehensively reviewed

by Hinsley *et al.*, (2018).

To provide a framework for the review, we use the orchid life cycle as an organising

principle (Figure 2; Table 1). Firstly, we highlight four unusual aspects of the biology

of orchids; (i) a high incidence of specialised pollination systems, (ii) pollinator

limited fruit set, but with high fecundity, (iii) dust-like seed and high dispersal, (iv)

dependence on mycorrhiza for germination and growth through the protocorm stage.

In this section we also provide a comprehensive review of the literature to quantify

the incidence of specialised mycorrhizal associations in orchids. We then establish if

these unusual aspects of orchid biology have been adequately addressed in studies

aiming to improve conservation outcomes. Finally, we identify current innovations

and future directions that if implemented could deliver large scale, effective orchid

conservation programs.

1 THE DEFINING FEATURES OF ORCHIDS

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3 Despite the vast array of shapes, colours and odours of orchid flowers (see Pridgeon

4 *et al*., 1999), a common set of floral traits characterise virtually all orchids. For

5 example, all orchid flowers are zygomorphic with the stamen(s) on one side of the

6 flower rather than in a symmetrical arrangement of separate anthers. Further, the

7 stamen and pistil are at least partly united, with most species bearing a single stamen

8 that is completely united with this pistil into one structure called the column (Dressler

9 1981). In the majority of orchids the petal opposite the column is modified into a lip

10 known as the labellum (though see Dafni and Calder, 1987). Due to the resupinate

11 development seen in most species, where the inferior ovary twists 180 degrees while

12 the flower is in bud, this petal is positioned on the underside of the column (though

13 see Peakall 1989). The pollen grains are numerous and typically bound in large

14 masses known as pollinia (Johnson and Edwards, 2000). In virtually all species of

15 orchid, the numerous seeds are tiny (mostly 0.05- 6 mm) and lack an endosperm

16 (Arditti and Ghani, 2000).

17

18 Orchids occupy a broad range of habitats, and their growth habits can be terrestrial,

19 epiphytic, lithophytic or even predominantly underground. Therefore, it is no surprise

20 that their growth forms show considerable variation across the family (Dressler 1981).

21 Nonetheless, one feature that is prevalent is the velamen, comprising one or more

22 layers of spongy cells on the outside of the roots (Dressler 1981). This structure

23 occurs in virtually all epiphytic orchids, as well as many terrestrial genera (Pridgeon

24 *et al*., 1999; Zotz *et al*., 2017). The presence of this spongy, water-absorbing layer

1   may have acted as a pre-adaptation to the evolution of epiphytism (Dressler, 1981;

2   Benzing, 1990; Gravendeel *et al*., 2004), which is so prevalent in the family.

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4   UNUSUAL FEATURES OF ORCHID BIOLOGY

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6   ***High incidence of specialised pollination systems***

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8   The Orchidaceae exhibits the full continuum of pollination systems from generalised

9   to highly specialised (e.g. Joffard *et al*., 2019), as well as various forms of autogamy

10   (Dressler, 1981). In orchids, highly generalised pollination strategies involving

11   multiple pollinator functional groups appear to be unusual (e.g. flies, bees and

12   butterflies as effective pollinators of a single species; Johnson and Hobbhahn, 2010).

13   Alternatively, many orchid species exhibit specialisation at the level of pollinator

14   functional groups (Argue, 2012). For example, several species of noctuid moths, but

15   not other insects, pollinate the North American orchid *Tipularia discolor* (Whigham

16   and McWethy, 1980; Argue, 2012). Further, as we show below, orchids are unusual in

17   that many species exhibit specialisation on only one or few pollinator species (i.e.

18   exhibiting ecological specialisation as defined by Ollerton *et al*., 2007; Armbruster,

19   2017). While highly specialised pollination strategies occur in many plant families,

20   the orchids may have been predisposed to the evolution of specialised pollination

21   strategies due to several floral features: the packing of pollen in pollinia means that

22   there can be efficient pollen transfer even at low visitation rates (Johnson and

23   Edwards, 2000); the positioning of pollinaria in close proximity to the labellum means

24   that only pollinators of a particular size may lead to pollen transfer (Schiestl and

25   Schluter, 2009; Li *et al*., 2008; Reiter *et al*., 2018a); the labellum of their

zygomorphic flowers can be extensively modified for positioning of the pollinator

(e.g. Phillips *et al*., 2014b; De Jager and Peakall, 2016) or for utilising different

methods of attraction (Johnson and Schiestl, 2016).

Several regional and global summaries of the number of known pollinator species in

orchid pollination systems are available (summarised in Table 2). While the number

of pollinator observations and study sites varies between studies, for several regional

and global estimates the number of pollinator species most commonly recorded for

any given orchid species was one (i.e. the modal value was one). As such, while the

average number of pollinator species varies among pollination strategies (e.g. more in

nectar rewarding than sexually deceptive systems; Joffard *et al.,* 2019) and

geographic regions (e.g. more generalist species in Europe than South Africa; Johnson

and Steiner, 2003), the literature currently shows that highly specialised pollination

predominates within the Orchidaceae. Thus, in many orchid species, reproductive

success in a given population depends on just one or a few pollinator species.

In part due to their high incidence of specialised pollination systems, the orchids

contain perhaps the most bewildering array of pollination strategies of any plant

family (Figure 3). In many orchids there is strong evidence of floral traits as

adaptations to attract specific pollinator species or pollinator groups (see examples in

Johnson and Schiestl, 2016). Even among orchid species that provide nectar reward

(perhaps the most common pollination strategy outside the orchids) there is evidence

for specific adaptation to pollen vectors drawn from a wide range of taxonomic

groups including birds, moths, long-tongued flies, solitary bees and wasps (Nilsson *et*

al., 1987; van der Cingel, 1995; Johnson *et al*., 1998; van der Niet *et al*., 2015; Reiter

et al., 2018a). One unusual rewarding pollination strategy, found in numerous

neotropical orchid species (approximately 600 species), is the provision of fragrance

to male euglossine bees that incorporate the compounds into a bouquet of chemicals

that they use in courtship (Ackerman, 1983; Ramirez *et al*., 2011).

Another unusual feature of the orchids is the very high frequency of deceptive

pollination strategies (Jersáková *et al*., 2006; Johnson and Schiestl, 2016). While

precise estimates are lacking for most regions (Srestha *et al*., 2020), it is commonly

cited that approximately one third of all orchids employ such strategies (see

references in Jersáková *et al*., 2006). Many deceptive strategies are specialised

through the use of floral signals that are attractive to particular pollinators (Johnson

and Schiestl, 2016). For example, deceptive pollination systems involving Batesian

mimicry of food plants (e.g. Nilsson, 1983; Peter and Johnson, 2008; Jersáková *et al*.,

2012), sexual deception by the mimicry of female insects (most recently reviewed by

Bohman *et al*., 2016), and mimicry of brood sites (Martos *et al*., 2015), all typically

involve the attraction of one or few pollinator species using chemical or visual

signals.

Pollinator limited fruit set, but high fecundity

While reproductive success has only been assessed for a very small fraction of the

Orchidaceae, for non-autogamous species fruit set within a flowering season appears

to be primarily limited by the number of flowers receiving pollen, a trend which holds

for both terrestrial and epiphytic species (Tremblay *et al*., 2005). Indeed, pollen

limitation in orchids is often substantial, with two to ten-fold increases in fruit set

commonly being reported following experimental hand pollination (reviewed in

Tremblay *et al*., 2005). While deceptive orchids have on average much lower fruit set

than rewarding species (Tremblay *et al*., 2005; mean for deceptive species = 20.7 %

(N = 130); mean for rewarding species = 37.1 % (N = 84)), and fruit set is on average

lower in tropical species (Tremblay *et al*., 2005), pronounced pollen limitation within

a flowering season appears to hold true regardless of geography or the pollination

strategy. Experimental investigations extending beyond a single flowering season

have shown that many orchids exhibit a subsequent cost of fruit formation, with

individuals often showing less vigorous growth or reduced investment in flowering

the following season (Snow and Whigham, 1989; Ackerman and Montalvo, 1990;

Primack *et al*., 1994; Sletvold and Agren, 2015). However, in these experiments the

number of artificially pollinated flowers is often well above natural pollination levels,

thus pollen limitation, not resource limitation, seems likely to dominate across the

lifetime of non-autogamous orchids (Calvo and Horwitz, 1990).

By virtue of the packaging of pollen as pollinia in orchids (though see Dressler, 1981

for some exceptions), when a pollination event does occur, vast numbers of pollen

grains are deposited on the stigma. The estimated number of pollen grains per

pollinia varies from approximately 5,000 – 4,000,000, depending on the species

(Nazarov and Gerlach, 1997; Johnson and Edwards, 2000). Therefore, even when just

a portion of the pollinium is deposited on a flower, subsequent fertilisation can yield

1000's of seeds per capsule (Darwin, 1877; Arditti and Ghani, 2000). Indeed, while

varying widely among orchid genera, reported maximum estimates range from

approximately 100 to 6,000,000 seeds per capsule (Arditti and Ghani, 2000;

Meléndez-Ackerman and Ackerman, 2001), with most species having well over a

1000 seeds per capsule. Therefore, even when a low number of fruits are produced in any given season, seed output by orchids can be exceptionally high.

A corollary of the *en masse* pollination of orchids is that the resulting seed is likely to be fathered by only a small number of pollen donors. While pollen-labelling studies have shown that there is some level of pollen carryover among flowers of different plants, confirming the potential for multiple fathers per fruit (e.g. Peakall, 1989; Peakall and Beattie, 1996; Johnson and Nilsson, 1999), such studies in multi-flowered species have also revealed moderate rates of geitonogamy (reviewed in Kropf and Renner, 2008). To date, the only two studies to conduct paternity analysis of orchid seed showed that there was either one father per fruit (Trapnell and Hamrick, 2004), or an average of 1.35 fathers per fruit (Whitehead *et al*., 2015). As such, many orchids may exhibit the unusual situation of high seed output, but with a low genetic diversity among the progeny, particularly among those species with solid rather than sectile or mealy pollinia.

Dust-like seed and high dispersal

While the dust-like seeds of orchids appear well adapted for wind dispersal (Beer, 1863), much of the seed falls close to the parent plant (e.g. Murren and Ellison, 1998; Nathan *et al*.; 2000; Jersakova and Malinova, 2007; Brzosko *et al*., 2017). Nonetheless, observations of orchid colonisation of distant areas of suitable habitat and remote oceanic islands (Dressler, 1981; Arditti and Ghani, 2000; Partomihardjo, 2003) demonstrate that such tiny seeds do have an exceptional capability for long-distance dispersal, with a small proportion of seeds presumably moving a very long

1 way. Given the colonising potential of many species, orchids are expected to exhibit

2 comparatively high levels of seed-mediated gene flow with low levels of population

3 genetic differentiation as a consequence (Phillips *et al*., 2012). Indeed, allozyme and

4 microsatellite data for terrestrial orchids supports this scenario, with most species

5 exhibiting low genetic differentiation, even across relatively large geographic

6 distances (see review of Phillips *et al*., 2012). While rare species, which presumably

7 have more geographically isolated populations, have on average greater genetic

8 variation between populations (Phillips *et al*., 2012), this is still typically less than that

9 seen in most other plant families (Hamrick and Godt, 1996; Phillips *et al*., 2012; but

10 see Arduino *et al*., 1996; Wong and Sun, 1999; Wallace 2002; Chung and Chung,

11 2007 for species with high differentiation).

12

13 Although most orchid population genetic studies have been based on terrestrial

14 species, the few studies of epiphytic and lithophytic taxa show a similar pattern of low

15 levels of differentiation across spatial scales of 10s to 100s of kilometres (Ackerman

16 and Ward, 1999; Borba *et al*., 2001; Trapnell and Hamrick, 2004; Avila-Diaz and

17 Oyama, 2007; Ribeiro *et al*., 2008; Kisel *et al*., 2012). From a conservation

18 perspective, it remains to be seen if following the decline of many orchid populations

19 via extensive vegetation clearing or inappropriate habitat management, whether there

20 are still sufficient orchids reproducing to maintain both regular gene flow among

21 populations and long-range colonisation.

22

Dependence on mycorrhiza for germination and protocorm growth

Given that orchid seeds typically lack an endosperm (Arditti and Ghani, 2000, Yeung, 2017), they are reliant on mycorrhizal fungi to provide the essential nutrition for germination and protocorm growth through to the green leaf stage (Smith and Read, 2008; Rasmussen and Rasmussen, 2009). Often this fungal association is maintained into adulthood, although the reliance of the adult plant on fungi is likely to vary between life forms (e.g. epiphytic vs terrestrial; Hadley and Williamson, 1972; Rasmussen and Rasmussen, 2009; Bayman *et al*., 2002). In adult photosynthetic terrestrial orchids, the plant exports sugars to the fungus (Cameron *et al*., 2006), while the plant receives Phosphorous across intact membranes, and fungal P, Nitrogen and Carbon from the lysis of the hyphae forming the pelotons (Cameron *et al*., 2006, 2008; Bougoure *et al*., 2013; Dearnaley and Cameron, 2017; Fochi *et al*., 2017). However, it is increasingly being recognised that some photosynthetic orchids are partial mycoheterotrophs, which in adulthood retain an ability to acquire some carbon from fungi (Gebauer *et al*., 2016). Interestingly, the retention of a fully mycoheterotrophic state, where the plant remains completely reliant on the fungus for carbon in adulthood, has also evolved sporadically across the Orchidaceae (Merckx, 2013).

Many of the orchid species studied thus far, including epiphytes and both photosynthetic and mycoheterotrophic terrestrial orchids, associate with a range of fungal species (e.g. Shefferson *et al*., 2005; Jacquemyn *et al*., 2010; De Long *et al*., 2012; Waud *et al*., 2017; Figure 4, see Table S1 for full list of studies and methodology). However, a number of orchid species have highly specialised

1   relationships, often using just one or few fungal species (e.g. Shefferson *et al*., 2005;

2   Otero *et al*., 2007; Bougoure *et al*., 2009; Swarts *et al*., 2010; Phillips *et al*., 2011a;

3   McCormick and Jacquemyn, 2014; Linde *et al*., 2014; Reiter *et al*., 2018b). Many of

4   the more extreme reported cases of specialisation are from the terrestrial orchids of

5   Australia. For example, based on our literature review of studies that have quantified

6   mycorrhizal specificity in detail via DNA sequencing of the ITS locus, terrestrial

7   Australian species associate with an average of 2.1 ± 0.3 (SE) (N = 10) fungal

8   Operational Taxonomic Units (OTUs) per orchids species, compared with an average

9   of 13.2 ± 2.7 (N = 32) for terrestrial European orchids (Figure 4; Table S1). These

10   findings highlight the potential for regional and/or taxonomic variation in the patterns

11   of specificity among orchid-mycorrhizal associations. However, at present a lack of

12   data of the relative effectiveness of the fungal associates detected in orchids makes it

13   challenging to draw generalisations about variation in mycorrhizal specialisation

14   among clades of orchid and geographic regions.

15

16   Despite epiphytes representing the majority of all orchid species, comparatively little

17   is known about their mycorrhizal ecology (Rasmussen *et al*., 2015). In fact, the

18   sporadic appearance and low abundance of pelotons in most adult epiphytic orchids

19   has led some researchers to question their importance for plant nutrition, at least for

20   adult plants (Hadley and Williamson, 1972; Lesica and Antibus, 1990; Bayman *et al*.,

21   2002). For example, in *Lepanthes rupestris* Bayman *et al*., (2002) found only a

22   solitary intact peloton in 300 root sections. Further, the *Rhizoctonia*- like fungi

23   isolated from the roots of *Lepanthes* were also frequently detected in the leaves of the

24   plant, raising the possibility that they may be non-functional endophytes (Bayman *et*

25   *al*., 1997). Nonetheless, while evidence for the importance of mycorrhiza in adults is

equivocal in epiphytic orchids, mycorrhizal fungi are present in protocorms (Zettler *et al*., 2011; Khamchatra *et al*., 2016; Izuddin *et al*., 2019) and fungi isolated from seedlings and/or adult plants can lead to increased germination and seedling growth *in vitro* compared with asymbiotic controls (Otero *et al*., 2005, 2007; Hoang *et al*., 2017*;* Meng *et al*., 2019). Furthermore, direct DNA sequencing of fungi from the roots of epiphytic orchids tends to recover a number of species of fungus from groups known to form mycorrhizal relationships with orchids more generally (e.g. Martos *et al*., 2012; Herrera *et al*., 2018; Rammitsu *et al*., 2019), hinting at their potential importance. Nonetheless, due to the lack of germination experiments testing for function (though see Meng *et al*., 2019), just how many species of fungal endophytes actually form mycorrhizal associations remains largely unknown in epiphytic orchids.

UNUSUAL ORCHID LIFE CYLE FEATURES AND CONSERVATION CONSIDERATIONS

Maintaining reproductive success - a key to effective conservation

Pollinator availability in specialised pollination systems

For the many orchids with specialised pollination, their reliance on just one or a few pollinator species for reproduction raises three key questions: (i) Does the geographic range of the pollinator(s) limit the distribution of the orchid? (ii) Does the abundance of the pollinator(s) limit available habitat for the orchid? (iii) What are the effects of anthropogenic habitat modification on specialised pollination systems? These questions apply equally when an orchid is reliant on one or few pollinator species

across its entire geographic range, and when despite geographic replacement of

pollinators there is high pollinator specificity for a given orchid population. In a study

investigating the potential for pollinator availability to limit the geographic range of

plants, Duffy and Johnson (2017) showed that the geographic range of the pollinator

was the best predictor of the environmental niche of the orchid for 11 out of 17

species of South African orchids with specialised pollination systems. At the scale of

suitable habitat patches within a species' geographic range, Phillips *et al*., (2014a)

found that rarity of *Drakaea* orchids is correlated with low occupancy of suitable

habitat patches by the respective pollinator species. These studies suggest that

pollinator availability may be a key process controlling the spatial distribution of

specialised orchid species. Further, Moeller *et al*., (2012) showed that this scenario is

also possible in plants with more generalist pollination systems. In this case, a

member of the Onagraceae had lower visitation by pollinating bees and greater pollen

limitation at its range margin (Moeller *et al*., 2012). As such, managing landscapes to

support populations of suitable pollinators could be critical for the persistence of

numerous species of rare orchids, irrespective of their pollination strategy.

In some orchid species, pollinator availability could also place a major constraint on

the sites suitable for conservation translocations. For example, of the 233 potentially

suitable sites surveyed for population establishment of the rare *Caladenia hastata*, the

pollinating thynnine wasp species was only detected at five sites (Reiter *et al*., 2017).

Remarkably, surveys to confirm that suitable pollinators are present prior to

conservation translocations are very rarely done (just 1 % of orchid conservation

translocations outside of Australia, Reiter *et al*., 2016). Furthermore, the failure to

consider pollinator availability is likely to be have been a key contributing factor to

1 the observation that half of the published conservation translocations that achieved

2 flowering, did not achieve any natural fruit set (Reiter *et al*., 2016).

3

4 *Declines of pollinator populations*

5

6 Pollinator declines are of growing global concern for flowering plants in general (e.g.

7 Potts *et al*., 2010; Sánchez-Bayo and Wyckhus, 2019), with subsequent reductions in

8 reproductive success and population size of non-orchid plant species already being

9 documented (Biesmeijer *et al*., 2006; Anderson *et al*., 2011). In many regions, the key

10 reason for the decline in the availability of pollinators has been the destruction of

11 habitat, and the fragmentation and degradation of the remaining habitat (Didham *et*

12 *al*., 1996). To date, within the orchids few studies have explicitly tested the effects of

13 habitat fragmentation on reproductive success and population persistence (but see

14 Pauw, 2007; Meekers and Honnay, 2011; Parra-Tabla *et al*., 2011; Phillips *et al*.,

15 2015b). However, the oil-producing members of the South African orchid genus

16 *Pterygodium* offer one of the best documented examples, to date. In this case, orchid

17 populations were rapidly lost from habitat fragments due to local extinction of the

18 pollinating bee, with the least clonal species most rapidly going extinct from small

19 habitat remnants (Pauw, 2007; Pauw and Hawkins, 2011).

20

21 The greater resilience of clonal *Pterygodium* species highlights the potential for

22 orchid communities to exhibit an extinction debt. This is the situation where, due to

23 clonality or individual longevity, extinction is yet to occur despite the population size

24 or habitat quality already falling below the threshold for persistence (Tilman *et al*.,

25 1994; Kuussaari *et al*., 2009). While the potential loss of pollinators from habitat

1   remnants is clearly an issue for any species that exhibit specialised pollination at the

2   population level, increased extinction risks could also apply to species with more

3   generalised pollination when total pollinator declines cause population growth rates to

4   fall below one. However, extinction debts in these more generalist cases may only

5   become evident over a longer time scale.

6

7   In addition to the pervasive threat of habitat destruction, there are many other factors

8   impacting pollinator availability, such as changes of habitat suitability through altered

9   management (Goulson *et al*., 2015), competition with invasive pollinators (Morales *et*

10   *al*., 2017; Vanbergen *et al*., 2018), shifts in phenology and local abundance through

11   climate change (Robbirt *et al*., 2014), the use of pesticides (Brittain *et al*., 2010), and

12   the spread of pathogens through human movement of pollinators (Graystock *et al*.,

13   2013). The best strategies for mitigating pollinator declines may vary between

14   regions, and depend on the surrounding land use. For example, in Europe sowing

15   wildflower strips has been shown to consistently increase the local abundance and

16   diversity of insect communities, although with a bias towards commonly occurring

17   insect species (Haaland *et al*., 2011; Johansen *et al*., 2019). Similarly,

18   supplementation of nest sites can increase bee pollinator populations in areas where

19   nesting sites are limited (Steffan-Dewenter and Schiele, 2008; Westerfelt *et al*., 2015;

20   Fortel *et al*., 2016). While it is presently unknown if such mitigation strategies also

21   aid rare pollinators or those with more complex life cycles (e.g. parasitoids), they

22   highlight the potential for manipulating habitat features to increase pollinator

23   availability.

24

1   For the large majority of orchids that are pollinated by insects, the targeted

2   management of orchid pollinators will often be hampered by the lack of biological

3   knowledge of the pollinator. However, for some nectar and pollen feeding pollinators,

4   information on diet is more readily attainable. In these cases, it may be possible to

5   manipulate the abundance of suitable forage plants to sustain populations of the

6   pollinators, thereby facilitating pollination of co-occurring orchids that are either

7   deceptive or provide a meagre reward (the 'magnet effect'; Laverty, 1992; Johnson *et*

8   *al*., 2003; Peter and Johnson, 2008; Menz *et al*., 2011). Indeed, the plant-pollinator

9   network approach (e.g. Pauw and Stanway, 2015; Phillips *et al*., 2020) could possibly

10   be extended to identify the range of co-occurring food plants that function to support a

11   population of the orchid pollinator. However, there is some experimental evidence

12   that abundant nectar producing plants can outcompete orchids for visitation by

13   pollinators (Lammi and Kuitunen, 1995), though these orchids still had higher than

14   average fruit set for a deceptive orchid (Lammi and Kuitenen, 1995; compare with

15   data reviewed in Tremblay *et al*., 2005).

16

17   *Pollination ecotypes and species management*

18

19   An interesting by-product of pollination strategies based on chemical attractants is

20   that orchid populations can exhibit similar colour and morphology while being

21   reproductively isolated by their chemical based attraction of different pollinator

22   species (Xu *et al*., 2011; Whitehead and Peakall, 2014). In sexually deceptive orchids

23   there are several instances of ecotypic variation in the semiochemicals involved in

24   pollinator attraction without obvious divergence in floral colour or morphology (e.g.

25   Bower, 2006; Breitkopf *et al*., 2013; Peakall and Whitehead, 2014; Menz *et al*., 2015;

1   Phillips *et al*., 2015a). Furthermore, cryptic floral ecotypes have even been found

2   within sexually deceptive species of varying distribution size and continuity of

3   populations (Menz *et al*., 2015; Phillips *et al*., 2015a), indicating that the presence of

4   ecotypes is not readily predictable.

5

6   It remains to be determined if species or floral ecotypes that are morphologically

7   cryptic are prevalent in chemical-based pollination systems outside of sexual

8   deception, although visually recognisable floral ecotypes are known in orchids using

9   other pollination strategies (e.g. references in Van der Niet *et al*., 2014). Should

10   ecotypes be present, such populations may need independent genetic management,

11   particularly if conducting hand pollinations to obtain seed for *ex situ* collections,

12   translocation or reintroduction, as hybrids may be of lower fitness (Phillips et al.,

13   2020). Further, different pollinators may have different habitat requirements, and the

14   use of ecotypes in conservation translocations would need to be tailored to reflect the

15   locally most effective pollinator species. In some cases ecotypes may prove worthy of

16   taxonomic recognition, and knowledge of the geographic range of ecotypes should be

17   incorporated into decisions for the protection of populations.

18

19   *Orchid pollination and the planting design for conservation translocations*

20

21   Larger translocated populations are more likely to be successful (Albrecht and

22   Maschinski, 2012; Godefroid *et al*., 2011; Silcock *et al*., 2019), in part because larger

23   populations are less susceptible to stochastic risks, Allee affects and inbreeding

24   depression (Allee *et al*., 1949; Lande, 1993; Armstrong and Seddon, 2008). In orchids

25   that secure pollination via rewards, reproductive success per plant is predicted to

1 increase with population size due to the increased ability of the population to attract

2 and sustain pollinators (Johnson *et al*., 2009; Meekers and Honnay, 2012). However,

3 in deceptive orchid species the reverse may be true. For example, in sexually

4 deceptive orchids the per plant rates of reproduction may actually decline at high

5 density or large population size, likely due to pollinators avoiding multiple visits to

6 deceptive flowers (Peakall and Beattie, 1996; Phillips *et al*., 2014a). A similar pattern

7 of higher fruit set in small patches has also been reported for food deceptive strategies

8 (Brundrett, 2019). Therefore, for such species, planting them in small sub-populations

9 or at low density may be the optimal design for conservation translocations. This

10 planting layout would also spread the risk of translocation failure between sites, and

11 create the potential for regular gene flow between populations and thus potentially

12 reduce the risks of inbreeding depression in small populations (Willi *et al*., 2006).

13 Indeed, this may be the first step towards replicating the natural meta-population

14 structure that appears to characterise many orchid species (Tremblay *et al*., 2006;

15 Winkler *et al*., 2009). While experimental tests of optimal population size are difficult

16 to achieve in animals and many plant groups (Armstrong and Seddon, 2008), orchids

17 are sufficiently fecund that even for many rare species an experimental approach is a

18 realistic possibility.

19

20 ***Pollinator limited fruit set, but high fecundity as an asset for conservation***

21

22 The typical high per capsule seed production in orchids means that pollinator limited

23 fruit set may not be a conservation issue if recruitment rates are sufficiently high to

24 maintain a stable population. However, if human intervention is required to keep

25 reproductive rates high enough to maintain populations (Phillips *et al*., 2015b), or if

seed needs to be collected for propagation or seed banks, then sampling of genotypes

from the population needs to be carefully considered. While a single fruit can provide

sufficient seed to propagate a large number of adult plants (Arditti and Ghani, 2000),

as already noted, the seeds may have been fathered by only one or few sires (Trapnell

and Hamrick, 2005; Kopf and Renner, 2008; Whitehead *et al*., 2015). As such, seed

needs to be collected from a variety of individuals, with a diversity of fathers, to avoid

any potential issues associated with low genetic diversity during later stages of the

conservation program. If hand pollinations are used to increase reproductive output,

pollen could be selected to maximise paternal diversity (e.g. pollen mixes from

multiple plants), or donors that may confer high fitness to the offspring. When

sourcing pollen from within populations, targeting plants beyond the distance over

which there is positive spatial genetic structure (usually less than ten metres for

orchids see Peakall and Beattie, 1996; Chung *et al*., 2004; Jacquemyn *et al*., 2007b;

though see Trapnell *et al.*, 2004) may lead to more fit seed.

Conservation consequences of dust-like seed and high dispersal

Genetic rescue as a viable management option for orchids

Genetic rescue - the artificial transfer of genes/individuals to counteract the negative

fitness effects of inbreeding depression - has been a successful management action for

rare species of plants and animals in both natural populations and those initiated

through conservation translocation (Frankham, 2015). However, when there is local

adaptation (Leimu and Fischer, 2008) and/or pronounced genetic differences among

populations, crosses between such populations could pose a risk of outbreeding

depression. Therefore, unless there is experimental data to the contrary, genetic rescue

is only advised in populations that are from broadly similar environments, have

experienced relatively recent gene flow (within last 500 years), do not have

chromosomal differences and are not autogamous (Frankham *et al*. 2011).

To our knowledge, very few published studies have considered the risks and benefits

of genetic rescue in orchids. One exception is a recent experimental study by Del

Vecchio *et al*., (2019) on *Himantoglossum adriaticum*. They showed that

experimental pollination of plants in small populations, using pollinia transferred

from large populations, lead to higher *in vitro* germination rates in the small

populations. This result indicates that genetic rescue may be effective in some of the

smaller populations of this species. Similar studies, that also track the fitness of

experimental crosses through to adulthood, would be of particular interest in groups

of orchids that are more likely to show outbreeding depression, such as predominantly

self-pollinating species.

We predict that genetic rescue will be a viable management option for many orchid

species given that low levels of genetic differentiation are the norm (Phillips *et al*.,

2012; see Figure 5 for an example where genetic rescue is likely to be beneficial).

However, as already noted above, some caution may be needed if chromosome

number variation exists. For example, recent estimates suggest that 12-16 % of plant

species exhibit cytotype variation (Soltis *et al*., 2007; Wood *et al*., 2009; Rice *et al*.,

2015), including several European and Australasian terrestrial orchids (Dawson *et al.*

2007; Trávníček *et al*., 2012; Pegoraro *et al*., 2016). Due to low seed production and

depressed offspring fitness in matings between cytotypes (Ramsey and Schemske,

1998), mixed cytotype populations can suffer reduced reproductive output that can

lead to the exclusion of one of the cytotypes (Levin, 1975; Fowler and Levin, 1984;

Husband, 2000). Similarly, crosses between plant ecotypes can lead to lower seed

viability or rates of protocorm formation (Jacquemyn *et al.,* 2018), as well as the

potential for maladaptation at later life history stages.

Given the uncertainties regarding outbreeding depression, when introducing

genotypes into an existing population, prior experiments should be undertaken to test

for any potentially adverse effects from the introduction of foreign genotypes (to at

least F2 generation, see Edmands, 2007). An exception to this would be when the

recipient population is already down to very low numbers, and the adverse effects of

inbreeding depression are already occurring or imminent. Alternatively, when

initiating new populations via conservation translocation, the process can be treated as

an experiment to test which genotypes are most fit in the recipient site, and if inter-

population crosses lead to increased fitness. This experimental approach is also more

likely to maximise evolutionary potential for anticipated future climate change.

Dependence on mycorrhiza for germination and protocorm growth

Use of effective mycorrhizal fungi for orchid conservation

Laboratory germination studies for both terrestrial and epiphytic orchids have

demonstrated that not all fungal species isolated from adult orchids are equally

effective at supporting germination (Otero *et al*., 2005; Bidartondo and Read, 2008;

De Long *et al*., 2013; Meng *et al.*, 2019; or even fungal individuals Huynh *et al*.,

1   2009), or have the same optimal conditions for germination in the laboratory (Reiter

2   *et al*., 2018b). Similarly, field studies have shown that the fungal species capable of

3   supporting germination are in some cases a subset of those that associate with the

4   adult (Bidartondo and Read, 2008; Jacquemyn *et al*., 2011), a different suite of fungal

5   species altogether (McCormick *et al*., 2004), or vary between habitats (Ruibal *et al*,

6   2017; Reiter *et al*., 2018b). As such, it follows that for those orchids that associate

7   with multiple fungal species, determining which fungi are most effective will be

8   critical to maximise the success of *ex situ* conservation, particularly when subsequent

9   relocation to the wild is planned.

10

11   Surprisingly, despite well-established techniques being available for the symbiotic

12   germination of terrestrial orchids for several decades (e.g. Clements and Ellyard,

13   1979; Clements *et al*., 1986), experiments to determine the most efficacious fungus

14   species appear to be rarely performed. Ideally, fungi would be isolated from wild

15   plants (preferably including protocorms or seedlings) and tested to determine which

16   fungal species support high germination rates or yield the most vigorous seedlings. To

17   account for genetic diversity of the symbiotic partner, multiple fungal individuals

18   should be maintained *ex situ* and used to generate plants for conservation actions.

19   Another interesting possibility that appears to have not yet been applied in orchid

20   conservation is to combine the use of relevant strains of endophytic bacteria with the

21   appropriate mycorrhizal fungi to promote germination and seedling growth. The

22   potential merit of this approach has been demonstrated in an experimental

23   germination study involving the terrestrial orchid genus *Pterostylis* (Wilkinson *et al*.,

24   1989), and more recently in a lithophytic species of *Dendrobium* (Wang *et al*., 2016).

25

1   *Not all microsites are equally suitable for germination*

2

3   In orchids, the suitability of sites for germination is likely to be determined by both

4   the spatial distribution of fungi, and the physiological requirements of both orchid and

5   fungus. In both terrestrial and epiphytic species orchid mycorrhizal fungi are

6   generally geographically widespread and often occur across a range of habitat types

7   (Otero *et al*., 2007; Davis *et al*., 2015; Phillips *et al*., 2016; Jacquemyn *et al*., 2017).

8   However, direct sequencing of fungi from the soil indicates that fungal distribution is

9   highly patchy within sites and is most often correlated with close proximity to orchids

10   and microsite scale environmental conditions (McCormick *et al*., 2009, 2018; Waud

11   *et al*., 2016; Rock-Blake *et al*., 2017). Furthermore, McCormick *et al*., (2012) showed

12   experimentally in some North American terrestrial orchids that increasing the

13   availability of rotting wood increased both fungal abundance and germination, but

14   that the effect differed depending on the stage of wood decomposition. In another

15   terrestrial orchid, *Isotria medeoloides*, higher abundance of fungi at the microsite

16   scale also appears to increase the probability that the plant emerges from dormancy

17   (Rock-Blake *et al*., 2017). Taken together, this evidence indicates that during orchid

18   translocation the choice of appropriate microsites could strongly increase the vigour

19   of adult plants. Therefore, managing sites to encourage growth of orchid mycorrhizal

20   fungi could lead to increases in orchid populations.

21

22   INNOVATIONS AND FUTURE DIRECTIONS

23

24   ***Improving survival in conservation translocations***

1   Given that conservation translocations are an increasingly common approach for the

2   preservation of endangered orchids, and that even in successful translocation projects

3   orchids still experience mortality in the year post planting (Reiter *et al*., 2016),

4   optimising the planting process could substantially increase the number of wild plants.

5   In general, there appear to have been few experimental tests of the role of size, age

6   and season of planting on the success of translocations of wild orchids (though see

7   Smith *et al*., 2009). However, in terrestrial orchids, the use of seed burial trials has

8   been a common strategy to investigate the suitability of particular microhabitats for

9   supporting germination (e.g. Rasumussen and Whigham, 1993; Batty *et al*., 2001;

10   Diez, 2007; Phillips *et al*., 2011). Unfortunately, germination does not guarantee

11   microsite suitability for the adult plant and, for many endangered orchids, insufficient

12   supplies of seed may severely limit the opportunity to use this approach. In Table 3,

13   we compare and contrast seed baiting with other approaches that have been employed

14   for investigating microsite suitability that may be less wasteful of seed. Some of these

15   alternatives, such as experimental plantings, also offer crucial insights into the

16   microsite ability to support both seedlings and adult plants. In addition, it would be of

17   interest to test if survival of plants is increased by deliberately inoculating the sites

18   with suitable OMF (e.g. Hollick *et al*., 2007), or if this is already achieved via the

19   introduction of symbiotically grown plants.

20

21   While most orchid conservation translocations have been attempted with terrestrial

22   species, a few have now been undertaken with epiphytes with some success, at least

23   as far as achieving flowering and fruiting (Izuddin *et al*., 2018). However, many of

24   these cases have been conservation translocations into heavily modified habitats

25   (Izuddin *et al*., 2018). Thus, more work remains to be done to optimise the process in

natural habitats, particularly with respect to optimising the placement of translocated

plants. For epiphytes in general, different strata within the trunk and canopy of the

tree represent different ecological niches to which different species are adapted

(Johansson, 1974; Gentry and Dodson, 1987). Similarly, while true host specificity is

rare among epiphytes (though see Tremblay *et al*., 1998), many epiphytic plants,

including some orchids, show a preference for particular host traits or surface

characteristics (Calloway *et al*., 2002; Crain, 2012; Gowland *et al*., 2013; Wagner *et al*., 2015; Timsina *et al*., 2016).

We predict that many translocated epiphytic orchids will exhibit variation in

recruitment and survival rates within the canopy, with the most favourable stratum

presumably matching the habitat of the adult plants. An experimental test of this

prediction was conducted by Kartzinal *et al*. (2013) for the orchid *Epidendrum*

firmum, where trials with seed packets demonstrated that germination was greatest

when the seed was in close proximity to adult plants. Germination was also found to

be primarily restricted to large native trees in microsites with high canopy cover.

Izzudin *et al.* (2019) also showed that in some epiphytic species germination was

associated with particular host tree traits, such as trunk diameter at breast height and

the presence of humus at the microsite. These findings highlight the necessity to

identify the microsite characteristics and canopy strata most favourable to germination

in order to optimise conservation outcomes in epiphytes. However, it would be of

interest to test if the presence of adult plants is a good indicator of germination

potential more generally, as the presence of adult plant plants could affect microsite

characteristics or be indicative of a shift in the epiphytic community since the

population was founded.

1

Combining experimental and demographic approaches to improve management

outcomes

4

5   Many threatening processes can impact multiple life history stages but predicting the

6   severity of impact across the different stages is a challenging task. Demographic

7   approaches can be powerful tools for determining which life history stages have the

8   greatest influence on population growth, and how they are affected by environmental

9   or ecological parameters (Schemske *et al*., 1994; Ehrlen *et al*., 2016). Not only do

10 these approaches provide the ability to test the effectiveness of different management

11 regimes, such studies can be used to test if a population is declining and so requires

12 additional conservation actions (Menges, 2008).

13

14 To maximise the benefits of demographic analysis, both experimental and control

15 treatments should be applied within the same study site. A good example of this

16 approach was the work of Sletvold *et al*., (2010), who investigated the effects of

17 different mowing regimes on the grassland orchid *Dactylorhiza lapponica* by

18 combining a long-term demographic study with treatments of different mowing

19 practices. While populations persisted in the absence of mowing due to the high rates

20 of adult survival, population sizes increased in the presence of traditional mowing.

21 Because many terrestrial orchids typically exhibit dormancy (Kery and Gregg, 2004;

22 Shefferson *et al*., 2005, 2011, 2018; Coates *et al*., 2006; Tremblay *et al*., 2009;

23 Hutchings, 2010), investigating the effects of different management regimes will

24 require the monitoring of populations over several years before experimental

1   treatments are applied, and then for several to many years thereafter to ensure that the

2   final census accurately reflects the number of individuals.

3

4   A major research gap is the need to identify the specific local or regional management

5   issues that adversely affect orchids. Among terrestrial orchids, some of the key local

6   factors that have been linked to population declines include a lack of grazing or

7   mowing of grasslands (e.g. Europe, Willems, 2001; Wotavová *et al*., 2004; Sletvold *et*

8   *al*., 2010), inappropriate fire regimes (e.g. Australia, Coates *et al*., 2006; Jasinge *et*

9   *al*., 2018; USA, Primack *et al*., 1994; Madagascar, Whitman *et al*., 2001), weed

10   invasion (e.g. Australia, Scade *et al*., 2006), herbivory (e.g. Australia, Faast *et al*.,

11   2009), trampling (e.g. Ballantyne and Pickering, 2011), and collection by humans

12   (Hinsely *et al*., 2018). Epiphytes are less well studied, but factors implicated in

13   population declines include herbivores (Winkler *et al*., 2005), a reduction in

14   precipitation (Zotz and Schmidt, 2006), and fire invasions from drier adjoining

15   habitats (Cribb *et al*., 2003). Unfortunately, many of the predicted drivers of orchid

16   population decline have not been fully investigated by combined experimental and

17   demographic approaches. However, such tests are urgently needed to confirm the

18   extent of the threat and how it is best mitigated.

19

20   ***Demographic data to inform assisted migration***

21

22   Assisted migration is the translocation of species beyond their natural range as a

23   conservation measure, and is often advocated as a potential strategy to help mitigate

24   the effects of climate change on threatened species (Thomas, 2011; see McLachlan *et*

25   *al*., 2007 and Ricciardi and Simberloff, 2009 for a debate over the merits of the

1   approach). For assisted migration to be effective, the introduced population must be

2   able to maintain positive growth rates in both current and future climatic conditions.

3   Therefore, wherever possible, long-term demographic and climatic data should be

4   used to predict suitable sites for orchid translocation (e.g. see Integrated Projection

5   Models, see Merow *et al*., 2014), as well as provide guidance on the optimal

6   conditions for the translocation itself. The widespread geographic ranges of orchid

7   mycorrhizal fungi (Jacquemyn *et al*., 2017), and the often wide range of fungal

8   species associated with orchids (e.g. Shefferson *et al*., 2005; Jacquemyn *et al*., 2010;

9   De Long *et al*., 2012; Waud *et al*., 2017), suggests that their availability should not

10   constrain the geographic region in which assisted migration could occur, assuming

11   that the symbiosis remains effective outside of the orchid's current geographic range.

12   On the other hand, for the many orchids with specialised pollination strategies the

13   geographic ranges of the pollinator, not the fungi (e.g. Phillips *et al*., 2014a, Davis *et*

14   *al*., 2015), may well constrain the geographic regions in which assisted migration will

15   be effective.

16

17   ***Integrating orchids into restoration programs***

18

19   The increasing number of large-scale restoration projects attempting to offset the

20   extensive worldwide habitat clearing of the past three centuries (e.g. Miller *et al*.,

21   2017), raises the question of whether these may offer opportunities to incorporate

22   orchids into restored landscapes? Such restoration projects can be grouped into three

23   broad categories that are likely to have different implications for orchids: (i) changing

24   management approaches to a particular vegetation community (e.g. re-instating

25   traditional grazing), (ii) attempting to recreate original habitat after vegetation

removal, but with the original abiotic soil properties largely intact, (iii) bringing

vegetation back to a cleared landscape with highly altered soil properties (e.g. a

tailings dump from mining). While there is some dispersal limitation if relying on

natural colonisation into restored sites (De Hert *et al*., 2013), in Europe there are

several cases of the successful restoration of orchid habitat following re-establishment

of mowing and/or grazing regimes (Willems, 2001; Wotavová *et al*., 2004; Sletvold *et

al*., 2010; Schrautzer *et al*., 2011; Gijbels *et al*., 2012). Further, some orchids are

known to colonise highly modified habitats (e.g. Shefferson *et al*., 2008). However,

evidence from Australia and Puerto Rico has found that some orchid species can

struggle to colonise habitats where restoration has been attempted (Grant and Koch,

2003; Bergman *et al*., 2006), highlighting the need to adapt restoration approaches to

local conditions and species.

Several key steps may be relevant for restoring orchids in highly modified landscapes

lacking natural vegetation (e.g. restoration types ii and iii above). Based on other

groups of soil fungi (e.g. Emam, 2016; Wubs *et al*., 2016), the use of topsoil could be

an effective source of inoculum of orchid mycorrhizal fungi. However, because

orchid seeds are short lived, with a relatively small (Whigham *et al*., 2006) or non-

existent soil seed bank (Batty *et al*., 2000), stored topsoil may be ineffective for

providing propagules. Germination rates of orchids are also typically very low, even

in the presence of suitable orchid mycorrhizal fungi (Hollick *et al*., 2007), meaning

that in most cases the introduction of symbiotically grown plants may be the most

effective method for establishing new populations. While the suitability of habitat for

germination and adult orchids can be manipulated at the micro-site scale (McCormick

et al., 2012), the dependency of orchids on other ecological partners (mycorrhizal,

pollinators, and phorophytes in epiphytes) means that many orchids may need to be introduced at later stages in the restoration process, once their ecological partners are already established. An interesting corollary of the observation that germination is highest near adult plants (e.g. Diez, 2007) is that during restoration it may be best to introduce additional orchids into microsites where establishment has already occurred.

The capacity for some tropical forests to rapidly regenerate suggests that at least partial restoration of epiphytic orchid communities will be possible. Furthermore, some epiphytic orchids regularly persist in remnant paddock trees (Koster *et al.*, 2009; Kartzinel *et al.*, 2013; Bohnert *et al.*, 2016), remnant edges, or secondary growth (Williams-Linera *et al.*, 1995; Hundera *et al.*, 2013). However, often the species of highest conservation concern will be those that occupy primary forest habitats (Hietz, 2005). Unfortunately, such species are likely to be susceptible to landscape modification, and their establishment in restored landscapes may prove to be particularly challenging. Reid *et al.*, (2016) demonstrated that in restored forest communities, epiphytic angiosperms reached their highest species richness when in close proximity to existing forest. Therefore, the retention of nearby intact forest may hold the key for achieving the rapid restoration of diverse orchid communities.

Conclusions – exceptional opportunities provided by orchids

Due to the unusual life cycle of orchids, their effective conservation can present unique challenges, but also novel opportunities. For example, while many orchid species exhibit highly specialised pollinator and fungal interactions, these relationships are often effective even at small population sizes. Therefore, given the

1   evidence in other organisms for persistence through surprisingly small genetic

2   bottlenecks, and the potential to use genetic rescue, we believe that conservation

3   biologists should not be deterred from working on orchid species with small

4   population sizes. Furthermore, orchids produce very large numbers of seed per

5   capsule and, with good horticultural practice, can provide the raw material for both

6   conservation programs and scientific experiments, even in particularly rare species

7   (e.g. Reiter *et al*., 2019).

8

9   The combination of long-term demographic data and experimental approaches to test

10 the effectiveness of alternative management strategies is potentially a powerful

11 approach for plant conservation (e.g. Sletvold *et al*., 2010). Among plants, the

12 collection of long-term demographic data should be particularly achievable for

13 orchids, as there is an exceptional capacity to use citizen scientists with a passion for

14 orchids to contribute to data collection (e.g. Reiter and Thomson, 2018). Given the

15 tremendous theoretical and practical inroads in our knowledge of orchid biology, the

16 challenge that remains for scientists and practitioners is how best to use this growing

17 knowledge to deliver large scale, effective orchid conservation programs.

18

6

7   SUPPLEMENTARY DATA

8

9   Supplementary data are available online at https://academic.oup.com/aob and consist

10   of the following. Table S1: A summary of studies that have quantified the diversity of

11   putative mycorrhizal associates in orchid species.

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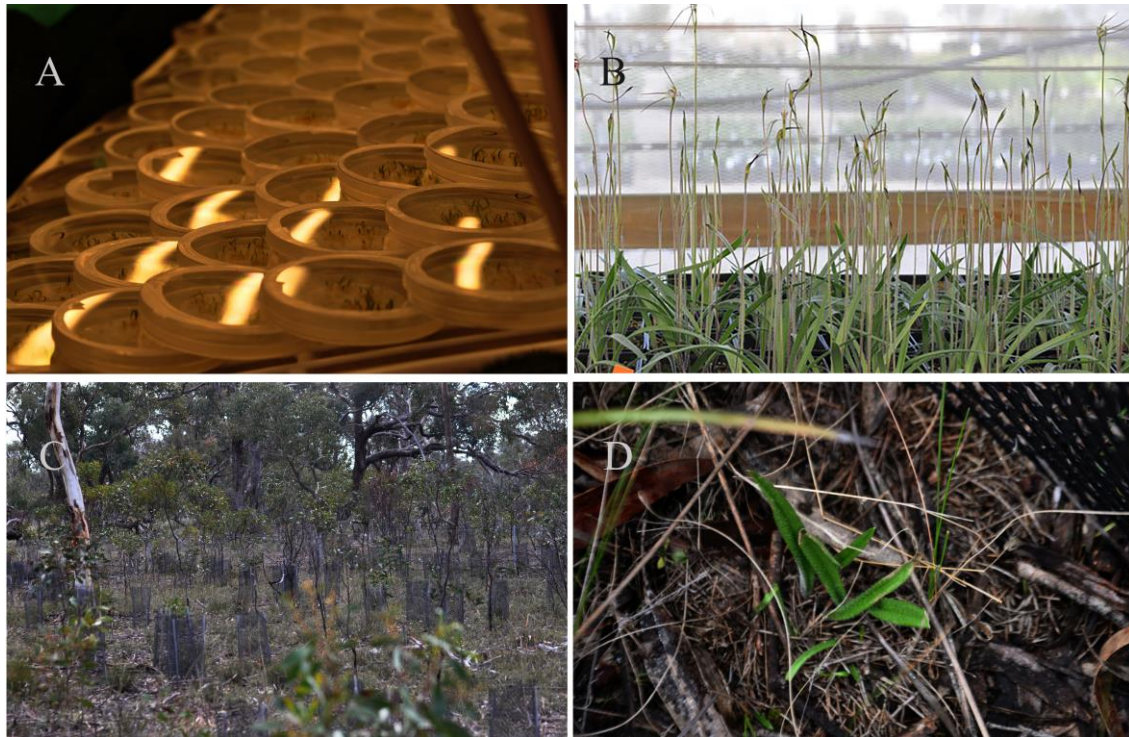
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3 **Figure 1:** Steps in the currently successful conservation translocation program of

4 *Caladenia colorata*, a threatened species from south-eastern Australia. (A) petri

5 dishes of seedlings germinated symbiotically using a specific species of *Serendipita*

6 mycorrhizal fungi (B) plants grown through to adulthood in glasshouse conditions (C)

7 translocation to wild sites that were selected based on a detailed assessment of the

8 vegetation community and confirmation of the presence of the primary pollinator

9 species (Reiter *et al*., 2018a) (D) wild recruits around the adult orchids that were

10 originally planted. A total of 883 plants were introduced between 2013 and 2017. As

11 of September 2018 there were 593 (67 %) of these plants surviving plus an additional

12 580 recruits, an increase of 65 % in the population beyond those initially planted and

13 97.8 % beyond those that survived translocation. Monitoring will now be conducted

14 to either confirm long-term viability of established populations, or alert managers to

15 life cycle stages that are limiting the maintenance of positive population growth.

16 Photos by Noushka Reiter.
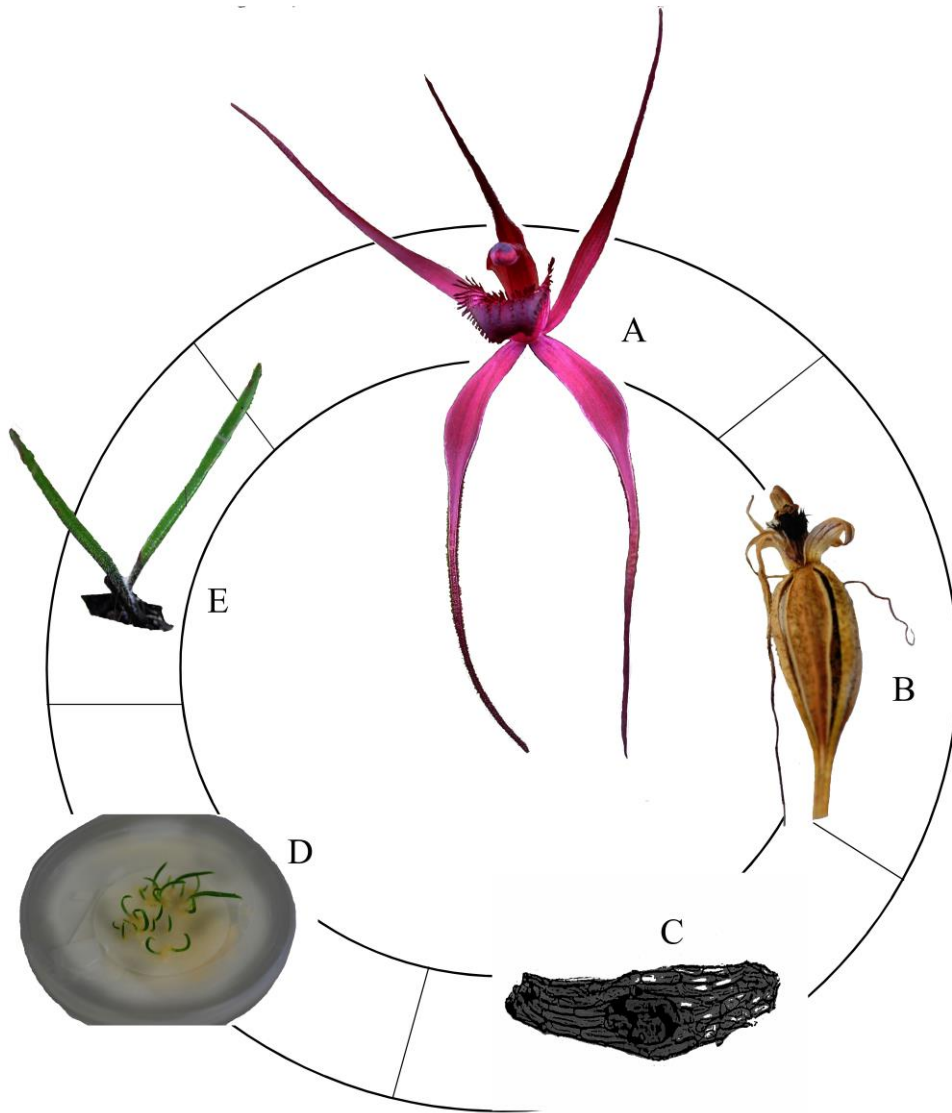
Figure 2: The life cycle of orchids. (A) flowering (B) fruit formation (C) seed

dispersal (D) germination through association with mycorrhizal fungi (E) recruitment

to adulthood. Features associated with these life cycle stages are elaborated upon in

Table 1.

Figure 3: Examples of pollination strategies in the orchids. (A) Pollination by fragrance collecting euglossine bees: A species of *Euglossa* bee pollinating a species of *Gongora*. The bees are attracted by fragrances that they collect and use in courtship bouquets (Ramirez *et al*., 2011). Photo: Santiago Ramirez. (B) Pollination by sexual deception: *Caladenia crebra* is pollinated by sexual deception of the thynnine wasp *Campylothynnus flavopictus* (Phillips *et al*., 2017). Long distance attraction is by mimicry of a blend of (methylthio)-phenol sex pheromones (Bohman *et al*., 2017). Photo: Rod Peakall (C) Pollination by oil collecting bees: *Corycium nigrescens* is pollinated by the melittid bee *Rediviva brunnea*, which collects oil to provision its brood. Photo: Michael Whitehead. (D) Pollination by sexual deception: *Drakaea glyptodon* is pollinated by sexual deception of the thynnine wasp *Zaspilothynnus trilobatus* (Peakall 1990). Long distance attraction is by mimicry of a blend of pyrazine sex pheromones (Bohman *et al*., 2014). Photo: Rod Peakall. (E) Pollination by nectar foraging hawkmoths: *Disa crassicornis* is pollinated by hawkmoths that feed on nectar produced at the end of the long nectar spur. Photo: Michael Whitehead.

1   (F) Pollination by brood site mimicry: *Gastrodia similis* mimics forest fruits using

2   chemical cues, which attract pollinating *Scaptodrosophila* flies searching for an

3   oviposition site (Martos *et al*., 2015). Photo: David Caron.

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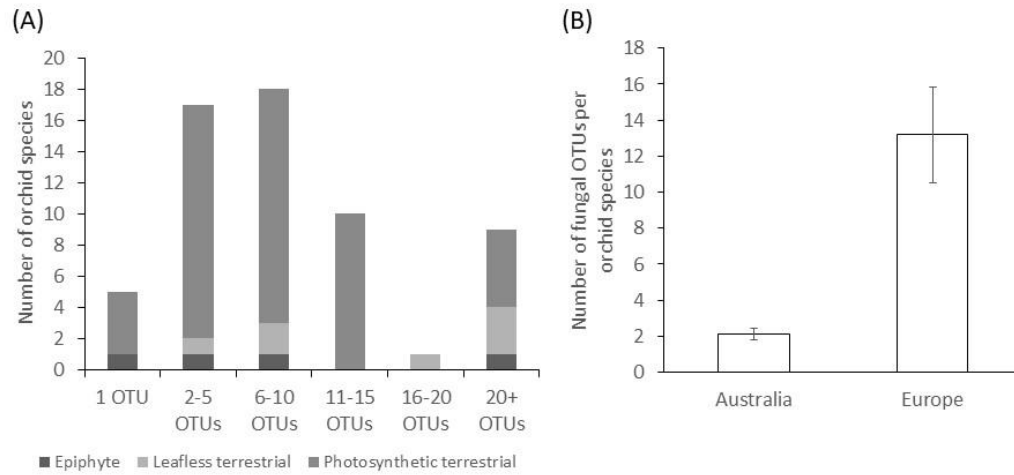
Figure 4: A summary of the number of fungal Operational Taxonomic Units (OTUs) that orchid species associate with based on the literature summarised in Supplement 1. Studies were included if they presented data on the ITS sequence locus, 15 or more orchid individuals were sampled, and orchids were sampled from two or more sites. For full methodology see Supplement 1. (A) The number of orchid species that exhibit varying levels of specialisation in mycorrhizal association, subdivided into species that are photosynthetic terrestrials, leafless terrestrials, and epiphytes. (B) The mean number (± SE) of fungal OTUs associated with photosynthetic terrestrial orchids in Europe compared with Australia.

Figure 5: An example of the potential benefits of genetic rescue in orchids. (A) The endangered *Thelymitra mackibbinii*, as of 2017 known from 40 wild plants across three populations (15, 22 and 1 plant per population) in Victoria, Australia. (B) *Thelymitra mackibbinii* plants grown from seed generated from the remaining wild plants via hand cross-pollination. Using the two largest remaining populations, plants on the left and right are from cross pollination within populations, while the plants in the centre exhibiting the most robust growth are from cross pollination between populations. All seedlings shown belong to the F1 generation. Photos and cultivation by Noushka Reiter.

1   Table 1: A summary of life cycle stages of orchids, their unusual features, and their

2   consequences for conservation. Life cycle stages are illustrated in Figure 2.

Life cycle stage	Features	Consequences
Pollination	Often exhibit a specialised strategy	Potentially limited by pollinator availability
	Often exhibit a deceptive strategy	Many species have low fruit set
	Chemicals can be crucial for pollinator attraction	Potential for cryptic taxa using different semiochemicals
Fruit formation	High seed output from a single capsule	High seed output for propagation and experiments; low genetic diversity of seed crop sourced from a single fruit
	Small number of seed parents per capsule	Low genetic diversity of seed crop sourced from a single fruit
Seed dispersal	Tiny, wind-dispersed seed	Capable of long-distance dispersal; low genetic differentiation among populations
Germination	Association with mycorrhizal fungi for germination	Not all fungi are equally effective; germination limited by fungal abundance and distribution; low per seed germination rates
	Tiny seed, usually lacking an endosperm	Low per seed germination and survival rates
Recruitment to adulthood	Terrestrial orchids require mycorrhizal association through to adulthood	Not all fungi are equally effective; persistence to adulthood limited by fungal abundance and distribution
	Some terrestrial orchids	Difficult to assess population numbers

	exhibit dormancy	and response to management without
		long term population monitoring

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1 Table 2: Estimates of the frequency of specialised pollination systems in orchid floras

2 at the global or continent scale based on pollinator records from published studies.

3 Mode and Mean/Median (specified in parentheses) refer to the number of pollinator

4 species per orchid species. *N* refers to the number of orchid species included in a

5 review. *summary statistics calculated in the present study using data from Gaskett

6 (2011). For the data from Gaskett (2011) we only included orchid species where

7 sexual deception was confirmed, and where pollinator(s) were identified below family

8 level. **only included studies where the pollination of more than one orchid species

9 had been presented.

Orchid flora	Mode	Mean/Median	*N*	Reference
Global	1	-	479	Tremblay (1992)
Global	1	3.97 ± 0.97 (mean ± SE)	424	Gravendeel *et al*., (2004); data from van der Cingel (2001)
Global**	-	2.30 ± 1.87 (mean ± SD)	186	Schiestl and Schlüter (2009)
Southern Africa	1	1 (median)	73	Johnson and Steiner (2003)
North America & Europe	3	5 (median)	41	Johnson and Steiner (2003)
Europe	1	7.44 (mean)	153	Joffard *et al*., (2019); data pre 2011 from Claessens & Kleyen (2011)
Global sexually deceptive*	1	1.29 ± 0.87 (mean ± SD)	288	Gaskett (2011)
Western Australian	1	1.05 (mean)	45	Phillips *et al*., (2017)

sexually deceptive

Caladenia

75

1 Table 3: Benefits and drawbacks of alternative methods for investigating microsite

2 suitability for the introduction of orchids. Note that the references were not

3 necessarily studies designed to identify introduction sites, but rather were chosen to

4 illustrate applicable methods.

Technique	Benefits	Drawbacks	References
Seed burial trials	Tests for germination in the wild	Uses large quantities of seed. May be capable of germinating but not surviving through to adulthood. Results affected by environmental conditions when experiment was done.	Batty *et al.*, (2001) Phillips *et al.*, (2011)
Use species that share mycorrhizal fungi as a guide	No wastage of seed	Need to confirm which species share fungi. Orchid species may show differences in habitat preferences.	
Introduce fungus with orchid	Fungus present at site. First generation of orchid contains effective fungus. No wastage of seed.	Site may not be suitable for fungus and/or plant in the long term.	Reiter *et al.*, (2016)
Microhabitat of adult plant	No wastage of seed.	Assumes knowledge of variables likely to be important for seed germination and adult growth	Menz (2013)
Experimental planting in microsites with different habitat	Experimental test	Assumes knowledge of variables likely to be important for seed	Reiter *et al.*, (2018b)

features		germination and adult growth. Spare seedlings needed for experimentation.	
DNA sequencing of soil	Can detect the presence of fungi in the absence of orchids.	Presence of fungus doesn't guarantee germination or survival to adulthood.	McCormick *et al*., (2019)

1