GLOBAL COLLABORATIVE RESEARCH ON THE POLLINATION BIOLOGY OF RARE AND THREATENED ORCHID SPECIES (ORCHIDACEAE)[1]

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ABSTRACT

Charles Darwin (1862, 1877) conducted field and lab research on orchid pollination mechanisms before any protocols or laws protected orchid species or individual populations. Information on the reproductive ecology of rare and threatened orchids remains intrinsic to their conservation as populations continue to diminish during the Anthropocene. We provide case studies of species representing five genera (*Calanthe* R. Br., *Corunastylis* Fitzg., *Cypripedium* L., *Spiranthes* Rich., and *Thelymitra* J. R. Forst. & G. Forst.) in three countries (the United States, Australia, and China). We compare differences in their pollination systems and seasonal variations in their fertility rates. Declining rates of fruit set and/or seed development are compared to breeding systems, pollinator life histories/foraging behaviors, and climate cycles. We conclude that, while some orchid species are pollinator limited, several interrelated factors influence rates of fecundity in terrestrial/lithophytic orchids.

Key words: Climate, conservation, fruit set, orchids, pollinator limited, pollinators.

We recognize Charles Darwin (1809–1882) as the first modern interpreter of how orchids are cross-pollinated. The first edition of his book (Darwin, 1862) was primarily a study of floral biomechanics and how these adaptations were derived by natural selection. The second edition (Darwin, 1877) contains far more information on the ecology of orchid pollination as related to reproductive success. For Darwin, reproductive success was a twofold process in which orchid flowers successfully dispersed whole pollinaria and then received whole or fragmented pollinia on their stigmas from members of the same species. By the second edition of his book, Darwin had examined the flowers of almost 110 orchid species, of which 28 were distributed throughout the British Isles. Reading Darwin's texts and letters, we note that his correspondents picked and dug specimens for him from British sites in an age without conservation laws or ethical restrictions. We are richer for it.

However, some modern orchid ecologists and conservationists pay little attention to reproductive effort in populations, although they may monitor plant numbers for years. These people seem to believe there is no need for continuing to record the data that so concerned Darwin. When examining populations of vulnerable, rare, or endangered species, some ecologists express interest only in population size, taking a census based on plant stages (sensu Gurevitch et al., 2006) and the longevity of individual genets. Without attention to interseasonal fluctuations in fertility, how can one expect to conserve an outbreeding population and increase both the numbers of recruits per generation and the levels of heterozygosity? For example, one of the most famous studies in recent years followed a population of *Ophrys sphegodes* Mill. in Britain for over three decades, but the author never found it relevant to record the annual number of flowers on inflorescences that had their pollinaria removed by insects and/or ovaries that matured into fruits (Hutchings, 2010).

This omission is important because the conversion rate of flowers into fruit in this species is reportedly less than 15% and its populations are declining in some regions in Britain (Jacquemyn & Hutchings, 1992). A similar census (1977–1985) of a restricted population of *Orchis militaris* L., another species in decline in Britain, showed much the same oversight in its failure to record the rates of pollinarium dispersal and capsule production (Hutchings et al., 1998). At least Darwin (1862) published a table on the natural rates of insect-mediated pollinarium removal in *Ophrys insectifera* L. (syn. *Ophrys muscifera* Huds.). In his second edition, Darwin (1877) released data on pollinarium removal rates in *Aceras anthropophorum* (L.) W. T. Aiton (= *Orchis anthropophora* Steud.); he also compared the low fruit set rates in *Ophrys* L. to those of five other species outside Britain (Bernhardt & Edens-Meier, 2014).

Although the Orchidaceae is regarded as one of the largest natural families of angiosperms (Dressler, 1993), its diversity has not protected thousands of species from poaching and environmental degradation (Endersby, 2016). Global decline in a number of orchid species had been well defined by institutions such as the International Union for Conservation of Nature (IUCN) long before the seminal publication of Koopowitz and Kaye (1983). The bilateral symmetry of orchid flowers limits the number of prospective pollinators in most species, especially those with pseudo-copulatory modes of presentation. Consequently, Dixon (2009) noted that the restoration of a species to its original distribution requires restoring the original components of its floral ecology, including its pollinators. Bernhardt and Edens-Meier (2010) concurred with Dixon but also pointed out that low rates of pollinator visitation were not the only reason for declining fruit set in a number of *Cypripedium* L. species. These authors argued for a more integrated approach to orchid conservation combining field and lab studies on pollination, breeding systems, and flower/fruit conversion ratios with genetics and floral demography. We now expand the original concerns of Bernhardt and Edens-Meier (2010) to incorporate recent observations on the reproductive ecology of threatened and endangered *Cypripedium* species and representatives of four additional genera based on recent research by the Bernhardt/Edens-Meier laboratory (Saint Louis University, St. Louis, Missouri, U.S.A.) and the Ren laboratory (Kunming Institute of Botany, Yunnan, China). Conservation efforts centering on the factors that influence orchid reproductive success should be a priority of all people interested in expanding orchid populations outside of greenhouses and the borders of botanical gardens.

REVIEW

CYPRIPEDIUM

This genus consists of 47 to 50 species confined to the Northern Hemisphere (Li et al., 2011), but its distribution remains highly disjunctive in both hemispheres. In Eurasia, most of its species are distributed from the Arctic Circle south through the Himalayas and west into northern Europe. In North America, species are found as far north as the Arctic Circle and south into tropical Mexico (Cribb, 1997). Not only are the species in this genus regarded as "poster children" for plant conservation, but they have also been interpreted as models of pollinator-limited systems. Indeed, a review of fruit set in this genus shows that populations of *Cypripedium acaule* Aiton, *C. calceolus* L., *C. fargesii* Franch., *C. japonicum* Thunb., *C. macranthos* Sw., *C. micranthum* Franch., and *C. reginae* Walter often experience seasons in which less than 5% of flowers are converted into capsules (Fig. 1; Edens-Meier et al., 2014b). Does a correlation exist between low fruit set and the identity and availability of primary pollinators in this genus? Edens-Meier et al. (2014b) noted that *C. acaule*, *C. macranthos*, and *C. japonicum* are pollinated almost exclusively by gynes of *Bombus* species. However, *Bombus* species are far too large to pollinate *C. reginae*, and these flowers appear to rely on medium-sized bees belonging to other genera in the families Apidae and Megachilidae (Edens-Meier et al., 2011). In contrast, drosophilid flies are the only known pollinators of *C. micranthum*. Due to the insufficient number of published field studies, it may be premature to blame low rates of fertility exclusively on overspecialized flowers dependent on a narrow range of pollinator species, insect castes, or genders.

Furthermore, the truism that flowers of *Cypripedium* species are rarely visited breaks down when we note the wide range of foragers entering the small, keeled labellum of *C. plectrochilum* Franch. (Li et al., 2008), the medium-sized labellum of *C. montanum* Douglas ex Lindl. (Bernhardt et al., 2014), and the large, rounded labellum of *C. reginae* (Edens-Meier et al., 2011). In some seasons, fruit set in *C. plectrochilum* is 45.9%, while two populations of *C. montanum* showed annual fruit set between 75% and 85%. In one season, fruit set jumped to 33% in *C. reginae*. Why do such discrepancies occur?

One partial explanation is based on differences in floral and insect morphometrics. While a broad

Figure 1.   *Cypripedium reginae* Walter from Lick Log Hollow in Shannon County, Missouri, showing scape blooming in May and the previous year's capsule. Photo by Retha Edens-Meier.

number of prospective foragers can enter the labella of most *Cypripedium* species, few can exit by one of the two rear exit holes (Darwin, 1877) and contact either of the dehiscent anthers during their escape. This contact is crucial to pollination in all genera of insect-pollinated species in the subfamily Cypripedioideae (Edens-Meier et al., 2014b). Some insects are too large to crawl under receptive stigmata and/or do not fit through the rear exit holes (Li et al., 2008; Edens-Meier et al., 2014b). Other insects are too small to contact receptive stigmata and/or dehiscent anthers as they escape (Li et al., 2008; Edens-Meier et al., 2011). In most acts of pollen dispersal in *Cypripedium* flowers, the critical dimensions are the insect's width at its widest point plus the depth of its hard and inflexible thorax versus the length and width of the orchid's escape holes (Li et al., 2006, 2008; Edens-Meier et al., 2011).

While the flower's comparatively larger dorsal entrance permits the broad entry of many anthophilous insects, those too large to exit via the rear openings must leave via the same dorsal entrance, chew escape holes in the labellum, or die of exposure during imprisonment (Bernhardt et al., 2014). All three scenarios fail to disperse pollen. Likewise,

frequent visitation by tiny bees, flies, and beetles to *Cypripedium* flowers with large, inflated labella only creates the illusion of cross-pollination. The capture and examination of these insects as they escape via the rear exits show an absence of orchid pollen on their bodies (Bernhardt et al., 2014; Edens-Meier et al., 2014b). These insects are not wide enough to contact dehiscent anthers and appear less likely to contact receptive stigmata on their next visits.

This situation suggests a second partial explanation for why pollinator visitation is so low in some *Cypripedium* species. Because all studied members of this genus fail to offer edible rewards, most *Cypripedium* species are best interpreted as generalist food mimics attracting polylectic and/or polyphagic foragers with supernormal stimuli incorporating color, scent, and labellum dimensions (see Edens-Meier et al., 2014b). Note that more than 75% of bees collected exiting the flowers of *C. montanum* also carried the pollen of one or more coblooming angiosperms at both sites in Oregon (GROWISER Reserve and the Deschutes National Forest Sisters Ranges District) (Bernhardt et al., 2014). When one *Cypripedium* species pollinated by small- or medium-sized bees representing several

Figure 2.   *Cypripedium margaritaceum* Franch. from Snow Mountain, Lijiang, Yunnan, China. This plant grew in a moist area. Photo by Zong-Xin Ren.

genera and families is in bloom, it is more likely to be pollinated when surrounded by many species of co-blooming wildflowers offering nectar and/or edible pollen. Conservation of *Cypripedium* species in their natural habitats must also involve conservation of the co-blooming pollen and nectar species preferred by the orchid's pollinators. Reproductive success is occasionally maintained when the *Cypripedium* species blooms in the presence of only a few nectar-/pollen-producing species with large populations (e.g., *C. plectrochilum* and *Fragaria* L. species; Li et al., 2008).

The annual climate and climate change must also be considered. Populations of *Cypripedium* species in the Himalayas distributed through parts of Yunnan, China, experienced 20 years of drought (1995–2015) and similar declines in spring snow melt. Our observations in 2015 showed that populations of some of these species were in retreat (Bernhardt, 2016a). The surviving plants did not bloom or showed arrested blooming with malformed flowers and the inability to inflate their labella (Figs. 2, 3; Bernhardt 2016b). Ren (in prep.) found that populations of *C. margaritaceum* Franch. and *C. lichiangense* S. C. Chen & P. J. Cribb on the Yulong Snow Mountain (Lijiang, Yunnan) failed to set any fruit from 2011 to 2015. A small population of *C. yunnanense* Franch., growing in a valley on a protected slope in Shangri-la (Yunnan), continued to flower and set fruit in 2015 (Bernhardt, 2016b).

CALANTHE YAOSHANENSIS Z. X. REN & H. WANG

With over 200 terrestrial and lithophytic species distributed through its warm temperate-tropical continent and islands, southeastern Asia remains the center of diversity for the genus *Calanthe* R. Br. The comparatively few species studied in this genus show a broad range of pollination systems, from mechanical self-pollination (autogamy) to pollination primarily by Lepidoptera, bees, or flies in the family Syrphidae. Speciation in this genus appears to be driven at least partially by generalist food mimesis, because the floral throat and/or hind spur lacks nectar. Those species pollinated by Lepidoptera deposit their pollinaria toward the bases of the proboscides of their vectors. In species pollinated by flies and bees, the pollinaria are usually deposited on the dorsum of the thorax (see review in Suetsugu et al., 2016), as occurs in most *Cypripedium* species (see above).

Calanthe yaoshanensis represents a comparatively recent discovery (Ren et al., 2011) and appears to be restricted to Yaoshan Mountain in Yunnan at elevations of 2850–2900 m. Because only 200 genets of this species have been counted so far (Ren et al., 2014), it must be regarded as rare and vulnerable. After three years of monitoring this population, Ren et al. (2014) found that natural rates of pollinarium removal differed significantly per annum but that the seasonal deposition of viable pollinia on receptive stigmas did not vary, remaining at less than 22%. The

Figure 3. *Cypripedium margaritaceum* Franch. from Snow Mountain, Lijiang, Yunnan, China, showing a malformed flower and undersized/undeveloped leaves in a drought-stricken area on the same mountain. Photo by Zong-Xin Ren.

flowers were self-compatible, but mechanical autogamy did not occur. The pollinia showed extended viability upon removal from the anther. Hand-manipulated cross-pollination of receptive stigmata with pollinia that were removed from anthers 24 hours prior produced as many fruits (100%) as did pollinations made with pollinia taken from anthers 30 minutes prior. Pollinia removed 48 hours prior to pollination continued to fertilize ovules at a reduced rate of 60%. When seeds produced by selfing and crossing were compared, however, the authors found that aborted seeds and undeveloped embryos were far higher in self-pollinated flowers.

As is common, fecundity in this species benefits from cross-pollination, but the cause of the low conversion rate of insect-pollinated flowers into fruit proved to be more complicated. Each plant usually produced only one inflorescence annually, each bearing one to 15 flowers. In 2009, all flowers on five inflorescences succumbed to a late freeze. In 2009 and 2010, herbivorous insects destroyed 15 inflorescences. In 2010, seven inflorescences were collected illegally for traditional medicine, belief in which remains strong in Yunnan (see below).

The most serious underexplored barrier to increased fertility in this orchid may be the limited efficiency, not frequency, of insects as pollinarium vectors. This species is pollinated by a large syrphid fly, *Eristalis tenax* L., and to a lesser extent by *E. cerealis* Fabricius and a species of *Criorhina* Meigen (all members of the Syrphidae). *Bombus patagiatus*

Nylander (Apidae) also visits the flowers but carries the pollinaria far less frequently than does *E. tenax*. Unfortunately, less than 45%–50% of all pollinaria transferred to insect bodies after anther contact were lost before the insects contacted the receptive stigma of a second genet. This loss occurred whether the prospective vector was a syrphid fly or a bumblebee. Most observations showed that visitations of appropriately sized insects to these flowers ended without the transfer of pollinaria to the insect's body or the deposition of pollinaria onto a receptive stigma. The authors interpreted this result as evidence of an evolutionary trade-off, a consequence of favoring male success in epidendroid orchids with hard, waxy pollinia and removable anther caps. Selection favors orchids that produce a pollinium that fathers all the seed in a second genet, which also requires a self-consistent modification of the column architecture (Ren et al., 2014). Pollinaria must compete for limited space on a receptive stigma, so deposition position on the pollinator must be precise. To father all the seeds in one ovary, the pollination mechanisms in *Calanthe yaoshanensis* may actually limit the efficiency of its own pollinators, causing insects to appear clumsy and inadequate when exiting these flowers.

SPIRANTHES SINENSIS (PERS.) AMES

This species is native to wet meadows, grassy slopes, and open woodlands from Siberia south

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Figure 4.   Traditional cabinet for preserved medicinal orchids preferred by the Han Chinese. Photo by Zong-Xin Ren.

through most of temperate China and up into the Himalayas from 100 to 3500 m (Chen et al., 1999). Earlier morphological treatments insisting that populations of pink-flowered plants were also distributed through the archipelagos of the Asian Pacific through Australasia are no longer in agreement with more recent molecular treatments (Tsukaya, 2005; Dueck et al., 2014). Future systematic studies will likely segregate insular populations of *Spiranthes sinensis* and either resurrect old names or redescribe them as new species.

It is unclear how many *Spiranthes* Rich. species occur in Yunnan, China, and this situation may confound conservation attempts. Both pink and white forms of *S. sinensis* s.l. are found above 3000 m, which may represent incipient speciation. The white form is most common in wet meadows and once formed populations in the hundreds, while the pink form is usually found in smaller and more discrete clumps on drier slopes above the perpetually wet peat. Nevertheless, the pink form may invade the edges of white populations, and pale pink forms (hybrids) have been recorded (Zhi-Bin Tao et al., in prep.).

Recent work by the Ren laboratory at the Kunming Institute of Botany, Chinese Academy of Sciences (Zhi-Bin Tao et al., in prep.) shows that the white form is pollinated primarily by large-bodied bees in the family Apidae, including *Apis cerana* Fabricius and various *Bombus* species. The molecule 2-phenyl-ethanol dominates the white form's scent but is also a common volatile found in a number of orchids and other unrelated angiosperms (Bernhardt et al., 2003;

Kaiser, 2010). In contrast, the pink form is pollinated by small- to medium-sized bees in the genus *Ceratina* Latreille (Apidae) and miner bees (Halictidae), and it is practically devoid of scent. As occurs in most outbreeding *Spiranthes* species studied to date, both forms are thought to secrete nectar. As in most nectar-producing orchids, the conversion rate of flowers into seed-filled capsules is higher (43.5%–68.5%, Ren, in prep.) than in species with mimetic flowers (Tremblay et al., 2005). However, ongoing analyses of seeds in the Ren laboratory have shown infertility rates of 16.2%–19.0% in the white form and 30.8% in the pink. Infertility is based on the number of empty seeds and/or abortive embryos in capsules, as in *Calanthe yaoshanensis* (Ren et al., 2011). This result suggests that, because bees visit more than one flower and linger on the same inflorescence, some self-pollination occurs via geitonogamy, leading to some inbreeding depression.

The declining status of both forms of *Spiranthes sinensis* s.l. in Lijiang (Yunnan) is a result of the traditional Chinese herbal industry. While these plants continue to reproduce sexually without human aid, they are harvested illegally and without scientific evidence as treatments for male impotence and diminished virility. Minority groups in Yunnan, including ethnic Tibetans and the Naxi people in Lijiang, do not use this plant. Instead, they are exploited for an illegal trade that supplies the Han majority throughout China. The plants are targeted as their scapes emerge in summer and flower buds become visible (Fig. 4); poachers remove the entire

Figure 5.   Two female poachers (pictured bag contains almost 100 plants of *Spiranthes sinensis* (Pers.) Ames) at 3300 m in Lijiang, Yunnan, China. Photo by Peter Bernhardt.

plant. In 2014, poachers targeted the pink form in wet meadows near the Lijiang field station at 3300 m. In July 2015, we failed to stop a team of three poachers (two women and one man; Fig. 5) from removing over 100 plants of the white form from a roadside wet meadow.

Corunastylis Fitzg. (syn. *Genoplesium* R. Br.) species

While the survival of many terrestrial species in temperate zones looks grim, the Australasian genus *Corunastylis* (approximately 45 species; see Jones, 2001) contains several taxa that have benefited from anthropogenic disturbance. Therefore, their success must be studied to see if it can be emulated or reproduced in other taxa. For example, some *Corunastylis* species are known to colonize trail paths throughout national parks in New South Wales (see <https://www.youtube.com/watch?v=ACG1iNk6k7M>). In January and February 2016, we counted and tagged 80 flowering stems of *C. ruppii* (R. S. Rogers) D. L. Jones & M. A. Clem. that were flowering along the Chase Track at Ku-ring-gai Chase National Park in New South Wales. In New South Wales and Victoria, other species are found in roadside verges

and under power lines where the vegetation is slashed but not frequently mown or sprayed with herbicides. Along one roadside in Kulnura, New South Wales, we found three species in bloom under the power lines, including *C. fimbriata* (R. Br.) D. L. Jones & M. A. Clem., *C. ruppii*, and *C. filiformis* (Fitzg.) D. L. Jones & M. A. Clem., of which we tagged 22 flowering stems. One site in Arcadia, New South Wales, was cleared of native vegetation for an orchard approximately two decades ago by scarifying the soil to at least 38 cm. The orchard was never planted, and some components of the surrounding native flora reinvaded the site. In 2016, from January through March, we counted over 200 flowering stems of *C. fimbriata* at this site.

All species in situ and under our observation from 2015 to 2016 continued to set fruit, and the conversion rate of flowers into fruit was very high for orchids, ranging from 50% to 80% (Bernhardt et al., in prep.). Why were pollination rates so high in these summer-flowering species? Bagged flowers did not set fruit in *Corunastylis filiformis*, *C. fimbriata*, and *C. ruppii*, suggesting that mechanical self-pollination (autogamy) did not occur in these species. Reviews by Bower (2001) and Bower et al. (2015), as

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Figure 6.   Unidentified female chloropid fly carrying the pollinarium of *Corunastylis ruppii* (R. S. Rogers) D. L. Jones & M. A. Clem. from the Chase Track at Ku-Ring-Gui Chase Track, New South Wales, Australia. Photo by John Martin.

well as photographic evidence provided by Kuiter (2016), showed that all species observed to date are pollinated almost exclusively by very small flies less than 2 mm in length. The majority of these insects belong to the Chloropidae (Fig. 6), with some pollinaria vectors in the Scatopsidae and Milichiidae. These insects are often mistaken for drosophilids but have different feeding preferences as winged adults. In Australia, milichiids feed on the wounds suffered by larger arthropods. Chloropids are also attracted to vertebrate wounds, consuming pus and blood; they also suck eye secretions and may be known as eye gnats (Borror et al., 2016). Because at least three *Corunastylis* species secrete nectar (Bower, 2001; Bernhardt, 2016c; Bernhardt, pers. obs.), we believe that their fluids may be unusually high in salts and amino acids. We observed hundreds of chloropids on these orchids, collected them with aspirators, and found that the flies carried pollinaria dorsally on the thorax, as cited previously (see Bower et al., 2015; Bernhardt, 2016c; Kuiter, 2016). The high numbers of these insects on these orchid flowers, combined with their ability to carry one or two pollinaria at the same time, explain the high conversion rate of flowers into fruit in the three *Corunastylis* species. While the fruit set of these species was far higher than that of all other species discussed in this paper except for *Spiranthes sinensis* (see above), the ratio of seeds with fully developed embryos to those with no embryos or aborted embryos (see above) in 2016 was less than 50% in *C. fimbriata* and *C. ruppii* but exceeded 70% in *C. filiformis* (Ren, in prep.). Only continued analyses will determine whether these initial data actually reflect inbreeding depression according to site and/or taxon.

THELYMITRA J. R. FORST. & G. FORST.

This primarily Australasian genus is represented by more than 100 species with breeding systems varying from obligately outcrossing to sub-cleistogamous (see review in Edens-Meier & Bernhardt, 2014). Most of these flowers open on warm-hot and sunny days, shutting by midafternoon, and are therefore known commonly as sun orchids. Unlike most orchids, the majority of *Thelymitra* species have radially symmetrical perianths because the labellum is the same shape and color as the lateral petals. Insects are attracted to the ornate column, where the two staminodes usually offer contrasting colors and highly ornamented lobes (Edens-Meier & Bernhardt, 2014). Over the past two centuries, children and naturalists have given species such descriptive common names as Queen of Sheba (*T. variegata* Lindl.), custard orchid (*T. villosa* Lindl.), blue lady (*T. crinita* Lindl.), rabbit ears (*T. antennifera* (Lindl.) Hook. f.), and shirt orchid (*T. campanulata* Lindl.).

As in most orchids, the flowers of previously studied *Thelymitra* species are self-compatible after

Figure 7. Inflorescence of *Thelymitra macrophylla* Lindl. (Mount Barker site, Western Australia). This species is a parent of the recurrent hybrid *T. antennifera* (Lindl.) Hook. f. × *T. macrophylla* Lindl. and shows how flowers bloom acropetally. Photo by Retha Edens-Meier.

hand pollination, including those taxa regarded as obligately outcrossing (Edens-Meier et al., 2013; see review in Edens-Meier & Bernhardt, 2014). Australia has declared a number of species as vulnerable or endangered on a state-by-state basis, and a recovery plan exists for five species found in Victoria and/or New South Wales. This plan includes a research program to understand mutualistic relationships between these terrestrial orchids, their fungi, and their pollinators (Coates et al., 2003–2007).

Although outcrossing species are food mimics (Edens-Meier & Bernhardt, 2014), some blue-purple flowers may also be regarded as Batesian or guild mimics of similarly colored, co-blooming angiosperms that offer pollen and/or nectar (Edens-Meier et al., 2013, 2014a). The pollination mechanism observed in the large-flowered, bee-pollinated species may be unique among the Orchidaceae. Instead of landing on the labellum and placing its head under the column, the prospective pollinator attempts to forage on the staminode lobes, which appear to mimic dehiscent anthers. With important exceptions, the majority of pollinaria vectors are female, polylectic bees in the

families Apidae, Colletidae, and Halictidae. Because these flowers are often buzz-pollination mimics, the flowers of one species may be pollinated by bees of vastly different sizes, in contrast to the size restrictions in *Cypripedium* (see above). We also found that the few bees we collected on *Thelymitra macrophylla* Lindl. (Fig. 7) and *T. crinita* differed vastly in size, although the floral dimensions were similar in both species (Edens-Meier et al., 2013, 2014a). Because bees of different sizes are poly-lectic, they may visit more than one *Thelymitra* species during the same foraging bout, thus producing a wide range of interspecific hybrids, often with columns unable to disperse pollinaria (Edens-Meier et al., 2013, 2014a; Edens-Meier & Bernhardt, 2014; Fig. 8). The annual rates of flowers pollinated were easy to determine in the large-flowered, insect-pollinated species because the pollinia were friable and whitish smears were easy to see on receptive stigmata. Furthermore, each bee- or hand-pollinated flower closed within less than 24 hours after pollination (Edens-Meier et al., 2013).

Unfortunately, despite the recovery plans for endangered/vulnerable species, there may be an
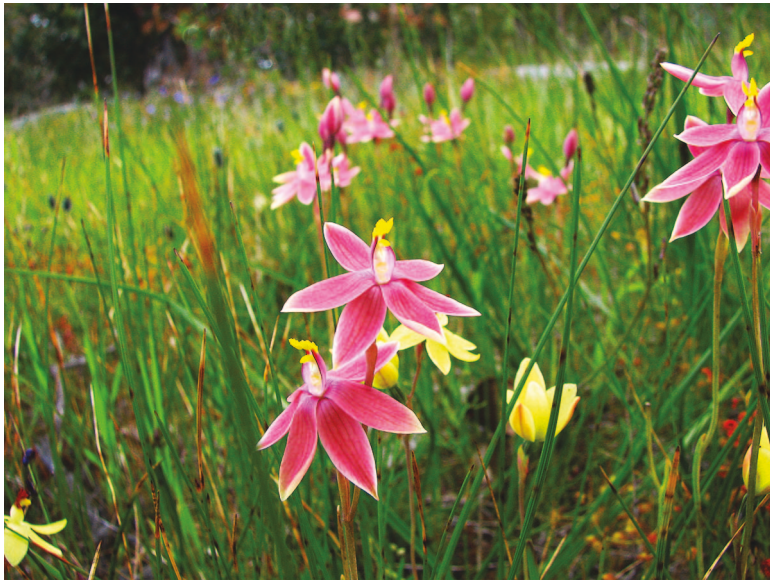
Figure 8. Pink- and apricot-colored first-generation hybrids of *Thelymitra antennifera* (Lindl.) Hook. f. × *T. macrophylla* Lindl. were numerous at the Mount Barker site, Western Australia, with the largest populations growing with the yellow-flowered parent species, *T. antennifera*. Photo by Retha Edens-Meier.

impending and unavoidable problem for large-flowered species that do not self-pollinate in the absence of insect pollinators. As occurs in many *Cypripedium* species (see above), the majority of flowers produced annually by obligately outcrossing species do not have their pollinaria removed, and even fewer flowers receive bee-deposited pollen on their stigmata. Over a single season, the ratio of pollinated stigmata (pollinia fragments on the stigma) in tagged inflorescences of *Thelymitra macrophylla* and *T. crinita* at our sites were 2% and 11%, respectively (Edens-Meier et al., 2013). Various authors have concluded that *Thelymitra* species with large, blue-purple flowers and staminode lobes terminating in trichome brushes are currently pollinator limited. Seed set remains low because pollinator visits between genets remain infrequent (Cropper & Calder, 1990; Sydes & Calder, 1993; see reviews in Edens-Meier & Bernhardt, 2014, and Edens-Meier et al., 2014b).

Several reasons for the decline in fruit set have been proposed. Bee populations may have decreased due to pesticide use and/or habitat fragmentation, and the non-systemic use of herbicides may have decreased pollen and nectar plants (Cropper & Calder, 1990; Fig. 9). Populations may also have been reduced by competition with the feral, non-native *Apis mellifera* L., and/or their eggs and larvae may have been consumed by introduced ant species (New, 2009). The problem may be even more specific

because these orchids are often identified as guild or Batesian mimics of co-blooming angiosperms, especially petaloid monocots in the Iridaceae and Asparagaceae with poricidal-porose anthers (Dafni & Bernhardt, 1989; Edens-Meier & Bernhardt, 2014). Similarities in pigmentation patterns and scent molecules between these orchids and their prospective mimics show convergent evolution (Edens-Meier et al., 2013). If the model species (e.g., blue species of *Orthrosanthus* Sweet; Iridaceae) are absent or regionally extinct where blue *Thelymitra* species continue to bloom, bees may avoid the mimics altogether (Edwards et al., 2012). Because at least 50% of all *Thelymitra* species now show some mode of mechanical self-pollination (Edens-Meier & Bernhardt, 2014), we ask whether it is selectively advantageous for bees to evolve discretionary foraging or whether the distributions and flowering seasons of food-offering models and food-mimicking orchids show less overlap in time and space due to a combination of natural selection and global warming.

DISCUSSION AND CONCLUSIONS

Bernhardt and Edens-Meier (2010) warned of the gap between positive efforts to protect orchid species in their natural habitats and the lack of information on their reproductive ecology and breeding systems. Our review of the taxa above shows that, while some species are pollinator limited (e.g., *Cypripedium* and *Thelymitra* species), this situation is a direct

Figure 9. *Thelymitra variegata* Lindl. (Queen of Sheba) was once common around Perth and Albany, Western Australia, but is now endangered due to sand mining and suburban expansion. Photo by Retha Edens-Meier.

consequence of the explosive speciation of the family (Tremblay et al., 2005). Our brief review suggests that we must seek species for which pollinators are plentiful and fruit set ratios remain high (e.g., *Cypripedium montanum*, *Spiranthes sinensis*, and some *Corunastylis* species) for comparison with species in which vector-mediated pollination seems to be in decline (most *Thelymitra* species). This search must become part of a much wider general effort in plant conservation to determine why some populations show high fecundity with observable recruitment and others do not. As may be expected, our review of reproductive ecology in a fraction of orchid taxa also indicates that climate fluctuations, herbivory, and poaching all lower reproductive success by lowering pollination rates.

Two additional factors reflecting the unique evolution of orchid life histories must be considered. First, increased fruit set does not guarantee a healthy or viable generation. Expanding ovary walls may be only an illusion of fertility and fitness. In the absence of a self-incompatibility mechanism (e.g., *Spiranthes sinensis* and *Calanthe yaoshanensis*), barriers to vector-mediated autogamy and geitonogamy may be weak. The plants may also be unable to recognize and reject pollinia transferred by legitimate pollinators among various siblings and parents. This situation could result in increased ratios of empty seeds and undeveloped embryos, increasing inbreeding depression within reduced populations. This issue may be more problematic in orchid species offering edible rewards (*S. sinensis*, some *Corunastylis* species) because pollinators may repeatedly return to the same flower or inflorescence or forage between closely related co-blooming genets growing in situ. Inbreeding depression may be one reason why floral mimesis is so common in orchids. Because pollinators receive no rewards from food mimics, these insects should leave the individual plant and/or immediate site and not return to the same plant or ramet (*Cypripedium montanum*; Bernhardt et al., 2014). By the time the pollinator is willing to forage on a second genet at another site, the potential for vector-mediated self-pollination or interspecific crosses is much lower, especially if pollen viability is long (Ren et al., 2014). Future comparisons of seed ratios in rewarding and non-rewarding orchid species will clarify this hypothesis. Meanwhile, we suggest that reproductive ecologists and conservationists work more closely with population biologists who have access to genetic markers.

Second, although orchid seeds lack endosperm, most orchids produce hundreds or thousands of seeds after successful pollination. This production must consume large amounts of water, hydrocarbons, and amino acids, and parents must support fruit that often takes months to mature and dehisce, especially in tropical epiphytes. Green fruits are targets of several herbivores (Edens-Meier et al., 2011). Therefore, while most orchids produce perfect (bisexual) flowers, they may be skewed toward fathering offspring instead of supporting the development of many

embryos, as in the case of *Calanthe yaoshanensis*. If all the grains in a pollinium are deposited on only one stigma as a compact and often solitary mass, there will be competition for limited receptive sites, which must influence column evolution and the precise attachment of the whole pollinarium for delivery to a receptive stigma (Ren et al., 2014).

All global conservation methods to conserve orchid species have merit, but mapping populations, DNA barcoding of remaining genets, and mass propagation in nurseries (to be followed by transplantation and reintroduction) are limited, even when used in concert. Conserving orchid species requires conserving a life history dependent on symbiotic fungi, sympatric flora, and pollinator guilds. An orchid species likely cannot be successfully restored in situ if its pollinator has also suffered regional extinction (Dixon, 2009). In the long term, protected or restored populations must produce recruits; otherwise, conservation techniques are little more than landscape gardening in disguise. Training and employing volunteers to hand pollinate flowers within a botanical garden, or even a small reserve, is worthwhile in the short term, but expecting people to cross-pollinate thousands of species around the world, in thousands of fragmented sites, is not feasible. Conservation education should emphasize both the recognition of vulnerable or rare orchids and the interrelationships between these orchids and the other species that share their habitats. This approach is not possible unless conservation-based institutions devote more time and funding to field and lab research on orchid life cycles and their regional ecology. Reproductive ecologists analyzing pollination and breeding systems should be present on every plant conservation team.

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