

## Orchid Sexual Deceit Provokes Ejaculation

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**ABSTRACT:** Sexually deceptive orchids lure pollinators by mimicking female insects. Male insects fooled into gripping or copulating with orchids unwittingly transfer the pollinia. The effect of deception on pollinators has been considered negligible, but we show that pollinators may suffer considerable costs. Insects pollinating Australian tongue orchids (*Cryptostylis* species) frequently ejaculate and waste copious sperm. The costs of sperm wastage could select for pollinator avoidance of orchids, thereby driving and maintaining sexual deception via antagonistic coevolution or an arms race between pollinator learning and escalating orchid mimicry. However, we also show that orchid species provoking such extreme pollinator behavior have the highest pollination success. How can deception persist, given the costs to pollinators? Sexually-deceptive-orchid pollinators are almost exclusively solitary and haplodiploid species. Therefore, female insects deprived of matings by orchid deception could still produce male offspring, which may even enhance orchid pollination.

**Keywords:** orchid pollination, deception, ejaculation, arms race, haplodiploidy.

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Most flowers attract and reward their pollinators with nectar, but sexually deceptive orchids lure their pollinators with counterfeit signals that mimic those produced by female insects (Schiestl et al. 1999; Schiestl 2005; Jersáková et al. 2006). When a male insect is fooled into gripping or copulating with an orchid flower, the pollinia adhere to his body, and he delivers them to other orchid flowers during subsequent deceptive liaisons. The signals that coerce male insects into visiting orchids are becoming well

studied. Several recent major discoveries indicate that the most important attractants are floral scents that mimic species-specific female-insect sex pheromones (Schiestl et al. 2000, 2003; Ayasse et al. 2003; Mant et al. 2005a; Schiestl and Peakall 2005). However, the factors driving the evolution and maintenance of sexually deceptive pollination signals and systems remain somewhat enigmatic.

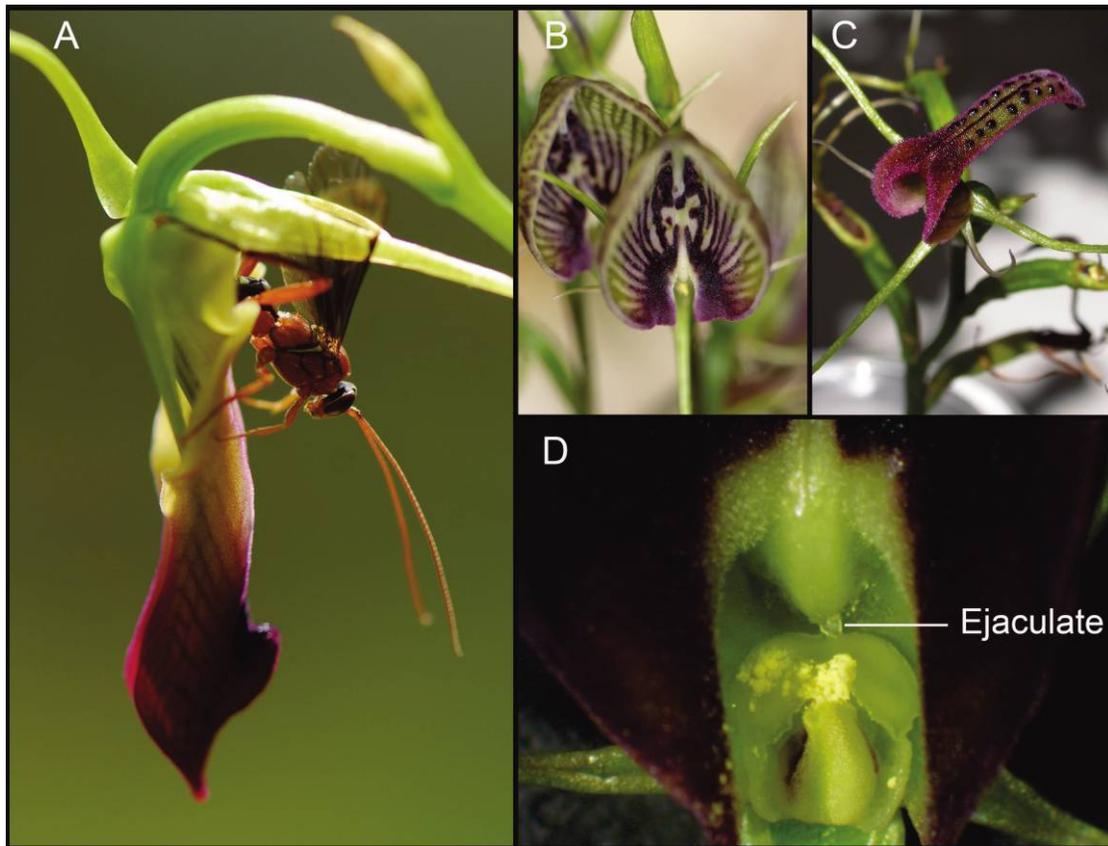
The evolution of deceptive pollination is generally considered unilateral; that is, although pollinators influence and select for orchid characters, orchids are not thought to influence the evolution of their pollinators (Schiestl 2005; Jersáková et al. 2006). Despite their exploitation of insect mating systems, sexually deceptive orchids are not thought to impose any significant costs on pollinators (Schiestl 2005). However, both male and female insects of pollinator species could suffer considerable reproductive fitness costs if orchids inhibit mating opportunities. Male pollinators can prefer orchids to real females (Schiestl 2004), prematurely end a copulation with a real female to visit an orchid (Coate 1965), or be unable to find real female mates among false orchid signals (Wong and Schiestl 2002). Crucially, pollinators would suffer significant costs if orchid deception causes pollinators to ejaculate and waste their sperm.

While some sexually deceptive orchid species require only pollinator gripping or brief entrapment for effective pollination (Peakall 1990; Alcock 2000; Lehnebach et al. 2005), other orchid species coerce their pollinators into energetic copulation (Peakall 1989; Singer et al. 2004; Blanco and Barboza 2005; Ciotek et al. 2006). Although these copulations are often described as “pseudocopulations” (Schiestl 2005; Jersáková et al. 2006), the vigorous response of pollinators suggests that true matings with ejaculation and costly sperm wastage may indeed occur (Coleman 1928; Blanco and Barboza 2005). Here we test whether two Australian sexually deceptive tongue orchids, *Cryptostylis erecta* and *Cryptostylis leptochila*, provoke ejaculation by their shared pollinator, male orchid dupe wasps (*Lissopimpla excelsa*, Ichneumonidae; fig. 1). We also test whether these wasps learn to avoid copulating with orchids after experience and present the results of meta-analyses for all sexually deceptive orchids, analyzing pollinator behavior, pollination success, and the incidence of pollinator

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**Figure 1:** Australian sexually deceptive tongue orchids (*Cryptostylis*) fool male orchid dupe wasps (*Lissopimpla excelsa*, Ichneumonidae) into copulation and inadvertent pollination. A, *Cryptostylis subulata* with pollinator in copula. B, C, Copulations with *Cryptostylis erecta* (B) and *Cryptostylis leptochila* (C) were used in this study. D, *Cryptostylis erecta* flower after pollinator visit. Note blob of pollinator ejaculate, plus removal of pollinia and deposition of pollen on stigma. Reproduced by permission, copyright Esther Beaton.

haplodiploidy, which could be crucial for pollinator resilience to deception.

## Methods

### *Identifying Pollinator Sperm from Testes*

Pollinators were caught with hand nets in the field near Sydney, Australia; *Cryptostylis erecta* and *Cryptostylis leptochila* orchids collected from nearby natural populations were used as bait. All pollinators were identified as male *Lissopimpla excelsa* (Ichneumonidae, Hymenoptera). Two pollinators were killed by freezing and dissected. The testes and seminal vesicles were removed, mounted on slides with 10  $\mu$ L of 0.1% DAPI (4'-6-diamidino-2-phenylindole) in 1  $\times$  phosphate buffer solution (pH 7.2), and viewed under an Olympus BH2 epifluorescence microscope at 40 $\times$  magnification. Photographs were taken with a high-resolution Nikon DXM1200F digital camera and imaging

facility. Scale bars were added with ImageJ software (Rasband 1997–2006).

### *Finding Ejaculate on Orchids*

Fresh *Cryptostylis* orchid flowers were exposed in the field (*C. erecta*,  $n = 20$ ; *C. leptochila*,  $n = 6$ ). After wild insects copulated with the flowers, the flowers were quickly taken to the laboratory. On some flowers, blobs of potential ejaculate were easily viewed without microscopy (fig. 1D). These blobs were removed with forceps, placed on a slide, and broken apart with entomological pins. Each blob was treated with DAPI and examined for fluorescent sperm as described above for sperm dissected directly from pollinator testes. When ejaculate was not visible, the whole flower was smeared across a slide. The smear was also treated with DAPI and examined for sperm.

*Pollinator Behavior after Experience*

We recorded how many pollinators approached, landed on, or copulated with *Cryptostylis* orchid flowers exposed in the field ( $n = 104$ ). Thirty of these pollinators were captured and housed separately in mesh cages (20 cm  $\times$  20 cm  $\times$  10 cm) in the laboratory. All were identified as male *L. excelsa* (Ichneumonidae, Hymenoptera). After at least 2 h, an orchid flower was placed into each cage for 10 min, and pollinator behavior was observed. After another interval, 14 pollinators received a fresh flower for another 10-min trial. A  $\chi^2$  analysis was used to compare the number of pollinators that approached, landed on, or copulated with an orchid in their first, second, and third trials.

*Data Collection for Pollination Success Analyses*

We monitored natural populations of *Cryptostylis subulata* ( $n = 28$  flowering plants; fig. 1A), *C. erecta* ( $n = 270$ ; fig. 1B), and *C. leptochila* ( $n = 43$ ; fig. 1C) near Sydney and Melbourne, Australia, and recorded the percentage of flowering plants in which both pollen was removed and fruit capsules were formed. We also surveyed the literature for data on pollination rates, pollinator behavior, and pollinator identity for *Cryptostylis* and other sexually deceptive orchids worldwide (Wolff 1950; Bino et al. 1982; Stoutamire 1983; Peakall 1989, 1990; Paulus and Gack 1990; Peakall and Handel 1993; Steiner et al. 1994; Bernhardt 1995; Peakall and Beattie 1996; Ayasse et al. 1997; Neiland and Wilcock 1998; Taylor 1999; Alcock 2000; Singer 2002; Wong and Schiestl 2002; Hopper and Brown 2004; Schiestl 2004; Schiestl et al. 2004; Singer et al. 2004; Blanco and Barboza 2005; Delforge 2005; Lehnebach et al. 2005; Mant et al. 2005b; Vereecken and Patiny 2005; Bower 2006; Ciotek et al. 2006; Dickson and Petit 2006; Gaskett and Herberstein 2006; Tyteca et al. 2006).

*Meta-Analysis 1: Pollination Success and Pollinator Sexual Behavior*

We combined our field data about *Cryptostylis* species with data for other sexually deceptive orchid species from the literature survey to consider how orchid pollination success interacts with the sexual behavior induced from the pollinator. Pollination success was defined as the percentage of flowering plants in which pollinia were both received and donated or in which there was fruit set. We used the percentage of flowering plants pollinated instead of the percentage of individual flowers pollinated to control for floral display size to avoid artificially overestimating pollination success in species with only one flower per plant, for example, *Chiloglottis*. We used pollination

rates per season because data on lifetime reproductive success of sexually deceptive orchid species were not available. When more than one study was available for a species, the pollination rates were averaged; for example, for *C. subulata*, our data were combined with those from Schiestl et al. (2004) and Gaskett and Herberstein (2006). Data were included only from orchid species in which the pollinator and its behavior were conclusively identified and natural pollination rates with sample sizes were provided. The 11 orchid species that fit these criteria were allocated to one of four hierarchical categories according to the intensity of pollinator sexual behavior they induced: (1) full copulation, often with ejaculation: *Cryptostylis* spp. (Schiestl et al. 2004; Gaskett and Herberstein 2006); (2) copulation only: European *Ophrys* (Ayasse et al. 1997; Neiland and Wilcock 1998) and South American *Geoblasta pennicillata* (Ciotek et al. 2006); (3) gripping of flower parts without necessarily involving copulation: Australian *Caladenia behrii* (Dickson and Petit 2006), *Chiloglottis trapeziformis* (Peakall and Handel 1993; Schiestl 2004), *Chiloglottis trilabra* (Peakall and Handel 1993), and *Drakaea glyptodon* (Peakall 1990); and (4) brief entrapment of male insects that collect pollinia on their escape: New Zealand and Australian *Pterostylis* species (Taylor 1999; Lehnebach et al. 2005). A nonparametric Spearman rank-order correlation (SPSS, ver. 15.0) was used to compare pollination rates across the four deception categories.

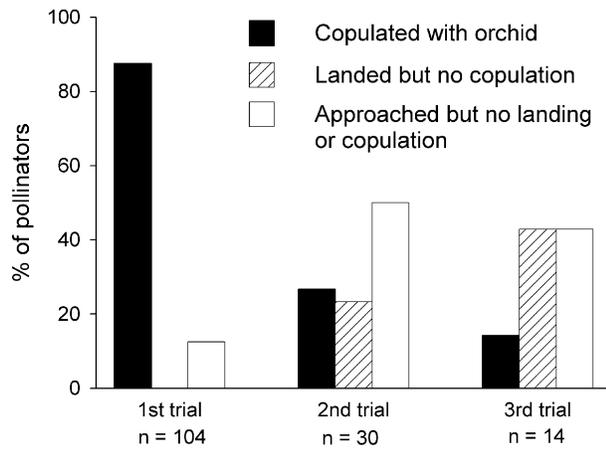
*Meta-Analysis 2: Pollinator Mating Systems*

For sexually deceptive orchid species with conclusively identified pollinators ( $n = 222$ ), the literature survey revealed only three pollinator mating systems: solitary and haplodiploid, social and haplodiploid, and diploid. We calculated the percentage of orchid species relying on each type of pollinator mating system, regardless of whether pollination rates were provided.

**Results**

During orchid pollination visits, male *Lissopimpla excelsa* copulate energetically with *Cryptostylis erecta* and *Cryptostylis leptochila* orchids (fig. 1A) and ejaculate large amounts of sperm (73%,  $n = 26$ ). The blob of ejaculate is often visible to the naked eye and consists of thousands of sperm identical to the sperm dissected from the wasp testes (fig. 1D). Sperm was also found in smears taken from some flowers without visible blobs of ejaculate. Pollinators copulated readily with the first *Cryptostylis* orchid they were offered (87.5%,  $n = 104$ ) but were significantly less likely to copulate with second (26.7%) and third orchids (14%;  $\chi^2 = 60.53$ ,  $df = 2$ ,  $P < .001$ ; fig. 2).

The first meta-analysis on pollination rates and polli-



**Figure 2:** With experience, male *Lissopimpla excelsa* wasps become less likely to copulate with and pollinate sexually deceptive *Cryptostylis* orchids.

nator behavior revealed that species with more extreme sexual deception have increasingly successful pollination (Spearman rank-order correlation:  $n = 11$ ,  $r = -0.71$ ,  $P = .014$ ; fig. 3). *Cryptostylis* species, which stimulate pollinator copulation and ejaculation, have the highest percentage of pollinated plants:  $70.35\% \pm 11.8\%$  for *C. erecta* ( $n = 402$ ),  $41.95\% \pm 1.03\%$  for *C. leptochila* ( $n = 121$ ), and  $66.6\% \pm 16\%$  for *Cryptostylis subulata* ( $n = 154$ ; data from our field survey, Schiestl et al. 2004, and Gaskett and Herberstein 2006; values are mean  $\pm$  SD). Pollination is intermediate for orchids that stimulate less intense pollinator sexual behavior, such as copulation without ejaculation or gripping the flower, and lowest for orchids that merely trap male insects briefly (fig. 3). The second meta-analysis on pollinator mating systems reveals that sexually deceptive orchids are almost exclusively pollinated by male haplodiploid insects with a solitary social system where males must patrol for mates (94.1%,  $n = 222$  orchid species; fig. 4).

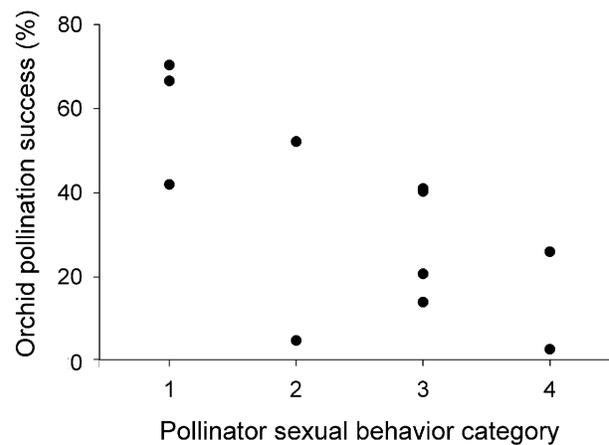
## Discussion

Our study provides the first conclusive evidence of insect ejaculation in response to floral stimuli. The high frequency of ejaculation is extraordinary and may even be an underestimate because the male wasps tested here that did not ejaculate (27%) could have copulated with orchids before, although no wasps carried orchid pollinia. Naive male insects may copulate and ejaculate even more readily than reported here.

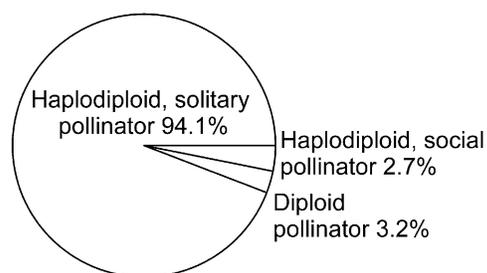
Orchids provoking more intense pollinator behavior have higher pollination success. Sexual deception involving copulation is relatively common (Schiestl 2005; Jersáková

et al. 2006) in comparison to less extreme sexual deception strategies such as entrapment, which are reported for very few genera (e.g., Singer 2002; Lehnebach et al. 2005). Stimulating vigorous or lengthy copulation may simply ensure the transfer of pollinia and thus higher pollination rates, but orchids with high pollination success are probably receiving more pollinator visits overall rather than just more effective visits. The pollination success of *Cryptostylis* orchids is considerably higher than that reported for other sexually deceptive and food-deceptive orchids in the temperate Southern Hemisphere ( $\sim 40\%$ : Neiland and Wilcock 1998) and globally ( $<10\%$ : Gill 1989;  $\sim 20\%$ : Tremblay et al. 2005). The success of *Cryptostylis* could be attributed to highly accurate and compelling mimicry that both attracts numerous pollinators and ensures pollinia transfer by stimulating vigorous copulation and ejaculation.

Unquestionably, producing sperm, ejaculate, or seminal fluids is costly for many animals. The energetic demands of sperm production can result in reduced body mass, a shortened life span, or limited lifetime sperm production (Wedell et al. 2002). Although we have no information about spermatogenesis in *Lissopimpla excelsa* wasps, evidence from similar parasitoids suggests peak sperm availability when males emerge and sperm depletion after multiple copulations (Damiens and Boivin 2006). In species that can replenish sperm, there can be refractory periods after ejaculation until sufficient sperm is produced for sub-



**Figure 3:** Orchid species causing ejaculation (pollinator behavior category 1: *Cryptostylis*; Schiestl et al. 2004; Gaskett and Herberstein 2006) have higher pollination success than orchids stimulating less extreme sexual behavior in which pollinators copulate without ejaculation (category 2: *Ophrys*, *Geoblasta*; Ayasse et al. 1997; Neiland and Wilcock 1998; Ciotek et al. 2006), grip the orchid's hinged major petal (category 3: *Caladenia*, *Chiloglottis*, *Drakaea*; Peakall 1990; Peakall and Handel 1993; Schiestl 2004; Dickson and Petit 2006), or become briefly trapped before collecting the pollinia on departure (category 4: *Pterostylis*; Taylor 1999; Lehnebach et al. 2005).



**Figure 4:** Almost all sexually deceptive orchid species are pollinated by solitary, haplodiploid insect species from 11 hymenopteran families. Six out of 222 orchid species are pollinated by social ants or bees: *Ophrys chestermanii*, *Ophrys normanii*, *Orchis galilea*, *Trigonidium obtusum*, *Leporella fimbriata*, and *Mormolyca ringens* (Bino et al. 1982; Peakall 1989; Singer 2002; Singer et al. 2004; Delforge 2005). Diploid beetles pollinate *Ophrys blitopertha* and visit *Ophrys fuciflora*, but pollination in the latter is primarily by other haplodiploid solitary Hymenoptera (Paulus and Gack 1990; Delforge 2005; Tyteca et al. 2006). Diploid dipterans pollinate *Pterostylis* spp. and *Lepanthes glicensteinii* (Bernhardt 1995; Taylor 1999; Blanco and Barboza 2005; Lehnebach et al. 2005).

sequent copulations (Wedell et al. 2002; Damiens and Boivin 2006). Pollinator reproduction may be severely compromised if males become sperm depleted during an orchid visit and then cannot copulate with real females or can transfer only seminal fluid without sperm (King 1987; Damiens and Boivin 2006). Copulation without sperm transfer would nonetheless reduce female sexual receptivity because monandry is common in Hymenoptera (Hardy 1994; Paxton 2005). Because the pollinator mating season coincides exactly with orchid flowering, there may be considerable competition between female wasps and orchids over attraction of males.

The cost of sperm production and the effects of sperm depletion drive the evolution of male behaviors such as prudent male mate choice and accurate identification of conspecifics (Wedell et al. 2002). Costly sexual deception by orchids could select for similar behaviors in pollinators. However, in regions without orchids, pollinators that respond promptly to any female insect signal may have a fitness advantage over males that are more discriminating. Therefore, selection for male discernment between real females and orchids may be compromised, and selection may instead strongly favor males that learn to recognize and subsequently avoid orchids after a deceptive experience.

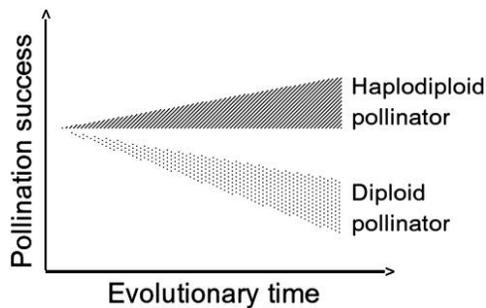
Pollinators of *Cryptostylis* and several other sexually deceptive orchids do learn to avoid sexually deceptive orchids (Peakall 1990; Alcock 2000; Wong and Schiestl 2002; Schiestl 2005). Pollinator learning would limit pollination and reproduction to only the most attractive orchids, thus selecting for increasingly persuasive orchid mimicry. In

turn, this would intensify selection for pollinator learning, thereby driving antagonistic coevolution between orchids and pollinators (Wong and Schiestl 2002). The direct relationship between pollinator and orchid reproductive success also suggests that there is sufficient impetus for an arms race.

How can the extraordinary pollination rates of *Cryptostylis* orchids be maintained, given the pollinator's frequent sperm wastage? It may be that the costs of sperm wastage and depletion are mitigated by pollinator migration into orchid populations from surrounding areas. A more intriguing hypothesis is that a steady supply of male insects available for pollination is maintained by the haplodiploid mating system of orchid pollinators in positive feedback with orchid sexual deception.

Almost all sexually deceptive orchids are exclusively pollinated by haplodiploid insects with a solitary social system, and this is not due to any phylogenetic constraint. The haplodiploid pollinators are from 11 hymenopteran families. Pollinators from the hymenopteran suborder Symphyta include Pergidae that pollinate *Caleana major* (Cady 1965) and Argidae that pollinate *Ophrys subinsectifera* (Delforge 2005). The other more modern and highly diverse hymenopteran suborder, Apocrita, includes all other sexually-deceptive-orchid pollinators, but these still represent two superfamilies: Ichneumonoidea (which has a single representative, *L. excelsa*, the pollinator of *Cryptostylis* orchids) and Aculeata, in which eight sexually-deceptive-orchid-pollinating families are split between Vespoidea (Pompilidae, Scollidae, Tiphiidae) and Apoidea (Sphecidae and four bee families: Andrenidae, Anthophoridae, Colletidae, and Megachilidae; Dowton and Austin 1994; Grimaldi and Engel 2005).

Haplodiploidy may provide pollinators with population-level resilience to orchid exploitation. If females of haplodiploid species do not mate, they can still produce haploid male offspring but no female offspring (King 1987; Hardy 1994). Females that receive insufficient sperm also produce unusually male-biased offspring sex ratios (King 1987). Therefore, haplodiploid pollinator species can still reproduce even if orchids prevent normal mating and insemination. Furthermore, orchids that inhibit sexual reproduction in haplodiploid pollinators could generate a ready supply of naive male insects to act as pollinators during future flowering seasons. Over evolutionary time, orchids exploiting haplodiploid pollinators could maintain or even improve their pollination success (fig. 5), as long as some normal pollinator sexual reproduction still occurred. A male-biased sex ratio could also increase competition over females (and orchids), thereby reducing male choosiness (Bonduriansky 2001) and making copulations with orchids more likely. The feedback between orchid deception and the production of male offspring would be



**Figure 5:** Orchid interference in haplodiploid pollinators' sexual reproduction could generate abundant male insects to act as pollinators because females deprived of males and matings due to orchid interference can produce only male offspring. Conversely, the mating system of diploid species would be less resilient to interference, so orchid exploitation could depress pollinator availability and reduce pollination success over time.

weakened if females derived other benefits from their mates, such as food or transport to oviposition sites, as for the thynnine wasps (Tiphidae) that pollinate sexually deceptive *Chiloglottis* and *Drakaea* orchid species (Peakall 1990; Peakall and Handel 1993). Conversely, sexual deception of diploid species would be unsustainable if deception significantly reduced pollinator reproduction, particularly if there was little or no pollinator migration (fig. 5). Over time, the decreased availability of pollinators would diminish orchid pollination success.

The maintenance of the high pollination rates in *Cryptostylis* may be possible precisely because this orchid genus interferes in its pollinator's reproduction and generates extra naive males. More generally, sexual deception could be maintained because haplodiploidy affords some longer-term population-level resilience against orchid subterfuge, despite the potential costs to individual insects.

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