

Osmia species (Megachilidae) Pollinate *Cypripedium parviflorum* (Orchidaceae) and *Packera paupercula* (Asteraceae): A Localized Case of Batesian Mimicry?

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The pollinator-non-rewarding Large Yellow Lady's-slipper (*Cypripedium parviflorum* var. *pubescens* [Willdenow] Knight) and the rewarding Balsam Ragwort (*Packera paupercula* [Michaux] A. Löve & D. Löve) exist together in some eastern Ontario alvars where they share a group of medium-sized, dark blue metallic pollinators in the bee genus *Osmia* (Mason Bees, Megachilidae). I provide evidence of floral mimicry of the ragwort by the orchid based on several observations: (1) *Osmia* visiting *P. paupercula* also visit *C. parviflorum* var. *pubescens*; (2) *Osmia* are more frequent visitors to *P. paupercula* than to other co-blooming plants; (3) *Osmia* are the primary pollinators of *C. parviflorum* var. *pubescens*; (4) the behaviour of *Osmia* on the flower of *C. parviflorum* var. *pubescens* involving landing on the staminode suggests mimicry; and (5) the largest populations of *C. parviflorum* var. *pubescens* in Ontario are in alvar landscapes where *P. paupercula* is abundant. Pollination in Large Yellow Lady's-slipper may vary geographically from non-model to species-specific Batesian mimicry, which is rare in orchids. This latter pollination mechanism may have evolved in ice-front environments during the Pleistocene, but then became isolated to unusual partial analogues of ice-front habitat during the Holocene with pollination in most of the current range appearing to be a generalist strategy.

Key Words: *Cypripedium parviflorum* var. *pubescens*; *Packera paupercula*; *Osmia*; Large Yellow Lady's-slipper; Balsam Ragwort; pollination; mason bee; floral mimicry; Ontario; alvar

Introduction

The Large Yellow Lady's-slipper (*Cypripedium parviflorum* var. *pubescens* [Willdenow] Knight, previously recognized as *C. calceolus* L. var. *pubescens* [Willdenow] Correll) is reported to be pollinated by a variety of small bees (Argue 2012). Observations of only Mason Bees (*Osmia* spp.) pollinating the yellow, pollinator-rewarding Balsam Ragwort (*Packera paupercula* [Michaux] A. Löve and D. Löve) and the yellow, non-rewarding (i.e., deceptive) Large Yellow Lady's-slipper in the Ottawa Valley led to consideration of the extent to which the orchid may be an example of localized Batesian floral mimicry, where a species with non-rewarding flowers has evolved floral characteristics of a rewarding species that confer an advantage of increased pollinator visitation (Dafni 1984).

Floral mimicry has been reported in a number of orchids (e.g., Dafni and Irvi 1981; Gigord *et al.* 2002; Pellegrino *et al.* 2008; Edens-Meier *et al.* 2014) and in some Asian species of *Cypripedium* (Sugiura *et al.* 2002; Li *et al.* 2006) and has been recently inferred in the North American Ram's-head Lady's-slipper (*Cypripedium arietinum* R. Brown; Catling and Kostiuik 2013).

For Large Yellow Lady's-slipper and Balsam Ragwort to be an example of Batesian floral mimicry, five criteria discernible in field study should be considered, apart from the fact that the Balsam Ragwort model and the orchid mimic occur locally together: (1) Mason Bees visiting the ragwort also visit the orchid; (2) Mason

Bees are more frequent visitors to Balsam Ragwort than to other co-blooming plants so that a specific model is suggested; (3) Mason Bees are primary pollinators of the orchid, i.e., the primary insect group transferring orchid pollen from one flower to another; (4) the behaviour of Mason Bees on the orchid flowers suggests mimicry; and (5) the orchid achieves higher abundance in the presence of Balsam Ragwort than in its absence, due in part to higher fecundity.

The present work focuses on the first four criteria listed above and considers support for the fifth. The work is centred on alvar landscapes in the Ottawa Valley that are diverse, rich in restricted species, and contain globally imperiled ecosystems (Catling 1995).

Study Area

The three study areas, all in eastern Ontario, included Marlborough Forest (45.0818°N, 75.8099°W), Braeside Alvar (45.4864°N, 76.4544°W), and Burnt Lands Alvar (Burnt Lands Provincial Park: 45.2530°N, 76.1486°W). The latitudes and longitudes represent approximate locations, and all observations and collections were made within 1 km of these sites. The specific study areas at each site were approximately 2 ha in extent and included 200–300 plants of Large Yellow Lady's-slipper and 200–700 plants of Balsam Ragwort. Large populations of Large Yellow Lady's-slipper occurred in the surrounding area, including up to 1000 plants within an area of 1 km². All three sites were dominated by more or less open woodlands of Eastern White Cedar (*Thuja*

occidentalis L.) with some Balsam Fir (*Abies balsamea* [L.] Miller) and White Pine (*Pinus strobus* L.).

Methods

Gathering pollination data and voucher specimens

I gathered data by direct observation and collection of bees on orchid and ragwort flowers. On all days when observations of pollinators were made it was sunny and 20–24°C. Areas where pollinators were observed or collected were less than 0.4 ha in extent.

Examining bees reveals pollination because the adhesive pollen of the orchid flower is smeared onto the dorsal surface of the thorax as the bee leaves one of the basal side openings of the flower and can be seen on the insect's thorax for several days afterward (personal observation) indicating that it was at least a visitor to a Large Yellow Lady's-slipper flower and a potential pollinator.

Bees with *Cypripedium* pollen smears in the study areas were assumed to be pollinators of Large Yellow Lady's-slipper. Although Ram's-head Lady's-slipper is present and blooms at the same time (or starts slightly earlier), robust bees (such as Mason Bees) that are over 8 mm long are likely too large to enter and exit the flowers of that species and have not been implicated in its pollination (Catling and Kostiuk 2013). The pollen of these two orchids cannot be differentiated by simple light microscopy using a double-staining technique with phloxine and methyl green (personal observation).

Specimens of bees collected as part of this study are in the collection of Dr. Laurence Packer at York University, Toronto, Ontario, Canada, and that of Dr. Cory Sheffield at the Royal Saskatchewan Museum, Regina, Saskatchewan, Canada. Dr. Sheffield identified *Osmia subaustralis* Cockerell, 1900 and *O. albiventris* Cresson, 1864 and Dr. Anna Taylor identified *Osmia proxima* Cresson, 1864.

Mason Bees visiting the orchid as well as the ragwort

On both 1 and 2 June 2011, I spent approximately an hour observing pollination of Balsam Ragwort in a patch of 400 plants at Braeside. On 3 and 4 June 2011, I observed a group of approximately 600 plants of Balsam Ragwort for approximately 1.5 h on the Burnt Lands. In all cases, ragwort flowers were within 100 m of Large Yellow Lady's-slipper plants. Observations were made between 11 a.m. and 12:30 p.m. I collected and examined bees visiting Balsam Ragwort for orchid pollen smears on the dorsal thorax and released them at the end of the observation period.

Mason Bees visiting other co-blooming plants

On 9 May and 1 and 2 June 2011, at each of the three study sites, I spent an hour between 10 a.m. and 2 p.m. determining whether Mason Bees were visiting the only other species blooming in the area: the introduced Common Dandelion (*Taraxacum officinale* F. H. Wiggers), Swamp Dandelion (*T. palustre* [Lyons] Symons), and Wild Strawberry (*Fragaria virginiana* Miller).

Mason Bees, the primary pollinator of the orchid

On 29 May 2011, I attempted to observe pollinators at Braeside. I spent 3 h watching a group of 30 flowers of Large Yellow Lady's-slipper during sunny, mild weather between 11 a.m. and 2 p.m. On 2 and 11 June 2008, I spent 2 h each at Marlborough Forest and the Burnt Lands observing pollination in a group of 25 and 50 flowers, respectively.

Behaviour of Mason Bees suggesting mimicry

On 4 June 2011, between 9 a.m. and noon, I conducted eight experiments with three *Osmia subaustralis* (one male, two females) and five unidentified *Osmia*. The bees had been isolated and maintained in captivity, resting at low light and low temperature (20°C) for 20 h. As a result of their inactivity, they were each easily transferred into a fine mesh cage containing two fresh flowers of Large Yellow Lady's-slipper. The 16 orchid flowers selected for the observations had lips 31–40 mm long with more or less circular distal openings with a maximum width of 8–11 mm (Figure 1) and showed no signs of visitation in the form of scales, hairs or displaced pollen. The maximum width of basal open-



FIGURE 1. *Osmia proxima* Cresson, 1864 exiting one of the basal lateral openings of a flower of Large Yellow Lady's-slipper (*Cypripedium parviflorum* var. *pubescens* [Willdenow] Knight). The anther is on top of the thorax of the bee. Photo by P. M. Catling, Burnt Lands Provincial Park, 11 June 2008.

ings was 2–3 mm becoming 3–5 mm when the lip was pressed downward. The flower stems were placed upright in water in a coffee cup, through a small opening in the lid; this prevented bees from falling into open water. Observations were made through the glass front after strong light and heat rising to 24°C was focused on the cage and led to activity. An experiment was considered to be completed when a bee became inactive or when it appeared intent on escape and showed little interest in the flowers.

Results

Of 76 bees captured while they were visiting Balsam Ragwort, 25 carried pollen likely originating from Large Yellow Lady's-slipper (Table 1).

Both co-blooming dandelion species were past peak flowering and infrequent. Wild Strawberry was infrequent but in peak bloom locally. Although many bees were seen on the flowers of these plants only one Mason Bee was seen visiting them — a flower of Wild Strawberry.

All observations of pollination are summarized in Table 2, which includes seven pollinator and three visitor records, all of which involved Mason Bees. In most cases, the bees were captured after they left one flower

and entered another; they were removed from the second flower. Apart from the fact that flowers were entered within a few seconds, there were no detailed observations of behaviour, except in the following two cases.

At Burnt Lands Alvar on 11 June 2008 on a sunny morning with air temperature 18°C, a bee was found inside the lip of a Large Yellow Lady's-slipper flower. The plant was covered with a net and within five minutes the bee exited the flower from a lateral basal opening with pollen on the thorax (Figure 1). It was later identified as *Osmia proxima*.

At Marlborough Forest on 2 June 2008 on a sunny late afternoon with air temperature 22°C, after a cloudy morning, a bee landed on the staminodium of a Large Yellow Lady's-slipper flower, then fell into the lip cavity. It emerged seven minutes later, with much pushing downward of the lip, from a lateral opening with pollen on its back. It was later identified as *Osmia proxima*.

During experiments to investigate mimicry, the bees generally became active within 10 minutes and flew around inside the cage. Within 1–18 minutes of flight, nine of ten bees landed on the staminodium of a centrally located Large Yellow Lady's-slipper flower. They then turned back and forth over the staminodium

TABLE 1. Observations of Mason Bees (*Osmia* spp.) carrying pollen of Large Yellow Lady's-slipper (*Cypripedium parviflorum* var. *pubescens* [Willdenow] Knight).

No. of bees with pollen (total bees observed)	Location	Collected on/in	Date
5 (20)	Braeside	Balsam Ragwort (<i>Packera paupercula</i> [Michaux] A. Löve and D. Löve)	1 June 2011
9 (20)	Braeside	Balsam Ragwort	2 June 2011
5 (20)	Burnt Lands	Balsam Ragwort	3 June 2011
6 (16)	Burnt Lands	Balsam Ragwort	4 June 2011

TABLE 2. Observations of Mason Bees (*Osmia* spp.) pollinating or visiting Large Yellow Lady's-slipper (*Cypripedium parviflorum* var. *pubescens* [Willdenow] Knight).

Bee species	Location/origin	Notes	Date
1 <i>Osmia proxima</i> Cresson, 1864	Burnt Lands	Pollinator. Found inside lip, exited with pollen.	11 June 2008
1 <i>Osmia</i> sp.	Burnt Lands	Pollinator. Leaving flower with pollen and entering another.	11 June 2008
1 <i>Osmia</i> sp. with pollen.	Marlborough 3 June 2008	Visitor, leaving flower	
1 <i>Osmia proxima</i> Cresson, 1864	Marlborough	Pollinator. Landed on staminodium with pollen then exited flower with pollen.	3 June 2008
2 cf. <i>Osmia albiventris</i> Cresson, 1864 (male)	Braeside	Pollinators. Both visiting two consecutive flowers with pollen taken to both flowers.	29 May 2011
3 <i>Osmia subaustralis</i> Cockerell, 1900 (1 female, 2 males)	Braeside	Pollinators. All three visiting two consecutive flowers with pollen taken to both flowers.	29 May 2011
1 <i>Osmia</i> sp.	Braeside	Visitor. Visiting a single flower and emerging with pollinia.	29 May 2011

probing it actively with the tongue. After 15–120 seconds of this, all nine bees fell off the staminodium into the lip. Time to exit was usually 5 minutes, but was 35 minutes in one case. In a few instances, a bee was inactive inside the lip for 5–10 minutes. Time spent struggling in the exit area below the stigma was 3–10 minutes and, during this time, the bee often pushed the lip downward, evidently with its back against the column to increase the space. Once the bee's head had reached the side opening, exit from the flower, as it passed under the stamen, required only 2–7 seconds. This was true for seven of nine trapped bees. Of the other two, one exited the side opening upside down with pollen attaching to the underside of the thorax and abdomen. Another bit a hole through one of the windows at the base of the lip and exited that way within 9 minutes after entering a flower. Most bees spent a few minutes grooming after leaving the flower and one had the wings stuck together, with pollen, over the back. Two bees went to, and through, the second flower within 5 minutes of visiting the first.

Discussion

The number of bees visiting Balsam Ragwort that carried pollen of Large Yellow Lady's-slipper seemed high, based on low levels of capsule development in many areas suggesting low levels of insect visitation. However, the orchid was common in both areas (Braeside and Burnt Lands) and generally flowered a little earlier than Balsam Ragwort; thus, it may have attracted attention when resources were limited.

Although the survey of Mason Bees on co-blooming plants included over 2 h of observation time, this is not considered an extensive survey. Nevertheless, it provides evidence that Mason Bees were very much associated with Balsam Ragwort and less so with other flowering plants.

The 11 observations of Mason Bees and no other species of bees on Large Yellow Lady's-slipper is evidence that they were the primary pollinators at these study locations (Braeside and Burnt Lands alvars and Marlborough Forest). Although Mason Bees were the only pollinators in the present study, studies of the similar, although not closely related (Li *et al.* 2011), European Lady's-slipper Orchid (*Cypripedium calceolus* L.) have revealed a number of different bee pollinators (Nilsson 1978, Antonelli *et al.* 2009). For Large Yellow Lady's-slipper in eastern North America, the only pollinators reported are halictid bees and small Carpenter Bees (*Ceratina* spp.), and these were based on few records (Argue 2012). I saw bees from both of these groups in the study areas and a significant bee fauna was present at least at the Burnt Lands site (Taylor and Catling 2011), although not all of these species would be active at the time of blooming of the orchid. Many species of bees, including Mason Bees, have been reported as visitors to Large Yellow Lady's-slipper (Argue 2012). Although this suggests that many bee

species may be involved in its pollination and some regional variation may be anticipated, Mason Bees may be the primary pollinators in some of the larger orchid populations in the study areas.

The pollination of slipper orchids is based on control of the path of the insect through the flower by morphologic attributes of the flower lip, where the insect enters the large frontal opening, deposits pollen, picks up a new pollen load, and then exits by one of the two small openings on either side of the lip base (Figure 1). This well-known and accepted phenomenon was first elaborated by Darwin (1862), but more correctly and in more detail in Darwin (1877) following help from Asa Gray, and it was later discussed by many others (e.g., Stoutamire 1967; Catling and Catling 1991; Argue 2012). What has been controversial is the attractant. Ideas have varied: the food value of hairs on the inner lip, the production of fragrances by the pollinating bees, the resemblance of the lip to a cavity nest site, and general food deception involving colour and nectar guides (Catling and Catling 1991; van der Cingel 2001). The latter of these has been the most agreed upon, but an interesting addition is that a flower may be more likely to be visited again if it has already been visited, as a result of accumulation of bee odours on specialized hairs (Nilsson 1978). The present work supports food deception, but further suggests that Mason Bees may be specifically attracted to the staminode due to its resemblance to the centre of a *Packera* flower. The darker and orange areas in open disc flowers within a mass of yellow disc flowers in Balsam Ragwort are similar to orange spots on a yellow staminodium of similar size in the orchid. The only other case in *Cypripedium* where bees contacted the staminodium first before falling into the labellum involved *C. guttatum* Swartz and mimicry was not implicated (Bänziger *et al.* 2005).

A distribution map for Balsam Ragwort in Ontario (Catling 1995, Figure 7) indicates a concentration in alvar landscapes along the edges of the Canadian Shield. Similarly, a map for Large Yellow Lady's-slipper (Whiting and Catling 1986, map 3c) shows concentrations in the same regions and absences from the Canadian Shield and parts of southwestern Ontario. Although the orchid is more widespread than the ragwort, it is similarly associated with limestone rock (Whiting and Catling 1986). The areas of high abundance of the orchid in southern Ontario are well known and include Manitoulin Island, the Bruce Peninsula, and limestone landscapes near Kingston and Ottawa. For example, with regard to the Bruce Peninsula, the Bruce-Grey Plant Committee (1997) notes that "it may be more common [here] than in any other part of Ontario." These same landscapes are the areas of abundance of Balsam Ragwort.

Conclusions

The following observations support the case for Batesian floral mimicry. A large proportion of Mason Bees visiting Balsam Ragwort also visited the orchid,

but not other co-blooming flowers. Mason Bees were the primary pollinators of the orchid and behaved on the orchid flowers as they did on the flowers of Balsam Ragwort by landing on the staminodium, which resembles the centre of the ragwort flower. Orchids were more abundant where the ragwort was abundant. Although it may be appropriate to consider ragwort species as important models in a generalized magnet species effect, pollination may vary geographically from non-model to species-specific Batesian mimicry where a single model can be readily identified. This latter possibility is of interest for three reasons: the rarity of floral mimicry in orchids, the existence of specialist and generalist strategies within one taxon, and the possibility of early evolution and mimicry in the past.

Floral mimicry is rare in orchids

Members of the genus *Cypripedium* have generally been regarded as generalist (non-model) food mimics (Catling and Kostiuk 2006; Pelligrino *et al.* 2008) like most food-deceptive orchids, which include a third of all orchids (Cozzolino and Widmer 2005), the largest family of vascular plants. Most deceptive orchids have not evolved species-specific Batesian floral mimicry (Johnson *et al.* 2003; Li *et al.* 2006), although it has been attributed to various species around the world, including Red Helleborine (*Cephalanthera rubra* [L.] Richard) from Europe (Nilsson 1983), Leopard Orchid (*Diuris maculata* Smith) from Australia (Beardsell *et al.* 1986), and Cluster Disa (*Disa ferruginea* [Thunb.] Swartz) from South Africa (Johnson 1994). Mimicry has been reported only recently in two of approximately 50 species of *Cypripedium*, a genus of the northern hemisphere (Li *et al.* 2011): a Japanese Lady's-slipper (*C. macranthos* Sw. var. *rebunense* (Kudo) Miyabe & Kudo (Sugiura *et al.* 2002) and Ram's-head Lady's-slipper (*C. arietinum* R. Brown) (Catling and Kostiuk 2013).

A specialist and a generalist within one taxon

Geographically based differences in food deception within taxa, ranging from specialization to generalist strategies, may be more frequent than is realized. The idea of such micro-ecological isolating mechanisms is not new and was discussed by Heslop-Harrison (1958) with regard to orchids and by Stoutamire (1967) with regard to lady's-slippers. This within-taxon variation may not always be as obvious as the anomalous white-flowered var. *rebunense* of *Cypripedium macranthos* on Rebut Island, Japan, which visually mimics the white-flowered *Pedicularis schistostegia* Vvedensky (Sugiura 2001, 2002). The pollinator-mediated mating restriction that has been shown between the varieties of *C. parviflorum* (Case and Bradford 2009; Case and Bierbaum 2013) may also occur between geographic races. There is also evidence for different pollination races in European *C. calceolus* (Antonelli *et al.* 2009). That different pollination races exist in Large Yellow Lady's-slipper is suggested by the 36 records of visitation and pollination by Mason Bees on alvar landscapes

and no records of other bees, as reported here, compared with reports of many other kinds of visiting bees in other locations (van der Cingel 2001). The possibility that some lady's-slipper species, and other deceptive orchids, are both specialists and generalists requires more study.

Early evolution and mimicry in the past

Mimicry might have originated as a general resemblance to co-occurring floral food sources, such as yellow blossoms. Although similar colours may not always indicate mimicry or pollinator sharing (Bierzychudek 1981), they might. Supporting this is the observation of the unusual abundance of Large Yellow Lady's-slipper (thousands of plants in a few hectares) along with several co-blooming, yellow-flowered species in remnant prairies along the railway lines in southeastern Saskatchewan (Catling and Kostiuk 2006). Corolla colour is sufficient to achieve floral mimicry, and pollinators can select for it (Gigord *et al.* 2002). Yellow-flowered species prominent in the Saskatchewan prairies are Hoary Puccoon (*Lithospermum canescens* [Michaux] Lehmann) and Heart-leaved Alexanders (*Zizia aptera* [A. Gray] Fernald), but species of ragwort are also present (personal observation). Following development of colour resemblance, the orchid may have evolved a more specific resemblance to Balsam Ragwort by developing reddish spots on the staminode and possibly in other ways. This may have occurred in the open, ever-changing ice-front environments that lasted for many thousands of years during the Pleistocene.

More recently, during the Holocene, the mimetic pollination system may have become isolated to unusual partial analogues of ice-front habitat with pollination in most of the current range appearing to be a generalist strategy. Thus we have a Batesian mimic that to a large extent became a generalist with only localized situations, as in the alvars studied here, that make the former pollination mechanism clear. Certainly, within the general range, there are areas of high abundance of Large Yellow Lady's-slipper, and many of these are in places that resemble ice-front and early postglacial habitats, such as alvars (Catling and Brownell 1995). Regardless of the likelihood of this hypothesis, it does draw attention to the possibility that pollination in temperate plants may sometimes be better understood through reference to past conditions.

Future research

Although this article presents evidence to suggest Batesian floral mimicry, the suggestion would be strengthened if the bees were found to carry only ragwort pollen, rather than observing bees visiting only ragwort flowers, as sampling based on a longer period would be achieved in this way.

Two more protocols for data collection that can be considered in future studies are: (1) flowers of the orchid and ragwort can be compared under ultraviolet (UV) light to determine whether similar patterns exist,

although floral mimicry is not entirely dependent on UV reflectance (Gigord *et al.* 2002); and (2) a biochemical analysis of scent would be helpful to determine the extent to which that factor plays a role, although studies have suggested that visual attributes may be more important than scent chemistry in deceptive orchids (Jersáková *et al.* 2012). The scent components may be general or contain those of the ragwort or even those of the pollinating bees (Volterová *et al.* 2007). These protocols have been effectively applied to studies of Batesian floral mimicry in other species (Edens-Meier *et al.* 2014).

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Literature Cited

- Antonelli, C., C. J. Dahlberg, K. H. I. Carlgren, and T. Appelqvist. 2009. Pollination of the Lady's slipper orchid (*Cypripedium calceolus*) in Scandinavia — taxonomic and conservational aspects. *Nordic Journal of Botany* 27: 266–273.
- Argue, C. L. 2012. *The Pollination Biology of North American Orchids: Volume 1 North of Florida and Mexico*. Springer, Berlin, Germany, 228 pages.
- Bänziger, H., H. Sun, and Y.-B. Luo. 2005. Pollination of a slippery lady slipper in south-west China: *Cypripedium guttatum* (Orchidaceae). *Botanical Journal of the Linnean Society* 148: 251–264.
- Beardsell, D. V., M. A. Clements, J. F. Hutchinson, and E. G. Williams. 1986. Pollination of *Diuris maculata* R. Br. (Orchidaceae) by floral mimicry of the native legumes *Daviesia* spp. and *Pultenaea scabra* R. Br. *Australian Journal of Botany* 34: 165–173.
- Bierzuchudek, P. 1981. *Asclepias*, *Lantana* and *Epidendrum*: a floral mimicry complex? *Biotropica* (suppl) 13: 54–58.
- Bruce-Grey Plant Committee. 1997. *A Guide to the Orchids of Bruce and Grey Counties, Ontario*. Owen Sound Field Naturalists, Owen Sound, Ontario, 105 pages.
- Case, M. A., and T. J. Bierbaum. 2013. Pollinator-mediated mating restriction between sympatric varieties of yellow lady's slipper orchids (*Cypripedium parviflorum* Salisb.). *Plant Systematics and Evolution* 299: 1721–1735.
- Case, M. A., and Z. R. Bradford. 2009. Enhancing the trap of lady's slippers: a new technique for discovering pollinators yields new data from *Cypripedium parviflorum* (Orchidaceae). *Botanical Journal of the Linnean Society* 160: 1–10.
- Catling, P. M. 1995. The extent of confinement of vascular plants to alvars in southern Ontario. *Canadian Field-Naturalist* 109: 172–181.
- Catling, P. M., and V. R. Brownell. 1995. A review of the alvars of the Great Lakes region: distribution, floristic composition, phytogeography and protection. *Canadian Field-Naturalist* 109(2): 143–171.
- Catling, P. M., and V. R. Catling. 1991. A synopsis of breeding systems and pollination in North American orchids. *Lindleyana* 6(4): 187–210.
- Catling, P. M., and B. Kostiuk. 2006. Tallgrass prairie in the Whitewood area of Saskatchewan. *Blue Jay* 64(2): 72–83.
- Catling, P. M., and B. Kostiuk. 2013. Pollination of Ram's-Head Lady's-Slipper (*Cypripedium arietinum*) in eastern Ontario and notes on the pollination system. *Native Orchid Conference Journal* 10(4): 20–28.
- Cozzolino, S., and A. Widmer. 2005. Orchid diversity: an evolutionary consequence of deception? *Trends in Ecology and Evolution* 20: 487–494.
- Dafni, A. 1984. Mimicry and deception in pollination. *Annual Review of Ecology and Systematics* 15: 259–278.
- Dafni, A., and Y. Irvi. 1981. Floral mimicry between *Orchis israelitica* Baumann and Dafni (Orchidaceae) and *Bellevalia flexuosa* Boiss. (Liliaceae). *Oecologia* 49: 229–232.
- Darwin, C. 1862. *On the Various Contrivances by which British and Foreign Orchids Are Fertilised by Insects, and on the Good Effects of Interbreeding*. John Murray, London, UK.
- Darwin, C. 1877. *The Various Contrivances by which Orchids Are Fertilized by Insects*. Second edition. John Murray, London, UK.
- Edens-Meier, R., R. A. Raguso, E. Westhus, and P. Bernhardt. 2014. Floral fraudulence: do blue *Thelymitra* species (Orchidaceae) mimic *Orthrosanthus laxus* (Iridaceae)? *Telopea* 17: 15–28.
- Gigord, L. D. B., M. R. Macnair, M. Stritesky, and A. Smithson. 2002. The potential for floral mimicry in rewardless orchids: an experimental study. *Proceedings of the Royal Society of London* 269: 1389–1395.
- Heslop-Harrison, J. 1958. Ecological variation and ethological isolation. *Uppsala Universitets Årsskrift* 1958(6): 150–158.
- Jersáková, J., A. Jürgens, P. Šmilauer, and S. D. Johnson. 2012. The evolution of floral mimicry: identifying traits that visually attract pollinators. *Functional Ecology* 26: 1381–1389.
- Johnson, S. D. 1994. Evidence for Batesian mimicry in a butterfly-pollinated orchid. *Biological Journal of the Linnean Society* 53: 91–104.
- Johnson, S. D., C. I. Peter, J. Ågren, and L. A. Nilsson. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. *Ecology* 84: 2919–2927.
- Li, J. H., Z. J. Liu, G. A. Salazar, P. Bernhardt, H. Perner, Y. Tomohisa, X. H. Jin, S. W. Chung, and S. W. Luo. 2011. Molecular phylogeny of *Cypripedium* (Orchidaceae: Cypridodioideae) inferred from multiple nuclear and chloroplast regions. *Molecular Phylogenetics and Evolution* 61: 308–320.
- Li, P., Y. Luo, P. Bernhardt, X. Yang, and Y. Kou. 2006. Deceptive pollination of the Lady's Slipper *Cypripedium tibeticum* (Orchidaceae). *Plant Systematics and Evolution* 262: 53–63.
- Nilsson, L. A. 1978. Anthecological studies of the Lady's-Slipper, *Cypripedium calceolus* (Orchidaceae). *Botanical Notiser* 132: 329–347.
- Nilsson, L. A. 1983. Mimesis of bellflower (*Campanula*) by the Red Helleborine Orchid (*Cephalanthera rubra*). *Nature* 305: 799–800.
- Pelligrino, G., F. Bellusci, and A. Musacchio. 2008. Double floral mimicry and the magnet species effect in dimorphic co-flowering species, the deceptive orchid *Dactylorhiza sambucina* and rewarding *Viola aethnensis*. *Preslia* 80(4): 411–422.
- Stoutamire, W. P. 1967. Flower biology of the lady's slippers (Orchidaceae: *Cypripedium*). *Michigan Botanist* 6: 159–175.

- Sugiura, N., T. Fujie, K. Inoue, and K. Kitamura.** 2001. Flowering phenology, pollination, and fruit set of *Cypripedium macranthos* var. *rebunense*, a threatened lady's slipper (Orchidaceae). *Journal of Plant Research* 114: 171–178.
- Sugiura, N., M. Goubara, K. Kitamura, and K. Inoue.** 2002. Bumblebee pollination of *Cypripedium macranthos* var. *rebunense* (Orchidaceae); a possible case of floral mimicry of *Pedicularis schistostegia* (Orobanchaceae). *Plant Systematics and Evolution* 235: 189–195.
- Taylor, A., and P. M. Catling.** 2011. Bees and butterflies in burned and unburned alvar woodland: evidence for the importance of postfire succession to insect pollinator diversity in an imperiled ecosystem. *Canadian Field-Naturalist* 125(4): 297–306.
- van der Cingel, N. A.** 2001. *An Atlas of Orchid Pollination, America, Africa, Asia and Australia*. A. A. Balkema Publishers, Brookfield, Vermont, USA. 296 pages.
- Volterová, I., J. Kunze, A. Gumbert, A. Luxová, I. Libikas, B. Kalinová, and A.-K. Borg-Karlson.** 2007. Male bumblebee pheromonal components in the scent of deceit pollinated orchids; unrecognized pollination cues? *Arthropod-Plant Interactions* 1: 137–145.
- Whiting, R. E., and P. M. Catling.** 1986. *Orchids of Ontario*. CanaColl Foundation, Ottawa, Ontario, Canada. 169 pages.

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