

BEE-MEDIATED POLLEN TRANSFER IN TWO POPULATIONS OF *CYPRIPEDIUM MONTANUM* DOUGLAS EX LINDLEY

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Abstract—The conversion rate of flowers into fruit in *C. montanum* at two sites over four seasons was 52-85%, unusually high for a food mimic orchid. Comparative measurements of the trap-like labellum of *C. montanum* showed it was intermediate in size compared to measurements of six other *Cypripedium* spp. found in North America and China. While visitors to flowers of *C. montanum* represented three insect orders, at two sites, over four seasons only small- to medium-sized, solitary bees (5-10 mm in length) carried the pollen massulae. Bee-visitation occurred at both sites and began within 24-48 hours following labellum expansion. Female bees in the genus *Lasioglossum* (Halictidae) were the most common carriers of massulae. However, species of visiting bees differed between sites and years. At both sites the majority of bees entered and escaped from the labellum in less than 180 seconds and there was no significant difference between the times bees spent in the flowers at both sites. At the site on the Eastside Cascades of Central Oregon, there was no correlation between the length and width of a bee and the time it spent escaping from the basal openings. There was no correlation between bee size and whether the bee carried massulae. Depending on site and year 41-58% of the bees exiting the orchids carried the orchid's pollen. Depending on site and year 75-100% of bees collected exiting the orchids via the basal openings also carried the pollen of at least one other co-blooming species.

Keywords: Basal openings, bees, *Cypripedium*, labellum, pollen masses (massulae), pollination, staminodium

INTRODUCTION

When pollen vectors are absent or infrequent during the flowering period of an out-breeding population this usually results in lower fruit and/or seed production. When this occurs consistently the plant population is usually categorized as pollinator-limited (*sensu* Committee on the Status of Pollinators in North America 2007). Low fruit and seed set are recorded often in orchid species that produce flowers lacking any edible or scent rewards for their pollinator(s). In fact, Tremblay et al. (2005) showed that orchid flowers with food deceptive modes of presentation (*sensu* Ackerman 1986) showed lower rates of fruit set than orchids with pseudocopulatory flowers (*sensu* Dafni & Bernhardt 1990). As floral mimesis dominates orchid speciation, the correlation between low fecundity and the absence of rewards remains of primary concern to conservationists Edens-Meier et al. 2014). Any attempt to protect dwindling populations of orchid species and/or reintroduction following regional extinctions requires the establishment and/or reestablishment of pollinator populations (Dixon 2009).

Cypripedium species (*sensu* Cribb 1999) produce flowers interpreted usually as food deceptive. With

important exceptions (e.g. *C. passerinum*; see Catling 1990), mechanical self-pollination in the absence of pollinators is uncommon within this genus. There are no modern references confirming any observations of flower-visiting insects consuming edible rewards and/or collecting nesting materials inside the inflated labellum (Bernhardt & Edens-Meier 2010; Argue 2011). Fragrance analyses of flowers of *Cypripedium* spp. show biochemical variation at the interspecific level (Barkman et al. 1997) but none of their insect pollinators, to date, have been observed collecting their scents unlike the male bees (Euglossini) associated with so many monandrous species of the Neotropics (Dressler 1968, 1981). None of the floral volatiles produced by *Cypripedium* spp. (Barkman et al. 1997) are unique to the genus. They are also produced by flowers of other angiosperm species, more likely to offer nectar and/or pollen as rewards.

It appears that the inflated labellum and diandrous column of at least 17 *Cypripedium* spp. function in the same way. In each case, the potential pollinator enters the labellum, passes under the receptive stigma, and then exits the flower via one of two exit openings. One anther is located above each exit. The anther contacts the escaping insect leaving a dorsal, often irregular, deposit of massulate pollen on the thorax and/or head. However, the diversity and density of pollinators in each *Cypripedium* species may vary broadly according to the combined elements of floral presentation including floral size, internal architecture,

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colour patterns, epidermal sculptures, scent, massulae morphology etc. (Bernhardt & Edens-Meier 2010; Edens-Meier et al. 2011, 2014).

Therefore, if all cross pollinated *Cypripedium* spp. are food mimics, the natural rates of pollinated pistils, and the conversion rates of pistils into fruit (see Edens-Meier et al. 2010, 2014), should be low (Tremblay et al. 2005). This is not always the case. Bernhardt & Edens-Meier, (2010) reviewed the literature on insect pollination in 15 *Cypripedium* spp. at 29 different sites. They noted that the conversion of flowers into fruit changed at the intraspecific level from year to year and from site to site. Many species suffered seasons in which the conversion rate of flowers into fruit was < 20% (see Primack & Stacy 1998; Lipow et al. 2002; Sugiura et al. 2002; Li et al. 2006; Bänziger 2008; Kull 2008; Sun et al. 2009). It was also true that some species including *C. calceolus* (Kull 2008), *C. fasciculatum* (Lipow et al. 2002), and *C. plectrochilum* (Li et al. 2008) bloomed at sites in which the conversion of flowers into fruit was 33–69%. These rates are entirely competitive with other obligately outcrossing herbs that offer edible pollen and/or nectar as rewards to pollen vectors (see Bernhardt & Montalvo 1979; and Bernhardt & Dafni 2000). Depending on the year, the conversion rate of flowers into fruit can be from 0–25% in a population of *C. acaule* (Primack & Stacy 1998), 33–57% in a population of *C. calceolus* (Kull 2008), and 9–26% in *C. tibeticum* (Li et al. 2006). Likewise, fruit production could vary tremendously between sites over the natural distribution of a single species. This included 0–25% between isolated populations of *C. acaule* (Primack & Stacy 1998) and 10–57% in *C. calceolus* (Kull 2008).

Some *Cypripedium* spp. experience higher fruit set ratios at specific locations in certain years. By following Tremblay et al. (2005) we should conclude that some insects are more common in some sites, over some flowering seasons, and are more likely to be deceived by the same food mimic more than once affecting cross-pollination. However, Bernhardt and Edens-Meier (2010) also noted that, “blaming low fruit set on the performance of a discrete lineage of anthophilous insects as poor pollinia vectors must be approached with caution.” The most fecund species including *C. calceolus*, *C. fasciculatum*, and *C. plectrochilum* depended on very different pollinators. *Cypripedium calceolus* s.s. (Kull 2008) was pollinated by a range of small bees in several families. In contrast, *C. plectrochilum* (Li et al. 2008) was pollinated exclusively by a few species in the genus *Lasioglossum* (Halictidae). *Cypripedium fasciculatum* was pollinated by female, parasitoid wasps in the genus *Cinetus* (Ferguson & Donham 2005). Likewise, when *C. acaule* (Primack & Stacy 1998) and *C. tibeticum* (Li et al. 2006) had an infrequent, “successful,” season of fruit set (25%) they were pollinated exclusively by gynes of a few *Bombus* spp.

In fact, there appears to be a *Cypripedium* species with pollination rates that may, at certain sites and years, be higher than any of the species discussed above. *Cypripedium montanum* is restricted to the Pacific and interior northwestern North America where it is found in mesic to dry forest clearings and slopes (Sheviak 2002; Vance 2007). Edens-Meier et al. (2011) showed that 94.0% of the pistils,

collected at random from withering flowers at two sites in Oregon, over two seasons ($N = 16$), contained germinating pollen on their stigmas and/or pollen tubes penetrating their styles. However, other studies showed that, as usual, the conversion rate of flowers into fruits varies within the same site over different years. A monitoring study on six plots of *C. montanum* in the Bighorn Mountains of Wyoming reported that in 2004 and 2005 average fruit set ($N = 5$, one plot lacked flowering stems) was 41% (range 0–67%) and 38% (range 4–77%) respectively (see Vance 2007). In contrast, Coleman (1995) followed fruit production in California populations over a four-year period and found that the average rate of fruit set was 61.0% over all sites. Huber (unpublished) monitored the rates of natural fructification at his wildflower reserve (GROWISER) in 2003 and again in 2004. He found that 75% stems ($N = 50$) produced at least one mature and dehiscent capsule in 2003 and 85% ($N = 50$) produced fruit in 2004.

Information on pollinators of *C. montanum* remained anecdotal through the 20th century. Luer (1975) observed that a *Bombus* sp. was unable to enter the labellum but small, unidentified, black bees were able to successfully enter through the large, dorsal opening on the labellum and exit via the basal openings (see above).

Therefore, this paper attempts to address five interrelated questions regarding insect-flower interactions in the pollination dynamics of two populations of *C. montanum*. These are the same populations that served previously as sources for observations on natural rates of pollination *in situ* (Vance 2007; Edens-Meier et al. 2010, 2014). First, how do labellum “trap” dimensions in *C. montanum* compare with the same dimensions in flowers of congeners measured in past publications? Specifically, how do labellum sac dimensions in *C. montanum* correlate with pollinator dimensions as in other congeners with different pollinators (Bänziger et al. 2005; Li et al. 2006, 2008ab; Bänziger et al. 2008; Edens-Meier et al. 2011, 2014)? Second, is this species pollinator-limited at either site or can it rely on mechanical self-pollination (see above) in the absence of pollinators? Third, which insect taxa are the most frequent vectors of pollen masses over different seasons and sites? Fourth, does the taxonomic composition of prospective vectors of pollen masses vary between seasons and sites? Fifth, if *C. montanum* lacks edible rewards, as in all other *Cypripedium* spp., which co-blooming species provide insects with edible rewards at different sites?

MATERIALS AND METHODS

Study sites and field states

The first site used from 6/4–6/16/03 and from 5/22–6/17/04 was in the Blue Mountains of Eastern Oregon (BMEO) at the GROWISER Reserve, Summerville. This site is found primarily on north and east facing slopes at 1050 m elevation with an annual precipitation of 50 cm, half of which falls as snow. The soils are deep volcanic ash with a woodland canopy of *Pseudotsuga menziesii*. There are approximately 700 stems of *C. montanum*, at various stages of maturity, growing in woodland gaps and glades but less

than 400 produce flowering stems annually (Huber, pers. obs.). While a portion of the property is developed for an *in situ* seeding program (Huber 2002) we performed no observations in these areas and did not use flowers from this program for measurement. In 6/2004, 6/30/2005, 7/06/2005 and from 6/5/2006–6/14/2006 we combined observations on two populations on the Eastside Cascades of central Oregon (ECCO) within the Deschutes National Forest Sisters Ranges District in Jefferson County, Oregon. Within the site the two colonies grow along or near Forest Services Road (FS1190) and are separated from each other by approximately 4/5 km. The site is located within a forest that burned in a 10-hectare wildfire in 2002. The soils derived from weathered tuff and andenite with shallow to deep sandy-gravelly loams formed from volcanic ash over colluvium and rendum. The total number of flowering stems in the two colonies totaled 139 in 2006.

Number of flowers and fruits per scape

The number of flowers on scapes of *C. montanum* appears to be dependent on the physical age of the plant (Huber 2002). As annual variation in flower numbers within the same population may influence visitation rates of potential pollinators we selected 40 flowering stems at random each year and counted the number of flowers/stalk at the BME0 site (2003, 2004). At ECCO we counted 103–206 flowering scapes each season (2004–2006) recoding the total number of flowers produced. We then returned to record the number of maturing capsules at ECCO.

Self-pollination in the absence and presence of pollinators

Flowers of *C. montanum* from both populations were found to be self-compatible (Edens-Meier et al. 2010). To determine whether pollen masses contacted viable stigmas in the absence of pollen vectors in 2004 and 2005, we tagged and covered eight mature buds on eight flowering stalks in tulle bags one or two days before the sepals and lateral petals released the labellum. The flower was considered open after the labellum expanded and opened its dorsal entrance. The flowers bloomed and withered under the tulle bags and we checked the stalks for fruits in July 2004, 2005. During the 2004 season at the BME0 site we also tagged an additional five flower buds on five stalks. The labellum on each bud was removed with cuticle scissors before it expanded to determine if self- or cross-pollination could occur in the absence of visiting insects entering the labellum. These flowers were also checked for evidence of fruit set in July 2004.

Labellum measurements

All terminology for floral dimensions (Fig. 1) and all labellum measurements taken at the BME0 and ECCO sites (2006) follow Li et al. (2006, 2008ab) with two exceptions. We did not record the length and width of the two, basal openings (rear orifices) as in the studies discussed in the Introduction (above) because our primary interest was in the “fit” of insects within the sac (see Luer 1975). We were not able to record the distance in mms of the receptive surface of

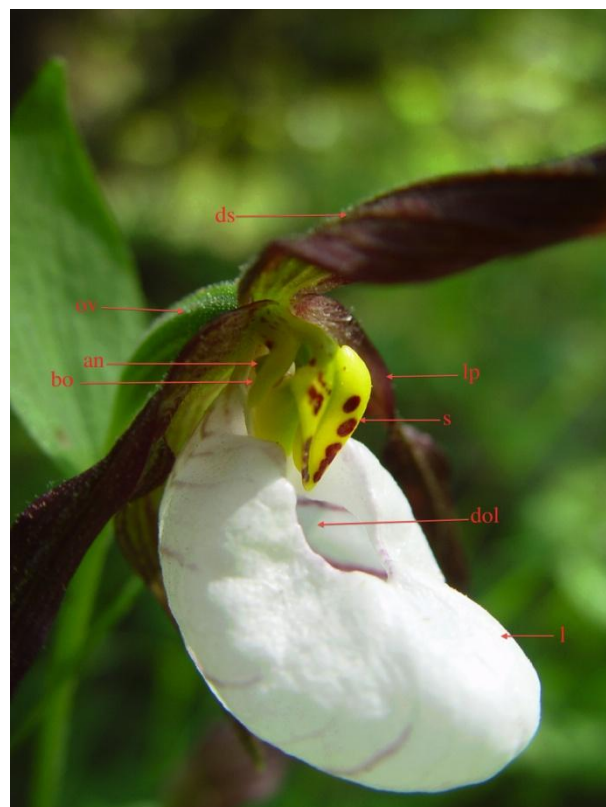


Figure 1. Flower of *C. montanum*: an = anther, bo = basal opening, dol = dorsal opening of labellum (note interior purple stripes), ds = dorsal sepal, l = labellum, lp = lateral petal, ov = ovary (Photograph by Nan Vance).

the stigma to the base of the labellum as in Li et al. (2006) as this would have meant splitting labella open longitudinally thereby reducing further floral presentation of the entire population of a conserved species. We used electronic digital calipers (Fisher Scientific Model I4-648-17) to record floral measurements. Flowers that were trampled, or eaten partially by unknown foragers, or had their labella punctured and deflated by leafcutter bees (*Osmia* spp., Megachilidae, see below) were excluded from all measurements. The dimensions of the *C. montanum* labella at BME0 ($N = 18$, 2004) and the ECCO sites ($N = 43$, 2006) were compared to measurements of six additional *Cypripedium* spp. in North America and China (Li et al. 2006, 2008; Banziger et al. 2008; Edens-Meier et al. 2008; Zheng et al. 2011; Ren unpublished).

Observations and timing of insect visitors

We observed the behaviour of insects on and in all flowers of *C. montanum* on sunny days at both sites (BME0 2003, 2004; ECCO, 2004, 2006) totalling approximately 110 field hours. Nocturnal visits by the first author were discontinued after an absence of activity following the setting of the sun. Wearing 3× magnification visors, we observed how and when insects entered the labellum through the central, dorsal entrance and whether they first landed on the labellum or on the contrastingly coloured staminode (see Chi et al. 2008). When an insect entered the labellum we observed whether it exited the flower through one of the two rear orifices (see Li et al. 2006, 2008ab). When insects

escaped from a flower we recorded whether they flew away and left the site or whether they were observed to visit a second flower on the same inflorescence, or a flower on another inflorescence in the same colony (see Li et al. 2008a). In 2004 we removed the labellum from five opening buds in the BMEO site before the labellum expanded (see above) to see if insects were attracted to the column of the flower in the absence of the labellum and whether they could remove pollen masses in the absence of the labellum.

We used stopwatches (Sports Time II Chronograph) to record how long it took individual insects to escape from a *C. montanum* flower via the rear exit openings at both sites. We started timing after we saw the insect fall into or fly into the labellum. Timing was stopped after we saw the same insect emerge completely from a rear exit.

Collection and processing of insect-visitor, cross-referencing their pollen loads and measuring the insect specimens

Insect visitors were collected as they visited flowers of *C. montanum* at both sites. Each specimen was killed separately in killing jars containing fumes of ethyl acetate. The specimen was then removed with forceps, placed on a glass slide and bathed in 2-3 drops of ethyl acetate washing pollen grains from the body and/or we scraped the body with the tip of a metal probe to dislodge sticky pollen masses. The solvent on the slide was allowed to evaporate and then the pollen residue was stained with Calberla's solution (Ogden et al. 1974) for five minutes before covering it with a glass cover slip. The dried insect was pinned and labelled. The glass slide was given the same label code as the insect specimen to co-reference insect and pollen identification. The slide was allowed to dry for a minimum of 24 hours before viewing and identifying contents under a light microscope. To facilitate identification of pollen, other than the grains of *C. montanum*, we made a pollen library of grains derived from anthers of co-blooming species flowering at and adjacent to the same study sites. As more than one insect was euthanized in the same jar on the same day, the pollen of a particular species was only scored as present on an insect when we counted >24 grains on the same slide that had the same shape, size, number of apertures and exine sculpturing (see Bernhardt & Weston 1996). There were four insect collection categories.

1) Insects Caught Outside the Flower. At the BMEO site in 2003 we noticed that a large number of insects either perched on the floral organs or hovered a few centimeters around a fully opened *C. montanum* flower, but were never observed actually entering the labellum. These insects were caught to determine whether any carried pollen masses of *C. montanum* during a previous but unobserved visit.

2) Dead, Dying, or Struggling Insects. At the BMEO site in 2003 and the ECCO site in 2006 we examined labella before 10:00 AM each morning and removed the dead and/or inert corpses of insects. The corpses were checked for the presence of *C. montanum* pollen.

3) Potential Pollinators. At the BMEO (2003, 2004) and ECCO (2004, 2006) sites we collected insects observed

to enter the labellum through the large, dorsal entrance and leave via one of the two basal openings. As specimens were collected only after they emerged from a basal opening, some were timed (see above) and their entrance-exit times were cross-referenced with their entomological identification, physical dimensions and pollen load analysis.

4) Visitors to *Fragaria vesca* var. *bracteata*. At the BMEO site on 6/6/03 we noted that small bees appeared to alternate their visits to the flowers of *C. montanum* with visits to clumped populations of *F. vesca* var. *bracteata*. We collected insects on flowers of *F. vesca* from 6/6/03 – 6/10/03 to determine how many specimens also carried pollen masses of *C. montanum*.

We measured some insect specimens caught at the ECCO site in 2006 using the same digital calipers used to measure floral architecture. This included the length of the insect from between its frons to the cercus (terminus) of its abdomen (mouth parts were not measured). As insects must squeeze through the opening of one of two basal openings, we also measured the width of the insect by measuring its widest part. In some specimens, we recorded the width of the widest segment at the base of its abdomen (e.g. *Lasioglossum*) while in others we recorded the width of the head or thorax (e.g. *Osmia*). All insect specimens were sent to C.D. Michener for identification and deposition in the Snow Entomological Museum at the U. of Kansas (Lawrence, Kansas).

Statistics

The mean number of flowers per scape at BMEO was compared between 2003 and 2004 using a t-test. The mean number of flowers and fruits (capsules) per scape at the ECCO site in 2004 and 2005 were compared in the same way. The same tests were used to compare the time bees spent inside *C. montanum* flowers at the BMEO site (2004) vs. the ECCO site (2006). Welch's variant was used to account for differences in the variance among sites, and two t-tests were run, one with an anomalously long data point at the BMEO site, and one with that point excluded.

Linear regression was used to determine if there was a relationship between the insect measurements and the time spent in flowers. T-tests were used to determine if there were relationships between the presence of orchid massulae (irregular smears or globs of pollen) on the body and bee length, and the presence of orchid massulae on the body and bee width. Bee lengths and widths were log transformed to follow a normal distribution.

RESULTS

Number of flowers/stem and reproductive success

In 2003 and 2004 stems produced one to three flower buds at the BMEO site. In 2003 the average number of flowers/scape was 2.0 ± 0.57 (mean $\pm$ sd, $N = 40$). In 2004 ($N = 40$) there was an average of 2.02 ± 0.53 flowers/stem. No difference was detected between the mean number of flowers/stem for two seasons ($t = -0.81$, $df = 77$, $P = 0.43$). Likewise, the mean number of flowers/scape at the ECCO site varied from as low as 1.46 in 2005 to as

Table 1. Census of flowering and fruiting scapes at ECCO (2004 – 2006).

Year	Scapes	Flowers	Flowers/Scape	Capsules	Capsules/Flower
2004	103	168	1.63	87	0.52
2005	206	301	1.46	164	0.54
2006	139	216	1.55	142	0.66

high as 1.63 in 2004 (Tab. 1). The conversion rate of flowers into fertilized capsules over three seasons (Tab. 1) ranged from 0.52–0.66.

Floral presentation

Flower buds on the same scape opened acropetally or subsynchronously at both sites. Flower buds nodded but, as the dorsal sepal unclasped itself from the labellum and became increasingly erect, the pedicel also bent upwards until the open flower was horizontal to its scape (Fig. 1). The transition from the full bud (labellum visible but un-inflated; sepals clasp the labellum) to the open flower phase occurred over a period of one to four days (24–96 hours) at both sites. We observed small bees and syrphid flies hovering around the flowers during this period but few actually landed on the flower and, of those that did, none were observed to crawl under the partially separated dorsal sepal to find the dorsal surface of the labellum. During this period, anthers examined under 3× were indehiscent.

In open flowers, at both sites, the sepals and lateral petals were greenish-yellowish brown. The staminode was usually yellow with clusters of red-brownish spots. The inflated labellum was white on the outside. Four to nine purple veins ran centrally and longitudinally along the inner surface of the floor of each labellum (Fig. 1). A strong, pleasantly fruity aroma was discernible from flowers on warm days while they stood in light gaps for 20–30 minutes. It was during this period we witnessed the earliest arrival of foraging insects

including those caught outside the flower and those that entered the labellum. Nectar glands and nectar secretions were not found in the labellum or on any other floral organ during examination under 3× magnification. Anthers examined under 3× were dehiscent often extruding greasy, hanging pollen masses.

Comparative labellum morphometrics

See Tab. 2 for measurements of the fully expanded labellum ($N = 18$ flowers from 18 scapes) at the BMEO site and at the ECCO site ($N = 43$ flowers from 43 scapes). These measurements suggested that the population of *C. montanum* was intermediate in size compared to the larger rounded sacs of *C. tibeticum* (Li et al. 2006) and *C. reginae* (Edens-Meier et al. 2011) and the smaller, keeled sac of *C. plectrochilum* (Li et al. 2008a). Labellum measurements in *C. montanum* were closer to Chinese species pollinated by small-medium sized bees (Banziger et al. 2008).

Self-pollination rates

None of the bagged flowers set fruit. None of the flowers set fruit if the labellum was removed (see below).

Insects caught outside the flowers

At the BMEO site in 2003 we caught 15 insects either perched on open flowers of *C. montanum* or hovering one or two centimeters around the flower (Tab. 3). The small,

Table 2. Comparative of the labellum in *Cypripedium* spp. (mean and standard deviation, in mms).

Species	N	La Length	La Width	La Depth	Entrance Length or Diameter	Entrance Width	Pollinators ¹	References
<i>C. fargesii</i>	20	NA	NA	4.72 (0.80)	6.16 (0.68) ²	NG ³	LS	Ren (unpublished)
<i>C. flavum</i>	30	NA	NA	4.77 (0.00)	8.56 (0.01)	NG ³	BW, Ca	Zheng et al. (2011)
<i>C. flavum</i>	NA	35.0–55.0	30–35.0	NA	10.0–13.0	5.0–9.0	AL	Banziger et al. (2008)
<i>C. montanum</i> (BMEO)	18	26.06 (2.81)	16.13 (2.81)	12.58 (0.97)	8.72 (1.30)	5.89 (0.85)	SMB	
<i>C. montanum</i> (ECCO)	43	25.54 (3.68)	15.87 (2.28)	13.33 (1.65)	7.91 (1.20)	5.89 (0.81)	SMB	
<i>C. plectrochilum</i>	50	NA	NA	1.22 (0.00)	2.80 (0.00)	NG ³	La	Li et al. (2008)
<i>C. reginae</i>	44	40.79 (3.30)	29.62 (2.92)	24.98 (3.28)	11.96 (2.04)	16.19 (2.92)	LMB	Edens-Meier et al. (2011)
<i>C. tibeticum</i>	20	49.67 (0.04)	37.01 (0.06)	30.37 (0.02)	12.87 (0.01)	NG ³	BG	Li et al. (2006)
<i>C. yunnanense</i>	NA	35.0–50.0	25.0–32.0	20	9.0–13.0	6.0–10.0	La	Banziger et al., (2008)

¹Pollinators = AL (*Andrena* spp.; large body), BG (*Bombus* gynae), BW (*Bombus* workers), Ca (*Calliphora vomitoria*; Calliphoridae), La (*Lasioglossum* spp.), LMB (Large, mixed bees), LS (*Lucilla* sp., Syrphidae), SMB (Small-medium sized bees); ²*C. fargesii* Entrance Length: $N = 32$;

³NG = Not given as Labellum entrance measurement presented in preceding column as a diameter

Table 3. Pollen load analyses of insects collected on or in *Cypripedium montanum* at the BMEO site in 2003.

Insect Taxon	Pollen Types*							
	N	Aster	Cl	Cm	Pi	Ros	UM	No Pollen
Insects Caught Outside Flowers of <i>C. montanum</i>								
Coleoptera								
<i>Anthaxia aenogaster</i>	1	0	0	0	0	0	0	1
Hymenoptera (Apoideae)								
<i>Andrena prunorum</i>	1	0	0	0	0	1	0	0
Eumeninae (wasp)	1	0	0	0	0	0	0	1
<i>Halictus tripartitus</i>	1	1	0	0	1	0	0	0
<i>Hylaeus ellipticus</i>	1	0	0	0	0	0	0	1
<i>Lasioglossum</i> (Evylaeus) sp. a	2	0	0	1	2	0	0	1
<i>L.</i> (Evylaeus) sp. b	1	1	1	1	0	1	1	0
<i>L. olympiae</i>	3	3	2	1	0	3	1	0
<i>Nomada</i> sp.	1	0	0	0	0	0	0	1
<i>Osmia</i> sp.	1	0	0	0	0	0	0	1
<i>Panurginus ineptus</i>	1	0	0	0	0	1	0	0
Totals	14	5	3	3	3	8	2	7
Dead, Dying, or Struggling Insects								
Diptera								
<i>Brachyopa</i> SP.	1	0	0	0	0	1	0	0
<i>Eulonchus</i> sp.	1	0	0	0	0	1	0	0
<i>Psilota</i> sp.	2	0	0	0	0	1	0	1
Hymenoptera								
<i>Osmia simillina</i>	1	0	0	0	0	0	0	1
Totals	5	0	0	0	0	3	0	1
Potential Pollinators (entered labellum dorsal crater and exited via rear orifice)								
Hymenoptera (Apoideae)								
<i>Andrena</i> (Micrandrena) sp.	1	0	0	0	0	0	0	1
<i>A.</i> sp. b	2	0	0	1	0	1	0	1
<i>Halictus rubicundus</i>	1	0	0	0	0	1	0	0
<i>Lasioglossum</i> (Dialictus) sp.	1	0	0	1	0	0	0	0
<i>L.</i> (Evylaeus) sp. a	3	0	1	1	0	2	0	0
<i>L.</i> (<i>Lasioglossum</i>) sp	1	0	0	1	0	0	0	0
<i>Lasioglossum olympiae</i>	4	0	1	4	0	4	0	0
<i>Panurginus ineptus</i>	4	0	0	0	0	4	0	0
Totals	17	0	2	8	0	12	0	2

*Aster = Asteraceae; Cl = *Clarkia*, Cm = *Cypripedium montanum*; Pi = *Pinus*, Ros = Mixed unidentified Rosaceae (including *Fragaria*, *Physocarpus capitatus*, *Potentilla*, *Rosa*, *Rubus parviflorus*, UM = Unidentified monocot (probably *Smilacina racemosa*)

black beetle, *Anthaxia aenogaster* (Buprestidae), was extremely common but was collected only once for identification purposes. They were often observed *in copula*, but we did not observe them foraging on floral organs. Bees were collected on the dorsal sepal or lateral petals and were often observed cleaning their legs. Only three bees captured in this way carried massulae of *C. montanum* and all three were bees in the genus *Lasioglossum* s.l. (Halictidae). These

three bees also carried the pollen of other co-blooming species. A single specimen of *Lasioglossum* (*Evylaeus*) sp. carried the pollinia of *C. montanum* mixed with the grains of five other pollen forms.

Dead, dying, or struggling insects

A total of nine dead insects, representing three Orders (Coleoptera, Diptera and Hymenoptera) were collected at

Table 4. Pollen load analyses of insects collected in the labella of *Cypripedium montanum* or as they exited via the rear orifices at the BME0 site in 2004.

Insect Taxon	Pollen Type*								
	N	Aster	Cal	Cm	Ger	Ros	Ss	UEU	No Pollen
Dead, Struggling Insects									
Coleoptera									
<i>Anthaxia aeongaster</i>	3	0	0	0	0	0	0	0	3
Hymenoptera (Apoideae)									
<i>Bombus fervidus</i>	1	0	0	0	0	1	1	0	0
<i>Eucera</i> (Synhalonia) sp.	1	0	0	0	0	0	0	0	1
Totals	5	0	0	0	0	1	1	0	4
Potential Pollinators (entered labellum dorsal crater and exited via rear orifice)									
Hymenoptera (Apoideae)									
<i>Andrena</i> (Micrandrena) sp	1	0	0	1	0	1	1	0	0
<i>Andrena</i> sp. 1	1	0	0	1	0	0	0	1	0
<i>Andrena</i> sp. 4	1	0	0	1	0	1	0	0	0
<i>Ceratina acantha</i>	8	0	1	3	0	2	1	0	3
<i>Halictus confusus</i>	2	0	0	0	0	2	0	0	0
<i>H. tripartitus</i>	7	4	0	3	1	6	1	0	0
<i>Lasioglossum</i> (Dialictus) sp.	3	0	0	1	0	0	0	0	2
<i>L.</i> (Evyllaeus) sp	11	2	0	4	0	8	0	0	2
<i>L. tegulariforme</i>	1	0	0	1	0	0	1	0	0
<i>Osmia</i> (Melanomia) sp	1	0	0	0	-	1	0	0	0
Totals	36	6	1	15	1	21	4	1	7

*Aster = Asteraceae, Cal = *Calochortus elegans*, Cm = *Cypripedium montanum*, Ger = *Geranium*, Ros = Mixed unidentified Roaceae, Ss = *Smilacina stellata*. UEU = Unidentified eudicotyledon

BME0 in 2003 and 2004 (Tables 3 and 4) while an additional 25 dead/inert specimens were removed from labella at the ECCO site in 2006 (Tab. 4). As above, the beetle, *Anthaxia aeongaster* (Buprestidae) was observed in great numbers at BME0 and collections presented here do not represent beetle density while the orchid population flowered. These beetles crawled inside the labellum through the dorsal opening and spent hours in the labellum. They were never observed to emerge from the flower via the two basal openings and never carried massulae. Pollen load analyses of inert or dying specimens showed they carried the grains of other co-blooming species at the site especially *Pinus* spp. True flies found dead in the labellum (Tab. 3) at BME0 and the ECCO site (Tab. 5) represented members of the flower-visiting family, Syrphidae, but they did not carry the massulae of the host flower.

On 6/12/2003 at the BME0 site we observed an 11 mm long female of *Osmia simillina* (Megachilidae) enter a labellum. After > six minutes the insect used its mandibles to tear a hole in the rear of the labellum. We caught it in a killing jar as it exited. It didn't carry massulae of *C. montanum*. The following day, this damaged labellum deflated. A 12 mm long male of *Eucera* (Synhalonia) sp. (Apidae) was collected at the BME0 site on 5/25/2004

while it was struggling within a labellum. It did not carry massulae. A dormant (legs still twitching), 6 mm long worker of *B. fervidus* (Apidae) was found in a labellum at BME0 on 5/25/2004 and it did not carry massulae. In 2003 and 2004 we observed that a few *Lasioglossum* spp. escaped from the labellum in a novel way. The bee grabbed the tip of the staminode, which protrudes downwards partway into the labellum. Using their first pair of legs to clutch the staminode, the insect climbed out of the labellum, thus avoiding any contact with the stigma or anthers. These bees immediately vacated the site.

On 5/18/2004 we found a withered flower at the ECCO site that contained a dead, female, bee identified as a *Lasioglossum* sp. The bee's head and thorax protruded from one of the flower's basal openings suggesting it died because it could not extricate its abdomen. Both the bee and the massulae it carried were infested with unidentified ascomycetes. The 19 dead, dying, or struggling insects collected at the ECCO site in 2006 (Tab. 5) were bees representing four families; Andrenidae (*Andrena*), Apidae (*Bombus*), Halictidae (*Lasioglossum*) and Megachilidae (*Megachile* and *Osmia*). All specimens were found dead in the flower except for the gyne of *Bombus bifarius*. This 14.32 x 2.54 mm insect landed on the labella of four flowers

Table 5. Pollen load analyses of insects collected at the ECCO site combining dead-struggling specimens with specimens that exited through the basal openings.

Insect Taxon	Pollen Types*								
	N	Aster	Cean	Cm	Pi	PL	UM	RM	No Pollen
Dead, Struggling Insects									
Coleoptera									
<i>Anthaxia aenogaster</i>	5	1	3	0	1	0	1	1	1
Diptera									
Unidentified syrphid	1	0	0	0	1	0	0	1	0
Hymenoptera (Apoideae)									
<i>Andrena</i> sp. I	3	1	3	0	3	1	0	2	0
<i>Bombus bifarius</i>	1	1	1	0	0	0	1	0	0
<i>Lasioglossum athabascense</i>	2	2	2	0	1	0	0	2	0
<i>L. (Evylaeus)</i> sp.	2	1	2	0	1	0	0	1	0
<i>L. sysmbrii</i>	2	1	1	0	1	0	1	1	0
<i>Megachile gilae</i> M	1	1	1	0	0	0	0	0	0
<i>Osmia albolateralis</i>	1	0	1	0	1	0	0	0	0
<i>O. ednae</i>	1	0	1	0	1	1	0	0	0
<i>O. malina</i>	1	1	1	0	1	1	0	0	0
Totals	19	9	16	0	11	3	3	8	1
Insects that Exited Via Basal Openings									
Hymenoptera (Apoideae)									
<i>Andrena</i> sp. I	2	0	2	1	0	0	1	0	0
<i>Lasioglossum athabascense</i>	4	2	4	1	1	1	1	3	0
<i>L. (Dialictus)</i> sp.	7	1	3	2	0	0	0	2	3
<i>L. (Evylaeus)</i> sp.	16	0	14	14	2	0	0	6	0
<i>Osmia ednae</i>	1	0	0	0	0	0	1	0	0
<i>O. (Melanosmia)</i> sp.	1	1	0	0	1	0	0	0	0
Totals	31	4	23	18	3	1	3	11	3

*Aster = unidentified members of the Asteraceae, Cean = *Ceanothus velutinus*, Cm = *C. montanum*, Pi = *Pinus*, PL = papilionoid legume (probably *Lupinus sericeus*) UM = Unidentified monocotyledon, RM = Mixed ticolporate grains (including *Aquilegia formosa*, *Fragaria vesca*, *Potentilla gracilis*, *Rosa gymnocarpa*, *Ranunculus* sp., *Rubus parviflora*).

on four plants before it squeezed itself into the labellum of the fourth flower and was unable to exit. All specimens collected were female excluding the male, *Megachile gilae*. While all the specimens carried the pollen of co-blooming plants at the Deschutes site none carried the massulae of *C. montanum* suggesting this was the first and last time most of these bees visited the labellum of this orchid.

Potential pollinators

Insects were observed entering the labellum of fully opened (first day) flowers of *C. montanum* at both sites over three seasons (Fig. 2 and 3). Bees were observed entering labella on warm, sunny, often cloudless days but only after the flowering stem bearing the flowers stood in a light gap for 20-60 minutes and produced discernible scent. We did not see insects enter a labellum while the flower stood in shade at either site. Depending on the site, and the amount

of canopy cover, a labellum of *C. montanum* could be entered by bees as early as 10:24 AM and as late as 4:30 PM. Clumped flowering stems remaining open over a seven-day period at BMEO received daily visits from bees entering labella over periods from one to five hours. Bee visitations to labella of plants at the ECCO site began as early as 11:25 AM and generally ceased prior to 4:30 PM.

The first time we observed an insect entering the large, dorsal entrance on a labellum and exiting via one of the two rear orifices was at BMEO on 6/6/2003. This bee carried a dorsal deposition of pollen mass on its thorax as it exited the flower (Fig. 3, 4). The specimen was later identified as a female *Lasioglossum (Dialictus)* sp. (Tab. 3). Combining four seasons of observations at both sites we observed this process >100 times. Bee visits were so numerous at the ECCO site between 10:24 AM-3:10 PM from 6/13-



Figure 2. Unidentified bee inside inflated labellum. Note that the bee's head is not visible as it is obscured by the staminode (Photograph by Nan Vance).



Figure 3. The same bee as in Fig. 1 emerges from the basal opening. Note the yellow pollen smear deposited on the thorax. Also, note that the bee escapes the interior of the labellum while clutching the lateral petal (Photograph by Nan Vance).



Figure 4. Pinned specimen of *Lasioglossum* sp (collected in 2003) wearing the entire contents of an anther cell on the dorsum of its thorax (Photograph by Andrew Huber).

6/15/2003, we commonly observed two bees in the same labellum at the same time. In all cases of “double” visits observed, the two bees exited the flower via the rear, basal openings. However, these bees left one-by-one as the escape path under the stigma did not appear to accommodate two bees at the same time.

Bees at the BME0 site entered the labellum and exited through the basal openings of the flower within an average of 222.84 ± 297.83 seconds ($N = 38$) when an abnormally long point (1945 seconds) was included and 176.30 ± 80.98 ($N = 37$) when that point was excluded. The mean time bees spent in flowers at the ECCO site in 2006 was 179.25 ± 133.17 seconds ($N = 28$). However, there was no difference in the time spent in flowers between these two sites whether that solitary and abnormally long point at BME0 was included (Welch's t -test, $t = -0.8$, $df = 54$, $P = 0.4271$) or excluded ($t = 0.10$, $df = 41$, $P = 0.9179$).

The behaviour of bees entering the labellum via the dorsal entrance and escaping via one of the two basal openings varied at inter- and intra-specific levels at both sites but we did not observe bees landing directly on the staminode, at either site, if the flower's labellum was intact (see below). The shorter bees (<7 mm in length) later identified as members of the Andrenidae (*Andrena*, and *Panurginus* spp.), Apidae (*Ceratina acanthi*) and Halictidae (*Halictus*; and some *Lasioglossum* [*Evyllaesus*] spp.) were more likely to enter the labellum by flying directly into the large, dorsal entrance and landing on the purple veins of the labellum floor (Figures 1, 2). Longer bees (7–10 mm) mostly

Lasioglossum spp. and *Osmia* spp. (Megachilidae), were more likely to land on the white outer surface of the labellum or, less frequently, on lateral petals before crawling onto the labellum. As these longer bees crawled towards the rim of the dorsal entrance the more likely they would fall into the labellum sac. In both shorter and longer bees, though, the added weight of the insect inside the labellum usually caused the flower to nod on its pedicel and the bee lost contact with the floral epidermis and rolled to the anterior (toe) of the labellum. Each bee usually turned around and crawled upwards. When it passed under the stigma it was lost from view but a bee could slide backwards into the labellum toe again. Every time a bee started over it had to pass under the stigma. When the largest bees (9–10 mm in length) crawled up through a narrow, hair-lined, exit canal (formed by the staminode and the incurved labellum) and attempted to push through one of the rear exit holes, they produced a distinctive buzz, reminiscent of the whining sound made when a female bee applies thoracic vibration to a cluster of porose-poricidal anthers (see Bernhardt 1996). On 6/7/2003 at the BME0 site, a bee later identified as a female of *Halictus triparitus* crawled under the stigma 12 times and poked its head through the basal holes four times before it finally escaped from the flower nine minutes later. In most cases observed, pollen was not deposited on the bee until the dorsal surface of the insect's thorax (Fig. 3 and 4) contacted the dehiscent anther while the insect extricated itself from a basal opening. Some bees <7 mm in length had pollen smeared on their heads and compound eyes instead of on the dorsum of the thorax. Using $3\times$ magnification we noted that anthers of each orchid flower appeared to be emptied of pollen masses within the first two days of bee visitation at both sites. Examination of specimens of long (9–10 mm long) specimens in the genus, *Lasioglossum*, showed that some bees carried off the entire contents of an anther locus following passive contact with the dehiscent anther as they attempted to escape from either of the basal openings (Fig. 4).

Two separate events could occur after the bee had fully extricated itself from the basal opening. In most cases the bee crawled onto one of the lateral petals or the dorsal sepal. We observed the insect making cleaning motions to its head, abdomen and first pair of legs before flying away. In a few cases the bee fell to the ground upon escape or fell onto one of the lower leaves of the orchid. In this case, the insect often remained motionless for 30 seconds to several minutes before it cleaned itself and flew away. After three seasons of fieldwork we have only one observation of a bee visiting and exiting more than one *C. montanum* flower in the same clump during the same visitation bout. This occurred at BME0 on 6/24/2004 from 11:24–11:42 AM. The unidentified and uncollected bee visited two flowers on the same inflorescence. All other bees, following their exit from the flower, flew away from the flowering scape until lost from view.

During all seasons we observed bee visitations, we never saw a bee land on the contrastingly coloured staminode before it entered the labellum. We only observed bees landing directly on the staminode after the labellum was removed by hand. These bees probed the upper surface of

the staminode with their mouthparts and then crawled around the column often contacting the exposed stigma. No transfer of pollen masses to these stigmas was ever observed. Female bees did not appear to recognize the dehiscent anthers as a potential source of pollen.

Mean bee lengths and widths

The mean length and width of bees ($N = 31$) of potential pollinators to *C. montanum* at the ECCO site was 6.85 (sd = 0.89) and 2.57 (sd = 0.49) respectively. For statistical analyses, bee lengths and widths at the Deschutes site (2006) were log transformed to follow a normal distribution. There was no relationship between insect size (length, width and length $\times$ width) and the time spent in flowers (linear regression, $F_{3,24} = 1.62$, $P = 0.2111$, $R = 0.17$).

Potential pollinators, bee diversity and pollen load analyses

A total of 84 potential pollinators were collected over four seasons at three sites (Tables 3-5) and 51 specimens (>0.60) belonged to the genus *Lasioglossum* sl. However, bee diversity varied at the BME0 site over two seasons and between the BME0 (2003 and 2004) and ECCO sites (Tables 3-5). *Panurginus ineptus* (all males), *Halictus rubicundus* (female) and *Lasioglossum olympiae* (all females) collected in 2003 were not found in 2004. Likewise, *Ceratina acanthi* (females), *Lasioglossum tegulariforme* (female) and *Halictus confusus* (females) collected in 2004 were not caught in 2003. While collections of *L. athabascense*, exiting flowers of *C. montanum* were made only at the ECCO site, in 2006 we failed to catch any members of the genera *Ceratina*, *Halictus*, *Panurginus* spp. at this site (Tab. 3). The ratio of potential pollinators carrying pollen masses of the host flower on their thoraces or heads was slightly higher at the ECCO site in 2006 (0.58) than at BME0 in 2003 (0.47) or 2004 (0.41). The presence of orchid massulae on a bee and the bee's length ($t = -0.99$, $df = 30$, $P = 0.3313$), or width ($t = -1.45$, $df = 30$, $P = 0.1584$) were not related at the ECCO site.

Of the potential pollinators the majority carried the pollen of at least one other co-blooming taxon that offered floral nectar and/or granular pollen (Tables 2-4; Fig. 5); 0.75 (BME0, 2003), 0.93 (BME0, 2004) and 1.0 (ECCO, 2006). Pooling results at BME0 in 2003 and 2004 we noted that >0.62 ($N = 53$) of the bees visited the pollen and/or nectar secreting taxa of at least one member of the Family, Rosaceae (*Fragaria vesca* var. *bracteata*, *Physocarpus capitatus*, *Potentilla glandulosa*, *Rosa* sp., *Rubus parviflorus*) and *Smilacina stellata* (Asparagaceae) before they began visiting flowers of *C. montanum*. On 5/25/04 we observed a *Lasioglossum*-sized bee foraging on flowers of *S. stellata* over a 20-minute period. It interrupted foraging on this species four times to land on labella of adjacent flowers of *C. montanum* but it never entered the labellum. In contrast, pollen load analyses of *C. montanum* collected at the ECCO site indicated that >0.74 of the potential pollinators to *C. montanum* also visited the flowers of the nectar-secreting shrub, *Ceanothus velutinus* (Rhamnaceae; Tab. 5).



Figure 5. Unidentified bee foraging in flower of *F. vesca* after it exited a flower of *C. montanum* approximately two meters away. Note the smear on the dorsum of the thorax (Photograph by Nan Vance).

Bee diversity on *Fragaria vesca* var. *bracteata*

Bees foraged on flowers of *Fragaria vesca* var. *bracteata* from 9:15 AM-3:50 PM depending on the time of day in which clumps stood in light gaps. A total of 30 bees were caught on flowers of *F. vesca* var. *bracteata* at BME0 in 2003 (Tab. 5). Seven bees (0.23) carried pollen masses of *C. montanum* (Tab. 6) including two females of *Nomada* sp. (Apidae), three males of *P. ineptus* (Andrenidae) and one female specimen of *Andrena* (*Microandrena*). While these three bee taxa were potential pollinators of *C. montanum* (Tab. 6) at the same site and season, specimens of the same species collected after they exited the orchid flowers failed to carry pollen masses.

DISCUSSION

Labellum of *C. montanum* vs. other *Cypripedium* spp.

Based on earlier and ongoing studies, labellum dimensions in the genus *Cypripedium* appear to correlate with the dimensions of their respective pollen vectors. In particular, they may reflect the canalization of some flower-insect interactions. While the big labellum of *C. tibeticum* accommodates *Bombus* gynes, and some of their smaller workers, the gynes appear to be the only pollinators of this species (Li et al. 2006). Likewise, *C. plectrochilum* has a much smaller and keeled labellum. It is well visited by insects but the only pollen vectors collected, to date, are a few of the smaller species in the genus *Lasioglossum* (Li et al. 2008a). *Apis cerana*, a large bee, couldn't enter the flower of *C.*

Bee Taxon	Pollen Types*				
	N	Cm	Frg	MRos	No Pollen
<i>Andrena</i> sp. a (f)	2	0	2	1	0
<i>Andrena</i> (<i>Microandrena</i>) sp. (f)	3	1	3	3	0
<i>Ceratina acantha</i> (m)	1	1	1	1	0
<i>Nomada</i> sp. (f)	10	2	8	5	2
<i>Osmia</i> sp. (m)	2	0	2	2	0
<i>Panurginus ineptus</i> (m)	12	3	11	11	1
Totals	30	7	27	23	3

Table 6. Bees collected on *Fragaria vesca* var. *bracteata* at the BME0 site in 2003.

*Cm = *C. montanum*, Frg = *F. vesca* var. *bracteata*, MRos = Mixed Rosalean/Ranalean pollen (but no *Fragaria*); (m) = male, (f) = female

plectrochilum while smaller, slenderer insects (e.g. ants) exited via the rear openings of the flower without contacting the dehiscant anthers. Labellum dimensions of *C. reginae* fall in between *C. tibeticum* and *C. plectrochilum* but are conspicuously larger and deeper than *C. montanum*. Only medium-large bees in the families Apidae and Megachilidae carried the pollen of *Cypripedium reginae*. When small-bodied, halictid bees exited *C. reginae* they always failed to contact the dehiscant anthers while the huge gynes of *Bombus* spp. couldn't fit through the rear exits and never contacted the dehiscant anthers either (Edens-Meier et al. 2011). Therefore, it's important to note that the dimensions of *C. montanum* did not permit the physically largest specimens of *Bombus*, *Osmia* and *Eucera* species to exit the flower. *Cypripedium montanum* is more likely to accommodate small-medium sized bees as in *C. yunnanense* (Banziger 2008).

Was *C. montanum* pollinator-limited at either site?

Results indicated that neither population was pollinator-limited over the seasons studied, even though a number of bees of appropriate size appeared to escape from the labellum sac at the ECCO site (see above). As *C. montanum* could not self-pollinate in the absence of insects we must conclude that the previous 94% rate of pollen deposited on stigmas reported by Edens-Meier et al. (2010), from both sites, was based on insect-mediated pollination. Of greater importance, as almost all bees left the site after freeing themselves via the basal openings we should conclude that the majority of bee-mediated pollinations at both sites were probably cross-pollinations (xenogamy) instead of vector-mediated, self-pollinations (to geitonogamy or autogamy). Small- to medium-sized female bees (5-10 mm in length) with polylectic and/or polyphagous foraging behaviour were the dominant pollen vectors at both sites. At both sites these massulae-carrying bees shared similar time periods for escaping from the rear of the flower although these bees represented a broad range of sizes and taxa. This appears comparable to a review of bee pollination in the North American species complex, *C. parviflorum* (see Argue 2011), closely allied to *C. montanum* s.s. (Li et al. 2011).

Were all visitors to *C. montanum* prospective pollen vectors?

At both sites, flowers of *C. montanum* attracted insects from as many as three insect orders. However, small beetles, flies and some Hymenoptera obviously either lacked the size, and/or behavioural patterns, and/or physical strength to exit the flower via the basal openings. Some bees (e.g. *Bombus*, *Eucera* and most *Osmia* spp.) were too large to make a legitimate, rear exit escape (see above). Other taxa (e.g. *Andrena prunorum*, eumenid wasp, *Hylaeus ellipticus* etc.), were infrequent visitors that rarely, if ever, entered labella. While the floral architecture of *C. montanum* accommodated an unusually wide variety of small-to medium-sized bees some species alternated between merely perching on the flower and actually entering and exiting the labellum (e.g. *Lasioglossum* spp., *Nomada* sp. and *Panurginus ineptus*). It's likely that some bees did not enter these flowers a second time due to the absence of rewards and any negative stimuli encountered during the escape period.

In this respect, the pollination ecology of *C. montanum* paralleled that of *C. plectrochilum* (Li et al. 2008a). While an unusually diverse range of insects visited both *Cypripedium* spp., all members of the Diptera, Lepidoptera and some Hymenoptera (ants, large bees) lacked appropriate physical dimensions and/or behavioural patterns and did not carry the orchid's pollen either. As *C. montanum* is bee-pollinated it is not surprising that the labellum sac is a death trap for some flies (Tab. 1). However, while the labellum dimensions of *C. montanum* accommodated an unusually broad diversity of small- to medium-sized bees, entrapment proved fatal to bees of varying sizes especially at the ECCO site.

Leaving a *C. montanum* flower via the basal opening did not guarantee successful transference of pollen masses to the bee each time. The flowers appeared to run out of massulae within one to two days after opening even though an individual flower could live from seven to 21 days depending on the site (Edens-Meier et al. 2010). As we could not measure bee depth vs. the distance between the receptive stigma and the floor of the labellum without destroying flowers of a protected species it was not possible to

determine which bee species were more likely to leave pollen on the receptive stigma as they crawled under it.

Our observations of bees landing on the staminode, only after the labellum was excised, tended to confirm the theory of Edens-Meier et al. (2014) that the pigmentation pattern on the interlocking staminode-labellum mechanism may represent part of a super-normal stimulus in some *Cypripedium* spp. That is, while the two, differently coloured patterns on the staminode both contrast with this white labellum, the bee probably sees both patterns as contiguous. Together, staminode and labellum floor patterns grade together forming an irregular blotch (*sensu* Kevan & Dafni 1996) often associated with flowers with bilateral symmetry. The pattern canalizes the bee's movements and it lands on, or near, the labellum floor dependably until the labellum is removed. Only then did the bee land on the colour pattern on the staminode as the now missing sac changed the floral symmetry to radial. We do not suggest that this interpretation fits all *Cypripedium* spp. as pigmentation patterns on the staminode and in the labellum vary broadly at inter- and intraspecific levels (e.g. Li et al. 2006, 2008ab and see colour plates in Edens-Meier et al. 2011).

Did pollen vector diversity vary between seasons and sites?

Female bees in the genus, *Lasioglossum* s.l. (Halictidae) carried pollen masses at both sites over three seasons and appeared to be the dominant, but not exclusive, dispersal agents of orchid pollen. Unfortunately, this genus consists of over 1100 species worldwide and it was not possible to identify each specimen to species (C.D. Michener, pers. comm.). We did find, though, that the *Lasioglossum* specimens, captured after they left the basal openings, represented at least three subgenera. Bees in the genus *Halictus* (Halictidae) and in the families Apidae and Andrenidae also carried pollen masses so *C. montanum* exploits both long tongue (Apidae) and short-tongue (Andrenidae, Halictidae) bees, of similar sizes, in the absence of floral nectar. It is clear, though, that the diversity of legitimate pollen carriers varied between 2003 and 2004 at the BMEO site and the diversity of pollen carriers at BMEO differed from the 2006 collection at ECCO. At BMEO, *C. acanthi*, *L. olympiae*, *L. regulariforme*, and *P. ineptus* were collected exiting the flowers for only one season each. *Halictus tripartius* was collected once outside the flowers of *C. montanum* in 2003, and it carried no pollen masses. However, seven specimens of *H. tripartius* were caught exiting the flowers in 2004 and three carried the orchid's pollen. Likewise, members of the genera *Halictus*, *Nomada* and *Panurginus* were never caught at ECCO in 2006. At that site, four specimens of *L. athabascense* made their only appearance as orchid pollen vectors while some *Osmia* spp. were physically small enough to pass through the rear exit of the flowers. The collection of bees on co-blooming *Fragaria vesca* showed that interpreting the role of a bee as a carrier of *Cypripedium* pollen should include specimens taken from co-blooming flora. It's possible to miss less frequent visitors to the orchid that can carry the pollen masses, when they visit, but prefer to forage on co-blooming species offering

nectar and/or pollen. Note, for example, that in 2003 three bee taxa (see above), caught while exiting *C. montanum*, did not carry the orchid's pollen but specimens of the same taxa, caught on *Fragaria vesca* did carry pollen of *C. montanum*.

The exploitation of a wider variety of small-to medium-sized bees must contribute to the increased frequency of pollination in *C. montanum* and its high fruit set assessed by Huber (unpublished) at BMEO. In contrast, we note that *C. plectrochilum* (small labellum), *C. henryi* (Li et al. 2008b; mid-sized labellum) and *C. yunnanense* (Bänziger et al. 2008; mid-sized) were pollinated exclusively by a few *Lasioglossum* spp. (Bänziger et al. 2008; Li et al. 2008b) and their conversion of ovaries into fruits peaked at 45%, 22% and 21%, respectively. Likewise, one population of *C. flavum* was pollinated only by a few *Andrena* spp. and its highest fruit set ratio was only 9.20% (Bänziger et al. 2008). In China, montane populations of *Cypripedium* spp. often show broadly overlapping distributions and flowering periods (Singchi et al. 1999; Perner & Luo 2007). While a narrower spectrum of pollen vectors may lower chances of interspecific hybridization (Bänziger et al. 2008), it might also depress reproductive success within sympatric *Cypripedium* spp. On temperate Chinese mountains most of the potential pollinators are either not attracted to the colours and odours of one *Cypripedium* species and/or are unable to fit into and/or navigate their floral interiors. As in other angiosperms, a *Cypripedium* species should be in danger of becoming pollinator-limited if resident populations of its few pollinator species decline and/or insect foraging seasons change (Committee on the Status of Pollinators in North America 2007).

What factors support a well-visited food mimic system?

Therefore, the high rate of visitation by prospective vectors of massulae in *C. montanum* was supported by two interlocking factors. First, as described above, the sheer number of flowering stems in bloom combined with their shared modes of floral presentation and architectural dimensions exploited a broad and variable diversity of polylectic/polyphagic foragers at both sites over several seasons. Exploitation of resident, small-to medium-sized bee faunas in *C. montanum* parallels results obtained from multiple sites and seasons in the pollination ecology of Eurasian, *C. calceolus* (Nillson 1979; Kull 1999; Bernhardt & Edens-Meier 2010).

Second, to exploit the broadest diversity of polylectic female bees *C. montanum* must appear to offer nectar and/or pollen as in the majority of non-specific (generalist) frauds (Ackerman 1986; Dafni & Bernhardt 1990). It's unlikely that successful pollination continues throughout the comparatively long floral lifespans of *C. montanum* in the absence of a dependable, co-blooming flora for generalist bees. We suspect that our frequent observations of potential pollinators at both sites were due, at least in part, to the diversity and density of co-blooming species. Flowers offering only pollen (Bernhardt 1996) and/or pollen and nectar rewards were always available at both sites, over the flowering seasons of *C. montanum*, even though floral diversity differed between sites. We also speculate that

habitats rich in food sources for bee offspring could encourage more nesting and increased populations of multi-voltine, bee taxa and one nectariferous species may be sufficient to support adult nutrition. Over 79% of the potential pollinators of *C. montanum* at ECCO collected pollen of *Ceanothus velutinus* (a mass flowering, nectar-secreting shrub). Different *Fragaria* spp. follow the distribution and overlapping flowering periods of some Chinese *Cypripedium* spp. (Li et al. 2008a) serving as food sources for orchid pollinators. Perhaps extensive populations of *Fragaria* spp. should be observed and regarded as insect collection sites more often and used as potential sources of pollinators of some *Cypripedium* spp. when their distributions and flowering periods overlap (see above).

However, we do not suggest that *Cypripedium* spp. with only a few specialist pollinators are always pollinator-limited and typified by low fruit set ratios. *Cypripedium fasciculatum* does not self-pollinate and is dependent on only a few *Cinetus* spp. (Diapriidae; Ferguson & Donham 1999). Lipow et al. (2002) compared natural rates of pollination (stigmas and pistils containing pollen tubes) in *C. fasciculatum*, at three disjunctive sites, over their respective flowering seasons. At one site in Oregon, the frequency of pollinated pistils was 69.2%. This, we argue, was and remains competitive with any *Cypripedium* spp. pollinated by many species of small-to medium-sized bees (Bernhardt & Meier 2010).

Variation of fruit set rates in *C. montanum*

We do not suggest that populations of *C. montanum* must always enjoy high rates of fruit set when their patchy populations co-occur with a diverse bee fauna and a co-blooming, flora offering nectar and/or pollen. The range of this species is from 0 – 2400 m throughout the Pacific Northwest and interior of Alaska, British Columbia and the continental United States (Sheviak 2002). The reproductive ecology of isolated populations is expected to vary between differing microhabitats and years. Nilsson (1979) and Kull (1999) found that, *C. calceolus* was also pollinated by a diverse assemblage of small-to medium-sized bees. The conversion ratio of pistils into capsules in *C. calceolus* was varied from 4–57% according to year and site (see review by Bernhardt & Edens-Meier 2010).

Previous results obtained by Edens-Meier et al. (2010) and Lipow et al. (2002) also suggest that natural rates of insect-mediated pollination in populations of *C. montanum* and *C. fasciculatum* may be higher than rates of matured fruit at the same site and year. There are several reasons why this occurs in *C. montanum*, *C. fasciculatum* and flowering populations of other *Cypripedium* species. First, some flowers in a population of *C. reginae* never set fruit because their maturing buds were ruined by late freezes (Edens-Meier et al. 2011). Second, the majority of orchid species postpone ovule fertilization. Megasporogenesis won't occur unless the flower is pollinated first and fertilization followed by fruit maturation and dehiscence usually takes weeks or months (Arditti 1992). This subjects slowly maturing ovaries to sudden fluctuations in climate, predation and human impact over extended periods. During that maturation period some ovaries of *C. reginae* are eaten by larvae of geometrid moths

(Edens-Meier et al. 2011). Luo Yi-bo (pers. comm.) observed domesticated yaks trampling *C. tibeticum*, *C. flavum*, *C. guttatum* and *C. yunnanensis* at higher elevations in Yunnan from 2003–2006. Browsing and trampling by North American ungulates (Karow, pers. comm. 2005) and cattle (Vance unpublished 2007) may also be severe on remaining populations of *C. montanum*.

Finally, we do not argue with the exhaustive review by Tremblay et al. (2005) that pollinator visits to sexual mimics surpass pollinator visits to food mimics resulting in higher rates of fruit set. We do suggest that floral mimicry in an orchid species needs to be evaluated on a species-by-species and population-by-population basis over several seasons before conservation policies can be established (Bernhardt & Edens-Meier 2010). *Cypripedium montanum* is a food mimic but has a conversion rate of flowers into fruits competitive or even surpassing some sexual mimics (Tremblay et al. 2005) within two sites over its natural range. Fruit set in *C. montanum* appears competitive with some other obligate, outcrossing, angiosperms that offer edible rewards (see review in Bernhardt & Meier 2010). Understanding the fine points of why this occurs (pollinator diversity, co-blooming nectar and pollen flora, interactions between pollinator dimensions and floral architecture, fruit predators, topography, and prevailing climate, etc.) should help us conserve and increase fecundity in remaining populations of this threatened species.

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