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# Pollinator Movement and Pollen Transfer in Hybrid Seed Canola

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UNIVERSITY OF CALGARY

Pollinator Movement and Pollen Transfer in Hybrid Seed Canola

by

Riley Joseph Waytes

A THESIS

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## **Abstract**

Production of hybrid seed canola requires the transfer of pollen from hermaphroditic ('male') to male-sterile ('female') plants, facilitated by managed pollinators (honey and leafcutter bees) and wild pollinators. I assessed pollinators in fields of hybrid seed canola located in Southern Alberta to determine what behaviours and conditions could enhance pollen transfer between male and female plants. I found that managed species were the most common pollinators, that all pollinators exhibited floral constancy to morph (male or female), and that pollinator type, time on flower, pollen on body, and flower of origin influenced pollination effectiveness. Pollinator movement between male and female flowers was positively affected by densities of conspecific pollinators, taxon richness, and relative floral profit. While hybrid seed canola morphs are sexually dimorphic, sufficient quantities of managed pollinators should reduce floral constancy. Wild pollinators can contribute to cross-pollination indirectly by increasing managed pollinator movement via increased diversity.

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## Epigraph

*No matter how intently one studies the hundred  
little dramas of the woods and meadows, one can never  
learn all the salient facts about any one of them.*

- Aldo Leopold, A Sand County Almanac

## Chapter One: **General Introduction**

### **1.1 Context**

Canola (*Brassica napus*), Canadian oilseed rape, is an economically important crop in Canada. Canola production ranged from 16.4 to 18.55 million metric tonnes in 2013-2016, and was second in field crop production only to wheat (Statistics Canada 2016). Two types of canola are grown in Canada—commodity canola, from which oil is extracted, and hybrid seed canola, which produces seeds for commodity canola production. The production of hybrid seed requires the hybridization of two parental lines. Hybridization is beneficial because can result in hybrid vigor, with hybrid plants producing higher yields than parent lines (as shown in foxtail millet, Siles et al. 2004). Because canola is naturally hermaphroditic and can self-fertilize, outcrossing for hybrid seed canola is controlled through the inducement of cytoplasmic male sterility in the ‘female’ parental line. This creates an artificially gynodioecious system of hermaphroditic and female flowers, which are spatially separated into bays. While commodity canola flowers are naturally self-compatible, pollination improves with insect visitation (Williams et al. 1987). Because female flowers in hybrid seed canola cannot pollinate themselves, they depend on pollen transfer from hermaphroditic (‘male’) flowers.

### **1.2 Pollinators in canola and their effectiveness**

The importance of insects facilitating cross-pollination between the parental lines in hybrid seed canola for yield makes it essential to understand the effectiveness of different types of pollinators. Traditionally, managed honey bees (*Apis mellifera*) and alfalfa leafcutter bees (*Megachile rotundata*) pollinate hybrid canola; they can be easily cultured and transported, and their allocation to crops can be controlled by beekeepers and growers. Wild pollinators may also be present; they include both social bees, such as bumble bees (genus *Bombus*), and solitary bee species (Zink 2013), as well as flies.

Previous studies have assessed pollinator contributions to pollination of both commodity and hybrid seed canola. In commodity canola, adding honey bees can increase the yield and quality of seeds (Fries and Stark 1983; Manning and Boland 2000;

Munawar et al. 2009). Wild pollinators, especially bee species, in canola (Ali et al. 2011) and pak choi (*Brassica rapa*; Rader et al. 2009) were at least as effective at delivering pollen to flowers as honey bees, although they were less common than honey bees. Hoverflies (family Syrphidae) can also increase canola yields (Jauker and Wolters 2008). Pollinators that are effective at pollinating commodity canola may also be effective at pollinating hybrid canola. However, the multiple floral morphs in hybrid canola (which are not present in commodity canola) and the inability of female flowers to self-pollinate may alter how pollinators interact with hybrid seed canola and affect how they contribute. This is especially true in the case of pollinators that contribute to commodity canola production by facilitating self-pollination.

Pollination research in hybrid seed canola primarily uses caged experimental plots, in which different pollinator taxa are confined within a tent and can only visit the enclosed lines of male and female flowers. This setup can inflate estimates of the contributions of pollinators. For example, Mesquida and Renard (1981) found that the foraging of honey bees on lines of hybrid seed canola was more intense within tents than outside of them. In their study, honey bees visited both male and female plants, but preferred male plants. Tent-enclosed leafcutter bees increased the seed yield of female canola at increased pollinator densities, but increasing densities of male leafcutter bees also interfered with female foraging in the enclosed space (Soroka et al. 2001). In the same study, bumble bees were weak pollinators of hybrid seed canola, but this was likely an artifact of their poor foraging response to confinement in a tent. Research by Jauker et al. (2012) suggests that solitary bees (such as *Osmia rufa*) could be as effective as honey bees in contributing to pollination, while hoverflies must be at comparatively higher densities than bees to contribute effectively.

Currently, there is a dearth of information on how well pollinators contribute to the pollination of hybrid canola in a natural field setting, as well as whether native pollinators contribute to its pollination. Honey bees and leafcutter bees are used in hybrid seed canola production, although how well they facilitate cross-pollination is not well-established. Wild bees and flies could be contributing to cross-pollination, but it is not clear whether they are even present in the fields (they likely are—see Morandin and

Winston 2006) and how important they are as pollinators. Differences in pollinator contributions are likely due to differences in pollinator morphology and behaviour, so it is also important to understand what factors lead to effective pollen transfer.

Another issue to address in the pollination of hybrid seed canola is the presence of multiple floral morphs. Pollen must travel from a male to a female morph. However, pollinators—especially bees—exhibit floral constancy, restraining their foraging activity to specific floral morphs (Waser 1986). For flower constancy, pollinators must distinguish between flowers, using cues such as colour or size. Sexual dimorphism in gynodioecious crops can decrease pollination efficiency by facilitating floral constancy, thereby decreasing pollinator transfer between parental lines (as in hybrid sunflower; Martin and Farina 2016). The additional separation of flower morphs in bays, rather than their intermixing, makes movement between types of morphs more energetically costly to the pollinator. It is therefore important to quantify the level of sexual dimorphism present between the floral morphs, and how this influences pollinator behaviour.

### **1.3 Factors driving cross-pollination in hybrid seed canola**

If hybrid seed canola promotes floral constancy to morph by pollinators, due to sexual dimorphism or spatial separation between the morphs, there could still be conditions that promote departure from floral constancy. Floral constancy can make foraging more profitable by allowing pollinators to identify and more quickly locate rewarding flowers (see Klosterhalfen et al. 1978 and Dukas and Waser 1994). Bees can—and should—‘sample’, i.e. occasionally visit flowers they are not constant to, to ensure they are not missing better rewards (Heinrich 1979). However, the cost of sampling alternatives in a system with spatial separation between morphs encourages constancy.

The importance of both habitat (resource) availability and the number of competitors is addressed by Fretwell and Lucas’ (1970) Ideal Free Distribution, an evolutionarily stable strategy which determines a distribution of fitness-maximizing competitors at equilibrium. This hypothesis predicts that pollinators should choose foraging places by responding to both habitat (resource) profitability and competitors. As the number of competitors in an area increases, the rewards available to any given pollinator decrease. A highly profitable patch with a correspondingly high number of

pollinators should have the same benefit to a pollinator as a less profitable, but less visited, patch. If foragers were omniscient, they could simply visit whichever patch had the best ratio of total floral rewards to competitors. They are not, so they must sample to determine whether they can improve their intake of rewards, or should stay where they are (Abrahams 1989; Gotceitas and Colgan 1991). This, combined with the constant flux of pollinators travelling in and out of hybrid canola, should lead to more crossing between different floral morphs, and therefore be more likely to lead to the transfer of pollen between the male and female morphs. In this study, the floral reward of interest is nectar, which is offered by both male and female flowers. Only male flowers produce pollen, and foragers specifically seeking pollen will gain nothing by visiting female flowers.

There are diverse taxa of pollinators in production fields of hybrid seed canola. Besides their individual contributions to cross-pollination, they may influence one another's movement of pollen through resource consumption. With fixed resources, an increase in conspecific pollinators can increase pollinator diet breadth, with pollinators more willing to visit multiple species of flowers (Fontaine et al. 2008), as predicted by optimal diet theory (Emlen 1966). Intraspecific competition is generally stronger than interspecific competition (Connell 1983; Gurevitch et al. 1992), because conspecifics have the most similar resource requirements. While interspecific competition alone would have comparatively weaker effects on pollinator movement than intraspecific competition, a combination of the two could have the greatest effect on resource consumption. This depends on the resource requirements of the pollinators—pollinators with more similar resource requirements will require more similar levels of resources, which means that they will more strongly effect each other. It could also be that diversity of pollinators drives resource consumption and therefore pollinator movement. A more synergistic use of resources could result in a more constant decline of resources.

The sexually dimorphic flowers of hybrid seed canola should promote some level of floral constancy among pollinators. Unlike naturally gynodioecious systems, in which female flowers are likely interspersed with males, the spatial separation of floral morphs in fields of hybrid seed canola provides an environment that further promotes floral

constancy and should allow a clearer understanding of what prompts abandonment of the behaviour. Finally, the presence of multiple types of pollinators (managed honey bees and leafcutter bees) allows a more nuanced look into how conspecific and heterospecific competitor abundance (and/or diversity) influence resource consumption and pollinator movement.

#### **1.4 Objectives**

With this research I sought to address how pollinators behaved naturally in a gynodioecious crop system, and how these behaviours, along with the morphology and presence of pollinators, influenced pollination. With the understanding that a system like hybrid seed canola likely encourages floral constancy, I was also interested in determining how pollinators changed their behaviours when in the presence of other pollinators (i.e., competitors).

The objectives of my thesis research were to 1) determine the presence, foraging preferences, and pollination effectiveness of pollinators visiting hybrid seed canola, and 2) determine whether increased competition leads to increased pollinator movement between morphs (as well as the form of competition—whether conspecific competitor abundance, heterospecific competitor abundance, and/or pollinator diversity affected pollinator movement). Other objectives were to establish the degree of sexual dimorphism between the floral morphs of hybrid seed canola, and to find what factors influenced visitation by leafcutter and honey bees. My goal is to help inform pollinator management within fields of hybrid seed canola.

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## Chapter Two: **Cross-pollination in Hybrid Seed Canola: Pollinator Effectiveness, Frequency, and the Consequences of Floral Constancy**

### **2.1 Abstract**

Production fields of hybrid seed canola in Alberta, composed of spatially separated hermaphroditic ‘male’ and male-sterile ‘female’ morphs, rely on insect pollinators for cross-pollination. Managed honey bees and alfalfa leafcutter bees are used for this purpose, although wild pollinators may also contribute. Pollinator taxa differ in size, behaviour, and the way in which they carry pollen, which may affect the effectiveness of pollen transfer. Other factors that could affect pollination include pollinator presence, and willingness to switch from male to female flowers. I measured pollinator presence in quadrats in a field. To test differences in pollination effectiveness, I used an interview bouquet to present unvisited female inflorescences to different pollinator taxa. Visited stigmas were collected and pollen deposition counted to determine pollination effectiveness. Pollinator taxa, the floral morph on which the pollinator was foraging on before being offered an inflorescence, the amount of pollen on a pollinator’s body, and time spent on the flower were all considered as possible predictors of pollination effectiveness. I also took videos of pollinator behaviour to compare between pollinator responses to interview bouquet flowers (avoidance, rejection, or acceptance). Managed pollinators were more likely to be present in the fields than wild pollinators were. Both honey and leafcutter bees were less likely to visit a female flower after foraging on male flowers. Pollinator type, the amount of pollen on the body, time spent on flower, and the type of flower pollinators originally foraged on determined pollen deposition on stigmas. Managed pollinators, especially female leafcutter bees, contributed the most to the pollination of hybrid seed canola in terms of presence and pollination effectiveness. The tendency of pollinators towards floral constancy to morph could reduce cross-pollination.

### **2.2 Introduction**

Pollination effectiveness is a measure of how well a pollinator contributes to a plant’s fitness. A pollinator’s effectiveness represents their ability to contribute to pollination success—specifically, the number of pollen grains transferred to the female

reproductive organs by a pollinator in a single floral visit. It has implications for yield, since the quantity of pollen not only affects whether a fruit is produced but also can affect the quantity of seeds per fruit (Mayfield et al. 2001). How effective a pollinator is depends on both their morphology and behaviour. Floral morphology also influences pollination effectiveness, as the shape and complexity of a flower affects what types of pollinators can access floral resources and how they do so (Faegri and van der Pijl 1971). There are additional factors beyond effectiveness that influence pollination, including pollinator availability and pollinator foraging preferences. Pollinator availability is important because the most frequent visitors to the flower likely have the most effect on its pollination (Stebbins 1970). A pollinator's foraging preferences will impact pollen movement between flowers, especially those in a multi-floral system; both generalist foraging and floral constancy can have consequences for pollen delivery. Consequently, to understand how pollinators contribute to a plant's pollination and eventual seed set, it is important to understand what pollinators are available for pollination, what their foraging preferences are, and how effective they are at pollen transfer (as well as which traits influence pollination effectiveness).

Pollinator effectiveness is an important consideration in agricultural systems that require the transfer of pollen by insects for seed set. This is especially true in the case of flowers with more complex morphology and pollen release mechanisms, such as blueberry (genus *Vaccinium*), but is still relevant for flowers with morphologies that are more accessible to pollinators, including apple (*Malus*), almond (*Prunus*), and canola (*Brassica*; Delaplane and Mayer 2000). Traditionally growers employ honey bees (*Apis mellifera*) when insect pollination is required for a crop, but honey bees are not always the most efficient pollinator. For example, while honey bees and bumble bees (genus *Bombus*) deposited equivalent amounts of pollen on almond stigmas, Thomson and Goodell (2001) found that bumble bees deposited more pollen on apple stigmas than did honey bees. Similarly, bumble bees, leafcutter bees and mining bees (family Andrenidae) all deposited more pollen on lowbush blueberry stigmas than honey bees, although the amount also depended on whether foragers were collecting pollen or nectar (Javorek et al. 2002).

Pollinator availability is a precondition for interest in pollination effectiveness. Sahli and Conner (2007) found that the less efficient pollinators of wild radish were still the most important contributors to its pollination due to their higher visitation frequency. Pollinator availability depends in part on how a crop is managed, as crops that require some level of insect visitation often use managed pollinators to ensure pollination. Wild pollinators, including bees and flies, may also contribute, although their presence is more uncertain and may be affected by landscape factors (Kremen et al. 2002) or competition with managed pollinators (Lindström et al. 2016).

Pollinator response, or how a pollinator interacts with the available floral resources, matters when there are multiple types of flowers (e.g., species, morphs). Self-incompatible flowers, as well as floral systems with dioecious (male and female flowers on separate plants) or gynodioecious (hermaphrodite and female) flowers, require pollen transfer between different morphs for pollination to occur (other than for hermaphrodites). Pollinators may exhibit floral constancy to one morph, which limits cross-pollination. In the case of gynodioecy, a failure to transfer pollen from the ‘male’ (hermaphrodite) to the female plants could result from males being more attractive or rewarding than females (Delph and Lively 1992; Ashman 2000), a pollinator preference for pollen-producing (Larsson 2005) or pollen-deficient flowers (Waller et al. 1985), or, in agricultural crops, spatial separation between patches of male and female flowers. Honey bees prefer male *B. napus* plants over female (Mesquida and Renard 1981), and are inefficient at pollinating gynodioecious hybrid sunflowers due to reduced movement between morphs (Martin and Farina 2016).

Pollinator morphology influences pollen deposition in two ways: 1) the physical manner in which pollinators contact the stigma and 2) how much pollen is available on the pollinator’s body for contact with and deposition on the stigma. Size influences how pollinators contact floral reproductive parts, with larger pollinators usually being more likely to come into contact with the stigma and therefore deposit pollen grains (Kandori 2002). Because larger pollinators also have more surface area on which pollen could cling, larger pollinators could carry more pollen for deposition. In pak choi (*Brassica rapa*) pollination, pollen deposition increased with an increase in pollen grains on a

pollinator's body (Howlett et al. 2011). Body size seemed to play a role in this, with the largest bee pollinator (*Bombus terrestris*) having the most pollen on its body and transferring the most pollen to pak choi stigmas. In addition to size, other morphological features may cause taxonomic groups to differ in their ability to hold pollen on their bodies and to transfer it to flowers. Hymenopterans (specifically bees) carry significantly more pollen than dipterans (Lindsey 1984; Orford et al. 2015). In addition to their more elaborate pollen-carrying structures (such as scopa or corbicula), bees possess dense branched hairs on their bodies that readily accumulate pollen (Thorp 1979). Pollinating flies such as hoverflies are comparatively sparsely haired and may not have branched or plumose hairs (Holloway 1976), which reduces their capacity to carry pollen.

Behaviours including time spent on a flower, flower choice, and resource choice (such as nectar or pollen rewards), can all affect the amount of pollen a pollinator deposits on a flower. Pollinators that spend a greater amount of time handling flowers often deposit more pollen (Thomson and Plowright 1980; Thomson 1986; Galen and Stanton 1989; but see Cresswell 1999). In a system that has multiple types of flowers (e.g., dioecious or gynodioecious plant systems), a pollinator travelling directly from a pollen donor will impart more pollen than one travelling from a female flower. Finally, whether a pollinator is foraging for pollen or nectar can affect pollen deposition. Foragers concentrating on pollen-gathering tend to remove more pollen while depositing less (Wilson and Thomson 1991), and visit inflorescences differently (Galen and Plowright 1985). Pollen foraging bees may groom pollen into corbiculae or scopae, which reduces pollen carryover (Thomson 1986) and makes pollen less available for deposition (Parker et al. 2015).

Seed production in hybrid canola (*Brassica napus*) depends on insect transfer of pollen between hermaphroditic ('male') and female morphs (Mesquida and Renard 1981). The flowers are cruciate and both pollen and nectar are easily accessible to pollinators (Delaplane and Mayer 2000). Both honey bees and leafcutter bees (*Megachile rotundata*) are used as managed pollinators in this system. Wild pollinators, including bees and flies, also contribute to commodity canola pollination (Morandin and Winston 2005), and likely contribute to seed production fields as well. Because they do not have

the resource or habitat support that managed pollinators do, wild pollinators may be present at lower numbers. Large fields of mass-flowering crops also tend to dilute the densities of native pollinators, making them less available overall for pollination (Holzschuh et al. 2016).

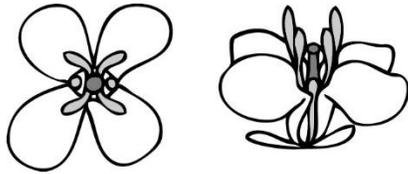
Previous studies on canola suggest that both bee and fly visitors can be effective contributors to pollination. Studies world-wide on commodity canola, which is entirely hermaphroditic and self-fertile, have found that the addition of honey bees increases pod yields (Manning and Boland 2000; Sabbahi et al. 2005; Oz et al. 2008; Munawar et al. 2009; Durán et al. 2010). Additionally, in commodity canola wild bees can deposit pollen at a level comparable to honey bees (Rader et al. 2009; Ali et al. 2011), and hoverflies can be efficient pollinators (Jauker and Wolters 2008). Caution should be taken in applying pollination conventions in commodity canola to hybrid canola, where the additional presence of female flowers in fields of hybrid canola can change how pollinators contribute to the crop. Studies on commodity canola could not assess pollinator response to different morphs (as would be present in hybrid seed canola) and commodity seed production likely includes pollination via self-fertilization, which is impossible for the female flowers in hybrid canola systems. Studies of pollinators visiting gynodioecious canola in caged tents using honey bees (Mesquida and Renard 1981), leafcutter bees (Soroka et al. 2001), and hoverflies (Jauker et al. 2012), suggest that these pollinators could be effective at transferring pollen (although hoverflies less so than bees). However, the contrived setup used in these studies could lead to behaviours that would not be observed in the uncaged world.

To understand how pollinators contribute to pollination in hybrid canola, I examined what pollinators were present, how they responded to female floral morphs, and how their behaviours and morphologies influenced pollination effectiveness. For pollinator frequency, I predicted that honey and leafcutter bees would be the most common pollinators because they are placed in the field by growers, whereas wild pollinators would be present at a much lower abundance. For pollinator response, I hypothesized that pollinators would exhibit floral constancy, and therefore pollinators foraging on male flowers would be less willing to visit a female flower than those

originally foraging on female flowers. This would be especially true for individuals foraging for pollen, for whom the female flower does not offer the desired food type. I predicted that pollination effectiveness would depend on both pollinator morphology and behaviour. Specifically, larger, more hirsute pollinators would have more body pollen and deposit more pollen on stigmas. Bees would be more effective at pollen deposition than flies. Among bees, size would be an important factor (for example, a bumble bee should deposit more pollen than the smaller leafcutter bee). I predicted that pollinators travelling immediately from a male flower would deposit more pollen than those moving from a female, and that more time spent on a flower would result in higher pollen deposition. I tested these predictions in fields of hybrid seed canola in Southern Alberta, Canada.

### **2.3 Methods**

Canola flowers are yellow and cross-shaped. Typically, flowers have a central stigma, around which are spaced four nectaries and six stamens (four long and two short) (Delaplane and Mayer 2000) (Figure 2.1). Male-sterile female flowers, present only in hybrid systems, have reduced, unviable stamens that present no pollen. Male flowers have significantly larger petals and a higher hourly nectar production than female flowers (Waytes unpublished data). Flowers open for about 3 days (Canola Council of Canada 2003), and are clustered on inflorescences. The number of receptive flowers per inflorescence ranges from 1-10, with an average of three per inflorescence (Waytes unpublished data), although this may vary by cultivar. Pollinated flowers produce seed pods containing an average of 15-40 seeds (Canola Council of Canada 2003), although the amount of pollen required for full seed set is higher than seeds produced, at around 100 grains (A Melathopoulos, personal communication). In hybrid seed fields, the flowers are separated into alternating bays (lines) of flowers by sex, and female bays are larger than male bays (6 m vs 1 m).



**Figure 2.1: Illustration of a hermaphroditic canola flower in top (left) and profile (right) view. The stigma (dark grey) is surrounded by anthers (light grey), four long and two short.**

Common flower visitors to canola are managed leafcutter and honey bees (Figure A.1). In Southern Alberta, potential wild bee visitors include bumble bees (*Bombus*) and solitary bees (including families Andrenidae, Colletidae, Halictidae, and Megachilidae) (Zink 2013). Previous studies on canola suggest that hoverflies (Syrphidae) contribute to pollination (Jauker et al. 2012). Butterflies and moths, some of them present as pest species in their larval stages, also visit flowers as adults (Ali et al. 2011).

### ***2.3.1 Study area and site selection***

My study sites were seed production fields of hybrid canola located in Southwestern Alberta in the Canadian Prairie region. Fields ranged from 26-94 ha in area, with an average area of 53 ha. Male flower density was on average about  $800 \pm 43$  flowers ( $\pm$ SE) per  $m^2$ , while the average female flower density was  $660 \pm 32$  flowers per  $m^2$ .

In 2015, I visited 21 hybrid seed fields. The data from these fields were used for the pollinator presence and response analyses. In 2016, I visited 18 hybrid seed fields, 15 of which were the same variety as the 2015 fields, and 3 of a different variety. I also visited one commodity field in 2016 to incorporate more wild pollinators into my pollination effectiveness analysis (see below). The data from all fields in 2015 and 2016 were used to analyze effectiveness.

### ***2.3.2 Pollinator presence***

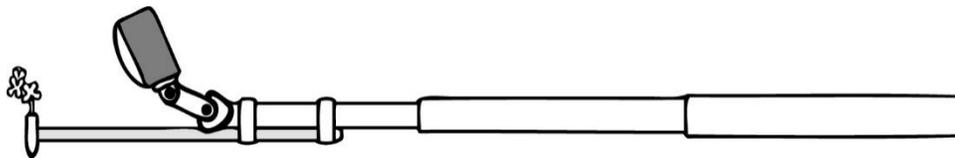
In 2015 I quantified pollinator visitation to establish how often different types of pollinators visited hybrid canola flowers. I established focal plots, composed of a paired section of male and female bay, in each field, with six plots per field (barring changes in weather or field conditions). Pollinators are central-place foragers, and distance away from their source constrains their foraging range. Both honey bees and leafcutter bees

have diminished foraging activity at greater distances from their hives or shelters (Ribbands 1951; Richards 1984; Jay 1986). To generate differences in visitation due to distance from source, I placed plots near to and far from leafcutter bee shelters, honey bee hives, and potential sources of wild bee habitat (such as a nearby fallow field or fence line). The range of distance from honey bee hives was 3-850 m, from leafcutter bee shelters was 1-161 m, and from wild bee habitat was 3-770 m.

I recorded pollinator visits to the female and male bays at each plot in the field using a 1 m<sup>2</sup> quadrat. During a ten minute observation period, I recorded the identities of floral visitors and the number of visits made to inflorescences by each group of pollinator. Pollinators were separated into different functional groups: honey bees, leafcutter bees, wild bees (described above), hoverflies (Syrphidae), calyptrate muscoid flies (Muscidae), and Lepidopterans.

### ***2.3.3 Measuring pollinator effectiveness and response***

I measured pollinator effectiveness and response using an interview bouquet technique (Thomson 1981). The apparatus consisted of a telescoping camera stick with a GOPRO Hero 4® camera, with macro lens, set up on the end. A microcentrifuge tube was placed at the end of a dowel extending about 15 cm in front of the camera (Figure 2.2). The apparatus, from the tip of the handle to the tube at the end of the dowel, was 1 m in length. During each offering I placed a virgin female inflorescence in the microcentrifuge tube, with the camera angled towards the flower for recording, and offered the inflorescence to pollinators visiting flowers within the crop. The inflorescence was placed close to the pollinator, usually within a few centimeters. I recorded the response of the insect and its interactions, if any, with the flower.



**Figure 2.2: Stylized drawing of an interview bouquet stick (1 m long). The camera (dark grey), with macro lens, is focused on a female canola inflorescence placed in a microcentrifuge tube.**

Two methods were used to ensure female flowers were not pollinated before being offered to floral visitors. One involved covering inflorescences with pollinator

exclusion bags at least a day before visiting a site, with any open flowers removed before covering. Another method was covering female hybrid canola plants at a separate site permanently with a wind- and pollinator-exclusion tent to prevent pollen deposition. Virgin female inflorescences were collected from these plants at the beginning of a field day and kept in a cooler to prevent insect visitation and loss of floral function due to heat. Control stigmas were collected daily to ensure no pollen deposition had taken place with either pollen exclusion method; measured pollen deposition was always zero.

Pollinators visiting flowers in the male or female bays were offered a female inflorescence. Pollinators that accepted (i.e., visited) the inflorescence were collected in-field with a Bioquip® Insect Vacuum, placed on ice until immobile, and then transferred to a microcentrifuge tube. They were frozen until processing (see below). After the pollinator was caught, I collected and mounted the stigmas of visited flowers on slides with glycerin fuchsin jelly, to be counted later. When pollinators visited multiple flowers on the virgin female inflorescences, I collected all stigmas but only the pollen deposition on the first was counted for consistency.

Leafcutter bees (male and female foragers were distinguished), honey bees, hoverflies, and bumble bees were collected for processing. Smaller solitary bees and Lepidopterans were both rare and unreceptive to the offered inflorescences, so they were not included in analyses. Due to a low sample size of wild pollinators in the 2015 season, the process was repeated in the summer of 2016 with more emphasis on non-managed pollinators and differentiating between pollen and nectar foragers on male canola. I expanded sampling efforts to another variety of canola in an effort to increase the number of wild pollinators, and a continued low frequency of bumble bee pollinators required the collection of bumble bees in a commodity canola field. While commodity canola is effectively the same as ‘male’ hybrid canola, my measurements represent pollination potential for bumble bees, rather than actual contributions to pollination in hybrid canola fields.

#### 2.3.3.1 Video analysis

I used videos of pollinators to obtain their reactions to the offered female inflorescences. The reactions offer insight into how their behaviours influenced pollen

deposition, as well as into how bees responded to female flowers in a gynodioecious crop system. Behaviours that could influence pollen deposition included the amount of time spent on a flower as well as the type of flower on which the pollinator was foraging before being offered a female inflorescence ('flower of origin'). I measured the amount of time a pollinator spent on a flower using the videos by timing how long the pollinator was in contact with the flower once the reproductive parts had been contacted. The flower of origin could be assessed in the field, but was also checked against the video records. I also recorded whether honey bees were nectar or pollen foragers (based on whether pollen was present on the corbicula). While honey bees may collect both pollen and nectar on a foraging trip, most foragers collect one or the other (Free 1960).

Pollinator responses on video were scored as avoidance, rejection, or acceptance. Avoidance implied that the pollinator did not visit the inflorescence and showed no visual recognition of it before flying away. This category is the broadest and least defined, as it is unclear whether the pollinator left due to fear of the video apparatus, rejection of the inflorescence, or simply did not notice the inflorescence. Rejection implies that the pollinator showed some visual recognition or inspection of the inflorescence, possibly even contacting a non-sexual part of a flower (such as the petal), and ultimately left without visiting or contacting the stigma. Acceptance included pollinators that came into contact with the reproductive parts of the flower. Low sample sizes of non-managed pollinators necessitated that only leafcutter bees and honey bees (which could also be separated by forager type) be used to compare between responses.

#### 2.3.3.2 Pollinator processing

To count the amount of pollen on a pollinator's body, I submerged pollinators in 2 mL of 70% ethanol (more ethanol was used for larger-bodied pollinators) in microcentrifuge tubes and sonicated them using a Branson Ultrasonic Bath Model 1800® to release the pollen from the pollinator's body in a non-destructive manner (as described in Kearns and Inouye 1993). Pollinators were sonicated for at least one minute, until any pollen visible on the pollinator's body was suspended into the ethanol. Honey bee and bumble bee pollen foragers had corbiculate loads removed prior to sonication, as pollen packed in this manner is not available for pollination (Thorp 2000). 10 µL of the pollen-

ethanol solution was placed upon a hemocytometer and the number of pollen grains within the grid was counted. I calculated the average number of grains per  $10^{-4}$  mL by multiplying the number of pollen grains by the degree of dilution, and then dividing by the number of hemocytometer squares counted. This number, multiplied by  $10^4$ , represented the average number of pollen grains per mL each pollinator had on their body. I repeated hemocytometer counts ten times for each individual, each time using the same sonicated sample.

#### ***2.3.4 Statistical analysis***

All analyses were performed in R v.3.2.2 (R Development Core Team 2015). Models were checked for collinearity of predictors, overdispersion, and outliers. Model residuals were checked for homogeneity of variance. Variable means and parameter estimates are presented  $\pm$  standard error; parameter estimates from negative binomial models are in log scale.

##### ***2.3.4.1 Visitation presence***

I analyzed visitation data using the glmmADMB package in R (Skaug et al. 2014). Limited visitation by non-managed pollinators necessitated that pollinator presence or absence in a ten minute period (rather than total visitation) be analyzed with the use of a binomial GLMM. A Wald  $\chi^2$  test was used to test for the effects of pollinator type on presence in plots. The variable ‘pollinator’ included honey bees, leafcutter bees, hoverflies, calyptrate muscoids, and native bees. The category ‘native bees’ included solitary bees as well as bumble bees, whose low presence (bumble bees were present in one quadrat out of 228 observations) prevented a more detailed assessment. Lepidopterans were excluded from analysis due to their lack of presence (four quadrats out of 228). Temperature was included as an extraneous variable. Site and plot nested within site were modeled as random effects. Post hoc comparisons between pollinators were made via a Tukey Test with the multcomp package in R (Hothorn et al. 2008).

##### ***2.3.4.2 Pollinator response***

Pollinator response was analyzed using a multinomial logistic regression using the R package VGAM (Yee and Wild 1995). Responses were taken from the 2015 field season videos, and included rejection, avoidance, or acceptance of female inflorescences.

The significance of each predictor was calculated using likelihood-ratio tests of nested models. One test examined differences between leafcutter bee and honey bee responses to female inflorescences (other pollinators were excluded due to insufficient sample size), while also considering possible interactions with flower of origin. A separate test compared the response of nectar-foraging and pollen-foraging honey bees. Because most pollen foragers (n=40 out of 43) were found on male flowers, forager choice was compared between nectar and pollen foragers only in the male bay. Julian day was included as an extraneous variable for both tests, because seasonal fluctuations in floral availability may affect pollinator responses to resources.

To parse out an interaction between pollinator type and flower of origin, I used post hoc G-tests to compare the likelihood of a pollinator exhibiting a response against the chance that responses occurred randomly (in which case actions would occur at a 1:1:1 ratio, where each was just as likely to occur as the other). I applied the Hochberg correction (Hochberg 1988) to account for the *a posteriori* contrasts.

Not all inflorescences used had the same number of flowers. Inflorescences with more flowers may be more attractive to pollinators (Willson and Price 1977), and pollinators may therefore alter their responses to inflorescences depending on the number of flowers. I checked to see whether the number of flowers on an inflorescence influenced pollinator response on a subsample of data (n=100 for both honey and leafcutter bees). The number of flowers on an inflorescence did not significantly affect bee response (df=2,  $\chi^2 = 1.61$ , p=0.45)

#### 2.3.4.3 Pollinator effectiveness

I analyzed pollen deposition (an integer, whose values frequently included zero) using generalized linear models with negative binomial error distributions to correct for overdispersion with the R package MASS (Venables and Ripley 2002). A likelihood-ratio  $\chi^2$  test was used to test for relationships between predictors and pollen deposition.

Pollinator taxa (honey bees, male and female leafcutter bees, bumble bees, and hoverflies), the type of flower the pollinator was originally foraging on (male or female), the amount of time spent on a flower, and the amount of pollen on a pollinator's body were all included as predictors of the effectiveness of pollen deposition (via pollen grains

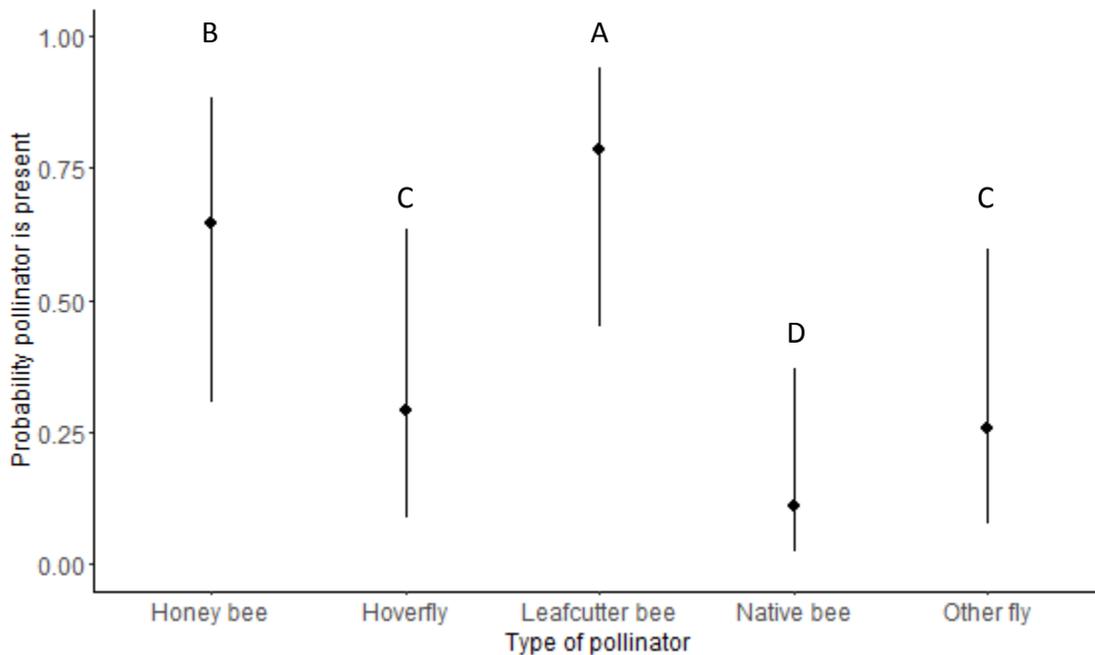
deposited on a stigma). Forager type (for honey bees) was considered as a possible predictor, but was eliminated due to a low sample size ( $n=3$ ) of pollen-foraging honey bees willing to visit a female flower. Post hoc Tukey tests evaluated differences between pollinators with the R package multcomp (Hothorn et al. 2008).

Small and unbalanced sample sizes necessitated my pooling of pollen deposition across variety and year. I recognize that this method may overlook varietal or yearly effects on pollen deposition.

## **2.4 Results**

### ***2.4.1 Pollinator presence***

Pollinator taxa significantly influenced pollinator presence ( $\chi^2=310.83$ ,  $df=4$ ,  $p<0.001$ ) (Figure 2.3), as did temperature ( $\chi^2=11.99$ ,  $df=1$ ,  $p=0.001$ ). Leafcutter bees were the most commonly observed flower visitors to plots (leafcutter bees versus honey bees ( $p=0.01$ ), native bees ( $p<0.001$ ), hoverflies ( $p<0.001$ ), and calyptrate muscoids ( $p<0.001$ )). Honey bees were the second most common (honey bees versus native bees ( $p<0.001$ ), hoverflies ( $p<0.001$ ), and calyptrate muscoids ( $p<0.001$ )), followed by flies (hoverflies were similar in abundance to calyptrate muscoids ( $p=0.96$ )). Both managed pollinators and flies were present more often than native bees (hoverflies versus native bees,  $p=0.006$ ; muscoids versus native bees,  $p=0.02$ ). As temperature increased, pollinators were more likely to be present in plots ( $\beta=0.06\pm 0.02$ ).



**Figure 2.3: The effect of pollinator taxa on whether the pollinator was present during a 10 minute observation of a 1 m<sup>2</sup> plot (n=228). Points represent means, and lines represent the 95% CI. Pollinator taxa included leafcutter bee (n=178), honey bee (n=147), native bee (n=26), hoverfly (n=68), and calyptrate muscoid (n=60). Letters indicate significant differences between means (p<0.05).**

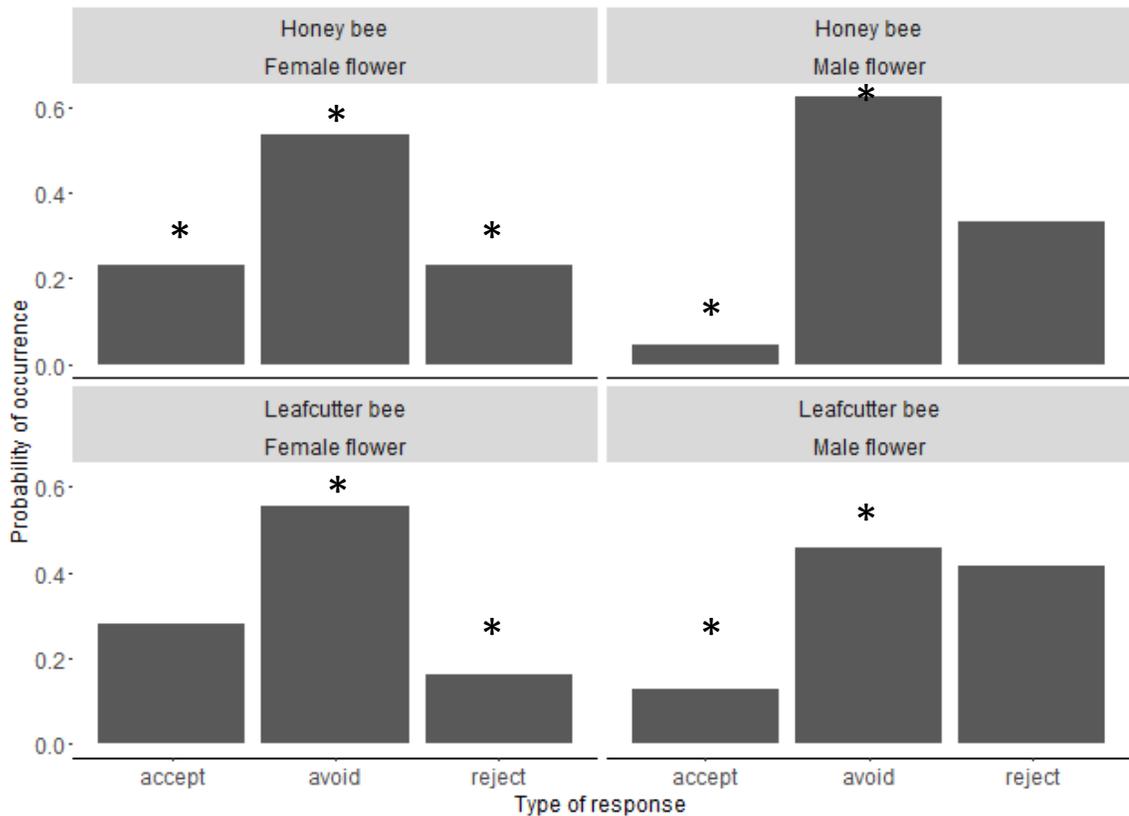
#### 2.4.2 Response

There was an interaction between flower of origin and pollinator taxa that influenced whether a pollinator would be willing to visit a female inflorescence ( $\chi^2=7.19$ ,  $df=2$ ,  $p=0.03$ ) (Figure 2.4). While foraging on female flowers, honey bees were less likely to accept or reject a female inflorescence than would be expected ( $G=6.31$ ,  $df=1$ ,  $p=0.012$ ). When foraging on male flowers, honey bees were less likely to accept a female inflorescence ( $G=72.37$ ,  $df=1$ ,  $p<0.001$ ), but 'reject' was not different from expected proportions ( $G=0.03$ ,  $df=1$ ,  $p=0.864$ ), suggesting that honey bees are more likely to reject a female inflorescence while foraging on male flowers but not female flowers.

While foraging on female flowers, leafcutter bees accepted female inflorescences with the expected frequency ( $G=2.48$ ,  $df=1$ ,  $p=0.12$ ), and were less likely to reject them ( $G=14.04$ ,  $df=1$ ,  $p<0.001$ ). While foraging on male flowers, leafcutter bees accepted female inflorescences less than the expected 1/3 frequency ( $G=28.93$ ,  $df=1$ ,  $p>0.001$ ) and rejected them with the expected frequency ( $G=3.64$ ,  $df=1$ ,  $p=0.06$ ). Leafcutter bees

therefore were less likely to accept female inflorescences while foraging on male flowers than while foraging on female flowers, and more likely to reject them.

Honey bees foraging on female ( $G=22.12$ ,  $df=1$ ,  $p<0.001$ ) and male ( $G=48.07$ ,  $df=1$ ,  $p<0.001$ ) flowers avoided inflorescences more than would be expected. This relationship was also true for leafcutter bees foraging on male ( $G=8.76$ ,  $df=1$ ,  $p=0.003$ ) and female ( $G=23.781$ ,  $df=1$ ,  $p<0.001$ ) flowers, suggesting that this relationship is an artifact of how pollinators interact with the interview bouquet apparatus.



**Figure 2.4: The probability of response to a female inflorescence being ‘accept’, ‘avoid’, or ‘reject’ based on pollinator type (honey bee (top;  $n=277$ ) and leafcutter bee (bottom;  $n=230$ )) and the type of flower that the pollinator was originally foraging on (female (left;  $n=216$ ) or male (right;  $n=291$ )), with Julian day held constant. Asterisks indicate responses that significantly deviate from an expected 1:1:1 ratio.**

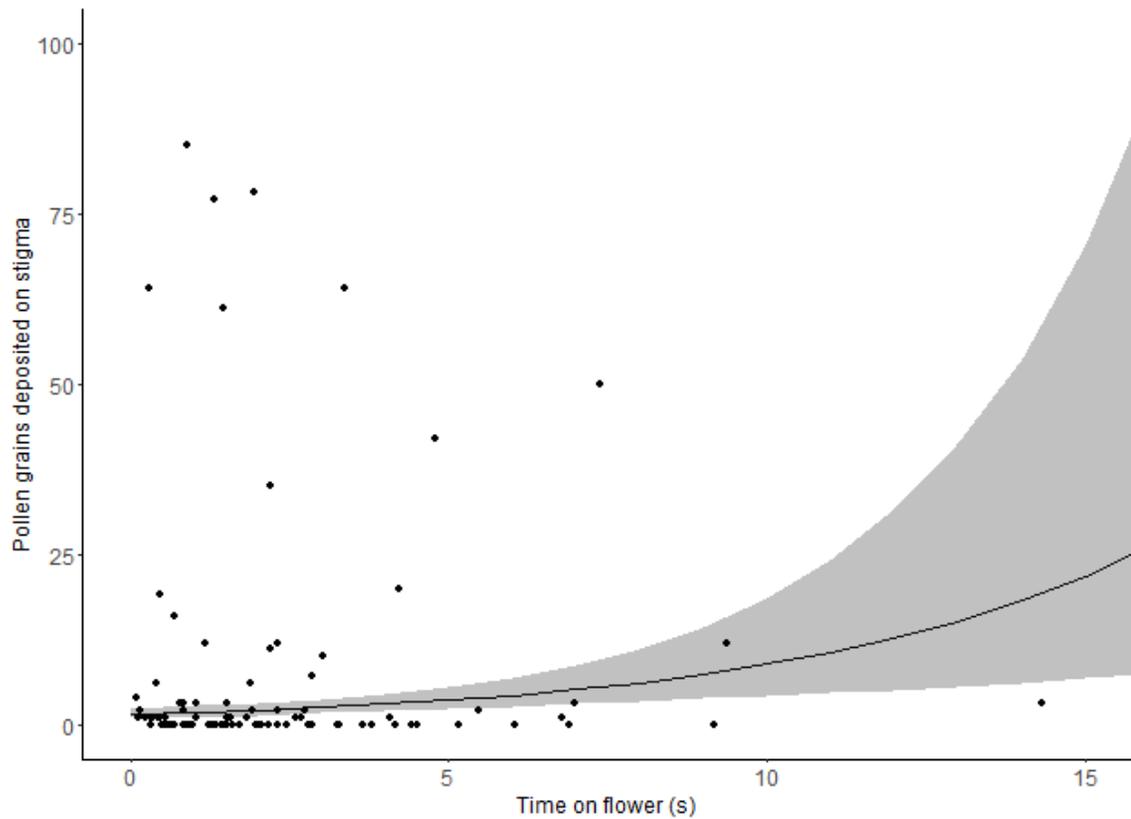
Julian day also influenced pollinator choice ( $\chi^2=9.77$ ,  $df=2$ ,  $p=0.01$ ). Bees were more likely to accept, rather than avoid, an inflorescence as the season progressed ( $\beta=0.06\pm 0.02$ ); for instance, the odds of a bee accepting rather than avoiding an

inflorescence at the end of the season was 5.57 times the corresponding odds at the beginning of the season.

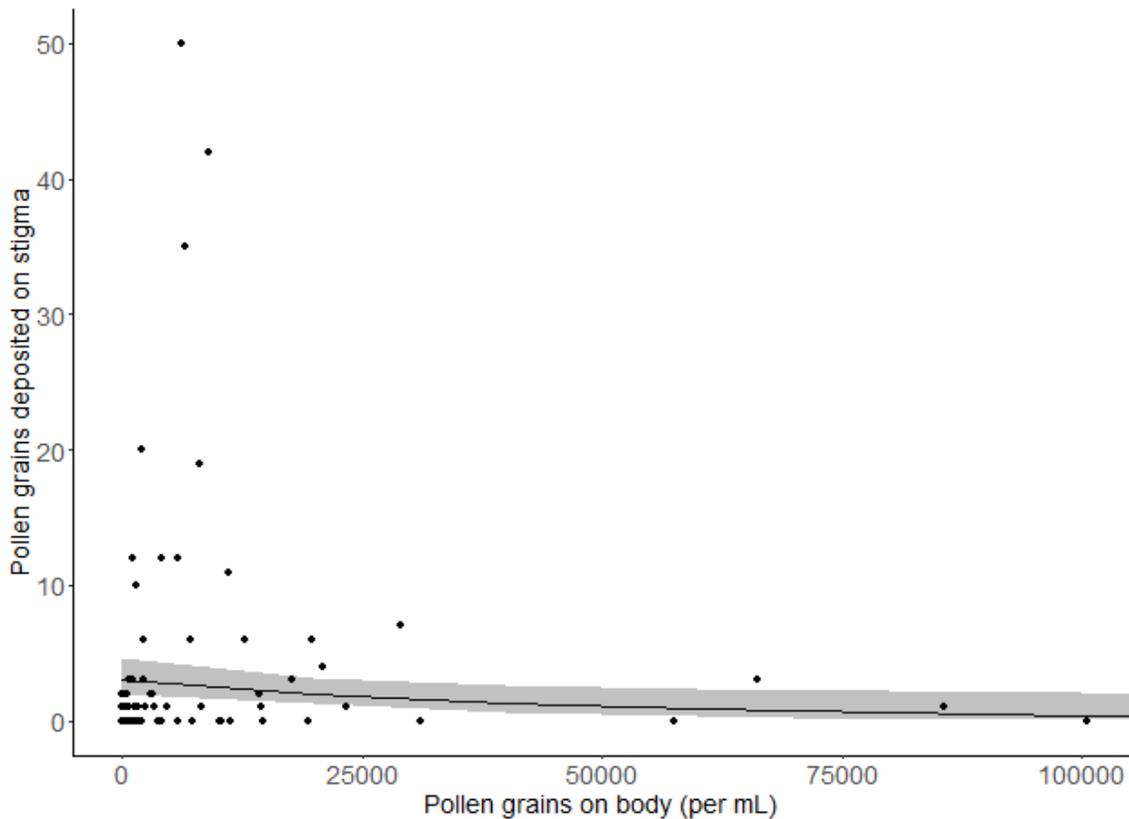
Although I expected that honey bees foraging for pollen would be less likely to visit a female inflorescence than nectar honey bee foragers, there was no significant effect of forager type on pollinator response ( $\chi^2=5.64$ ,  $df=2$ ,  $p=0.06$ ). Julian day also did not affect response by honey bee foragers in the male bay ( $\chi^2=1.83$ ,  $df=2$ ,  $p=0.40$ ). Honey bees foraging on male flowers showed similar responses to female flowers regardless of what resource they were foraging for (pollen or nectar).

#### **2.4.3 Pollinator effectiveness**

Pollinator type ( $\chi^2=44.79$ ,  $df=4$ ,  $p<0.001$ ), flower of origin ( $\chi^2=41.94$ ,  $df=1$ ,  $p<0.001$ ), time on flower ( $\chi^2=15.77$ ,  $df=1$ ,  $p<0.001$ ), and the amount of pollen on a pollinator's body ( $\chi^2=4.17$ ,  $df=1$ ,  $p=0.04$ ) all significantly influenced pollen deposition on canola stigmas. Pollinators travelling directly from male flowers deposited higher pollen loads on female stigmas ( $32.2\pm 10.1$  grains) than pollinators travelling from female to female flowers ( $1.7\pm 1.2$  grains). Pollinators handling flowers for longer times deposited more pollen grains ( $\beta=0.18\pm 0.05$ ) (Figure 2.5). It is important to note the removal of an outlier at  $> 60$  s, which will be discussed below. Unexpectedly, the relationship between pollen on body and pollen deposited was a negative one ( $\beta=-0.20e-05\pm 0.09e-06$ ) (Figure 2.6).



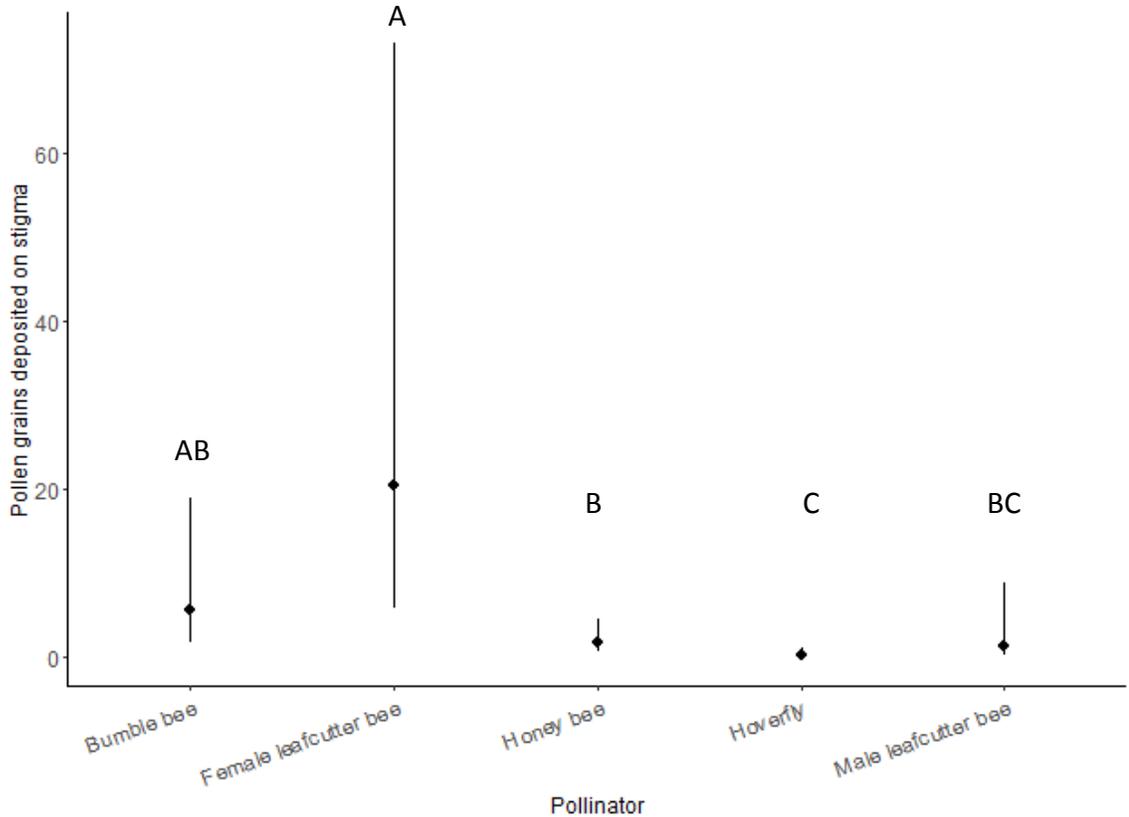
**Figure 2.5: Relationship between the amount of time spent on a flower and pollen grains deposited, with predicted trend line plotted against observed (non-adjusted) points. For the trend line, variables pollinator type (5 levels), flower of origin (2 levels), and pollen on body were held constant. Shaded area represent the 95% CI.**



**Figure 2.6: Relationship between the average number of pollen grains on a pollinator's body (per mL) and pollen grains deposited on stigma, with predicted trend line plotted against observed (non-adjusted) points. For the trend line, variables pollinator type (5 levels), flower of origin (2 levels), and time spent on flower were held constant. Shaded area represent the 95% CI.**

While flies did show the lowest pollen deposition (Figure 2.7), differences in pollination effectiveness among bees was not as expected. Female leafcutter bees ( $n=20$ ), with an average pollen deposition of  $38.17 \pm 14.44$  per stigma per visit, did not deposit a significantly different amount of pollen than bumble bees did ( $n=13$ ,  $27.23 \pm 14.11$  grains,  $p=0.29$ ), but were significantly better at pollen deposition than honey bees ( $n=43$ ,  $8.40 \pm 4.47$  grains,  $p<0.001$ ), male leafcutter bees ( $n=6$ ,  $1.17 \pm 0.54$  grains,  $p=0.03$ ), and hoverflies ( $n=21$ ,  $1.29 \pm 0.72$  grains,  $p<0.001$ ). The number of pollen grains deposited by bumble bees, intermediate between female leafcutter and honey bees, was not significantly different than either honey bee deposition ( $p=0.20$ ) or male leafcutter bee deposition ( $p=0.60$ ), but was greater than hoverfly pollen deposition ( $p<0.001$ ). The number of pollen grains deposited by honey bees was not significantly different than the

amount deposited by male leafcutter bees ( $p=1.0$ ) but was greater than hoverfly pollen deposition ( $p=0.03$ ). Male leafcutter bees and hoverflies deposited the least pollen per visit, and were not significantly different in pollen deposition from one another ( $p=0.50$ ).



**Figure 2.7: The effect of pollinator taxon on the number of pollen grains deposited on stigmas. Pollinator taxa included bumble bees ( $n=13$ ), female leafcutter bees ( $n=20$ ), honey bees ( $n=43$ ), hoverflies ( $n=21$ ), and male leafcutter bees ( $n=6$ ). Points represent means and lines represent the 95% CI. Pollen deposition was averaged between flower of origin; time spent on flower and pollen on body were held constant. Letters indicate significant differences between means ( $p<0.05$ ).**

## 2.5 Discussion

### 2.5.1 Pollinator presence

Managed pollinators were much more likely to be present in plots than wild flies or bees. This result was not unexpected; managed pollinators are actively cared for and

their numbers can be controlled, while wild pollinators depend on the availability of nesting and floral resources in the environment. It is somewhat surprising that leafcutter bees were more likely to be present in plots than honey bees, as their smaller size should restrict their movement more so than honey bees (Greenleaf et al. 2007). However, my analysis compared presence, not visitation, and it might be that honey bees still had higher overall visitation. Because leafcutter shelters were dispersed throughout hybrid canola fields, and honey bees were located only on field edges, it was more difficult to get 'far' from leafcutter bee shelters (160 m, versus 850 m from honey bees). This also possibly influenced visitation by the two pollinators. It is important to note that leafcutter bee sex was not distinguished for presence/absence measurements, which may have implications for pollination effectiveness because the sex ratio is typically male-biased (Richards 1984), especially at the beginning of the season.

Flies were the next most abundant pollinators after managed pollinators. As larvae, some species of hoverflies prey on crop pests such as aphids (Sarwar 2013), and so may be present in canola at multiple life stages. The presence of canals and ditches near canola fields could be breeding places for flies with an aquatic larval stage, such as hoverflies in the genus *Eristalis* (Buckton 1895). Feedlots located near some of the fields may have also provided resources for the larvae of muscoid flies.

The least frequent floral visitors in fields of hybrid seed canola were native bees, with a mean probability of encounter of only about 11%. Mass-flowering crops such as hybrid seed canola can increase local densities of bees (Westphal et al. 2003); however, if requirements for floral and nesting resources by native bees are not met, large fields of flower crops may simply serve to dilute already existing populations of wild pollinators (Holzschuh et al. 2016). The presence (and contributions) of native pollinators may be therefore higher in smaller agricultural fields. Isaacs and Kirk (2010) found that native bees (bumble bees) contributed most to pollination in small-sized blueberry fields, but that honey bees dominated pollination in large fields. Another aspect to the low native bee presence might be competitive displacement of native pollinators by managed bees (Lindström et al. 2016). The high numbers of managed pollinators in seed fields of hybrid canola could actually be preventing wild bee visitation, especially if native bees are able

to seek alternative resources from semi-natural habitat in the surrounding environment (Zink 2013).

### **2.5.2 Response**

Managed pollinators showed slightly different responses to female inflorescences depending on what they were foraging on prior to being offered a female inflorescence. However, honey and leafcutter bee responses to the female inflorescences indicated that the pollinators could distinguish between flower types (male or female) and may preferentially visit one over the other. Honey bees were more likely to reject a female inflorescence while foraging on male flowers than while foraging on female flowers, and leafcutter bees were both more likely to reject and less likely to accept a female inflorescence when foraging on male rather than female flowers. The ‘avoid’ category may have also included pollinator rejections of inflorescences, but the ambiguous nature of the category makes it difficult to parse this out from bees who did not see the proffered inflorescence or who were afraid of the interview bouquet apparatus.

The tendency for bees to exhibit floral constancy to morph has important implications for flower breeding systems that require pollinator movement between floral morphs. Male and female canola flowers are visually similar in colour and shape, but males have larger petals and present pollen, which bees may use as visual cues to distinguish them from females. To assist with foraging, bees may categorize certain stimuli—such as colour—and use those as a tool to more quickly locate rewarding floral types (Klosterhalfen et al. 1978; Dukas and Waser 1994). It is possible that pollinators associate the larger petal size and presence of pollen with the more rewarding male flowers; females, with smaller petals and lacking pollen, may be ignored. The spatial separation of morphs may further promote floral constancy to morph. Bees should abandon floral constancy when inconvenient, such as when flowers that are as rewarding as those they are constant to are more accessible; however, when flower types are equally accessible, bees will more likely behave in a floral constant manner (Marden and Waddington 1981). The spatial separation of bays used in the production of hybrid canola seed means that pollinators are not bypassing easily accessible and similarly rewarding flowers when exhibiting floral constancy. This study is not the first that has shown that

pollinators can distinguish between morphs in gynodioecious crops and selectively visit only one morph; Mesquida and Renard (1981) showed that honey bees preferred male-fertile oilseed rape plants, and this behaviour has been observed in sunflower as well (Martin and Farina 2016).

Honey bees foraging for nectar or pollen on male flowers showed similar responses to female inflorescences. Honey bees tend to forage for pollen or nectar, and are less likely to collect both in one foraging trip (Free 1960). Honey bees foraging for pollen are therefore limited to male flowers, while nectar foragers have no such restrictions. One possible explanation for the lack of difference between nectar and pollen foragers is, again, floral constancy. I found that both types of honey bee foragers tended to avoid or reject female inflorescences while foraging on male flowers (as opposed to female); although they are not limited to foraging on males, nectar foragers may still be taking visual cues from male flowers to find resources. It is important to note that the presence of a small number of pollen foragers in the female bays suggests that not all pollen foragers are collecting only pollen. These results suggest that the floral morph may determine foraging behaviour more strongly than the resources a pollinator is foraging for.

### ***2.5.3 Pollinator effectiveness***

Pollinators originally foraging on male flowers deposited more pollen on female stigmas than those originally foraging on female flowers. Female flowers lack pollen and cannot directly contribute to its distribution. Once a pollinator has contacted an anther, pollen deposition will decline with subsequent floral visits (Levin and Berube 1972). Even if a pollinator had switched from the male to female bay before visiting the female inflorescence, the amount of pollen available for deposition would still be less than that coming directly from a male flower. The willingness of a pollinator to switch between flower morphs is therefore essential for pollen deposition.

Pollinators that spent greater time on flowers deposited more pollen overall. This result agrees with previous studies that suggest that more time on a flower leads to higher pollen deposition (Thomson and Plowright 1980; Thomson 1986; Galen and Stanton 1989). There was one outlier that had to be removed, which involved a female leafcutter

bee spending over 60 seconds motionless on a flower. The lack of activity may have led to a lower-than-expected pollen deposition, so the time a pollinator spends on a flower should represent when pollinators actively handle resources (rather than simply standing idle). Cresswell (1999) found a similar relationship, where *Bombus lapidarius* pollinators accessing *B. napus* nectaries stayed fairly immobile while doing so, and as such did not contribute any more to pollination than pollinators spending a shorter amount of time on the flowers.

I predicted that higher amounts of pollen on a pollinator's body would result in higher pollen deposition (Howlett et al. 2011). Instead, higher amounts of pollen on a pollinator's body resulted in lower pollen deposition. Depending on what resource pollinators were foraging for, the pollen on a flower visitor's body may not be available for pollination. Pollinators that groom pollen can make it inaccessible for pollination (Thomson 1986), and pollen foragers are more likely to groom and pack pollen. I attempted to address this issue by removing corbiculate loads from honey and bumble bees; however, pollen residue could have been left on the legs. In the future, hind legs should be removed entirely for processing. I also did not remove pollen from leafcutter bee scopae, which are located ventrally on the bee's abdomen. This pollen is packed dry and should still be available for pollination. However, a leafcutter bee foraging primarily for pollen may groom it from the rest of their body and take greater care to preserve it, exhibiting behaviours such as arching their abdomen away from the flower while visiting. It is likely that pollinators primarily foraging for pollen have more pollen on their bodies but that it is less accessible for pollination. The study by Howlett et al. (2011) included both pollen and nectar foragers, but did not directly measure stigma deposition; instead, they referenced an earlier study by Rader et al. (2009) that measured insect pollen deposition on *B. rapa* (but not body pollen). This indirect association between body pollen and stigmatic deposition may have overlooked complications arising from type of forager (pollen or nectar).

I expected that a pollinator's ability to hold pollen on their bodies (based on hairiness and size) would positively influence their pollination effectiveness, although this was based on the assumption that higher amounts of pollen on a pollinator's body

would result in higher deposition. Bees (excepting male leafcutter bees) were significantly better at pollen deposition than flies. This agrees with previous studies in canola that found that flies had to be present at much higher densities than bees to achieve similar levels of pollination (Jauker et al. 2012).

Among bees, female leafcutter bees were the most effective at pollen deposition, comparable only with bumble bees in their ability to deposit pollen. Leafcutter bees are smaller than both honey and bumble bees, but canola flowers are also relatively small, so there is no morphological mismatch in terms of size. It is possible that the abdominal scopae of female leafcutter bees pick up pollen grains better than the comparatively sparser ventral abdominal hair on bumble bees and especially honey bees; the positioning of pollen on the underside of the body may make it more likely that it contacts the stigma than pollen located laterally or dorsally. The leafcutter bee population is composed of both female and male bees, so it is important to consider the abilities of male leafcutter bees to deposit pollen as well. Male leafcutter bees deposited much lower amounts of pollen than female leafcutter bees, although the amount was comparable to honey bee and bumble bee pollen deposition. That supports the idea that the position of the leafcutter bee scopae is important for pollination, as males lack scopae.

Bumble bees and honey bees were similarly effective at depositing pollen, although bumble bee pollen deposition was more variable than honey bee pollen deposition (thus the overlap with female leafcutter bee deposition). The variation in bumble bee deposition could be due to differences in effectiveness among species (which, due to the limited sample size, were grouped together for bumble bees), or the type of resources the pollinator was foraging for (pollen or nectar). The small sample size prevented comparing pollen deposition between forager type (for example,  $n=3$  for honey bee pollen foragers). The lower pollen deposition by honey bees, as compared to female leafcutter bees, may be due to how the pollinators groom and carry their pollen, rather than their size or amount of hair.

There may have been interactions between variables that the data could not address due to limited sample sizes. For example, flower handling time or the amount of

pollen on a pollinator's body might differ depending on the type of morph they were originally foraging on.

Pollination success is an essential component to plant reproductive success. While I did not measure the latter, A Melathopoulos reported that around 100 grains of pollen were necessary for a full seed set per flower (personal communication). On average, no pollinator deposited that many pollen grains in a single visit; however, it would take a female leafcutter bee the least number of visits to fully pollinate a canola flower, compared to all other pollinators (except bumble bees). Honey bees, male leafcutter bees, and hoverflies would require multiple visits to achieve a similar level of pollination.

One behaviour this study did not address, but which could affect pollination effectiveness, is the 'side-working' behaviour exhibited by honey bees visiting canola (Delbrassinne and Rasmont 1988). Honeybees engaging in side-working on canola approach the flower from the side, rather than the top, to access the nectaries; this behaviour allows them to access nectar without coming into contact with the stigma. This act of floral larceny results in a loss of resources with likely no or low pollen deposition. Although I offered female inflorescences to side-working bees, none visited and so the effect this behaviour has on pollen transfer could not be measured. Previous studies found that side-working behaviour led to poorer pollen deposition (Thomson and Goodell 2001).

#### **2.5.4 Conclusion**

While the effectiveness of pollinating taxa is important, frequency of floral visits and pollinator behaviour need to be considered in conjunction with effectiveness to gain a full understanding of how pollinators interact within a system. For example, in my study system, bumble bees showed the potential to contribute to the pollination of canola on par with managed leafcutter and honey bees. However, they were present at such low numbers (to the point where I had to seek them out in commodity fields) that their actual contribution to the pollination of hybrid canola is minimal. Likewise, solitary bees such as those in the family Halictidae have been previously shown to have a high pollination efficiency in commodity canola (Ali et al. 2011), but they were not present frequently enough to contribute meaningfully to the pollination of hybrid canola. Even if solitary

bees were superb pollinators of hybrid canola, the modest contributions of hoverflies would likely have a larger impact because they are present more frequently.

I found both that pollinators needed to be willing to switch between flower types to deliver higher amounts of pollen, and that in this system pollinators exhibited floral constancy that made them unwilling to switch between flower types. Floral constancy is therefore a behaviour in gynodioecious crop systems that can detrimentally impact pollen transfer to females. The spatial separation and sexual dimorphism of hybrid seed canola morphs likely encourages floral constancy, since alternative morphs are not only less attractive but also take more energy to seek out due to the spatial separation of floral morphs.

Floral constancy is one type of foraging strategy, and pollinators may abandon it if it becomes costly or non-profitable. It is important to understand what conditions can promote bees abandoning floral constancy in systems such as hybrid canola, because a more generalist foraging behaviour should promote pollen transfer from male to female flowers. Flowers with less sexual dimorphism (Martin and Farina 2016) or that are intermixed rather than separated in bays would be less likely to promote floral constancy. Alternatively, foraging decisions made by pollinators depend upon resource availability, which is subject to the foraging decisions of other pollinators. As competitors consume resources, pollinators may have more incentive to find alternatives to what they are foraging on. Increased pollinator density can therefore increase pollinator diet breadth (Fontaine et al. 2008).

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## Chapter Three: **Competitor density and diversity facilitate pollinator movement in hybrid canola fields**

### **3.1 Abstract**

Male-sterile (lacking pollen, ‘female’) flowers in gynodioecious pollination systems require the transfer of pollen from hermaphroditic (pollen-donors, ‘male’) flowers to produce fruit. Sexual dimorphism between the floral morphs promotes floral constancy by pollinators to a particular morph, which reduces cross-pollination. My study system was hybrid seed canola, which is an artificially gynodioecious crop with sexually dimorphic male and female flowers spatially separated into bays. To understand what might influence pollinator movement in this system, I examined factors that underlie the Ideal Free Distribution (IFD), which addresses how mobile consumers (pollinators) should respond to habitat (resources) and competitors. More competitors should decrease local rewards, resulting in more pollinators sampling or switching to floral alternatives. I addressed the mechanisms underlying an IFD to test if pollinators would be more willing to switch bays as local competitor density increased, or as local relative profitability decreased. I measured both overall pollinator movement between bays and individual pollinator movement between inflorescences in male bays and compared this to 1) conspecific pollinator density, 2) conspecific and heterospecific pollinator density, and 3) taxon richness, as well as relative floral profit, all of which were measured in both bays, to determine what conditions would lead to more movement between the floral morphs. I determined that higher local conspecific pollinator density and lower local floral profit increased overall movement between the bays, while taxon richness influenced whether an individual pollinator switched floral morphs. Increasing the density of conspecific pollinators or pollinator diversity could promote cross-pollination of females in gynodioecious systems.

### **3.2 Introduction**

In estimating profitability while foraging, pollinators must consider both their resource intake rate and their costs of foraging, and modify their behaviour accordingly. Pollinators can use different foraging strategies to optimize their foraging returns. Floral constancy is one such foraging strategy, in which pollinators restrict their visits to one

type of flower, even in the presence of alternative rewards (Waser 1986). It can be a beneficial behaviour in that it allows pollinators to more efficiently forage for resources by associating rewards with certain patterns or cues. Both honey bees (Klosterhalfen et al. 1978) and bumble bees (Dukas and Waser 1994) identify and selectively visit floral morphs with higher rewards. Floral constancy does inflict a potential cost, since pollinators visiting only one resource may miss other, potentially more rewarding resources. When a floral-constant forager encounters consistently low rewards, or high costs, they should sample other resources, and potentially switch to more rewarding, alternative morphs (Heinrich 1979).

An important factor that affects resource availability—and therefore should affect pollinator foraging behaviour—is density of competitors. Competitors deplete resources through their consumption. The foraging strategy of a pollinator should therefore reflect not only the potential rewards offered by flowers but also the behaviours of other pollinators in the system, since the behaviour of individual pollinators will affect floral reward availability. Fretwell and Lucas' (1970) Ideal Free Distribution (IFD) solves how optimal foragers should respond both to habitat (resource) profitability and the number of competitors, and visit habitats (resources) accordingly. At an IFD, pollinators should match resources, with more competitors on more highly rewarding patches.

The implications of pollinator behaviour are particularly interesting in animal-pollinated gynodioecious systems, which are composed of hermaphroditic and male-sterile plants. Hermaphroditic flowers (hereafter referred to as 'male', emphasizing their role as pollen donors) can be either cross-pollinated or self-pollinated. Male-sterile ('female') flowers require pollen transfer from male flowers. The pollination of female flowers therefore requires pollinator movement between male and female flowers. There is sexual dimorphism between floral morphs in this system, which may be visually apparent (e.g., reduced anthers in females, different petal sizes) or reflected in rewards (e.g., amount of pollen, nectar volume, nectar sugar content) (Delph and Lively 1992; Ashman 2000; Shykoff et al. 2003).

Pollinator movement between morphs in gynodioecious systems is essential for female reproductive success, and yet sexual dimorphism between the morphs might

reduce inter-morph pollinator movement. If female flowers are less attractive or rewarding than male flowers, as is often the case in gynodioecious systems (Delph and Lively 1992; Ashman 2000), then pollinators should prefer to visit males. Depending on the level of sexual dimorphism, even with equally rewarding males and females, differences in appearance could cause visiting pollinators to discriminate between the morphs. In this case, pollinators might show constancy, restricting their visits to one type of flower (or morph). For example, honey bees switched between morphs less often in cultivars of hybrid sunflower that exhibited greater levels of sexual dimorphism (Martin and Farina 2016). Such behaviour would not necessarily affect the pollination of male-fertile flowers, but would reduce female flower pollination.

When a pollinator perceives sub-optimal foraging in its current habitat—due to inferior floral resources and/or competitor consumption—it should switch to a better habitat. In this way, fitness-maximizing pollinators foraging in a gynodioecious system might choose to move between morphs. When habitats (resources) are spatially separated, a system at equilibrium (i.e., at an IFD) might prevent movement between them. However, pollinators are not omniscient, and lack perfect knowledge of foraging alternatives. Resource sampling is required to reach an IFD (Abrahams 1989; Gotceitas and Colgan 1991). In an open system where both pollinator abundances and resources vary with time, pollinators likely must continually reassess their environment.

The production of hybrid seed canola (*Brassica napus*) is accomplished in an artificially gynodioecious system that requires animal pollination. In this system, the high densities of floral morphs are spatially separated in bays. A high floral density and interspersed of both floral types should promote floral constancy (Kunin and Iwasa 1996); the spatial separation of the canola flower morphs, where pollinators must expend extra effort to visit alternative morphs, could further contribute to floral constancy. When local conditions are not at an IFD, higher pollinator densities on a floral morph should lead to higher resource consumption, resulting in more pollinators sampling and moving between morphs. This would be especially true at bay edges, where travel between morphs is the least costly. Movement between bays could reflect differences in the relative floral profit between them.

Both the European honey bee (*Apis mellifera*) and the alfalfa leafcutting bee (*Megachile rotundata*, hereafter referred to as ‘leafcutter bee’) are used together to pollinate production fields of hybrid canola. Wild pollinators (such as native bees or flies) may also be present. The presence of multiple pollinators in the system adds another layer to competitive pressure. Intraspecific competition is typically the strongest form of competition (Connell 1983; Gurevitch et al. 1992), because conspecifics have the most similar resource requirements. The density of conspecific pollinators should therefore influence movement. Heterospecific pollinators also consume resources, however, and contribute to the overall decrease of resources in an area. Exploitative interspecific competition, for instance, influenced the presence and behaviour of bees foraging on *Vicia dasycarpus* (Soltz 1987). Abundances of heterospecific pollinators with similar resource requirements (such as different species of bees), in addition to conspecific pollinators, could have a larger impact on resource depletion and therefore pollinator movement than conspecific abundance alone.

While pollinator abundance can influence pollinator movement, pollinator diversity could as well. A diverse assemblage of pollinators may enhance pollination (Hoehn et al. 2008). This is because diversity can lead to a more synergistic use of resources, as different types of pollinators might use resources at different times of the day, show different sensitivities to weather conditions, or forage at different levels within the crop canopy. In a system with multiple pollinators, it is therefore important to consider the effects of heterospecific abundances and diversity alongside conspecific abundances on pollinator movement.

I hypothesized that pollinator movement between morphs in fields of hybrid canola is influenced by the two factors that matter for achievement of an IFD: density of competitors, and the profitability of resources. I predicted that, when densities of pollinators are low, there should be little or no pollinator movement between floral bays, because resources are not limiting and competition is essentially non-existent. As the density of pollinators increases, resources will be depleted, and I predicted that there should be increased movement between bays, if only to monitor the quality of the alternative morph type. The type of competitor should be important; conspecific

competitors will affect each other, but the abundance or diversity of heterospecific pollinators will also affect resource consumption and therefore encourage movement alongside conspecific abundance. I predicted that pollinators would respond to differences in floral profit between bays, and move from areas of lower profit to those of higher profit.

### **3.3 Methods**

I visited 21 hybrid seed canola fields in Southern Alberta in 2015. Fields ranged from 26-94 ha, with an average area of 53 ha. Because the production of hybrid canola seed requires insect pollination, all sites were stocked with managed honey bees and leafcutter bees. All fields grew the same variety of seed canola. The floral morphs were spatially separated into alternating bays, or lines, of plants. On average, male flower density was about  $800 \pm 43$  flowers ( $\pm$ SE) per  $m^2$  and female flower density was about  $660 \pm 32$  flowers per  $m^2$ . The female bays were wider than the male bays (about 6 m vs 1 m); at the end of the season the male rows were removed by mowing and the female rows were allowed finish forming pods and then harvested. Most fields were circular, with bare field corners (Figure A.2).

I considered the effects of two main classes of traits that might influence crossing between bays, competition and floral rewards. The level of competition in each bay was represented through the visitation of pollinators to each type of bay (see Section 3.3.3). ‘Competition’ was construed as visitation by conspecific and heterospecific pollinators, as well as taxon richness. Floral rewards, or profit, were estimated as a combination of the nectar production (J/h) and density (per  $m^2$ ) of each floral morph in an area. I predicted that competition would have a comparatively stronger effect on movement than profit, because the uniformly high availability of flowers in hybrid seed canola fields would likely preclude any dramatic shifts in resource availability that alone could prompt crossing between bays. This is supported by differences in variability between visitation and profit variables, with leafcutter and honey bee visitation having coefficients of variation of 127.8 and 137.9, respectively, while the CV of male and female profit were 59.5 and 54.2.

### ***3.3.1 Plot selection***

I selected six plots within each field in an attempt to vary the abundances of three different types of pollinators (honey bees, leafcutter bees, and wild pollinators), recognizing that pollinator visitation should decrease with increasing distance from pollinator source in agricultural fields ( Ribbands 1951; Richards 1984; Jay 1986). Each plot contained a paired section containing both a male and a female bay. Plots were established ‘near’ to and ‘far’ from identified sources of pollinators (honey bee hives, leafcutter bee shelters, and semi-natural habitat (a potential source of wild pollinators)). I used a Nikon Laser 800S Rangefinder® to measure the distance (to the nearest meter) from the closest source of pollinators to the plot. The placement of managed pollinators influenced the maximum distance possible for ‘far’ plots. The range of distance near to and far from honey bee hives and potential wild bee habitat was large (3-850 m, and 3-770 m, respectively) in comparison to the range of distance near to and far away from leafcutter bee shelters (2-161 m). This was due to the location of the sources, as potential wild bee habitat and honey bee hives were located outside of the field, and leafcutter bee shelters were dispersed regularly throughout the field.

### ***3.3.2 Floral data***

I took floral measurements, including petal size, nectar volume, nectar sugar content, and floral density, to calculate an estimate of floral energy profit and the level of sexual dimorphism between the male and female morphs (Figure A.3). Floral measurements, with the exception of density, were taken on the field edge.

To measure petal size, I measured five petals, collected individually from plants at least 1 m away from one another, of each floral morph per field. Petals were measured to the nearest 0.05 mm from the base to the tip using a set of callipers.

I measured nectar volume at each site from five separate flowers of each morph, immediately upon arrival to the site (between 8:30 and 9:30 am), and again after a period of about seven hours. I used the initial nectar volume measurement to represent what would be initially available to the pollinators as they began foraging (morning nectar standing crop), and the measurements after seven hours to find the nectar production ( $\mu\text{L/hr}$ ) for each floral morph. After the seven hour period I also measured nectar sugar

concentration. Between measurements the flowers were marked and each inflorescence was covered with a fine-mesh Umiwe® white organza bag to prevent insect visitation. I measured nectar volume using 5  $\mu\text{L}$  microcapillary tubes (Drummond Microcap®), and nectar sugar concentration with an Atago 2340 HSR-500 Handheld Brix Refractometer®.

I measured floral density at each plot in all fields in both the male and the female bays with the use of a 0.25  $\text{m}^2$  section of a 1  $\text{m}^2$  quadrat placed against the edge of the bay. I counted the total number of inflorescences within the section, as well as the number of flowers per inflorescence for five representative inflorescences. It should be noted that the use of representative, rather than random, sampling does introduce the potential for sampling bias. The mean number of flowers per inflorescence was then multiplied by the number of inflorescences and then quadrupled to give the average density of flowers per square meter.

Flower density is one measure of resource availability, but pollinators should also respond to differences in nectar production between the two morphs. I therefore multiplied floral density at the plot level (flowers/ $\text{m}^2$ ) by the average nectar energy production of each of the floral morphs (J/h), resulting in a measure of plant profit (J/h $\cdot\text{m}^2$ ). To find the energetic production of each floral morph, I multiplied the density of sucrose at 20°C (1.177 mg/ $\mu\text{L}$ ) by the energy value of sucrose (16.49 J/mg), the adjusted sugar concentration of the flower (1.252, converting from g solute per g solution to g solute per mL solution (Bolten et al. 1979)), and the volume per hour of nectar produced ( $\mu\text{L}/\text{h}$ ). The amount of profit calculated was considered representative of the profit produced per  $\text{m}^2$  per hour in each bay.

### ***3.3.3 Pollinator visitation***

I recorded pollinator visitation in the female and male bays at each plot in the field using a 1  $\text{m}^2$  quadrat (Figure A.4). I placed quadrats opposite to each other in each bay, with their edge located on the bay edge. Quadrats were observed for a ten minute period in each bay in each plot. During an observation period, all pollinator visits to inflorescences within the quadrat were recorded. Each pollinator visit to a new inflorescence was counted, regardless if the pollinator had visited previous inflorescences within the quadrat. Only visitation to inflorescences, and not visitation to flowers within

an inflorescence, was quantified. Pollinators were identified without capture to the most specific degree possible: managed non-native bees to species, and wild bees to family (with the exception of the genus *Bombus*). All foraging honey bees were female, whereas leafcutter bees were both male and female. Hereafter, my use of the term ‘visitation’ refers to the total number of visits made by pollinators to separate inflorescences in a 1 m<sup>2</sup> quadrat within a ten minute time period. Hence a visitation of ‘10’ could be obtained from a single bee visiting 10 different inflorescences, or 10 different bees visiting a single (and identical) inflorescence, and all cases between these extremes. While visitation is not a perfect measure of pollinator abundance, it is representative of the pollinator activity at each bay in a plot. In this chapter, I use visitation as a proxy for local density of competitors.

I measured pollinator visitation primarily to understand its impact on movement. Distance from source should constrain the visitation of both leafcutter bees and honey bees (Ribbands 1951; Richards 1984; Jay 1986). The small body size of leafcutter bees is predicted to limit their foraging range more so than honey bees (Greenleaf et al. 2007). Interspecific competition could also affect pollinator visitation. Currie (1997) showed that honey bee abundances declined around leafcutter bee shelters. This could be due to scramble competition, as heterospecific competitors will be most numerous around their source, or interference competition due to nest defence or mating harassment. If this relationship exists, there would be a positive effect on visitation by distance from heterospecific pollinator source. I therefore used distance from source populations (honey bee, leafcutter bee, wild pollinator) as a means of choosing plots within a field to produce variation in densities of the different pollinator types.

Understanding what might affect visitation (the surrogate for competitor density) is important if visitation affects movement between morphs. I tested the effects of distance from source on bee visitation to confirm its importance. I also examined distance to heterospecific managed pollinator sources for its influence on pollinator visitation in conjunction with distance from conspecific source, either through an interaction or as two separate variables. These tests were limited to the two managed pollinators and their associated hives/shelters. I predicted that the managed pollinators would be less abundant

at greater distances away from their source, with leafcutter bee visitation declining more quickly compared to honey bee visitation. I predicted that the effect of distance from heterospecific sources would be positive, with greater visitation at greater distances away from heterospecific sources.

### ***3.3.4 Bee movement***

Bee movement was measured using two different methods, transect-crossing counts and following foraging individuals.

To quantify pollinator movement between bays, I counted bees crossing a 2 m length of rope set between two stakes at the height of the canola flowers, placed parallel to the break between the male and female bays (Figure A.4). An observer stood at one end of the transect line, viewing down its length, and recorded the number, type, and direction of crossing insects. Each transect was observed for a total of five minutes, broken into 2.5 minutes of monitoring a single crossing direction. I measured wind and temperature at shoulder height with a Kestrel 2000 Pocket Wind Meter®.

I also followed individual pollinators foraging in the male bay to measure their tendency to switch to the female bay, as this movement is required for pollination of the female plants. At each plot individual honey bee and leafcutter bees were followed for up to 60 seconds to determine their propensity to switch to the female bay. Honey bees often restrict their foraging to collecting either nectar or pollen (Free 1960), and so I distinguished pollen foragers (carrying pollen on their corbicula) and nectar foragers (carrying no corbicular pollen). The category ‘nectar’ foragers therefore may contain pollen-foraging individuals early in their foraging trip, and ‘pollen’ foragers includes those individuals collecting both pollen and nectar. Direct interference between pollinators may also cause more movement (as has been shown with honey bees; Greenleaf and Kremen 2006), so I also noted any inter- or intraspecific interactions between pollinators (i.e., incidents where a pollinator physically contacted another insect, either incidentally through flower sharing or through more aggressive behaviour). If a pollinator was lost (i.e., flew out of sight) during the 60 s, the total time followed and the sequence of inflorescences it visited until it was lost was recorded.

Movement across transects was measured simultaneously with visitation surveys, and the individual pollinator follows were conducted immediately after, so that observed switching could be compared with the number of pollinator visits. Transects started in the immediate area of the quadrat visitation counts, while individual follows took place within 10 m of the quadrat locations.

### ***3.3.5 Statistical analysis***

All analyses were performed in R v.3.2.2 (R Core Team 2015). Models were checked for multicollinearity, and residuals for overdispersion, normality, and outliers. Variable means and parameter estimates are presented  $\pm$  standard error.

*A priori* candidate models for bee visitation and bee movement were assessed using Akaike's Information Criterion (AIC) (Akaike 1973). The smaller sample size of the transect-crossing data necessitated the use of corrected AIC (Sugiura 1978; Hurvich and Tsai 1989). I calculated  $\Delta$ AIC or  $\Delta$ AICc by subtracting the lowest AIC(c) value from all other values. Within the top-ranking models, those with a  $\Delta$ AIC(c) of  $<2$  were considered to have substantial support, and those of  $<10$  were kept for inference (Burnham and Anderson 2002).

When dealing with model selection between nested models, there is a potential for models with uninformative parameters to be selected due to their similarity to better-fitting models. In these cases, the addition of a parameter does not improve model fit (as represented by model log-likelihood). However, because the additional parameter only incurs a penalty of +2 AIC, it is possible for the model to be within 2  $\Delta$ AIC of a better-supported model despite the uninformative parameter (Burnham and Anderson 2002, page 131). Arnold (2010) shows how models with an extra parameter composed of randomized variables can still be selected if they share parameters with legitimately well-fitting models. Models within 2  $\Delta$ AIC of the best-fitting model that share all of its characteristics but contain an additional parameter should be scrutinized before being used for inference. I therefore performed follow-up likelihood ratio tests on all models within this category to check for uninformative parameters, and excluded from inference those with parameters that did not improve the log-likelihood.

#### 3.3.5.1 Floral data

To accommodate the non-linear relationship between the predictor and one of the response variables, floral data were analyzed using generalized additive mixed models with a normal distribution in the `gamm4` package in R (Wood and Scheipl 2014). Response variables included petal size, nectar volume per hour, and nectar sugar content; sex was a fixed effect, with extraneous covariates Julian day (splined), temperature, and hours covered (nectar data only). Site was a random effect.

#### 3.3.5.2 Pollinator visitation

To test whether distance from source affected pollinator abundance, I analyzed the visitation of leafcutter bees and honey bees using generalized linear mixed effects models with a negative binomial error distribution in the `glmmADMB` package in R (Skaug et al. 2014). The models included a full model with an interaction between leafcutter bee shelters and honey bee hives, a model without the interaction, a model with only the conspecific pollinator source, and a model containing only extraneous covariates. Distance from leafcutter bee shelters was ln-transformed to explain leafcutter bee visitation, but distance from honey bee hives was left untransformed. Extraneous variables were those whose effect I was not interested in but still affected the response variable. Julian day was used as an extraneous covariate for both types of pollinators; temperature and time of day were also used for leafcutter bees, and wind speed for honey bees. Site and plot nested within site were included as random effects. Coefficients of top-ranking models were standardized to allow for comparison.

#### 3.3.5.3 Bee movement

For the transect line crossings, I constructed candidate models to predict the conditions under which leafcutter bees and honey bees would switch from one bay to another (Table 3.1). Recall that I predicted that competition would be the strongest from conspecifics, but that heterospecific visitation or taxon diversity (in conjunction with conspecific visitation) could still influence changes in movement. To distinguish these alternatives, I separated competitors into four categories: 1) conspecific visitation, 2) conspecific and heterospecific visitation, 3) conspecific visitation and taxon richness, or 4) conspecific and heterospecific visitation and taxon richness. Visitation was used as a

representation of pollinator abundance. Because the crudity of species classifications precluded species richness from being measured, a more general taxon richness that encompassed broader insect classifications was used. This included honey bees, leafcutter bees, native bees (all non-managed bee species), hoverflies (*Syrphidae*), bee flies (*Bombilyiidae*), calyprate muscoids, wasps, and lepidopterans. Taxon richness was rarefied to a sample size of two to allow for comparisons between areas of different sample sizes with the R package *vegan* (Oksanen et al. 2016).

**Table 3.1: Candidate models to explain the number of pollinators crossing transects. Each competition term, with the exception of the null model and the richness variable, was run with three different bay conditions, with and without one of two profit terms. Extraneous covariates (Julian day and temperature) and site as a random effect were included in every model.**

Competition	Bay	Profit
Conspecific	Current * Opposite	$\Delta$ Profit + Current profit
Conspecific + heterospecific	Current + Opposite	$\Delta$ Profit
Conspecific + richness	Current	
Conspecific + heterospecific + richness		
[Null (extraneous covariates only)]		

While the potential effects of competitors in the same bay on foraging gain seem clear, the more difficult to detect presence of pollinators in the opposite bay could dampen foraging prospects in the alternative patch, and act as a deterrent to movement. If pollinators associate higher abundances of competitors with decreased rewards (rather than simply responding to decreased rewards), they should avoid areas with more competitors. Visitation to the opposite bay could modify the tendency of a pollinator to switch bays, either through interactive effects between bays (e.g., an increase in pollinator visitation in the opposite bay acting to deter movement to that bay), or just by decreasing the attractiveness of the opposite bay due to the presence of competitors. Unlike pollinator abundance, taxon richness in the opposite bay would be more difficult for pollinators to assess at a distance and would likely not deter crossing. I therefore included potential effects of visitation of the opposite bay in movement measures, but not taxon richness.

Floral profit ( $J/h \cdot m^2$ ) of each bay could also affect pollinator movement. Bays with higher profit relative to where pollinators are foraging should attract pollinators. It is

also possible that the absolute profit of the bay pollinators were foraging in could modify their tendency to move, since higher absolute profit should deter pollinators from leaving an area. Pollinators could just respond to  $\Delta$  profit, the difference in profit between the bay being crossed to and the bay currently occupied, or modify their response to  $\Delta$  profit depending on the absolute value of the profit of the bay they were already in.

To explain frequency of crossing between bays, I fit models that included the influence of competitor visitation to the bay being currently occupied and the opposite bay (of a different floral morph) as 1) an interaction (current\*opposite), 2) non-interacting variables (current + opposite), and 3) just the current bay. Models were also compared with and without the effect of either relative profit alone, or relative profit and the profit of the bay the pollinator was currently in. A null model (with extraneous covariates and site as a random effect) was also included.

I analyzed transect line crossings using generalized linear mixed effects models with negative binomial error distributions in R with the package glmmADMB (Skaug et al. 2014). Because of the non-linear nature of the leafcutter bee crossing response, all pollinator visitation predictors were ln-transformed for analyses of leafcutter bees. Site was included as a random effect. Julian day and temperature were included as extraneous covariates. Predictor variables in the top-ranking models were standardized to obtain standardized coefficients.

The propensity of a pollinator to switch bays during individual follows was analysed using Cox proportional hazards models with the survival package in R (Therneau 2015); *a posteriori* contrasts were analysed with the use of the rms package (Harrell 2016). A low number of switching events (39, out of a sample size of 728) for individual travels necessitated that the pollinator data be analysed as a whole (rather than separating pollinators by foraging type). To simplify interpretation, the types of competition were limited to total pollinator visitation, rarefied taxon richness, or a combination of the two (Table 3.2). Combining competitors in this manner allows for more simplified inferences, but is also likely to lower statistical power, given differences in competitive effects between and among species. By combining visitation among all pollinators into a single variable, I imply that all individuals are competitively similar, so

caution must be used in making inferences about this variable. Similar to transect crossing models, the influences of bay (either the additive or interactive effects of visitation to both the male and female bays, or just visitation to the female bay) and the two profit variables (male profit, or the absolute profit of the male bay, and  $\Delta$  profit) were included. Along with competition and profit, I included whether or not there were physical interactions between pollinators (pollinator encounters), and pollinator type. Switching should vary between pollinator type, as honey bees often specialize on either nectar or pollen foraging (Free 1960), whereas female leafcutter bees must provision their offspring with both pollen and nectar. Pollen-foraging honey bees should therefore be less likely than nectar foragers or leafcutter bees to cross to the female bay.

**Table 3.2: Candidate models to explain individual pollinators switching from foraging on male to female flowers. Each competition term, with the exception of the null model, pollinator-only model, and the richness variable, was run with three different bay conditions, with and without  $\Delta$  profit and male profit. Pollinator type was included in every model but the null model. Extraneous covariates (Julian day and temperature) were included in every model, as well as site as a frailty term, and interactions between pollinators as a stratified term.**

Competition	Bay	Profit
Total abundance	Current * Opposite	$\Delta$ Profit + male profit
Richness	Current + Opposite	$\Delta$ Profit
Total abundance + richness	Current	
Pollinator (pollinator term only)		
[Null (extraneous covariates only)]		

Observations were right-censored, as individuals may have switched after being followed for the full minute or after being lost before the minute was up. The amount of time followed as well as whether the bee switched bays were both included in the model response to account for this. Preliminary tests revealed incidences of pollinator encounters to be a non-constant hazard (the likelihood of encounter was not constant over time), and so the term was included as a stratified variable. I used a frailty approach to account for non-independence among bees within each site. Extraneous covariates were Julian day and temperature.

## 3.4 Results

### 3.4.1 Floral data

The petals of male flowers were larger than those of female flowers, with the average male petal length being  $12.5 \pm 0.10$  mm ( $n=86$ ), and the average female petal length  $8.64 \pm 0.09$  mm ( $n=86$ ) ( $\Delta AIC=350.14$ ,  $df=7$  without the variable sex). Male flowers had a rate of nectar secretion of  $0.06 \pm 0.004$   $\mu\text{L}/\text{h}$  ( $n=90$ ), while female flowers' hourly nectar secretion rate was  $0.04 \pm 0.003$   $\mu\text{L}/\text{h}$  ( $n=86$ ) ( $\Delta AIC=9.13$ ,  $df=8$  without the variable sex). This translated into a difference of profit of  $1.47 \pm 1.00$  J/h ( $n=90$ ) for male flowers and  $1.08 \pm 0.75$  J/h ( $n=86$ ) for female flowers.

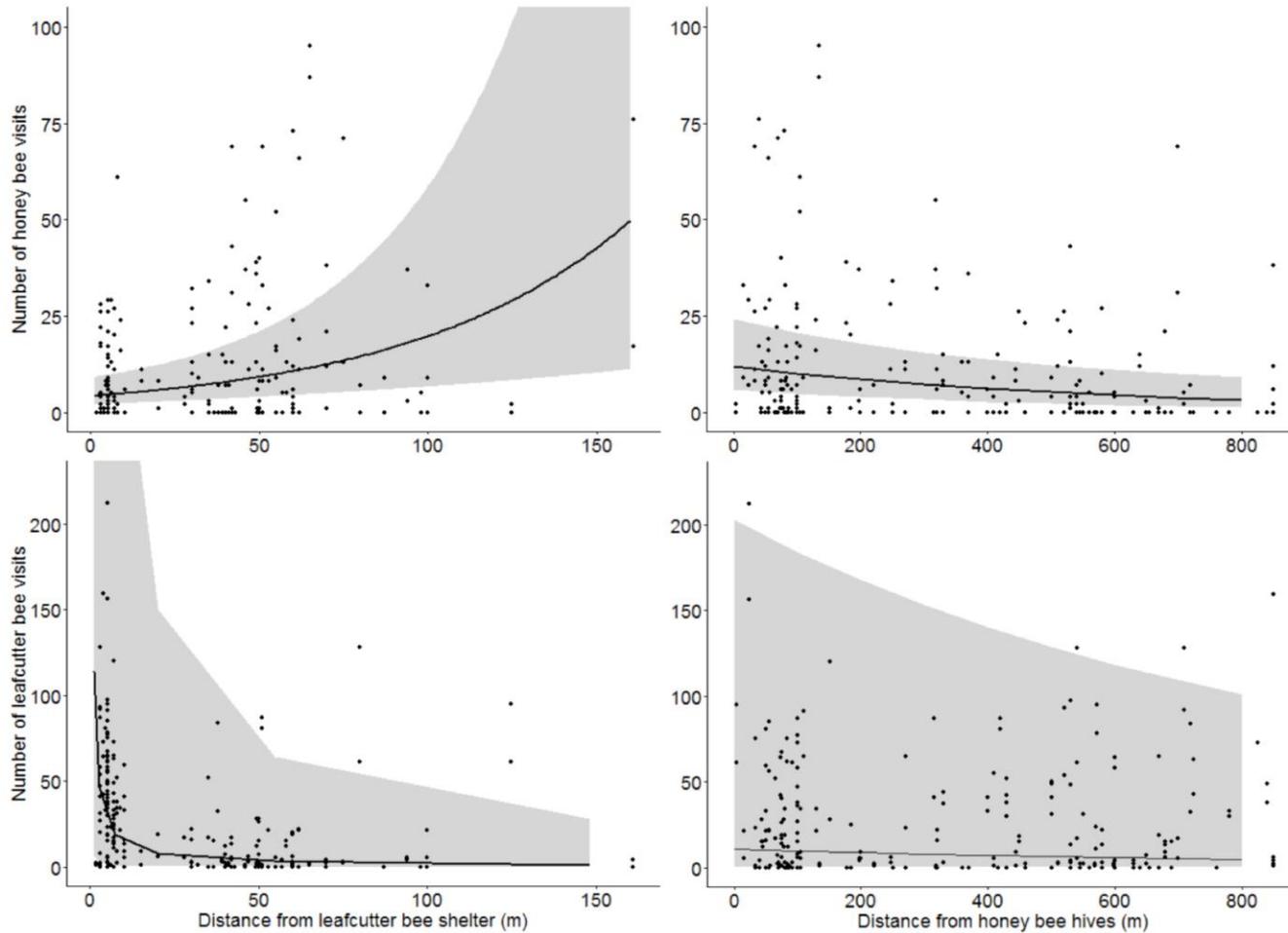
In contrast, nectar sugar concentration ( $df=8$ ,  $\Delta AIC=0.84$ ) and morning nectar standing crop ( $df=3$ ,  $\Delta AIC=4.02$ ) were not influenced by the sex of the floral morph. Overall, the nectar sugar concentration was  $54 \pm 0.34\%$  ( $n=70$ ) and morning nectar standing crop was  $0.12 \pm 0.02$   $\mu\text{L}$  ( $n=217$ ).

### 3.4.2 Pollinator visitation

Distance from both conspecific and heterospecific sources influenced visitation by leafcutter bees ( $df=7$ ,  $\Delta AIC=0$ ) (Figure 3.1), although leafcutter bee visitation could still be explained without considering the effect of distance from honey bee hives ( $df=6$ ,  $\Delta AIC=3.96$ ). This is likely because the negative effect of distance from leafcutter bee shelters on leafcutter bee visitation was much stronger ( $\beta=-1.08 \pm 0.12$ ) than the negative effect of distance from honey bee hives ( $\beta=-0.24 \pm 0.12$ ). While leafcutter bee visitation declined much more rapidly away from leafcutter bee shelters than from honey bee hives, they also experienced considerably shorter physical scales over which competition was evaluated (mean distance from leafcutter bee shelters was  $31.2 \pm 2$  m, while from honey bee hives was  $332.6 \pm 16.6$  m).

Honey bees were also strongly influenced by distance from honey bee hives and leafcutter bee shelters ( $df=7$ ,  $\Delta AIC=0$ ) (Figure 3.1); the effect of distance from a heterospecific source mattered more for honey bee visitation than it did for leafcutter bee visitation (distance from honey bee hives alone was  $> 10 \Delta AIC$ ). The relationship was negative for distance away from honey bee hives ( $\beta=-0.42 \pm 0.13$ ) and, as expected, positive for distance away from leafcutter bee shelters ( $\beta=0.47 \pm 0.13$ ). Honey bee

visitation declined more gradually away from hives than leafcutter bee visitation did from their shelters.



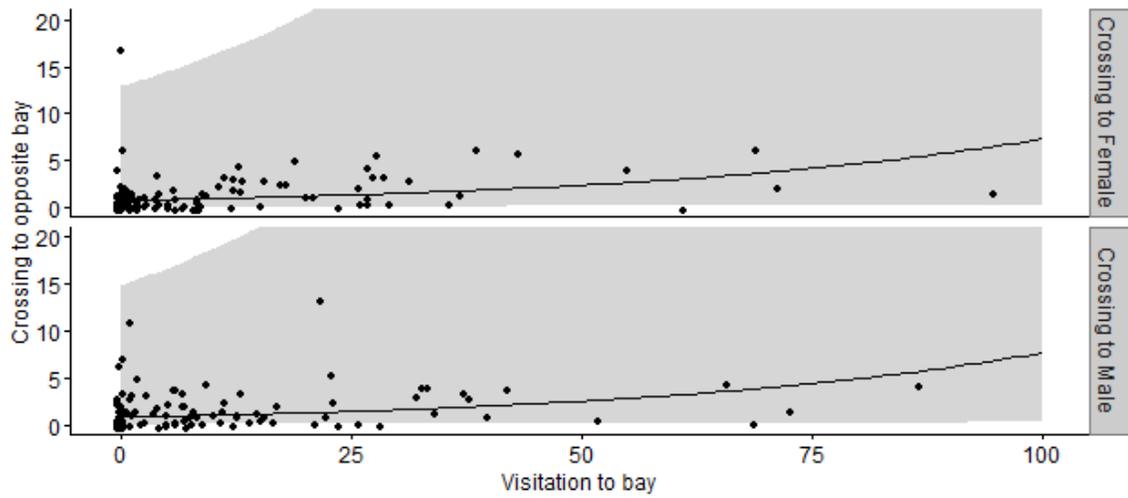
**Figure 3.1: The influence of distance from the nearest leafcutter bee shelter (left) and honey bee hives (right) on honey bee (top) and leafcutter bee (bottom) visitation (n=234), with predicted trend line plotted against observed (non-adjusted) points. Extraneous variables (Julian day, temperature, and time of day for leafcutter bees; Julian day and wind speed for honey bees) were held constant for trend lines. Shaded area represents the 95% CI.**

### **3.4.3 Bee movement**

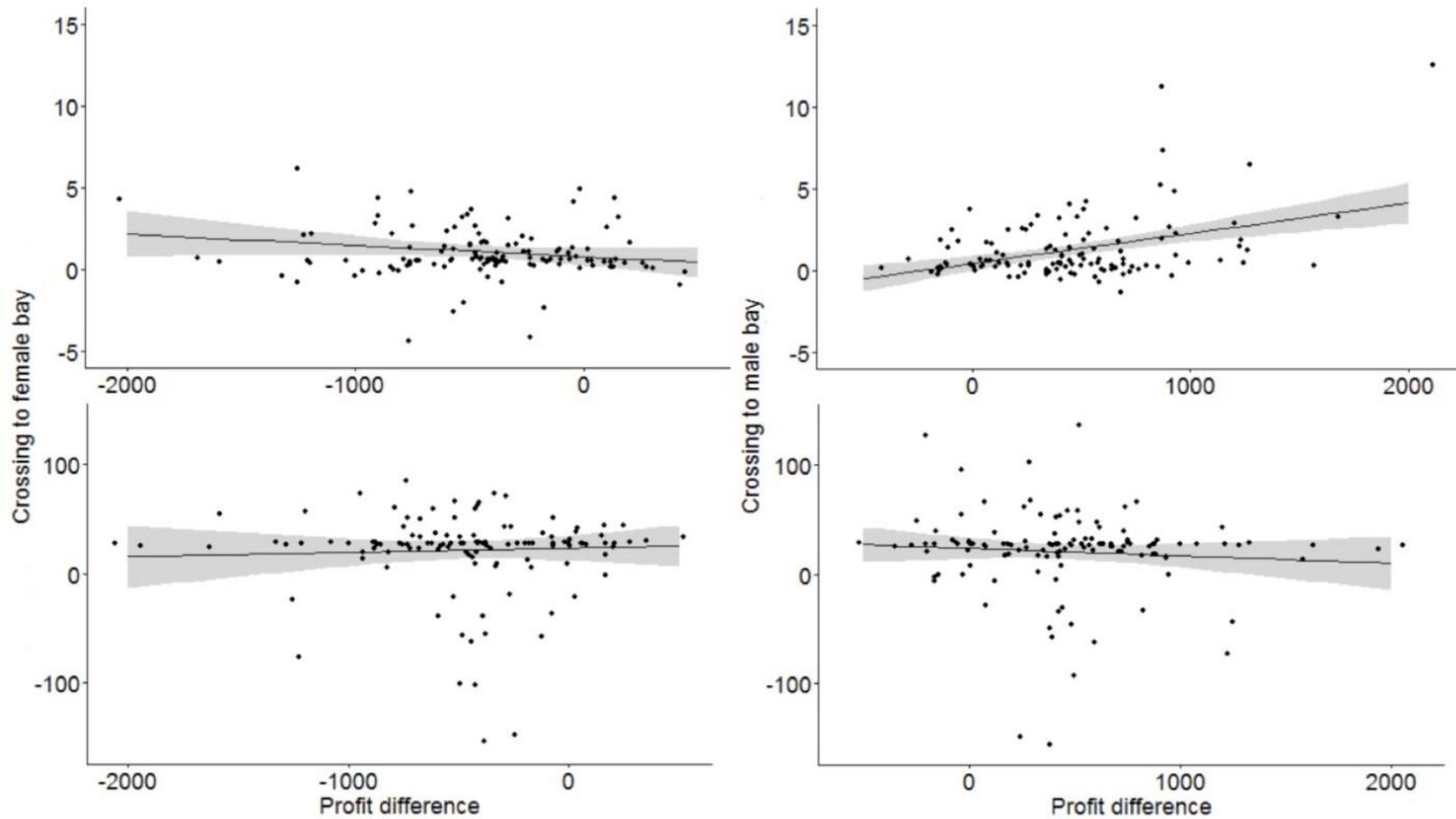
#### 3.4.3.1 Transect line crossing

Visitation by conspecific pollinators in the current bay and  $\Delta$  profit best explained pollinator crossing frequency between bays for both leafcutter and honey bees (Tables B.1—B.4). The relative difference in profit, rather than the absolute profit of the bay pollinators were crossing from, most influenced movement. With the exception of taxon richness for honey bees crossing to the male bay, visitation by heterospecific pollinators and taxon richness were not largely influential in causing pollinators to cross between bays. The abundance of pollinators in the bay being crossed to did not affect honey bee crossing, although it was important for leafcutter bee crossing. I first consider pollinator type, and then crossing type, in the following results.

As expected, higher visitation by honey bees to the male bay led to an increase in the number of honey bees crossing from the male to the female bay ( $df=6$ ,  $\Delta AICc=1.49$ ) (Figure 3.2).  $\Delta$  profit also played a role ( $df=7$ ,  $\Delta AICc=0$ ), although its partial effect on crossing was unexpectedly negative; honey bees were less likely to cross to the female bay as profit in the female bay increased relative to the amount in the male bay (Figure 3.3). However, the effect of both honey bee visitation and  $\Delta$  profit on crossing was relatively weak, especially in comparison to the effects of Julian day and temperature (Table 3.3), which alone could account for honey bee movement to the female bay ( $df=5$ ,  $\Delta AICc=3.39$ ). As the season progressed, honey bees were more likely to cross from the male to the female bay; likewise, as temperatures increased, more bees crossed to the female bay. Greater floral profit in the male bay was not a deterrent to crossing towards the female bay.



**Figure 3.2: Relationship between honey bee visitation to the male or female bay (X) and crossing to the female (top) or male (bottom) bay (Y), with predicted trend line plotted against observed (non-adjusted) points (n=121).  $\Delta$  profit, temperature, and Julian day were held constant for trend line. Shaded area represents the 95% CI.**

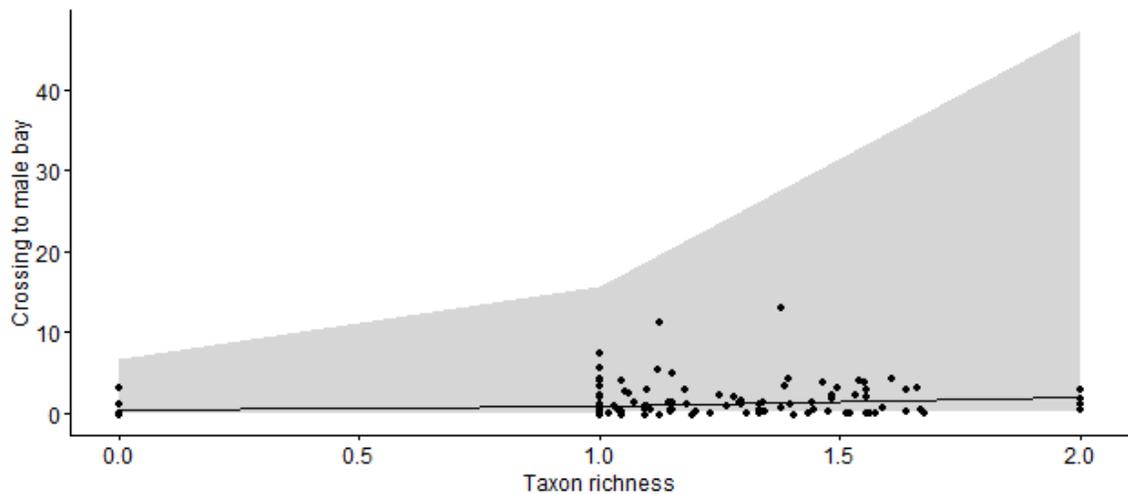


**Figure 3.3: Partial regression plots of honey bees (top; n=121) and leafcutter bees (bottom; n=122) crossing between bays as influenced by  $\Delta$  profit between the bays. The number of bees crossing between bays (Y) was adjusted to account for the effects of visitation, temperature, and Julian day. The figures on the left represent female profit minus male profit, while the figures on the right represent male profit minus female profit. Shaded area shows the 95% CI.**

**Table 3.3: Parameter estimates (SE) for top-ranked honey bee transect-crossing models (n=121). Variables have been standardized to allow for direct comparison of coefficients. The coefficients show the likelihood of a honey bee crossing to the male or female bay as a response to honey bee (HB) visitation (v) to the male (M) or female (F) bay, rarefied taxon richness (R), and relative profit ( $\Delta P$ ). Julian day and temperature are included as extraneous variables in every model, and site is present as a random effect.**

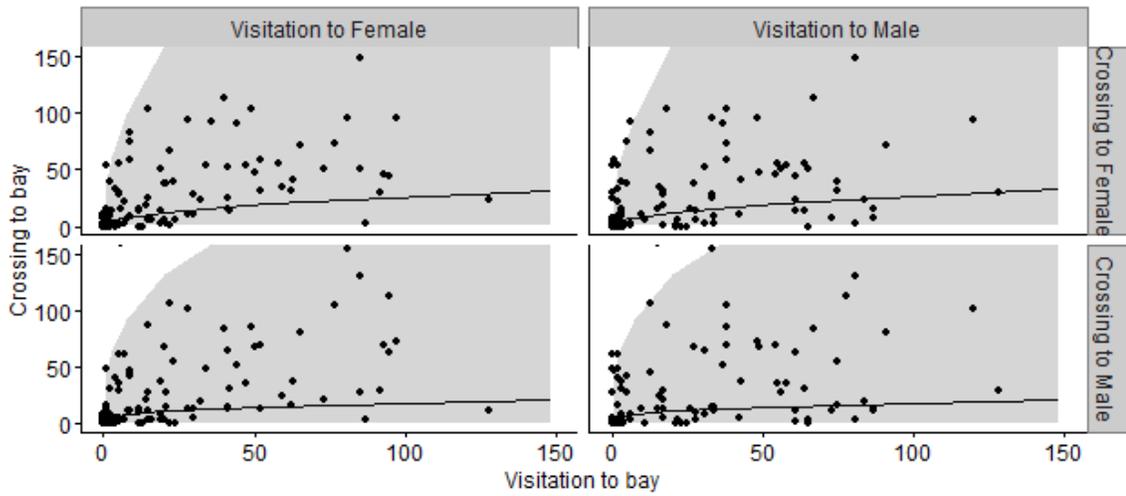
Crossing to	Model	Conspecific visitation		Richness	$\Delta$ Profit		Julian day	Temperature
		To male	To female		M-F	F-M		
Female	HBvM	0.30 (0.15)					0.46 (0.20)	0.47 (0.19)
	HBvM+ $\Delta P$	0.37 (0.16)				-0.27 (0.15)	0.40 (0.20)	0.44 (0.19)
	Null						0.63 (0.18)	0.49 (0.19)
Male	HBvF+ $\Delta P$		0.40 (0.13)		0.52 (0.13)		-0.12 (0.16)	-0.08 (0.16)
	HBvF+RF+ $\Delta P$		0.36 (0.13)	0.30 (0.17)	0.53 (0.13)		-0.06 (0.16)	-0.21 (0.17)

In a similar fashion, honey bee visitation to the female bay and  $\Delta$  profit had the largest impact on honey bee crossing to the male bay ( $df=7$ ,  $\Delta AICc=1.16$ ), while absolute profit in the female bay did not affect their tendency to cross. When there was higher honey bee visitation to the female bay, more movement was observed towards the male bay (Figure 3.2); increasing profit in the male bay relative to the female bay resulted in more honey bees crossing to the male bay (Figure 3.3). Both results support my predictions. Along with honey bee visitation and  $\Delta$  profit, increases in taxon richness in the female bay resulted in more honey bees crossing to the male bay ( $df=8$ ,  $\Delta AICc=0$ ) (Table 3.3; Figure 3.4). The effects of Julian day and temperature were largely negligible in influencing honey bee movement to the male bay.



**Figure 3.4: Frequency of honey bees crossing to the male bay as influenced by rarefied taxon richness ( $n=121$ ), with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables  $\Delta$  profit, visitation, temperature, and Julian day were held constant. Shaded area represents the 95% CI.**

For leafcutter bees, increased visitation to both the male and female bays led to more leafcutter bees crossing to the female bay ( $df=7$ ,  $\Delta AICc=1.5$ ), as did an increase in relative female profit ( $df=8$ ,  $\Delta AICc=0$ ). The positive effect of leafcutter bee visitation to both bays (Figure 3.5) was stronger than the positive effect of  $\Delta$  profit (Figure 3.3; Table 3.4). Higher temperatures resulted in more leafcutter bees crossing to the female bay, while there was less crossing later in the season. Increasing profit in the male bay did not influence the movement of leafcutter bees to the female bay.



**Figure 3.5: The relationship between leafcutter bees crossing to the female (top) and male (bottom) bays, as influenced by leafcutter bee visitation to the female (left) and male (right) bay, with predicted trend line plotted against observed (non-adjusted) points (n=122).  $\Delta$  profit, temperature, and Julian day were held constant for the trend line. Shaded area represents the 95% CI.**

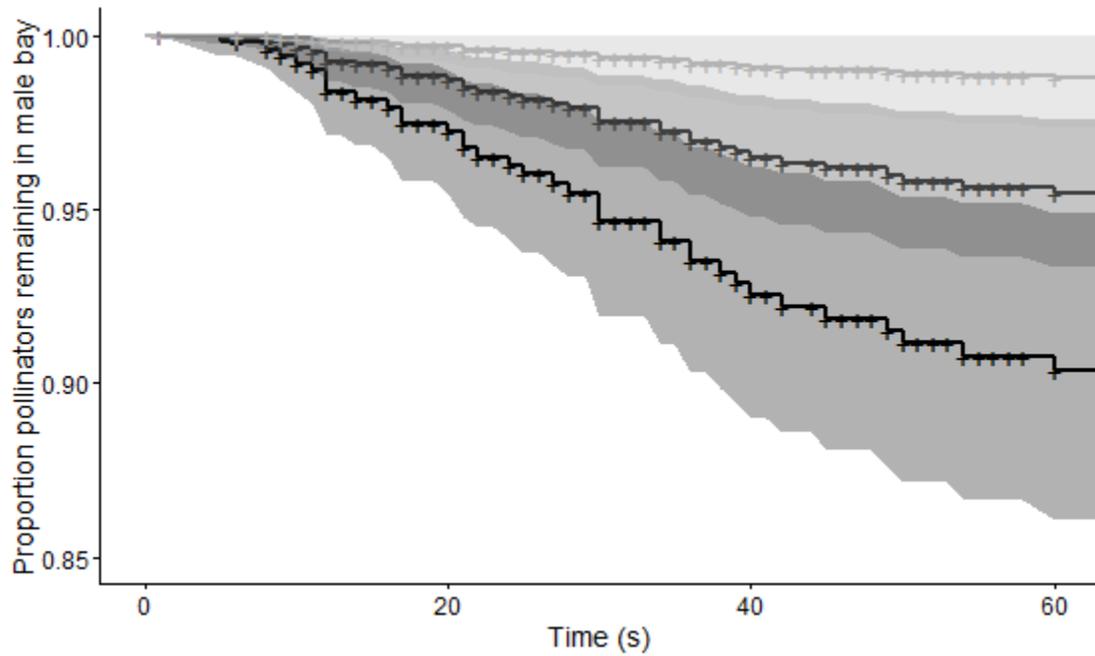
**Table 3.4: Parameter estimates (SE) for top-ranked leafcutter bee transect crossing models (n=122). Variables have been standardized to allow for direct comparison of coefficients. The coefficients show the likelihood of a leafcutter bee crossing to the male or female bay as a response to leafcutter bee (LCB) and heterospecific bee (HetB) visitation (v) to the male (M) or female (F) bay, as well as relative profit ( $\Delta P$ ). Julian day and temperature are included as extraneous variables in every model, and site is present as a random effect.**

Crossing to	Model	Conspecific visitation		Heterospecific visitation	$\Delta$ Profit		Julian day	Temperature
		To male	To female	To female	M-F	F-M		
Female	LCBvF+LCBvM	0.81 (0.18)	0.71 (0.17)				-0.81 (0.18)	0.55 (0.17)
	LCBvF+LCBvM + $\Delta P$	0.82 (0.18)	0.73 (0.18)			0.23 (0.11)	-0.74 (0.17)	0.56 (0.16)
Male	LCBvF+LCBvM	0.58 (0.17)	0.84 (0.17)				-0.70 (0.17)	0.58 (0.16)
	LCBvF+LCBvM + $\Delta P$	0.57 (0.17)	0.87 (0.17)		-0.23 (0.12)		-0.63 (0.16)	0.60 (0.15)
	LCBvF+HetBvF + $\Delta P$		1.22 (0.12)	-0.21 (0.12)	-0.28 (0.12)			
	LCBvF+ $\Delta P$		1.27 (0.12)		-0.25 (0.12)		-0.45 (0.16)	0.67 (0.15)
	LCBvF+HetBvF		1.20 (0.13)	-0.19 (0.13)			-0.55 (0.17)	0.68 (0.16)
	LCBvF		0.76 (0.08)				-0.51 (0.16)	0.66 (0.16)

An increase in leafcutter bee visitation to both the male and female bays resulted in increased leafcutter bee movement towards the male bay ( $df=7$ ,  $\Delta AICc=1.42$ ) (Figure 3.5; Table 3.4). Leafcutter bee visitation to the female bay alone ( $df=6$ ,  $\Delta AICc=9.91$ ), or with  $\Delta$  profit ( $df=7$ ,  $\Delta AICc=8.23$ ), also explained the tendency of leafcutter bees to cross from the female to the male bay, although this relationship was improved with the addition of leafcutter bee visitation to the male bay. Unlike leafcutter bees crossing to the female bay, they were unexpectedly less likely to cross to the male bay as it became relatively more profitable ( $df=8$ ,  $\Delta AICc=0$ ) (Figure 3.4). The profitability of the female bay did not influence movement to the male bay. The visitation of heterospecific pollinators to the female bay, along with leafcutter bee visitation to the female bay with ( $df=8$ ,  $\Delta AICc=7.24$ ) or without ( $df=7$ ,  $\Delta AICc=9.75$ )  $\Delta$  profit, had a potentially negative, if comparatively small, effect on the tendency of leafcutter bees to cross between bays (Table 3.4). Temperature and Julian day had similar influences on whether leafcutter bees crossed to the male bay as they did on leafcutter bees crossing to the female bay: increased temperatures resulted in more observed crossing, while less crossing was observed as the season progressed.

#### 3.4.3.2 Individual follows

Bees generally did not switch between bays during 60 s of observation. Out of 728 observations, only 39 switching events were recorded (5.4%). After controlling for extraneous covariates, pollinator type was important in determining whether an individual switched from foraging on male to female inflorescences ( $df=4$ ,  $\Delta AIC=4.47$ ). Honey bees, regardless of whether they were foraging for nectar or pollen, were less likely than leafcutter bees to switch to the male bay (Figure 3.6; Table 3.5). Post hoc contrasts showed that nectar-foraging honey bees were more likely to cross between the bays than were pollen-foraging honey bees ( $p=0.04$ ).



**Figure 3.6: Propensity of leafcutter bees (black line), honey bee nectar foragers (grey line), and honey bee nectar foragers (light grey line) to remain in the male bay for 60 s (n=728). Slopes are Cox model coefficients (see Table 3.4). Shading around lines represents the 95% CI.**

**Table 3.5: Cox analysis for top ranking models, with hazard ratios  $\pm$  SE (n=728). Variables include total pollinator visitation (Total) to the male (M) and female (F) bay, rarefied taxon richness (R; rarefied sample=2), and the type of pollinator. The tendency of leafcutter bees to switch acts as a reference level for nectar and pollen honey bee forager movement. Site was included as a frailty term and a term for interactions between pollinators was included as a stratified variable.**

Variables	Total visitation		Richness	Honey bee forager			
	To male	To female		Nectar	Pollen	Julian Day	Temperature
TotalM+TotalF+R+Pollinator	0.999 (0.01)	0.99 (0.01)	3.47 (0.67)	0.46 (0.35)	0.12 (0.63)	0.94 (0.02)	1.05 (0.04)
R+Pollinator			4.6 (0.68)	0.50 (0.35)	0.13 (0.63)	0.94 (0.02)	1.04 (0.04)
TotalM+RM+Pollinator	0.99 (0.01)		3.47 (0.66)	0.47 (0.35)	0.12 (0.63)	0.95 (0.02)	1.04 (0.04)
TotalM+TotalF+Pollinator	0.997 (0.01)	0.99 (0.01)		0.47 (0.35)	0.14 (0.62)	0.94 (0.02)	1.05 (0.04)
TotalM+Pollinator	0.99 (0.01)			0.49 (0.35)	0.14 (0.62)	0.94 (0.02)	1.04 (0.04)
Pollinator				0.54 (0.35)	0.15 (0.62)	0.94 (0.02)	1.04 (0.04)

More pollinators switched from foraging on male to female flowers over time as taxon richness increased in the male bay ( $df=5$ ,  $\Delta AIC=0.99$ ). In contrast, the effects of pollinator visitation to both the male and female bay—either in addition to taxon richness ( $df=7$ ,  $\Delta AIC=0$ ) or alone ( $df=6$ ,  $\Delta AIC=2.11$ )—were largely negligible in increasing the likelihood of switching (Table 3.5). Excluding the effects of visitation to the female bay and focusing on the effect of pollinator visits to the male bay, either in conjunction with taxon richness ( $df=6$ ,  $\Delta AIC=1.22$ ) or alone ( $df=5$ ,  $\Delta AIC=3.39$ ), showed negative but marginal effects of the total number of pollinator visits compared to taxon richness and pollinator type (Table 3.5). Neither the amount of profit in the male bay nor the relative difference in profit between bays influenced the tendency of a pollinator to switch to foraging on female inflorescences (Table B.5). As the season progressed (measured as Julian day), pollinators were less likely to switch. Temperature had a positive, if small, effect on crossing (Table 3.5).

### **3.5 Discussion**

#### **3.5.1 Floral data**

Hybrid canola is gynodioecious, and therefore sexually dimorphic. Along with the obvious absence of pollen on female flowers, there were both visual (petal size) and rewards-based (nectar production) differences that distinguished the morphs. This sexual dimorphism influenced pollinator visitation behaviour in this study. This was also the case in gynodioecious hybrid sunflower, in which visual heterogeneity between parental lines was thought to reduce crossing between parental lines and therefore efficacy of pollination (Martin and Farina 2016).

Despite this sexual dimorphism, and higher nectar production by males, the early morning nectar standing crop showed no difference in volume between morphs. This implies that pollinators are still visiting both sexes, rather than preferentially visiting the more rewarding male flowers. Delph and Lively (1992) found a similar situation in the gynodioecious shrub *Hebe stricta*, in which pollinators preferred to visit the more rewarding male flowers, but the natural standing nectar crop did not differ significantly between floral morphs. This supports the IFD theory that floral visitors deplete the rewards in males until their profitability equals that of female flowers.

### **3.5.2 Pollinator visitation**

The data supported my prediction that the visitation of honey and leafcutter bees was affected by distance away from both honey bee hives and leafcutter bee shelters. This agrees with previous findings that suggest that honey and leafcutter bee foraging is limited by distance away from their source (Ribbands 1951; Richards 1984; Jay 1986). This has obvious implications for pollination, since flowers located outside of foraging ranges will have reduced or no visitation. Leafcutter bees have comparatively greater visitation near their shelters than honey bees have near their hives, but also show a dramatic drop in visitation by around 25 m away from their shelters, whereas the number of honey bee visits decline more gradually with distance away from their hives.

My study also confirmed a relationship suggested by Currie (1997), who found that honey bee abundance dropped around leafcutter bee shelters, suggesting that honey bees likely avoid leafcutter bee shelters. Declines in honey bee visitation around leafcutter bee shelters could be due to competition or avoidance of interactions with leafcutter bees. Leafcutter bee visitation is highest around their shelters compared to elsewhere in the field, so resource consumption would likely be higher around shelters. Honey bees may avoid the area around leafcutter bee shelters because the foraging is relatively poor.

Besides scramble competition for resources, it is also possible that honey bees were avoiding behavioural interactions with leafcutter bees that could interrupt their foraging (i.e., interference competition). Although I did not quantify these behaviours, I commonly observed aggressive behaviour towards competitors by leafcutter bee females (in which a female bee would fly and forcefully hit another pollinator with her body). Territoriality of female bees, especially when involving nest defence, has been seen before in solitary bees (Batra 1978). *M. rotundata* females are typically gregarious (Richards 1984), but I did observe honey bees intruding into leafcutter bee nesting cavities during cold days when leafcutter bees were inactive, likely to steal resources. If leafcutter bees identify honey bees as a threat to their nests or local foraging, they may display more aggressive behaviour towards honey bees.

Another potentially disruptive behaviour was sexual harassment (often in the form of grappling) initiated by male leafcutter bees towards other pollinators. This behaviour is common among these males, and can disrupt flower visitation; increases in male density reduce the number of foraging trips made by females (Rossi et al. 2010). Incidences of male grappling were not limited to female leafcutter bees, but were also observed against honey bee foragers.

Leafcutter bees were also affected by distance from the closest honey bee hives, but unlike the increase in honey bee visitation at greater distances away from leafcutter bee shelters, leafcutter bee visitation declined at greater distances from honey bee hives. This is likely not due to a facilitative relationship between the pollinators, as they are both competing for the same resources. Rather, it could be due to the location of honey bees hives on field boundaries. For this study these hives might represent a proxy of the field edge. Because of their smaller foraging ranges, I assumed leafcutter bees would stay within the canola field. However, besides food resources, leafcutter bees also require leaves for nesting material, and must compete with other leafcutter bee females for access to nesting cavities within the shelters. If the material in canola leaves or flowers is deficient as nest material, some leafcutter bees may have moved to the field edge in search of nesting material. They may also seek out alternative nesting cavities outside the field.

### **3.5.3 *Bee movement***

#### 3.5.3.1 Transect line crossing

According to the IFD (Fretwell and Lucas 1970), foragers should maximize their returns by responding to both profit and competitors (which decrease profit), such that all foragers obtain identical gains. Male flowers in this hybrid canola system were more rewarding and visually distinct from female flowers. This, along with the spatial separation between bays, could deter movement between morphs. However, the local consumption of resources by competitors and the relative differences in profit between bays should motivate pollinators to sample and move to alternative foraging resources. Greater competitor pressure and floral profit should therefore motivate more movement between bays.

After statistically controlling for differences in profitability, the number of competitors was important in driving foraging behaviour. At low levels of pollinator visitation (and therefore low levels of competition), little crossing to the adjacent bay was observed. As local competition increased, pollinators increasingly crossed. Competition in the opposite bay had no effect on honey bee movement (and a positive effect on leafcutter bee movement, discussed below), suggesting that pollinators modify their foraging habits in response to the consumption of local resources (encountering poor rewards, and responding accordingly), but perhaps not directly as a response to competitor abundances.

After controlling for competition, the relative difference in profit between bays also prompted pollinator response. As predicted, honey bees crossing to the male bay and leafcutter bees crossing to the female were more likely to cross when the opposite bay had a relatively higher amount of profit. However, while the difference in profit still mattered for honey bees crossing to the female bay and leafcutter bees crossing to the male, the effect was negative. As profit increased relative to the bay the foragers were in, they were less likely to cross towards it. This relationship seems nonsensical from the perspective of optimizing foraging gain. One possible explanation is that there was an interaction between  $\Delta$  profit and visitation—higher profit resulted in more foragers, which decreased local rewards. In this case, areas with lower profit but less competition would give comparable returns (i.e., an IFD), and pollinators should move less. However, visitation of pollinators to the opposite bay was not an obvious deterrent to honey or leafcutter bee crossing. While it is possible pollinators could assess density of competitors and associate them with resource consumption, there was no clear indication that either type of bee did this.

The degree of response to  $\Delta$  profit differed between pollinators. It was a less influential driver of crossing for leafcutter bees than for honey bees. This could be due to a difference in conspecific visitation between the two bee species. Leafcutter bee visitation was comparatively greater than honey bee visitation (mean visitation was  $49 \pm 6$  bees per plot for leafcutter bees, compared to the mean honey bee visitation of  $20 \pm 3$ ).

While profit was still important, visitation could have exerted a stronger pressure on resource use due to the generally greater abundances of leafcutter bees.

Given that higher pollinator visitation leads to more crossing, and that honey bees and leafcutter bees collect similar resources, it is surprising that visitation by heterospecific pollinators and taxon richness (with the exception of honey bees crossing to male) had so little effect on crossing. It is possible, if unlikely, that leafcutter bees and honey bees do not exert strong enough pressure on each other to affect each other's foraging. Another explanation could be the limited spatial overlap between the two species of pollinators. From the visitation data we can see that honey bees tend to avoid areas around leafcutter bee shelters, and that leafcutter bees are very limited in their range. The limited effects of heterospecific visitation could be because the frequency of heterospecific encounters in this system is low enough that it is not comparable to conspecific visitation. Native bee pollinators, which could potentially also affect resource consumption, had a minimal presence in this system, which again limited the number of heterospecific encounters.

In the one case the number of heterospecific pollinator visits had the potential to affect movement (of leafcutter bees crossing towards the male bay), the effect was surprisingly negative. An increase in the number of heterospecific visits led to a decrease in crossing to the opposite bay. The effect was weak compared to other variables, however, and only mattered when a more important effect (visits by leafcutter bees to the opposite bay) was excluded.

While visitation by heterospecific competitors had little influence over movement, taxon richness, along with conspecific abundance, had a positive influence on honey bee movement to the male bay. This is evidence that heterospecific pollinators do have some influence on movement, just not directly through greater numbers of pollinators. The effect of taxon richness was also an important factor for bay switching in the individual-follow analyses, and is discussed in more depth in the next section.

While leafcutter bees exhibited more movement to the opposite bay with an increase in visitation of conspecific foragers, the bees also demonstrated an attraction to higher densities of conspecifics. Visitation of leafcutter bees in both bays was not

collinear, so it was not just a function of overall higher area visitation leading to more movement. Possible explanations for this apparent attraction towards higher densities of conspecifics include conspecific cuing (using congregations of conspecifics to determine resource quality), predation avoidance, or for mating opportunities.

Kiester and Slatkin (1974) suggested that organisms (in the case of their study, lizards) could use congregations of conspecifics as cues to find better quality resources more quickly than alternative foraging methods. Even though conspecific organisms can serve as a source of competition, they also might be good indicators of quality foraging, as they will have similar resource demands. This behaviour has been shown in bumble bees, where naïve foragers were more likely to choose resources where a conspecific forager was present than those without (Kawaguchi et al. 2007). Congregations of leafcutter bees on one type of floral morph might indicate that it is a good resource and worth visiting, and therefore motivate visitation by other leafcutter bees.

Predator or parasitoid avoidance is another possible reason leafcutter bees might move towards congregations of conspecifics, although an unlikely one. Flocking behaviour has been thought to reduce predation through group detection (Pulliam 1973), or to lower the chance of individual predation through taking shelter within congregations (Williams 1964). Leafcutter bees have a number of potential predators and parasites, and it is possible that they could seek out conspecifics to avoid these. However, because predation and parasitisation generally happen at the nest and brood level (Pitts-Singer and Cane 2011), avoidance through grouping would be less necessary for adult foragers.

A third potential explanation is mate-seeking. Unlike honey bees, where foragers are entirely female, a large percentage of leafcutter bee floral visitors are male. Sex ratios vary based on environmental factors, but tend to be male-biased (Richards 1984). While females tend toward monogamy, males do not (Eickwort and Ginsberg 1980); indeed, they should try to maximize their fitness by mating with as many females as possible. The attraction of leafcutter bees to areas with high leafcutter bee visitation might be a reflection of male movement. If this is true, you would expect to see a higher proportion of males as leafcutter bee density increases. This cannot be parsed out with the data in this study, as leafcutter bees were recorded only as a species, and not by gender.

However, because male leafcutter bees can negatively affect female foraging, it is an important issue that should be considered in the context of pollination effectiveness (Rossi et al. 2010).

Finally, while the movement of honey bees to the female bay could be described by visitation to the male bay, temperature and Julian day had comparatively larger effects. The weather and time of season are therefore important considerations for foraging behaviour. An increase in crossing later in the season might reflect reduced resource availability and therefore decreased choosiness. Weather has been shown to impact pollinator visitation, especially in honey bees (see Ribbands 1964). While it was not a focus of this study, weather could have a large impact on pollinator behaviour in agricultural crops.

#### 3.5.3.2 Individual follows

The strongest influence on whether a pollinator foraging on male flowers would switch to female flowers over time was taxon richness. Taxon richness took into account not only other bee pollinators, but pollinators such as flies and butterflies that might not compete at the same level as bees. While different pollinator taxa may not consume resources at the same rate or competitive level, a greater diversity of taxa might result in more synergistic resource use. Hoehn et al. (2008), who found that pollinator diversity and not abundance best explained pumpkin yield, cited differences in spatial and temporal flower use by different groups of pollinators. Pollinators who are more robust to poor weather, who forage earlier in the day, or who forage at different levels of the crop contribute to the depletion of resource levels even when other pollinators are not accessing those resources. Areas with high taxon richness could have a more constant resource drain than those with only a few species, where resources would have more time to replenish in their absence. This finding agrees with other studies, such as Greenleaf and Kremen (2006), which found that a greater richness of wild pollinators increased the efficiency of honey bees in hybrid sunflower.

Pollinators showed different degrees of willingness to switch over time. Leafcutter bees were the most likely to switch from the male to the female bay, followed by honey bees foraging on nectar, and finally honey bees foraging on pollen. A low

tendency to switch from male flowers can be expected from pollen-gathering honey bees. It is somewhat surprising that leafcutter bees are more likely to switch inflorescences over time than nectar-foraging honey bees, since both are subject to nectar availability. However, honey bees have shown a reluctance to switch from floral constant behaviour in other systems (such as a study by Kay et al. (1984), in which honey bees showed floral constancy to male *Silene dioica* flowers even as female flowers produced comparatively higher levels of nectar throughout the day). This is not necessarily a bad foraging strategy, so long as other pollinators in the system are switching. There is also the potential that honey bees are more likely to exhibit floral constancy to limit competition between hive mates. As suggested by Heinrich (1979), workers in social bee colonies might exhibit cooperative foraging behaviour, taking lower individual rewards but contributing more to the colony stores by avoiding competitive behaviour. However, not all honey bee foragers in hybrid canola are from the same colony, so this is unlikely.

Pollinator visitation, which was an influential variable for transect crossing, was generally unimportant in influencing an individual pollinator's tendency to switch over time. While it was included in the top-ranking models, its lack of effect on crossing suggest that the selection of those models may have been due to the extra parameters inflating their value, rather than them having any notable effects on movement. The lack of effect of pollinator visitation could be because it represented total pollinators, and was not broken down by pollinator type like it had been for transect crossing. Some of the pollinators, like pollen-foraging honey bees, would also be less responsive to increases in nectar consumption.

Similar to visitation, profit influenced transect crossing, but was not important in explaining the tendency of individual pollinators to switch bays. It is possible that bees were less aware of differences in profit for the individual follows. Transect crossings took place between the male and female bays; pollinators on bay edges could more easily appraise resources in the opposite bay, and there would be less distance to travel between the bays. Pollinators followed for the individual travel portion were selected throughout the male bay. Pollinators foraging in the middle of the bay might find the other bays less accessible, or not be aware of the alternative resources.

It is important to note that out of 728 observations, only 39 actual switching events were recorded. Over a 60 second time period, more than 85% of pollinators remained foraging on male flowers (Figure 3.6). Some of this could be due to pollinators switching after being lost, or after the sixty seconds, thus the necessity of using censored data. It is also possible that for the pollinators to be followed, they needed to be slow enough for an observer to record. The data in this section might be biased towards slower pollinators, and those that are more reluctant to move.

#### **3.5.4 Conclusion**

The tendency of a pollinator to switch floral morphs has important implications for pollination in floral systems with male-sterile flowers. While pollinators might exhibit floral constancy, this is dependent upon how rewarding the behaviour is; they should change their behaviour in response to resource availability. Pollinator behaviour was responsive to resource use by competitors (crossing between morphs more in areas with more conspecific visitation) as well as to differences in profit availability between the morphs, although there may be interactions between profit and competition. While the abundance of heterospecific pollinators did not greatly affect crossing, taxon richness did have a positive influence on crossing in some circumstances. Areas with higher abundances of conspecific pollinators should exhibit a higher crossing frequency of these pollinators between morphs, as should areas with a more diverse pollinator assemblage. The results of this study suggest that similar gynodioecious systems could benefit from higher conspecific pollinator abundance and/or pollinator diversity to facilitate pollen transfer to female morphs.

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## Chapter Four: **General discussion**

In this study I sought to determine the presence, foraging preferences, and pollination effectiveness of pollinators foraging in hybrid seed canola in Southern Alberta. With the understanding that the sexual dimorphism between the male and female morphs, along with their spatial separation, would promote floral constancy, I also looked at what factors motivated movement between morphs (separated in patches). These factors included floral profit and competitors (conspecific and heterospecific abundance, as well as diversity).

Managed pollinators, both leafcutter and honey bees, were the primary pollinators in hybrid seed canola. They were the most frequently encountered pollinators in plots. Female leafcutter bees were more effective at transferring pollen than honey bees, but were more limited in their foraging range. Male leafcutter bees deposited pollen on canola stigmas at similar levels to honey bees. Female flowers likely require multiple visits from pollinators for sufficient pollination (especially in the case of honey and male leafcutter bees), but it is easier to incorporate more managed pollinators (which can be stocked at sufficiently high numbers to ensure adequate pollination) than it would be to bolster wild pollinator populations.

One correlate of movement between bays is an individual pollinator's floral constancy (i.e., its willingness to switch floral types). Although they were frequent floral visitors, both types of managed pollinator showed strong floral constancy to morph, being less likely to visit a female flower (when offered it) while foraging on male flowers. The sexual dimorphism between the types of floral morphs, as well as the spatial separation of the morphs, likely encourages floral constancy. However, when statistically controlling for floral profitability, both types of bees were more likely to move with increasing local visitation by conspecific competitors, seemingly responding to competitor-driven declines in resource availability. Tendencies towards floral constancy can therefore be overcome through higher densities of conspecific competitors, who decrease local rewards and encourage movement to alternative resources.

Interestingly, heterospecific abundance (measured with my 'visitation' metric) did not affect movement. This is likely because of the more restricted foraging range of

leafcutter bees, and avoidance of leafcutter bee shelters by honey bees. To effect an increase of crossing by either type of pollinator, management emphasis must therefore be placed on increasing conspecifics: changing the density of leafcutter bees will little influence the bay-crossing tendency of honey bees, and vice versa. Leafcutter bees were more limited in their foraging range than honey bees, and had a high visitation around shelters. Because the increase in leafcutter bee crossing brought about by leafcutter bee visitation begins to asymptote at higher levels of visitation (at around 50 visits per plot), and because leafcutter bees are relatively local in their foraging ranges (most visits being within 25 m or less of the shelter), placing more leafcutter bee shelters in a field may encourage more crossing than would increasing abundance in each shelter. Similarly, for honey bees, dispersing hives around the field edges (e.g., at each pivot corner) could make up for range limitations.

An increase in pollinator visitation can increase pollen deposition (as well as, in hybrid canola, motivate the necessary movement between male and female flowers). However, association between fruit set and pollinator visitation is not always positive. An overabundance of floral visitors can decrease fruit set (Young 1988). For instance, Sáez et al. (2014) found that, while increases in bee visitation increased pollen deposition on flowers, it also led to a lower fruit-set in *Rubus idaeus* due to style damage. In this case, over-visitation led to the damage of reproductive structures of flowers. The benefits from increased pollinator visitation should be weighed against the potential detriments of over-visitation, something that my study did not measure.

Based on their effectiveness of pollen transfer, wild pollinators, especially bumble bees and hoverflies, can contribute directly to pollination of hybrid seed canola, with a pollination effectiveness at least on par with honey bees. However, wild pollinators were not present in sufficient numbers to pollinate at a level comparable to that of managed pollinators. Wild pollinators do not receive the support that managed pollinators do, and depend entirely on resource and nesting availability in their landscape. Intensive agricultural landscapes reduce the abundance and diversity of wild bees (Kremen et al. 2002). Wild pollinators may also face competitive displacement by managed pollinators (Lindström et al. 2016). While there are ways to support wild pollinator populations, such

as using uncropped or enhanced field margins as extra resources (Kells et al. 2001, Carvell et al. 2007), it is unlikely that wild pollinator populations would grow to the point where they could provide sufficient pollination services in Southern Albertan fields of hybrid seed canola.

Rather than contributing directly through pollination services, the importance of wild pollinators might be through facilitating changes in the foraging behaviour of managed pollinators. While I found that abundance of heterospecific pollinators did not result in more movement, a higher diversity of pollinators (taxon richness) resulted in managed pollinators being more willing to switch from foraging on male to female inflorescences, an effect that increased as the flowering season progressed. This is supported by other studies which found that higher wild pollinator diversity improves pollination efficiency of honey bees in hybrid sunflower (Greenleaf and Kremen 2006) and pollination effectiveness in varietally self-incompatible almond (Brittain et al. 2013). A greater diversity of pollinators can lead to a more synergistic use of resources, which could prompt managed pollinators to seek out alternatives in the environment.

My study provides insight into how pollinators behave in and move throughout fields of hybrid canola, a gynodioecious crop system. By tracking pollinators individually and as a group, I was able to address what motivates individuals to visit flowers, how their actions and morphology influence pollination effectiveness, and how pollinators alter their behaviour based on both the available floral resources and the behaviour of other pollinators. By using a mobile interview bouquet technique to study pollinator effectiveness, I was able to circumvent some of the limitations of caged experimental studies. The ability to record pollinator interactions with flowers also allowed me better insight into how certain behaviours affect pollen deposition. There are still some issues to be addressed with the interview bouquet technique, including the problem of smaller solitary bee species avoiding the interview apparatus (thus preventing me from measuring their effectiveness of pollination), and the use of cut flowers preventing yield from being measured. However, it offers an alternative to caged tent studies or stationary sentinel plants. The results of this study can be applied to other gynodioecious crop systems, and

potentially to naturally gynodioecious systems (with caveats, as floral morphs may be interspersed in natural systems).

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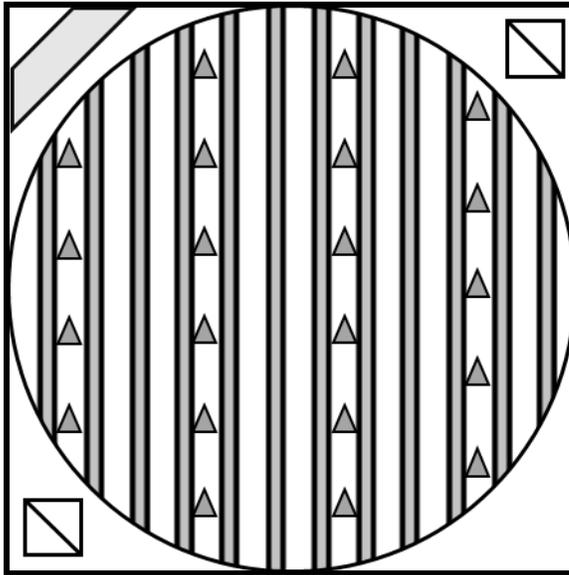
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## Appendices

### Appendix A: Field photos and illustration of field layout



**Figure A.1:** Clockwise, from upper left, a bumble bee (*Bombus* spp), honey bee (*A. mellifera*), hoverfly (Syrphidae), and leafcutter bee (*M. rotundata*) visiting canola flowers.



**Figure A.2: Illustration of the field layout for production of hybrid canola seed, with female bays represented by white bands and male bays represented by grey bands (field not to scale). Placement of honey bee hives (white boxes with diagonal stripe) and potential wild bee habitat (light grey trapezoid) varied by field, but were always located on a field edge or corner. Leafcutter bee shelters (grey triangles) were distributed throughout female bays. Fields were generally square with 0.8 km sides, with a circular irrigated area planted to the crop, and ‘corners’ with honey bee hives either left bare or planted with a non-canola crop.**



**Figure A.3: Female (left) and male (right) canola inflorescences.**



**Figure A.4: Visitation and transect crossing were measured simultaneously. Visitation was measured by the number of visits to each inflorescence made by each type of pollinator to a 1 m<sup>2</sup> quadrat in 10 minutes (quadrat in lower left). Transect crossings were the number of pollinators crossing to the male (left bay) or female (right bay) in 2.5 minutes over a 2 m transect line (upper left, red poles denote transect ends). Photo by Ralph Cartar 2015.**

**Appendix B: Lists of  $\Delta\text{AICc}$  and  $\Delta\text{AIC}$  for transect crossing and individual travel models**

**Table B.1: Models explaining the frequency of honey bees (n=121) crossing from the male to the female bay, as influenced by honey bee (HB) or heterospecific bee (HetB) visitation (v) to the male (M) or female (F) bay, rarefied taxon richness in the male bay (RM), relative profit ( $\Delta P$ ), or the profit of the male bay (PM). The  $\Delta\text{AICc}$  of top-ranking models is in bold; models with 10  $\Delta\text{AICc}$  or less that contain uninformative parameters are italicized.  $\Delta\text{AICc}$  is the difference between the  $\text{AICc}$  of the model and the  $\text{AICc}$  of the best performing model;  $\log(L)$  represents model log-likelihood.**

Model	$\Delta\text{AICc}$	$\log(L)$	df
<b>HBvM+<math>\Delta P</math></b>	<b>0.00</b>	<b>-169.57</b>	<b>7</b>
<i>HBvM+HBvF+<math>\Delta P</math></i>	1.42	-169.18	8
<b>HBvM</b>	<b>1.49</b>	<b>-171.40</b>	<b>6</b>
<i>HBvM+RM+<math>\Delta P</math></i>	1.87	-169.41	8
<i>HBvM+HetBvM+<math>\Delta P</math></i>	1.88	-169.41	8
<i>HBvM*HBvF+<math>\Delta P</math></i>	2.01	-168.36	9
<i>HBvM+<math>\Delta P</math>+PM</i>	2.18	-169.57	8
<i>HBvM+HBvF</i>	3.39	-171.27	7
<b>Null</b>	<b>3.39</b>	<b>-173.41</b>	<b>5</b>
<i>HBvM+HBvF+RM+<math>\Delta P</math></i>	3.47	-169.10	9
<i>HBvM+RM</i>	3.56	-171.35	7
<i>HBvM+HetBvM</i>	3.59	-171.37	7
<i>HBvM+HBvF+<math>\Delta P</math>+PM</i>	3.63	-169.17	9
<i>HBvM+HetBvM+<math>\Delta P</math>+RM</i>	3.77	-169.24	9
<i>HBvM+RM+<math>\Delta P</math>+PM</i>	4.10	-169.41	9
<i>HBvM+HetBvM+<math>\Delta P</math>+PM</i>	4.11	-169.41	11
<i>HBvM*HBvF+RM+<math>\Delta P</math></i>	4.24	-168.34	10
<i>HBvM*HBvF+<math>\Delta P</math>+PM</i>	4.24	-168.34	10
<i>HBvM*HBvF</i>	4.31	-170.63	8
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math></i>	5.33	-166.55	12
<i>HBvM+HBvF+RM</i>	5.53	-171.24	8
<i>HBvM+HBvF+HetBvM+HetBvF+<math>\Delta P</math></i>	5.67	-169.06	10
<i>HBvM+HetBvM+RM</i>	5.68	-171.32	8
<i>HBvM+HBvF+RM+<math>\Delta P</math>+PM</i>	5.73	-169.09	10
<i>HBvM+HetBvM+<math>\Delta P</math>+PM+RM</i>	6.05	-169.24	11
<i>HBvM*HBvF+RM+<math>\Delta P</math>+PM</i>	6.52	-168.32	11
<i>HBvM*HBvF+RM</i>	6.54	-170.63	9
<i>HBvM*HBvF+HetBvM*HetBvF</i>	7.19	-168.66	11
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math>+RM</i>	7.58	-166.47	13
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math>+PM</i>	7.72	-166.54	13
<i>HBvM+HBvF+HetBvM+HetBvF</i>	7.72	-171.22	9

<i>HBvM+HBvF+HetBvM+HetBvF+ΔP+RM</i>	7.75	-168.94	11
<i>HBvM+HBvF+HetBvM+HetBvF+ΔP+PM</i>	7.97	-169.05	12
<i>HBvM*HBvF+HetBvM*HetBvF+RM</i>	9.45	-168.61	12
<i>HBvM+HBvF+HetBvM+HetBvF+RM</i>	9.92	-171.18	10
<i>HBvM*HBvF+HetBvM*HetBvF+ΔP+PM+RM</i>	10.02	-166.47	14
<i>HBvM+HBvF+HetBvM+HetBvF+ΔP+PM+RM</i>	10.10	-168.93	12

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**Table B.2: Models explaining the frequency of honey bees (n=121) crossing from the female to the male bay, as influenced by honey bee (HB) or heterospecific bee (HetB) visitation (v) to the male (M) or female (F) bay, rarefied taxon richness in the female bay (RF), relative profit ( $\Delta P$ ), or the profit of the female bay (PF). The  $\Delta AICc$  of top-ranking models is in bold; models with 10  $\Delta AICc$  or less that contain uninformative parameters are italicized.  $\Delta AICc$  is the difference between the  $AICc$  of the model and the  $AICc$  of the best performing model;  $\log(L)$  represents model log-likelihood.**

Model	$\Delta AICc$	$\log(L)$	df
<b>HBvF+RF+<math>\Delta P</math></b>	<b>0.00</b>	<b>-175.96</b>	<b>8</b>
<i>HBvF+HetBvF+<math>\Delta P</math>+RF</i>	1.13	-175.41	9
<b>HBvF+<math>\Delta P</math></b>	<b>1.16</b>	<b>-177.64</b>	<b>7</b>
<i>HBvF+HetBvF+<math>\Delta P</math></i>	1.54	-176.73	8
<i>HBvF+RF+<math>\Delta P</math>+PF</i>	2.04	-175.86	9
<i>HBvM+HBvF+RF+<math>\Delta P</math></i>	2.13	-175.91	9
<i>HBvM+HBvF+<math>\Delta P</math></i>	2.98	-177.45	8
<i>HBvF+HetBvF+<math>\Delta P</math>+PF+RF</i>	3.11	-175.26	11
<i>HBvF+<math>\Delta P</math>+PF</i>	3.11	-177.52	8
<i>HBvF+HetBvF+<math>\Delta P</math>+PF</i>	3.40	-176.54	11
<i>HBvM+HBvF+RF+<math>\Delta P</math>+PF</i>	4.11	-175.76	10
<i>HBvM*HBvF+RF+<math>\Delta P</math></i>	4.40	-175.91	10
<i>HBvM+HBvF+<math>\Delta P</math>+PF</i>	4.76	-177.22	9
<i>HBvM*HBvF+<math>\Delta P</math></i>	5.02	-177.35	9
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math></i>	5.50	-174.12	12
<i>HBvM+HBvF+HetBvM+HetBvF+<math>\Delta P</math>+RF</i>	5.57	-175.33	11
<i>HBvM+HBvF+HetBvM+HetBvF+<math>\Delta P</math></i>	5.62	-176.52	10
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math>+RF</i>	6.31	-173.32	13
<i>HBvM*HBvF+RF+<math>\Delta P</math>+PF</i>	6.42	-175.75	11
<i>HBvM*HBvF+<math>\Delta P</math>+PF</i>	6.84	-177.13	10
<i>HBvM+HBvF+HetBvM+HetBvF+<math>\Delta P</math>+PF</i>	7.29	-176.19	12
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math>+PF</i>	7.30	-173.82	13
<i>HBvM+HBvF+HetBvM+HetBvF+<math>\Delta P</math>+PF+RF</i>	7.47	-175.10	12
<i>HBvM*HBvF+HetBvM*HetBvF+<math>\Delta P</math>+PF+RF</i>	8.32	-173.10	14
HBvF+RF	14.50	-184.31	7
HBvF	14.85	-185.56	6
HBvF+HetBvF	15.88	-185.00	7
HBvF+HetBvF+RF	16.05	-183.99	8
HBvM+HBvF+RF	16.56	-184.24	8
Null	16.95	-187.67	5
HBvM+HBvF	16.99	-185.55	7
HBvM*HBvF+RF	18.77	-184.23	9
HBvM*HBvF	19.13	-185.53	8

HBvM+HBvF+HetBvM+HetBvF	20.27	-184.98	9
HBvM+HBvF+HetBvM+HetBvF+RF	20.44	-183.92	10
HBvM*HBvF+HetBvM*HetBvF	20.80	-182.95	11
HBvM*HBvF+HetBvM*HetBvF+RF	21.61	-182.17	12

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**Table B.3: Models explaining the frequency of leafcutter bees (n=122) crossing from the male to the female bay, as influenced by leafcutter bee (LCB) or heterospecific bee (HetB) visitation (v) to the male (M) or female (F) bay, rarefied taxon richness in the male bay (RM), relative profit ( $\Delta P$ ), or the profit of the male bay (PM). The  $\Delta AICc$  of top-ranking models is in bold; models with 10  $\Delta AICc$  or less that contain uninformative parameters are italicized.  $\Delta AICc$  is the difference between the  $AICc$  of the model and the  $AICc$  of the best performing model;  $\log(L)$  represents model log-likelihood.**

Model	$\Delta AICc$	$\log(L)$	df
<b>LCBvM+LCBvF+<math>\Delta P</math></b>	<b>0.00</b>	<b>-413.45</b>	<b>8</b>
<i>LCBvM*LCBvF+<math>\Delta P</math></i>	0.80	-412.73	9
<b>LCBvM+LCBvF</b>	<b>1.50</b>	<b>-415.30</b>	<b>7</b>
<i>LCBvM+LCBvF+<math>\Delta P</math>+PM</i>	1.71	-413.19	9
<i>LCBvM*LCBvF</i>	2.10	-414.50	8
<i>LCBvM+LCBvF+RM+<math>\Delta P</math></i>	2.16	-413.42	9
<i>LCBvM*LCBvF+<math>\Delta P</math>+PM</i>	2.70	-412.55	10
<i>LCBvM*LCBvF+RM+<math>\Delta P</math></i>	2.87	-412.63	10
<i>LCBvM+LCBvF+RM</i>	3.64	-415.27	8
<i>LCBvM+LCBvF+RM+<math>\Delta P</math>+PM</i>	3.83	-413.11	10
<i>LCBvM*LCBvF+RM</i>	4.13	-414.40	9
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math></i>	4.35	-413.37	10
<i>LCBvM*LCBvF+RM+<math>\Delta P</math>+PM</i>	4.74	-412.41	11
<i>LCBvM+LCBvF+HetBvM+HetBvF</i>	5.86	-415.26	9
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+PM</i>	6.16	-413.12	12
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+RM</i>	6.61	-413.35	11
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math></i>	7.54	-412.63	12
<i>LCBvM+LCBvF+HetBvM+HetBvF+RM</i>	8.05	-415.22	10
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+PM+RM</i>	8.42	-413.07	12
<i>LCBvM*LCBvF+HetBvM*HetBvF</i>	8.70	-414.39	11
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+PM</i>	9.60	-412.46	13
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+RM</i>	9.71	-412.52	13
<i>LCBvM*LCBvF+HetBvM*HetBvF+RM</i>	10.74	-414.23	12
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+PM+RM</i>	11.76	-412.32	14
<i>LCBvM+<math>\Delta P</math></i>	14.27	-421.68	7
<i>LCBvM</i>	14.84	-423.05	6
<i>LCBvM+RM+<math>\Delta P</math></i>	15.83	-421.36	8
<i>LCBvM+<math>\Delta P</math>+PM</i>	16.34	-421.62	8
<i>LCBvM+RM</i>	16.39	-422.74	7
<i>LCBvM+HetBvM+<math>\Delta P</math></i>	16.46	-421.68	8
<i>LCBvM+HetBvM</i>	17.00	-423.05	7
<i>LCBvM+RM+<math>\Delta P</math>+PM</i>	17.84	-421.25	9
<i>LCBvM+HetBvM+<math>\Delta P</math>+RM</i>	17.95	-421.31	9

LCBvM+HetBvM+RM	18.43	-422.66	8
LCBvM+HetBvM+ $\Delta$ P+PM	18.56	-421.61	11
LCBvM+HetBvM+ $\Delta$ P+PM+RM	20.03	-421.21	11
Null	88.98	-461.18	5

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**Table B.4: Models explaining the frequency of leafcutter bees (n=122) crossing from the female to the male bay, as influenced by leafcutter bee (LCB) or heterospecific bee (HetB) visitation (v) to the male (M) or female (F) bay, rarefied taxon richness in the female bay (RF), relative profit ( $\Delta P$ ), or the profit of the female bay (PF). The  $\Delta AICc$  of top-ranking models is in bold; models with 10  $\Delta AICc$  or less that contain uninformative parameters are italicized.  $\Delta AICc$  is the difference between the  $AICc$  of the model and the  $AICc$  of the best performing model;  $\log(L)$  represents model log-likelihood.**

Model	$\Delta AICc$	$\log(L)$	df
<b>LCBvM+LCBvF+<math>\Delta P</math></b>	<b>0.00</b>	<b>-411.93</b>	<b>8</b>
<i>LCBvM+LCBvF+<math>\Delta P</math>+PF</i>	0.29	-410.96	9
<i>LCBvM*LCBvF+<math>\Delta P</math></i>	1.14	-411.39	9
<b>LCBvM+LCBvF</b>	<b>1.42</b>	<b>-413.74</b>	<b>7</b>
<i>LCBvM*LCBvF+<math>\Delta P</math>+PF</i>	1.77	-410.57	10
<i>LCBvM+LCBvF+RF+<math>\Delta P</math></i>	2.20	-411.92	9
<i>LCBvM*LCBvF</i>	2.42	-413.14	8
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math></i>	2.52	-410.94	10
<i>LCBvM+LCBvF+RF+<math>\Delta P</math>+PF</i>	2.55	-410.96	10
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+PF</i>	3.31	-410.18	12
<i>LCBvM*LCBvF+RF+<math>\Delta P</math></i>	3.41	-411.38	10
<i>LCBvM+LCBvF+RF</i>	3.53	-413.70	8
<i>LCBvM*LCBvF+RF+<math>\Delta P</math>+PF</i>	4.09	-410.57	11
<i>LCBvM+LCBvF+HetBvM+HetBvF</i>	4.17	-412.90	9
<i>LCBvM*LCBvF+RF</i>	4.62	-413.13	9
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+RF</i>	4.73	-410.89	11
<i>LCBvM+LCBvF+HetBvM+HetBvF+<math>\Delta P</math>+PF+RF</i>	5.62	-410.15	12
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math></i>	5.62	-410.16	12
<i>LCBvM+LCBvF+HetBvM+HetBvF+RF</i>	6.29	-412.83	10
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+PF</i>	6.79	-409.54	13
<i>LCBvM*LCBvF+HetBvM*HetBvF</i>	6.98	-412.01	11
<b>LCBvF+HetBvF+<math>\Delta P</math></b>	<b>7.24</b>	<b>-415.56</b>	<b>8</b>
<i>LCBvF+HetBvF+<math>\Delta P</math>+PF</i>	7.78	-414.71	11
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+RF</i>	8.00	-410.15	13
<b>LCBvF+<math>\Delta P</math></b>	<b>8.23</b>	<b>-417.14</b>	<b>7</b>
<i>LCBvF+<math>\Delta P</math>+PF</i>	8.78	-416.33	8
<i>LCBvF+HetBvF+<math>\Delta P</math>+RF</i>	9.02	-415.33	9
<i>LCBvM*LCBvF+HetBvM*HetBvF+<math>\Delta P</math>+PF+RF</i>	9.22	-409.54	14
<i>LCBvM*LCBvF+HetBvM*HetBvF+RF</i>	9.28	-411.99	12
<i>LCBvF+HetBvF+<math>\Delta P</math>+PF+RF</i>	9.69	-414.53	11
<b>LCBvF+HetBvF</b>	<b>9.75</b>	<b>-417.91</b>	<b>7</b>
<b>LCBvF</b>	<b>9.91</b>	<b>-419.06</b>	<b>6</b>
<i>LCBvF+RF+<math>\Delta P</math></i>	10.22	-417.04	8

LCBvF+RF+ $\Delta$ P+PF	10.88	-416.26	9
LCBvF+HetBvF+RF	11.37	-417.62	8
LCBvF+RF	11.71	-418.88	7
Null	83.48	-456.91	5

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**Table B.5: Models explaining the frequency of honey and leafcutter bees switching from male to female inflorescences in 60 s, as influenced by total pollinator visitation (TBv) in the female (F) or male (M) bay, taxon richness (R), relative profit ( $\Delta$  profit), male bay profit (PM), or pollinator type (Pollinator) (n=728, n<sub>events</sub>=39). The  $\Delta$ AIC of top-ranking models is in bold; models with 10  $\Delta$ AIC or less that contain uninformative parameters are italicized.  $\Delta$ AIC is the difference between the AIC of the model and the AIC of the best performing model; partial  $\log(L)$  represents model partial log-likelihood.**

Model	$\Delta$ AIC	partial $\log(L)$	df
<b>TBvM+TBvF+RM+Pollinator</b>	<b>0.00</b>	<b>-214.14</b>	<b>7</b>
<i>TBvM*TBvF+RM+Pollinator</i>	0.94	-213.61	8
<b>RM+Pollinator</b>	<b>0.99</b>	<b>-216.63</b>	<b>5</b>
<i>TBvM+RM+Pollinator</i>	1.22	-215.75	6
<i>TBvM+TBvF+RM+<math>\Delta</math>P+Pollinator</i>	1.83	-214.05	8
<b>TBvM+TBvF+Pollinator</b>	<b>2.11</b>	<b>-216.20</b>	<b>6</b>
<i>RM+<math>\Delta</math>P+Pollinator</i>	2.64	-216.46	6
<i>TBvM*TBvF+RM+<math>\Delta</math>P+Pollinator</i>	2.69	-213.48	9
<i>TBvM*TBvF+Pollinator</i>	2.74	-215.51	7
<i>TBvM+RM+<math>\Delta</math>P+Pollinator</i>	3.01	-215.65	7
<b>TBvM+Pollinator</b>	<b>3.39</b>	<b>-217.83</b>	<b>5</b>
<i>TBvM+TBvF+RM+<math>\Delta</math>P+PM+Pollinator</i>	3.75	-214.01	9
<i>TBvM+TBvF+<math>\Delta</math>P+Pollinator</i>	3.86	-216.07	7
<i>TBvM*TBvF+<math>\Delta</math>P+Pollinator</i>	4.41	-215.34	8
<i>RM+<math>\Delta</math>P+PM+Pollinator</i>	4.47	-216.37	7
<b>Pollinator</b>	<b>4.48</b>	<b>-219.38</b>	<b>4</b>
<i>TBvM*TBvF+RM+<math>\Delta</math>P+PM+Pollinator</i>	4.62	-213.45	10
<i>TBvM+RM+<math>\Delta</math>P+PM+Pollinator</i>	4.78	-215.53	8
<i>TBvM+<math>\Delta</math>P+Pollinator</i>	5.10	-217.69	6
<i>TBvM+TBvF+<math>\Delta</math>P+PM+Pollinator</i>	5.82	-216.05	8
<i>TBvM*TBvF+<math>\Delta</math>P+PM+Pollinator</i>	6.36	-215.32	9
<i>TBvM+<math>\Delta</math>P+PM+Pollinator</i>	6.97	-217.62	7
Null	14.02	-226.15	2