The Ecological Context of Pollination: Variation in an Apparent Mutualism

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Ecology and Evolutionary Biology
University of Toronto

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Abstract

What makes a good pollinator? As pollinators perform the function of pollination, exporting and delivering grains between conspecific plant species, the floral visitor's foraging interests and the messy process of pollination interfere; many pollen grains are removed but not delivered. “Conditionally parasitic” floral visitors “waste” so much pollen (from a plant perspective) that they sometimes reduce total pollen delivery, the total number of pollen grains transferred to conspecific plant stigmas. Most pollinators visit plants with a diverse assemblage of other floral visitors; as pollinators remove, transport, and deposit pollen, they interact indirectly with one another through the supply of available pollen. These interactions can result in conditional parasitism because they affect the number of pollen grains that are successfully transported to stigmas.

In this thesis, I explore how pollinator wastefulness and variation in pollinator assemblages may affect pollen export and delivery. I document a particular ecological situation in which a pollen-specialist (“oligolectic”) bee and a nectar-collecting fly visit concurrently. In this system, pollen removal by the oligolectic bee results in substantial and rapid pollen depletion, which suggests that pollen wastefulness may be costly. The assemblage of pollinators varies widely throughout the geographic range of the plant; citizen scientists and I documented a consistent geographic pattern. These geographic differences correspond with variation in floral traits that are linked to pollen transfer by bees and flies. Finally, I use a simulation model to explore what ecological contexts will cause a floral visitor to increase or decrease overall pollen delivery. As a whole, these data provide substantial evidence that conditional parasitism may occur, and help elucidate aspects of plants, pollinators, and mechanisms of pollen transfer that may make it more likely. Moreover, I document plant traits that may be a result of selection mediated by conditionally parasitic floral visitors.
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I dedicate this “happy bee book” to my niece Ginny, who observes and investigates all plants and animals.
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A bee is worth a thousand flies

Spanish proverb
1. Context

1.1. Introduction

Biotic pollination results from the merging of the disparate goals of widely different taxa – namely, pollen and nectar foraging by flower visiting animals and pollen transfer for the reward-providing plant; it is a classic example of a mutualistic interaction (Willmer 2011, Bronstein 2009). In mutualisms, the goals of different taxa often coincide, and it is from these coincidences that we derive the common perception of mutualisms generally, and pollination specifically: that the interactions are always pairwise and always beneficial for both partners (Waser 1986, Bronstein 2001). We assume that relationships are consistent and positive across geographic and temporal scales. Much research in mutualism and pollination biology implicitly takes this view; for example, studies often examine the role of selection of one partner on another, at one time and in one location (as reviewed by Herrera et al. 2006, Waser 2006). Comparisons between mutualistic partners are generally defined by species-specific characteristics that are considered to be consistent wherever and whenever these partners converge (e.g. Larsson 2005, Sahli et al. 2007, Motten et al. 1981). However, modern researchers recognize that relationships are usually complicated and involve multiple players, that the interacting species face variable conditions and fluctuating rewards, and that “mutualistic” relationships are not necessarily positive in every ecological context (e.g., Thomson 2003, Willmer 2011, Hargreaves et al. 2009). In mutualistic interactions, benefits are usually not accrued without paying a cost, and the magnitudes of both will vary (Bronstein 2001). In order to better understand the complexity of mutualistic relationships, we need to take a closer look at the cost of mutualistic interactions and closely examine biological factors that may affect the ratio of costs and benefits in the relationship (Bronstein 2001). In plant-pollinator relationships specifically, we need to measure the cost that plants pay for pollination service, consider attributes of pollinator biology that affect their role as pollinators, and investigate pollinator value to plants within the ecological context (e.g. Hargreaves et al. 2009, Herrera et al. 2006, Larsson 2005, Lau and Galloway 2004, Thomson 2003, Thomson and Thomon 1992).

This thesis joins other recent studies in considering pollinator value to plants within the context of complex ecological circumstances (e.g. Brittain et al. 2013, Forrest et al. 2011, Brosi and Briggs 2013). How important is the ecological context in determining pollinator value to plants? Under what circumstances does a floral visitor decrease plant fitness, rather than increasing it? How variable are plant-pollinator relationships in space and time?

1.1.1. The cost of pollinator service

By calling animals that transfer pollen “pollinators”, we emphasize the plant-centric, mutualistic view of pollination; that the primary role of pollinators is to deliver pollen grains from one plant individual to
another individual of the same plant species (i.e., “export” and “deliver” pollen grains). However, pollinators – more circumspectly called floral visitors – deliver pollen only as an accidental by-product of their primary motivation, the collection of resources from plants. Pollinators actually interact with pollen grains by actively or passively removing grains from one plant individual (pollen “removal”) and then carrying those grains (pollen “transport”) until they are placed on conspecific floral stigmas (pollen “deposition”) or lost to the environment. The process of pollination is messy and wasteful, and most pollen grains that leave flowers do not participate in pollination; in species with no pollen aggregation, less than 1% of grains reach conspecific stigmas. The aggregation of pollen into groups (e.g. tetrads, polyads, viscin threads, and pollinia) increases pollen transfer efficiency, but pollen aggregation is relatively rare, occurring in only 42 of 457 plant families (Harder and Johnson 2008). Biologists tend to focus on the positive interactions between pollinators and plants, by measuring pollen deposition and the number of seeds produced as a result of a pollinator visit; pollinators increase plant fitness. However, we tend to ignore the cost of pollen transfer. A pollinator visit benefits plants through the export and delivery of pollen grains, but this interaction also results in grains that are exported but not delivered. From the plant perspective, these undelivered grains are a waste of floral resources. Some pollinator visits are more wasteful than others, and wasteful pollinator visits can intercept pollen that would otherwise be delivered, reducing total pollen delivery. Therefore, pollinator visits can concurrently increase pollen delivery in the short term by depositing pollen, yet also decrease pollen delivery in the long term by wasting pollen. In a possibly extreme case, a wasteful pollinator visit may decrease total pollen delivery more than it increases it through pollen export and deposition; this pollinator acts as a parasite (Thomson 2003, Castellanos et al. 2003).

1.1.2. Measurements of pollinator value

Traditionally, pollinator effectiveness and efficiency have been assumed to be constants, and defined as an intrinsic attribute of a particular pollinator species or group (e.g. Wilson and Thomson 1991, Thomson and Goodell 2001, Larsson 2005, Sahli and Conner 2007). We relate pollinator characteristics at the species level to a pollinator species’ visitation rates, its “effectiveness” (the per visit contribution to female plant reproductive success), its “efficiency” (the per visit contribution to female plant reproductive success in relation to plant resources used), and additional species-specific measurements of pollinator value to plants (as reviewed by Ne’eman et al. 2010). For example, pollinator effectiveness and efficiency are correlated with body size in the insect pollinators visiting Raphanus raphanistrum (Sahli and Conner 2007), and the bat Anoura geoffroyi is a more effective pollinator of the plant species Aphelandra acanthus than the hummingbird Adelomyia melanogenys, because the bat’s fur can contain and transport more pollen than the hummingbird’s feathers (Muchhala and Thomson 2010).

However, the value of a pollinator visit also depends on extrinsic factors, such as the “pollinator climate”, the diversity and abundance of the whole pollinator assemblage visiting a plant species (Grant and Grant 1965). The pollinator climate is important because all visitors deplete the same pool of pollen that is
available on plants. Most plant species are visited by a variety of floral visitors who interact directly and indirectly within a framework of fluctuating plant rewards; individual foragers consume, collect, or displace rewards, rendering them unavailable for future visitors within the pollinator assemblage. The amount of pollen ultimately delivered to plant stigmas may be the result of many individual pollinators—each with differences in removal and deposition rates—interacting with the supply of available pollen. For example, a pollinator foraging on a plant with an abundance of pollen will go on to pollinate more productively than a pollinator foraging immediately following a pollen-depleting female bee. While species-specific, intrinsic pollinator characteristics certainly play a role in defining pollinator value to plants, the ecological context may be as important as intrinsic characteristics or even more so. Therefore, measurements of pollinator value to plants should consider both intrinsic and extrinsic variables, including the pollinator climate.

We can use “pollen depletion models”—mathematical models that track pollen movement through plant populations based on rates of pollen removal, pollen deposition, and visitation by floral visitors—to estimate how total pollen delivery $G$ varies with the pollinator climate (Harder and Thomson 1989, Thomson and Thomson 1992, Harder and Wilson 1994, Thomson 2003). Pollen depletion models incorporate both species-specific factors and the ecological context, and these models consider pollinator value to plants at the level of the visit. Instead of a widely applicable constant, the graph for pollen delivery is better presented as a multidimensional response surface or “delivery surface” of combinations of visitors (Fig. 1.2). When comparing pollinators, we can use the measurement $\Delta G_i$, the number of additional grains that would be delivered by one additional visit by pollinator species $i$. Pollen delivery surfaces and pollen depletion models can expose cases in which the addition of a pollinator visit actually lowers overall pollen delivery. In these cases, a floral visitor may decrease plant fitness, rather than increasing it; the relationship may be more parasitic than mutualistic.

1.1.3. Context-dependence and conditional parasitism

We suspect that some pollinators may increase fitness within one ecological context, but decrease it in another. Hargreaves et al. (2010) documented decreased pollen deposition with the addition of *Apis mellifera* to primarily bird-pollinated *Aloe maculata* plant individuals, and Wilson and Thomson (1991) found higher pollen transfer with only nectar-collecting *Bombus* spp. than with both nectar-collecting *Bombus* spp. and pollen-collecting *Apis mellifera* in patches of *Impatiens capensis*. These “conditional parasites” may be misleading to pollination biologists, because a parasitic interaction may look like a mutualistic one. If conditionally parasitic interactions between plants and pollinators are prevalent in nature, then pollination biologists will need to investigate the nature of the plant-pollinator relationship before continuing with their studies. Conditional parasites also provide an interesting opportunity for the study of pollinator-mediated selection on plant traits. When conditional parasites act as mutualists by increasing pollen delivery, selection may act on floral traits that increase visits by these pollinators; when conditional parasites act as parasites by decreasing total pollen delivery, selection may act on floral traits.
that decrease visits by these pollinators or increase visits by alternative pollinators. Selection may also act on floral traits that decrease pollen wastefulness by conditional parasites.

1.1.4. The variable pollinator climate

Biotically-pollinated plants are commonly exposed to a pollinator assemblage that varies widely over space and time. There are a number of examples of geographic variation in pollinator diversity and abundance (Miller 1981, Robertson and Wyatt 1990, Arroyo and Dafni 1995, Johnson and Steiner 1997, Price et al. 2005, Gomez et al. 2008). Variation in the pollinator climate is probably much more common than the small number of studies implies; documenting geographic and temporal variation requires a substantial investment in labor, identification expertise, and destructive sampling, and it may be documented only when it accompanies interesting and obvious changes in floral traits. Although modern researchers recognize that biotically-pollinated plants are commonly visited by a variety of animals that vary in diversity and abundance, our current understanding of plant-pollinator interactions is based almost entirely on studies conducted in one place and at one time (as reviewed by Herrera et al. 2006).

Geographic variation in the pollinator climate provides an interesting framework in which to study variation in pollinator value to plants. We understand that the pollinator climate often varies geographically, and we also have theoretical evidence that the value of pollinators to plants depends on the ecological context. Therefore, the direction of the interaction – whether a pollinator visit increases or decreases plant fitness – is likely to change through space and time.

1.1.5. Pollen collection by bees

The amount of pollen that pollinators waste depends partly on the reward that they seek. Among pollinator groups, the vast majority primarily seek nectar, pollen, or both; however, floral visitors vary in the relative quantity of each. In this thesis, I refer to “passive” and “active” pollen collectors to categorize pollinator species’ usual interactions with pollen. “Passive pollen collectors” include those groups of pollinators that collect mostly nectar in an effort to fuel their own activities, such as many flies and butterfly species. These floral visitors interact with pollen mainly in a passive way, by accumulating grains on their bodies during nectar foraging. Although some of these visitors consume some pollen, the amounts are relatively small and limited to their own individual nutritional needs, not those of their offspring. “Active pollen collectors” collect large quantities of pollen in addition to nectar. I categorize most female bees this way because they are highly proficient at collecting pollen to provision offspring from a range of plant resources (“pollen-generalist bees”) or from only one plant species and its close relatives (narrowly “oligolectic” bees) (Cane and Sipes 2006). The conflict of interest between plants and active pollen-collecting species is especially interesting; active pollen collectors are motivated – and selected – to deliver pollen from plants to larvae. From a plant perspective, this pollen does not contribute to plant reproduction and is therefore “wasted.”
Attributes of pollinators that increase pollen deposition may also increase pollen wastefulness. For example, actively pollen-collecting bees are generally considered to be the most highly valuable pollinators of a wide variety of plant species (Vogel and Machado 1991, Freitas and Sazima 2003, Hoffmann and Kwak 2005, McIntosh 2005). Bee foraging is efficient, systematic and often individually constant to one plant species (i.e., in a sequence of visits, visiting exclusively one flower species) (Waser 1986, Harder 1990, Chittka et al. 1997). Moreover, bees are proficient at handling flowers, both because of adaptations for higher efficiency (Cane and Payne 1993, Minckley et al. 1999, Moeller and Geber 2005) and a striking ability to learn (Raine and Chittka 2006, 2008). However, those attributes of bees that increase their proficiency at pollen transfer may also increase the cost of bee visitation, by increasing the amount of pollen that is delivered to bee nests rather than floral stigmas. For example, proficiency in flower handling will hypothetically also increase pollen removal, so the adaptations that bees exhibit and their ability to learn increases the amount of pollen that bees collect rather than deliver.

1.1.6. Solitary bee pollination

While many of the well-known bee species (honey bees in the genus *Apis* and bumble bees in the genus *Bombus*) are eusocial, the vast majority (94%) of bee species are solitary; each female is fertile and maintains her own nest (Michener 2000). There is enormous diversity in the nesting behavior of these species, including many that nest communally in some manner that is not fully eusocial or fully solitary (Michener 2000). Cleptoparasitic species (cuckoo bees), which make up about 20% of bee species, lay their eggs in the nests of other bees (Michener 2000). Foraging differs in social and solitary bee species in that social workers are required to collect pollen and nectar to provision the whole colony, while solitary bee foragers collect pollen and nectar for only their own offspring. Social and solitary bees also differ in their phylogenetic history and behavior; most importantly to this thesis, most social bees are corbiculate, meaning that they groom pollen into a flat plate surrounded by long hairs (the “corbicula”), often moistening the pollen into a concrete pellet. Most solitary bees, in contrast, are non-corbiculate, meaning they groom pollen into a group of elongated hairs (the “scopa”), where it is held more loosely (Thorp 1979). The corbiculate bees are a monophyletic group composed of over 1000 species in the tribes Apini (honey bees), Meliponini (stingless bees), Bombini (bumble bees), and Euglossini (orchid bees). The first two are highly eusocial, the third primitively eusocial, and the fourth solitary, communal, or weakly eusocial (Michener 2000).
1.1.7. Fly pollination

*This flower abounds in this district, and is very abundantly visited by short-tongued flies; one at least, and often as many as six or eight, may usually be seen on every head. They feed upon pollen, manipulating the anther-tube with their forelegs and proboscis. Though so numerous they are comparatively of little importance to the work of fertilization.*

*Willis and Burkill (1895)*

Flies (Order Diptera) are second only to bees in their commonness as flower visitors, yet we know very little about fly behavior and their importance as pollinators (Kearns 2001, Larson et al. 2001, Willmer 2011, Woodcock et al. 2014). Species in seventy-one families of flies are flower-feeding, and flies have been reported as regular visitors of at least 555 plant species (Larson et al. 2001); probably many more are unreported. Though often numerous on flowers, flies are often assumed to contribute only minimally – if at all – to pollen export and delivery (Faegri and van der Pijl 1966, Larson et al. 2001, Willmer 2011). Their value as pollinators may be obscured because they are often part of a generalist assemblage of several different types of insects (Kearns 2001). Very few studies have examined fly effectiveness, efficiency, or importance as floral visitors, but those that have show that they are more effective than their reputation suggests (as reviewed by Larson et al. 2001). Ssymank et al. (2010) argue that fly pollinators are valuable because their abundance at flowers makes up for the relatively low numbers of grains that they transfer. In this thesis, I argue that fly pollinators are underappreciated because although they interact with relatively fewer grains than bees, they are much less wasteful from the plant’s perspective. Only some fly species collect pollen, and only in the adult stage, whereas all larval and adult bees depend on pollen. In addition, the vast majority of species lack specialized structures for transporting pollen grains (Ssymank et al. 2010).

Hover flies (in the family Syrphidae) and bee flies (in the family Bombyliidae) are the most common of the anthophilous flies. Both forage for pollen and nectar. Bombyliid flies are conspicuous nectar foragers that hover over flowers during feeding (Larson et al. 2001). Though rarely studied, there are a few examples in the literature of bombyliid flies acting as effective pollinators (Beattie 1971, Motten et al. 1981, Motten 1986, Grimaldi 1988).

1.1.8. Pollination in early spring

Much of this thesis is set in the context of the early spring wildflower communities in North American temperate woodlands, which are defined by relatively cool temperatures, frequent rains, few plant species in flower, and foraging by only the earliest emerging insects. Early spring plants are vulnerable to inadequate pollination, because many bee species are not yet active, the flowering season is short, and
frequent spring rains interfere with pollinator foraging (Motten 1986). This setting is convenient for studies of plant-pollinator interactions because the smaller communities simplify the complexity of interspecific interactions. Common early spring wildflowers include *Claytonia virginica*, *Erythronium* spp., *Hepatica americana*, *Sanguinaria canadensis*, *Thalictrum thalictroides*, and *Cardamine angustata*. Solitary bees in the family Andrenidae are prevalent in early spring, along with solitaries in the families Halictidae and Apidae, various species of *Bombus* (bumble bee) queens, and occasionally the honey bee *Apis mellifera*. Flies and butterflies are also common.

1.2. Study systems

This thesis focuses on a certain type of plant-pollinator relationship, one in which a plant is visited by a diverse and abundant pollinator assemblage with relatively high visitation rates by both active and passive pollen-collectors. I use two generalist plant-pollinator systems to represent this type of plant-pollinator relationship: in my primary system, *Claytonia virginica* is visited by a variety of nectar-collecting and pollen-collecting insects, including the oligolege *Andrena erigeniae* and the primarily nectar-collecting bee-fly *Bombylius major* (Fig. 1.1). The bee-fly *B. major* is a parasite of solitary bees, including *Andrena*, and therefore may be parasitizing *A. erigeniae* populations. In a secondary system, the highly generalist species *Brassica rapa* is visited by, among other insects, the highly generalist bumble bee *Bombus impatiens* and the cabbage white butterfly *Pieris rapae*. The *C. virginica* system models natural variation in the ecological context for observational study, while the *B. rapa* system allows for pollinator manipulation and provides a tractable representation for experimental work; within this thesis, *B. rapa* and its pollinators is a model system for highly generalist plant-pollinator relationships.

Motten et al. (1981) observed the visitation rates and pollination effectiveness (number of seeds set resulting from one pollinator visit) of visitors to *C. virginica* in North Carolina forests. *Andrena erigeniae* and *B. major* made the majority of visits (75%) and had similar pollination effectiveness (4 and 3.4 seeds after one visit, respectively). The authors observed no pollen-limitation of *C. virginica* reproduction, except in the first flowers to open and during inclement weather when visitation was low. They estimate visitation rates of *A. erigeniae* and *B. major* to be 4.1 and 2.4 visits per flower per day, respectively. The authors did not consider pollen removal or pollen depletion in *C. virginica*, so I investigated these aspects.

1.3. My approach

My approach centers around the quantification of pollen grains, because the delivery of pollen grains is a component of plant fitness. I observe pollen grains within the ecological context; I measure visitation rates of pollinators, the number of grains that pollinators remove and deposit, and the rate that pollen grains in a plant population deplete over time. This work is basic, foundational pollination biology, but it has so far been limited by difficulties in tracking pollen grains; moreover, it is necessary to resolve these issues to better understand the complexity of plant-pollinator interactions. When quantification in real
systems becomes impossible, I use simulation models to track the hypothetical movement of quantities of pollen in plant populations.

Hermaphroditic plants attain fitness through both female function (the production of fruits and seeds) and male function (pollen donation). The quantity of pollen grains delivered to plant stigmas is important for both female and male function, because a plant that receives pollen depends on the delivery of pollen to fertilize its own ovules, and a plant that donates pollen will be able to fertilize the ovules of other plant individuals. Historically, most research in plant reproductive biology and pollination has focused on female fitness, using seed or fruit production as sole measurements of plant fitness. Plant female fitness is assumed to be limited either by pollen deposition or resource availability, and the extent to which these factors limits plant reproduction in natural populations is a controversial topic in plant reproductive biology (Harder et al. 2010, Aizen and Harder 2007, Knight et al. 2005, Ashman et al. 2004). Despite the theoretical result that pollen limitation should be rare, the current consensus is that there is extensive variation in pollen limitation depending on the ecological context, and that pollen limitation can be fairly common in plant populations (Burd 1994, Knight et al. 2005, Richards et al. 2009). Pollen delivery is not a foolproof estimate of plant female fitness, because there is not always a direct relationship between stigmatic pollen deposition and the production of seeds; however, the quantity of pollen grains delivered may have important implications for female fitness when there is a relationship between pollen deposition and seed production.

I do not focus on plant female fitness; instead, this thesis deals primarily with quantitative aspects of pollen delivery and the effect of pollinators on plant male fitness. I measure and simulate the quantity of pollen delivered as a proxy for a pollinator's contribution to plant fitness. The quantity of pollen grains delivered is a strong proxy for plant male fitness, because the ability to export and deliver pollen grains should directly increase siring success.

The visitation rates of pollinator species are an important consideration. When visitation rates are low, pollen delivery is more likely to impact female fitness; however, conditional parasitism is more likely to occur when there are multiple pollinators that each visit often. Conditional parasitism is more likely to impact pollen export and delivery than the production of fruits and seeds; therefore, we are more interested in the value of pollinators to plant male function. This thesis focuses on pollen delivery and plant male function in the context of high visitation rates.

1.4. Overview of the chapters

In the chapters that follow, I investigate the effect of the ecological context on pollinator value to plants; I seek to understand the importance of the pollinator climate in determining whether a pollinator visit increases or decreases plant fitness. I use observational studies and simulation models to explore how the pollinator climate varies in space and time, and how that variation affects the pollination partnership.
I begin in Chapter 2 with a comparison of two pollinators in a single ecological context; I measure benefits – and more uniquely, costs – of visits by an active pollen-collector and a passive pollen collector. The benefits of a single visit by the oligolege *Andrena erigeniae* and a single visit by the passive pollen collector *Bombylius major* are similar in that they deliver approximately the same number of grains. However, *A. erigeniae* remove many more grains, and that removal results in significant and rapid pollen depletion in Pennsylvania populations of *Claytonia virginica*. This chapter introduces the importance of the pollinator climate: although *C. virginica* is well pollinated by *A. erigeniae* in these Pennsylvania populations, *B. major* would provide similar benefits at a lower cost if the bee fly visited more often.

In Chapters 3 and 4, I document variation in the pollinator climate visiting *C. virginica* and explore the effect of such variation on plant evolution. Chapter 3 describes the results of a citizen science project in which volunteers documented interesting changes in the pollinator climate throughout *C. virginica*’s range. The oligolege *A. erigeniae* visits more often in Western populations of *C. virginica* and sister species *C. caroliniana*, while the primarily nectar-collecting *B. major* visits more often in Southern populations. These results correspond with Chapter 4, in which I describe my own observations of the pollinator climate in Pennsylvania, Maryland, and North Carolina. I link observed visitation rates of the oligolege *A. erigeniae* to the rates of pollen depletion in these plant populations. In addition, I measure geographic variation in plant traits – the amount of pollen produced, and the rate of pollen presentation – that may affect pollen removal, transport, and delivery depending on the pollinator climate.

In Chapter 5 I explore one aspect of bee biology that may have important implications for pollinator wastefulness and the nature of bee-plant relationships. Female bees gather grains from their bodies by grooming, stereotypical movements that concentrate pollen grains into morphological structures for transportation back to the bee nest. Grooming behavior varies between pollinator groups; members of the corbiculate Apidae, which I refer to as the “corbiculate” bees (such as honey bees and bumble bees) manipulate pollen extensively, moistening it to form pollen pellets in the corbiculae, a structure on the hind tibae. Bees not in the corbiculate Apidae – which I refer to as “non-corbiculate” bees (such as *Andrena erigeniae* and other solitary bee species) mainly gather pollen more loosely into local concentrations of long hairs, known as “scopae” (Michener 1998). We measured the function of groomed and ungroomed *Brassica rapa* pollen on the bodies of two corbiculate bees, *Apis mellifera* and *Bombus impatiens*, and two non-corbiculate bees, *Megachile rotundata* and *Halictus* spp. Grooming by corbiculate bees appears to impair pollen function, in that groomed pollen resulted in lower seed set than ungroomed pollen, but grooming by non-corbiculate bees did not have an effect. Because their groomed pollen is impaired and therefore wasted, corbiculate bees may be more wasteful than non-corbiculate bees. Moreover, non-corbiculate bees may be more valuable pollinators than previously thought.

Finally, I explore the circumstances that may result in conditional parasitism in natural populations, and evaluate whether conditional parasitism is likely to be an important part of plant-pollinator interactions in nature (Chapter 6). I describe a simulation model that represents the *C. virginica* system; then, I vary
pollinator characteristics such as the removal and deposition rates of each pollinator group and plant characteristics such as the schedule of pollen presentation. Using these simulations, I describe the characteristics of a plant-pollinator relationship that may reveal conditional parasitism – high visitation rates by active and passive pollen collectors, high removal rates or low deposition rates by the active pollen collector, and simultaneous pollen presentation.

1.5. Figures

![Figure 1.1. Photos of the *Claytonia virginica* pollination system. (a) A *C. virginica* female-phase flower. (b) A *C. virginica* male-phase flower. (c) The bee-fly *Bombylius major* visiting *C. virginica*. (d) The oligolectic bee *Andrena erigeniae* visiting *C. virginica.*]
Figure 1.2. Example of a pollen delivery surface.

Rather than thinking about pollinator value to plants as a species-specific constant (a), we should consider it to be a multidimensional response surface, or “delivery surface” (b).
Specialist pollinators deplete pollen in the spring ephemeral wildflower *Claytonia virginica*

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Contribution of authors: Study conceived and designed by AJP and NMW, data collected by AJP and NMW, data analysis by AJP, article drafted by AJP, revisions by AJP, NMW, and JDT.

2. **Abstract**

Pollinators that collect pollen – and specifically, pollen-specialist or “oligolectic” bees – are often considered to be the best pollinators of a (host) plant. While pollen-collectors and oligolectic bees often provide substantial benefits to host plants, especially in the amount of pollen that they deliver, they can also exert substantial costs because they are motivated to retain as much pollen as possible. We measured the costs and benefits of nectar-collecting, pollen-collecting, and oligolectic pollinators to the spring ephemeral *Claytonia virginica*. While all pollinators delivered roughly the same number of grains per visit, the oligolege contributed most to *C. virginica* pollen delivery because of high visitation rates. However, the oligolege also removed many grains; this removal may be especially costly because it resulted in the depletion of pollen grains in *C. virginica* individuals. While *C. virginica* appears to be reliant on pollen transfer by the oligolege in these populations, nectar-collecting visitors could provide the same benefit at a lower cost if their visitation rates increased.

2.1. **Introduction**

What makes a good pollinator? Pollination involves pollen transfer: animals remove, transport, and deposit pollen between conspecific plant individuals. In generalized pollination systems, one plant species is often visited by a variety of potentially pollinating species with diverse attributes and characteristics; these qualities can affect the removal of grains from the pollen-donating plant individual, the transport of those grains to another conspecific plant individual, and the deposition of those grains on the plant stigma. Plant individuals benefit most from animal pollination when pollen transfer is efficient, i.e. when most of the pollen that they produce is transferred to receptive, compatible stigmas. However, plant individuals pay a cost for pollination service in that not all pollen that is produced is successfully deposited. From the plant perspective, pollen grains that do not pollinate conspecific stigmas are “wasted”, and potentially costly (Castellanos et al. 2003). We know that animals vary extensively in their “pollination efficiency”, defined as the proportion of pollen removed that is subsequently deposited.
(LeBuhn and Holsinger 1998, Larsson 2005); this measurement estimates the proportion of pollen that is wasted. However, we do not yet understand what determines the cost of this wasted pollen to plants. Wasted pollen will be more costly when the supply of pollen is limited (Thomson 2003); thus, pollinator visits have higher relative costs when they result in “pollen depletion”, a reduction in the pollen standing crop remaining in an open flower or population with many open flowers in nature. However, pollen depletion has rarely been measured empirically.

Floral visitors can include primarily nectar-collecting species (e.g., most flies and butterflies), those that collect both nectar and pollen (e.g., most female bees), and sometimes one or more floral visitors that collect pollen from only that plant species and close relatives (e.g., pollen-specialist or oligolectic bees). There are many reasons why pollen-collecting bees – and in particular oligolectic bees – may be considered valuable pollinators to a plant. Bees forage efficiently and systematically (Harder 1990, Chittka et al. 1997), which will increase the number of flowers that they visit. Bees frequently exhibit some level of floral constancy, so they temporarily preferentially visit one floral type (as reviewed in Waser 1986), and oligolectic bees, by definition, focus their pollen foraging effort primarily on their host plant; these characteristics can also result in disproportionately high numbers of visits to a (host) plant (Cane and Payne 1993, Minckley et al. 1999, Moeller 2005). Oligolectic bees learn how to handle flowers quickly (Raine and Chittka 2006, 2008), and some oligoleges exhibit adaptations to more efficiently handle host plant flowers (Strickler 1979, Thorp 1979, Laverty and Plowright 1988, Schlindwein and Wittmann 1997, Müller and Bansac 2004). Due to these factors, a number of studies have considered bees generally, and oligolectic bees in particular, to be the most important pollinators to a (host) plant (Vogel and Machado 1991, Freitas and Sazima 2003, Hoffmann and Kwak 2005, McIntosh 2005).

However, there is a substantial cost to bee pollination – and especially in oligolectic bee pollination – in that adult female bees are selected to efficiently deliver pollen from anthers to larvae, not to stigmas; from a plant perspective, this pollen is wasted. Bee larvae require substantial quantities of pollen (Yeo et al. 1996) and there is evidence that bee reproduction is limited by pollen (Strickler 1979, Müller et al. 2006, Steffan-Dewenter and Schiele 2008, Roulston and Goodell 2011). Many of the same factors that may increase the value of bees to plants – and in particular, the value of oligolectic bees to their host plant – may also increase the amount of pollen that they are able to use to provision larvae. For example, bee learning and oligolectic adaptations to a host plant, such as adaptations to more efficiently handle host plant flowers, increase the amount of pollen that the bees can sequester (Schlindwein and Martins 2000). There is experimental evidence that oligolectic bees collect more pollen per foraging effort (Strickler 1979, Laverty and Plowright 1988, Cane and Payne 1993). Bees, and especially oligolectic bees, may also learn sophisticated behaviors for exploiting plant resources, e.g. by foraging preferentially on male or male-phase flowers (Ågren et al. 1986, Bierzychudek 1987, Eckhart 1991, Ashman and Stanton 1991, Wilson and Thomson 1991, Eckhart 1992a, Tollsten and Knudsen 1992, Delph and Lively 1992, Ashman 2000). Plants can manipulate the number of grains a pollen-collector can remove by controlling the pollen presentation schedule, i.e. the rate at which pollen is made available for removal by pollinators.
Separating pollen into packages or dispensing pollen from those packages over time can limit pollen collection, potentially mitigating the cost of pollen collection to plants (Harder and Thomson 1989).

Measuring the costs of pollen collection is difficult, and there are few studies that are both quantitative and thorough. Several studies have compared the amount of pollen removed by different pollinators by measuring the number of grains that each floral visitor removes in a single visit (i.e., “single-visit pollen removal”) (e.g., Larsson et al. 2005, Sahli et al. 2007, Wilson and Thomson 1991). This research shows that species that actively collect pollen (both generalist and oligolectic female bees) sometimes remove a great deal more pollen than species that only passively transport grains (Larsson 2005), but not always (Sahli and Conner 2007). Often, generalist and oligolectic species remove a similarly high percentage of grains, e.g. at least half of available grains (Strickler 1979, Strickler and Cane 2001, Larsson 2005). Pollen collected by pollen-collecting and oligolectic bee species that is delivered to larvae rather than stigmas – wasted pollen from the plant perspective – may diminish fitness through male function; the total cost of wasted pollen will depend on how much pollen remains because wasted pollen will also diminish plant fitness through female function if female function is limited by pollen receipt (Castellanos et al. 2003).

When pollen collecting bee species – and oligolectic bee species in particular – have evolved to maximize their collection effort, it follows that they may deplete the supply of pollen in a plant population. This pollen depletion may affect an individual plant’s male fitness because of lost opportunities for pollen export by future flower visitors. Pollen depletion at the population level may also decrease pollen transfer in the population as a whole. Predicting the effects of pollen depletion on pollen delivery when multiple floral visitors are visiting has inspired a set of theoretical “pollen depletion models” (Harder and Thomson 1989, Thomson 2003, Castellanos et al. 2003). These models use basic empirical data such as the visitation rate, single-visit pollen removal, and the number of grains delivered in a single visit (“single-visit pollen deposition”) to calculate pollen depletion and hypothetical pollen delivery when multiple floral visitors are visiting; they demonstrate that a pollen-collecting floral visitor may reduce pollen delivery by depleting pollen and impacting the pollen transfer of future floral visitors. Despite this theoretical focus, pollen depletion at the population level is rarely measured empirically in natural systems. We have found only three examples of pollen depletion measurements of natural plant populations in the literature (Wilson and Thomson 1991, Minckley et al. 1994, Raine et al. 2006), and each study measured population pollen depletion for only one day. Empirical measurements of pollen depletion will help assess if pollen depletion models are realistic and relevant to natural plant-pollinator systems.

Here, we compare the costs and contributions of different pollinator groups, including primarily nectar-collecting individuals, primarily pollen-collecting individuals, and oligoleges, to the pollination of a spring ephemeral wildflower. We link oligolectic bee pollen removal to substantial pollen depletion in plant populations, providing a more comprehensive view of the cost of oligolectic bee visitation.
2.2. Methods

*Claytonia virginica* L., “Spring Beauty” (Portulacaceae) is a spring ephemeral wildflower native to North American eastern woodlands, ranging from Georgia to Ontario and from the East Coast west to Kansas and Nebraska. *Claytonia virginica* is protandrous; pollen and nectar are offered on the first day, in the male phase, although only nectar is produced in the female phase. (In this study we refer to all first day flowers as “male-phase” and second and third day flowers as “female-phase” even though the flower may no longer be acting as a male or female). Nectar production of male- and female-phase flowers is roughly equivalent in *C. virginica* (Motten et al. 1981). *Claytonia virginica* has six ovules, and is self-compatible but not self-pollinating (Motten et al. 1981), and self-pollinated flowers produce fewer seeds (Schemske 1977). *Claytonia virginica* is visited by a variety of insects, among them the oligolectic solitary bee *Andrena erigeniae*, which collects pollen exclusively from *C. virginica* and the closely related *C. carolinana* (Davis and LaBerge 1975); for simplicity, our study considers *A. erigeniae of C. virginica* only. The range and phenology of *A. erigeniae* match that of *C. virginica* (Davis and LaBerge 1975). A number of generalist species also visit, collecting pollen, nectar, or both from *C. virginica* and other sources. The most frequent generalists are the bee-fly *Bombylius major*, which does not actively collect pollen, and small generalist bees in the genera *Lasioglossum* and *Ceratina*, who collect both pollen and nectar from *C. virginica*. We chose to group the latter bee genera into one functional group (“small generalist bees”) because they are difficult to distinguish in the field and because they are primarily generalist pollen and nectar collectors with similar size and behavior.

Male-phase *C. virginica* flowers have five anthers with two locules each, therefore separating pollen into ten “packages” that dehisce one at a time. Anthers usually dehisce during the first hour or two of the flower’s opening; dehiscence occurs faster in warmer temperatures.

The data included in this study were collected in a number of sites in suburban Philadelphia, Maryland, and North Carolina in Spring 2009 and Spring 2010. The majority of the data were collected in 2009 on five populations of *C. virginica* in suburban Philadelphia; these populations were located on the grounds of Lankanau General Hospital, Andorra Park, Ridley Creek State Park, and two private woodlots. All pollen depletion data are from these Pennsylvania populations. To supplement sample sizes for pollen removal and deposition, we included additional data collected in Spring 2010 at Mason Farm Biological Reserve in Chapel Hill, North Carolina and Patuxent National Wildlife Refuge in Laurel, Maryland.

### 2.2.1. Single-visit pollen removal

To measure “single-visit removal” of visiting insects, we estimated the number of pollen grains remaining within the anthers of a flower after a single visit by a floral visitor compared to that remaining in a sample of unvisited control flowers. Before male-phase flowers opened, we covered flowers with cages with mesh small enough to prevent visitor entry. These structures were fashioned from plastic milk jugs; we removed the bottoms and inserted screened “windows” on the sides to increase air flow, and then
staked the structures into the ground with wire. At the onset of pollinator activity and throughout the course of the day, we uncovered these unvisited flowers and allowed a single visit from a free foraging insect visitor. All visited and control flowers had anthers that were totally dehisced — therefore, our data on pollen removal come from flowers with maximal pollen availability. Many single visits were conducted in situ; however, in order to encourage visitation and increase sample sizes, we often inserted our prepared flowers into an “interview stick” (Thomson 1988), an approximately 1.5m stick with a flower pick attached to the end, into which a flower could be inserted and presented to foraging insects. After the visit, we carefully removed the anthers from the flower into centrifuge tube with 1.00 mL 70% ethanol for storage. At the end of each day, we collected the anthers from leftover male-phase flowers under the pollinator exclusion structures; these were our unvisited controls. Therefore, if pollen is lost passively from male-phase flowers throughout the day, our unvisited controls represent counts of pollen after that loss has occurred. In the lab, we counted the number of pollen grains in each sample (both visited and unvisited) using a Coulter Multisizer 3 particle counter (Beckman Coulter Inc., Brea, California). We prepared samples by adding 0.9% saline and then weighed the samples. We counted four 1 mL subsamples using the particle counter and took the average of the subsample counts to obtain a mean count for the subsample. Sample counts were not highly variable; generally we obtained less than a 10% difference between counts. We then multiplied the mean of the subsample counts by the weight of the sample (including the added saline) to obtain the final estimate of the whole sample.

To estimate the mean amount that a given pollinator removes in a single visit, we compared the mean number of grains remaining after all single visits by that floral visitor group to the mean number of grains in unvisited controls. ((Grains in an unvisited flower – grains remaining)/Grains in an unvisited flower) (as in Wilson and Thomson 1991).

2.2.2. Single-visit pollen deposition

To estimate the “single visit pollen deposition” of visiting insects, we also collected data on the number of pollen grains deposited during a single visit by an individual pollinator on previously unvisited female-phase flowers. To prevent contamination by self-pollen, we emasculated the flowers during the male-phase the day before; as often as possible, we removed the anthers before anther dehiscence to further reduce pollen contamination. We obtained visits to female-phase flowers using the same methods as the removal samples. After a visit, we collected the flower and placed it in a flower pick with water in a cooler for 24 hours to allow deposited pollen to adhere to the stigma and begin pollen tube growth. Then we removed the stigma with forceps and placed it in a microcentrifuge tube filled with 70% ethanol for storage. In the lab, we mounted each stigma on a slide with fuschian stain, and counted the number of pollen grains deposited (Kearns and Inouye 1993).
2.2.3. Visitation

To determine representative visitation rates to *C. virginica*, we conducted observations of specialist and generalist insect visitation to *C. virginica* male- and female-phase flowers. We first determined a sample of fresh flowers that we could observe simultaneously and recorded the number of male- and female-phase flowers. We then observed this flower set for five minutes and identified visitors to visitor group. We conducted a set of visitation observations approximately every hour during periods of bee activity.

2.2.4. Depletion

We measured pollen depletion in five plant populations in suburban Philadelphia on seven days in late April 2009, for a total of eleven pollen depletion curves.

To assess the rate of pollen depletion in plant populations, we collected the anthers from a set of eight flowers in the population every hour during insect activity. We began collecting anthers at the start of anthesis, when the first few anthers began to dehisce (approximately the same time as the beginning of bee activity). On warm days this occurred as early as 8:45am, but on cool days or after rain this often did not occur until late morning or early afternoon. We then collected one set of anthers every hour until female-phase flowers began to close or insect visitation ended; on most days we collected 2-4 sets of anthers. Anthers were placed into separate microcentrifuge tubes (one per individual) filled with 1 mL of 70% ethanol. We chose flowers haphazardly, regardless of how many anthers had dehisced, but attempted to collect flowers from various parts of the population during each collection period. We counted these using the same method as the removal samples.

During the counting process, undehisced anthers were opened and emptied; therefore, our counts represent the full number of pollen grains in the flower, including those from anthers that had not yet dehisced at the time of collection. Therefore, the rates of pollen depletion in our data start with the total number of grains that will be available during that day, and plot how that number is reduced through time due to pollen removal. Our data do not measure pollen availability, but instead represent the number of grains remaining in a flower.

In order to compare hypothetical and empirical pollen depletion rates, we also modeled a hypothetical depletion curve based on the mean number of grains in an unvisited male-phase flower, the mean per-visit removal by oligolectic *A. erigeniae* females from our empirical data (Fig. 2.2), and the visitation rate of *A. erigeniae* females to male-phase flowers (2.1 visits/hour, Fig. 2.1). We assumed that *A. erigeniae* females would remove a constant proportion, estimated as 61% of the pollen remaining when each male-phase visit occurred. Because *A. erigeniae* females dominated the other floral visitors in visitation rate, no other floral visitors were included in this calculation. As in our empirical data, we did not include the effect of gradual presentation of pollen due to the sequential dehiscence of anthers, but instead modeled as if all grains were available at the start of anthesis. The number of grains available ($A$) after one visit is given
by the following equation (Harder and Wilson 1994), with pollen production or number of grains available before any visits \( (A_0) \), the number of grains available after one visit \( (A_i) \), and the proportion of remaining pollen removed during each visit \( (p_i) \).

**Equation 2.1**

\[ A_i = A_0 (1-p_i) \]

We calculated \( A_i \) for 10 visits, the number of visits that would occur in just over five hours (2.1 visits/hour, Fig. 2.1) with pollen production \( (A_0) \) of 2764 grains, the mean of our empirical data (Fig. 2.2), and the proportion removed \( (p_i) \) of .61, calculated from our empirical data (Fig. 2.2). We then plotted \( A_i \) against time, assuming a constant visitation rate of 2.1 visits/hour.

### 2.2.5. Data analysis

All statistical analyses were done using R 3.0.1 (R Core Team 2013). We compared the number of pollen grains deposited and the number of grains remaining in anthers after a single visit among visitor groups using generalized linear models (GLMs). For each, the predictor variable was the pollinator group \( (A. arisingae females, B. major, or small generalist bees) \) and the response variable was the number of grains deposited or the number of grains remaining after a single visit by that pollinator. We used a negative binomial error distribution for both the removal and deposition models because the response variable in both data sets was overdispersed (Lindén and Mäntyniemi 2011), to do so we used the R function `glm.nb` in the library `MASS` (Venables and Ripley 2002). We conducted multiple comparisons using the `glht` function in the library `MULTCOMP` (Hothorn et al. 2008).

To evaluate how the hour of collection affected the number of grains remaining in male-phase flowers, we used generalized linear mixed models (GLMMs). The predictor variable was time since anthesis and the response variable was the total number of pollen grains in an open flower. Because depletion could vary due to factors of the specific day of data collection, we included the day of data collection as a random effect. We used the function `glmmADMB` in the `glmmADMB` library (Fournier et al. 2012) because it allowed us to include random effects and account for overdispersion using a negative binomial distribution. We observed no autocorrelation structure in the average of the residuals over time. We calculated the effect size of the time since anthesis using the function `r.squaredLR` in the library `MuMIn`.

In addition, we conducted the same model analyses for each individual day independently. For these individual analyses, we used the `glm.nb` function in the library `MASS` because we did not need to include random effects.
2.3. Results

2.3.1. Visitation

*Andrena erigeniae* females were by far the most common visitor, with a mean visitation rate of 1.05 visits per female-phase flower and 2.10 visits per male-phase flower per hour (Table 2.1, Fig. 2.1). *Bombylius major* visited female-phase flowers 0.07 times and male-phase flowers 0.03 times per flower per hour, and small generalist bees visited female-phase flowers 0.21 times and male-phase flowers 0.45 times per flower per hour (Table 2.1, Fig. 2.1). *Andrena erigeniae* males also visited, but very rarely. Both *A. erigeniae* females and small generalist bees showed a preference for male-phase flowers, visiting male-phase approximately twice as often as female-phase flowers (Table 2.1, Fig. 2.1). Insect activity began as flowers opened. *Andrenaerigeniae* females visited more often in the morning hours, slowing as floral rewards were depleted. *Bombylius major* visitation was consistent throughout the day and continued into the afternoon, well after resource depletion.

2.3.2. Single-visit pollen removal

An individual male-phase *C. virginica* flower contained on average 2764 ± 952 grains (mean ± SD). There were 1078 ± 918 pollen grains remaining after a single visit by an *A. erigeniae* female, which is significantly fewer than the number in an unvisited control (Table 2.1, Fig. 2.2, z= 11.320, *P* < 0.001), the number remaining after a single visit by *B. major* (2053 ± 623 grains, Table 2.1, Fig. 2.2, z= 6.066, *P* < 0.001) and the number remaining after a single visit by a small generalist bee (2203 ± 812 grains, Table 2.1, Fig. 2.2, GLM, z= 5.924, *P* < 0.001). The number of pollen grains remaining after a single visit by *B. major* was significantly lower than the number of grains in an unvisited flower (Table 2.1, Fig. 2.2, GLM, z= 3.436, *P* = 0.003). There was no significant difference between the number of grains remaining after a single visit by a small generalist bee and the number of grains in an unvisited flower (Table 2.1, Fig. 2.2, GLM z= 2.191, *P* = 0.12).

2.3.3. Single-visit pollen deposition

*Andrena erigeniae* females deposited an average of 39.43 ± 52.07 (mean ± SD) grains in a single visit, while *B. major* deposited 30.00 ± 18.97 grains and small generalist bees deposited 14.97 ± 12.96 grains. There is no significant difference between the number of grains deposited by an *A. erigeniae* female and *B. major* (Table 2.1, Fig. 2.2, GLM, z=0.928, *P* =0.98), and no significant difference between the number of grains deposited by *B. major* and a small generalist bee (Table 2.1, Fig. 2.3, GLM, z=2.119, *P* =0.33). The number of grains deposited by an *A. erigeniae* female is significantly higher than the number of grains deposited by a small generalist bee (Table 2.1, Fig. 2.3, GLM, z=3.621, *P* =0.005).
2.3.4. Depletion

Pollen depletion appears to be rapid in these *C. virginica* populations. In the first hour, the mean number of grains per flower was 2761 grains, which drops to 1509 grains in the second hour and 804 grains in the third hour. Including the time since anthesis significantly improved the model fit, indicating that time since anthesis is a significant factor in the model describing pollen depletion (Fig. 2.4, GLMM, $r^2=0.34$, $z=-12.1$, $P<2e-16$).

On each day of data collection, the number of pollen grains remaining in a male-phase flower showed a significant negative relationship with the time of collection (Table 2.2).

2.4. Discussion

In Pennsylvania populations of *C. virginica*, *A. erigeniae* females visited four times as often as all other visitors combined. In a single visit, an *A. erigeniae* female removed on average 61% of the remaining pollen on a male-phase flower. *A. erigeniae* females also visited male-phase flowers approximately twice as often as female-phase flowers (1.05 visits and 2.10 visits per hour, respectively). In these populations of *C. virginica*, pollen is depleted quickly; one hour after anthesis, only 55% of pollen remains, and 29% remains after two hours. We measured depletion in natural populations visited by a community of naturally occurring pollinators. Therefore, the observed pollen depletion is the result of visitation by the whole community of floral visitors, rather than *A. erigeniae* females only; however, our evidence suggests that the oligolege is the primary driver of the observed pollen depletion.

We expected the oligolege to remove more grains than pollen-collecting generalists in single visits, and all bees to remove more grains than the primarily nectar-collecting fly. However, the small generalist bees removed surprisingly few grains. This result indicates that small generalist bees are collecting mostly nectar and – due to their small size – avoiding contact with anthers when they land on the corolla to collect nectar. In this system, the cost of pollination service by nectar-collecting flies and the small generalist bees is minimal. However, our data show a clear difference in pollen removal between oligolecic *A. erigeniae* females and the primarily nectar-collecting fly *B. major*; therefore, the relative cost of oligolecic visitation is high.

Our estimate that *A. erigeniae* females remove 61% of remaining grains is surprisingly low compared to other measurements of pollen removal by bees – both oligolecic and generalist pollen-collector – in the literature. Our rates of pollen removal are estimations based on the mean number of grains in unvisited flowers and the mean number of grains after single visits, so perhaps this method of estimation is not as accurate as those measurements of pollen removal that compare visited and unvisited anthers on the same flower (Williams and Thomson 2001, Sahli and Conner 2007) or from an unvisited flower in the same location on the stem as the visited flower (Strickler 1979). Moreover, we measured pollen removal by comparing visited and unvisited flowers that both had completely dehisced anthers, which is unlikely.
to occur in natural *C. virginica* populations; perhaps when less pollen is available, the percentage removed would be higher. In any case, the percentage of pollen removed by specialists and pollen-collecting generalist bee groups does not show a clear pattern across studies. In the *Knautia arvensis* pollinator community, generalist bumble bees remove fewer grains than females of the pollen specialist *Andrena bhattorfiana* (62% and 79%, respectively), perhaps because the generalist bumble bees are foraging for nectar (Larsson 2005). Oligolectic *Hoplitis anthocopoides* removes 78% of *Echium vulgare* grains, while pollen-collecting generalists *Megachile relativa*, *Osmia coerulescens*, *Hoplitis productiva*, and *Ceratina calcarata* remove a similarly substantial 79%, 77%, 84%, and 72% of grains (Strickler 1979). Even though the (surprisingly low) removal rate of the oligolectic bee is significantly higher than the removal rate of generalist species in our study, we are not as certain that oligolectic bees are always superior at pollen collection than generalist bees; another factor (such as bee size) may be more important.

Despite relatively low removal rates by *A. erigeniae* females, *C. virginica* pollen is depleted quickly. From this apparent contradiction, we conclude that pollen can deplete quickly in plant populations even when removal rates are not extraordinarily high, as long as the visitation rate is high; pollen depletion may be even more substantial when pollen removal rates are higher. The only other studies that measure pollen depletion found similarly extreme rates of pollen depletion in one day of visitation (Wilson and Thomson 1991, Minckley et al. 1994, Raine et al. 2006, Raine and Chittka 2008). Moreover, our measurements of pollen depletion included grains in as-yet undehisced anthers; we measured the number of grains remaining in a male-phase flower, rather than the number of grains available. *Claytonia virginica*'s gradual anther dehiscence probably delays pollen depletion because pollen cannot be removed until it is made available. The rate of pollen depletion would be even more extreme if all of the pollen in a male-phase flower was presented at once. Measuring pollen depletion for additional pollination partnerships may reveal a more widespread pattern that pollen-collecting and oligolectic bees often substantially deplete the supply of pollen in the populations that they visit.

When we calculated hypothetical pollen depletion given the empirical measurements of visitation and removal rates of *A. erigeniae* females, the depletion rate is similar to the observed pollen depletion rate (Fig. 2.4). Our hypothetical calculations are an example of the types of calculation commonly used in pollen depletion models (Harder and Thomson 1989, Thomson 2003, Castellanos et al. 2003). This consistency between empirical measurements and theoretical predictions provides evidence that the foundation of these theoretical models is realistic to natural plant-pollinator systems. The hypothetical pollen depletion rate showed more drastic pollen depletion in the first few hours of visitation than our empirical data; this may be because *C. virginica* anthers do not dehisce simultaneously, but sequentially over the course of the first hour or two of insect visitation. Despite this difference, these results justify the use of parameter estimation and simple models to estimate population pollen depletion, rather than investing the time and effort to collect these data empirically.
From our measured values of visitation and pollen transfer, there appear to be substantial costs and substantial benefits to oligolectic *A. erigeniae* female visitation relative to the other floral visitors. *Andrena erigeniae* females appear to be monopolizing the pollen supply through high visitation rates to male-phase flowers and high single-visit removal values relative to the other floral visitors. However, although these bees deposit an unremarkable quantity of grains per visit (not significantly different from *B. major*), their mutualistic contribution is multiplied through numerous female-phase visits. The primarily nectar-collecting *B. major* doesn’t remove as many grains as do *A. erigeniae* females. *Bombus major*’s contribution to pollen deposition is as high as *A. erigeniae* females’ on a per-visit basis, but cannot compete in visitation frequency; as a result, *B. major* cannot be considered more valuable than *A. erigeniae* in these Pennsylvania populations. The pollen-collecting generalist species do not deposit as many grains as do *A. erigeniae* females in single visits, but they also do not remove a significant number of grains. Combined with their relatively low visitation rates, there is no evidence that pollen-collecting generalist species have a substantial impact – either positive or negative – on *C. virginica* pollination in these populations.

Combined with relatively high single visit deposition rates, the oligolectic *A. erigeniae* seems irreplaceable in these Pennsylvania populations because of high visitation rates; though we did not collect data to address these factors, these visits may have been more consistent and more efficient as well (Cane and Payne 1993, Minckley et al. 1999, Moeller 2005). Anecdotally, *A. erigeniae* females appeared to exhibit high fidelity to *C. virginica*, in that we never observed *A. erigeniae* foraging on another floral species and visitation by *A. erigeniae* females seemed to span the entire time period in which *C. virginica* flowers were open. However, because of *C. virginica*’s early spring flowering time, not many other flowers were available and visitation by all floral visitors also seemed fairly consistent. The bees in our study – both *A. erigeniae* females and the small generalist bees – also foraged efficiently and systematically, while *B. major* traveled farther between visits (pers. obs.). This feature may increase the visitation rate of the bees (especially *A. erigeniae*), but the foraging behavior of *B. major* may affect the pollen grains that it delivers by increasing their diversity and distance traveled; in other systems, flies have been shown to carry more diverse pollen larger distances than small-bodied bees (Rader 2011) These factors deserves further study.

Although *C. virginica* appears to be reliant on *A. erigeniae* visitation in these Pennsylvania populations, we can speculate that under different circumstances, visits by *A. erigeniae* females may be more detrimental than beneficial. On average, *A. erigeniae* female bees remove over one thousand pollen grains in a single visit to a male-phase *C. virginica* flower, and then deposit only 39 of them in a female-phase visit; many of the remaining grains probably provision offspring and are wasted from the plant perspective. *A. erigeniae* females also make approximately two male-phase visits for every female-phase visit. Thus because of *A. erigeniae* pollen collection, the male function of *C. virginica* individuals is reduced. If the wasted grains have the potential to be delivered to *C. virginica* stigmas by a future pollinator, than visits by *A. erigeniae* females could be lowering overall pollen delivery in *C. virginica* populations. In these Pennsylvania populations, there is potential for future pollinators to more efficiently transfer wasted grains because a diversity of other floral visitors are present, visit with high frequency, and have the potential to contribute
substantially to *C. virginica* pollination if their numbers increased. The best candidate for this role is *B. major*, which delivers similar numbers of pollen grains to *C. virginica* female-phase flowers but at a lower cost. With higher population size or visitation rates, or both, *B. major* could intercept pollen from *A. erigeniae* females and deliver more of it to *C. virginica* stigmas; therefore, the addition of visits by *B. major* can only improve pollen delivery.

Moreover, there may be benefits to *C. virginica* pollen transfer by a diverse insect assemblage visiting over time rather than immediate pollen transfer by the dominating *A. erigeniae*. Diversity in those individuals that transfer pollen may mean that the cohort of grains fertilizing ovules may also be more diverse. Pollen diversity may increase the number of seeds produced or increase the fitness of those seeds, or both (e.g. Kron and Husband 2006). A diverse insect assemblage visiting over time may also deliver pollen from a greater distance. The distance that grains travel may also have an effect on fitness, e.g. by counteracting inbreeding depression (Ellstrand 1992, Richards 2000).

Although *A. erigeniae* females are the dominant pollinator in this context, the value of a pollinator to a plant can change because the context of visitation by floral visitors is highly plastic. Insect populations are known to drastically fluctuate, so *A. erigeniae*, *B. major*, and small generalist bee populations may vary stochastically, or with changes in geographic, climatic, and seasonal patterns. For example, Motten et al. (1981) report much higher visitation by *B. major* in North Carolina populations. Moreover, *A. erigeniae* females may be competitively excluding other floral visitors by monopolizing *C. virginica* nectar and pollen, meaning that a decrease in *A. erigeniae* visits could increase visits by other floral visitors; this is especially reasonable considering *C. virginica* is a valuable floral resource in an otherwise sparse floral community in the early spring. These kinds of changes to the pollinator context may reduce the value of *A. erigeniae* females to *C. virginica* because they increase the potential for future pollinators to more efficiently transfer pollen to conspecific stigmas.

At the individual level, the context of visitation by floral visitors can also change through variation in floral traits that increase or decrease visits. Our study was designed to investigate the value of *A. erigeniae* females to populations of *C. virginica*, in that we measured individuals haphazardly within plant populations and used our measurements as a proxy for the population as a whole. However, individuals within these populations may be experiencing selection to increase male fitness through floral traits, by manipulating the visitation rate of particular floral visitors or mitigating the cost of pollinator visitation. Our data show that visits by *A. erigeniae* females are potentially costly but with a high reward to *C. virginica*, while visits by *B. major* are less costly but with a similarly high reward and visits by small generalist bees are less costly but with a relatively low reward. Therefore, if selection is acting on *C. virginica* individuals to increase pollen transfer, selection may act to either increase or decrease visits by *A. erigeniae* females, depending on the relative importance of the costs and benefits of visits by *A. erigeniae* females. However, if selection is acting to increase pollen transfer, selection should always act to increase visits by *B. major* and small generalist bees, because there is consistently little or no cost to these visits. Selection may also
act to mitigate the cost of oligolectic bee visitation by reducing the amount of pollen that oligolectic bees can remove in a single visit by manipulating the pollen presentation schedule (Harder and Thomson 1989). The packaging of pollen into ten units may be a response to selection to mitigate *A. erigeniae* pollen collection; there may be continued selection pressure for further pollen packaging or increasingly delayed anther dehiscence.

2.5. Tables
Table 2.1

Measurements of visitation rate, single-visit removal and deposition, and calculated pollen transfer efficiency for common floral visitors of *C. virginica*.

<table>
<thead>
<tr>
<th>Floral visitor</th>
<th>Visitation rate</th>
<th>Removal sample size</th>
<th>Number of grains remaining ± SD</th>
<th>Mean proportion removed</th>
<th>Deposition sample size</th>
<th>Number of grains deposited ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. erigeniae</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td>1.05 (to female)</td>
<td>50</td>
<td>1078 ±918</td>
<td>61%</td>
<td>53</td>
<td>39.43±52.07</td>
</tr>
<tr>
<td></td>
<td>2.10 (to male)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><em>B. major</em></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.07 (to female)</td>
<td>45</td>
<td>2053±623</td>
<td>23.7%</td>
<td>22</td>
<td>30</td>
<td>30±18.97</td>
</tr>
<tr>
<td>0.03 (to male)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small generalist bee</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.21 (to female)</td>
<td>34</td>
<td>2203±812</td>
<td>20.31%</td>
<td>30</td>
<td>14.97±12.96</td>
<td></td>
</tr>
<tr>
<td>0.45 (to male)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2. Summary of *C. virginica* pollen depletion data.

<table>
<thead>
<tr>
<th>Date</th>
<th>Site</th>
<th>$r^2$</th>
<th>$P$</th>
<th>Line on Fig. 2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 23</td>
<td>Lankanau</td>
<td>0.30</td>
<td>5.38e-05 ***</td>
<td></td>
</tr>
<tr>
<td>April 24</td>
<td>Lankanau</td>
<td>0.36</td>
<td>0.000108 ***</td>
<td></td>
</tr>
<tr>
<td>April 24</td>
<td>Ridley Creek</td>
<td>0.42</td>
<td>2.77e-05 ***</td>
<td></td>
</tr>
<tr>
<td>April 25</td>
<td>Lankanau</td>
<td>0.61</td>
<td>6.14e-12 ***</td>
<td></td>
</tr>
<tr>
<td>April 26</td>
<td>Andorra Park</td>
<td>0.63</td>
<td>3.66e-12 ***</td>
<td></td>
</tr>
<tr>
<td>April 27</td>
<td>Linsey</td>
<td>0.67</td>
<td>3.56e-12 ***</td>
<td></td>
</tr>
<tr>
<td>April 27</td>
<td>Patio Store</td>
<td>0.13</td>
<td>0.0852</td>
<td></td>
</tr>
<tr>
<td>April 28</td>
<td>Andorra Park</td>
<td>0.58</td>
<td>6.15e-11 ***</td>
<td></td>
</tr>
<tr>
<td>April 28</td>
<td>Ridley Creek Bowl</td>
<td>0.30</td>
<td>0.00206 **</td>
<td></td>
</tr>
<tr>
<td>April 29</td>
<td>Andorra Park</td>
<td>0.37</td>
<td>0.00329 **</td>
<td></td>
</tr>
</tbody>
</table>
2.6. Figures

**Figure 2.1.** Visitation rates by *C. virginica* floral visitors in Pennsylvania.

Rates are means ± 95% CI.
Figure 2.2. Plot of pollen grains remaining after a single visit to *C. virginica*.

Small points are individual data points. Large points are means ± 95% CI. Boxes not sharing a letter are significantly different at $P=0.05$. 
Figure 2.3. Plot of pollen grains deposited during a single visit to *C. virginica*.

Small points are individual data points. Large points are means ± 95% CI. Boxes not sharing a letter are significantly different at $P=0.05$. 
Sample Depletion Curve

Pooled Depletion Curves

Number of pollen grains remaining in a flower

Hours since anthesis

Statistical model from empirical data

Pollen depletion model
**Figure 2.4.** Regression of pollen depletion over time.

a) Points are actual measurements; lines are our statistical model fit to each individual day. b) The pink line is hypothetical pollen depletion calculated using the mean number of pollen grains in unvisited flowers (2764 grains), the oligolectic bee visitation rate of 2.1 times per hour and a removal rate of 61%. The grey line is the fit of the statistical model.
3. Abstract

The pollinator climate for a particular plant species is often variable, and this variation may lead to the formation of pollination ecotypes, plant populations that are morphologically and genetically distinct from other plant populations because of adaptation to the local pollinator climate. Linking the pollinator climate to established gradients may uncover patterns in the pollinator climate and subtle pollinator ecotypes, but biologists often lack the resources and time to investigate the pollinator climate across species’ entire ranges. To study the community of pollinators visiting the spring ephemerals *Claytonia virginica* and *Claytonia caroliniana*, we assembled a team of citizen scientists to monitor pollinator visitation to plants throughout the species’ ranges. Citizen scientists documented some interesting differences in pollinator communities; specifically, that western *C. virginica* and *C. caroliniana* populations are visited more often by the pollen specialist bee *Andrena erigeniae* and southern populations are visited more often by the bombyliid fly *Bombylius major*. Differences in pollinator communities throughout the plants’ range may affect the male fitness of individual plants or the reproductive success of plant populations, or both; these differences may affect selection on floral traits and potentially result in pollinator-mediated local adaptation and pollination ecotypes.

3.1. Introduction

Since Darwin, biologists have focused on the significant role of animal pollinators in shaping floral traits and even in angiosperm diversification and speciation (Darwin 1877, van der Niet et al. 2014). A rich history of research has explored the role of a pollinator species in determining the reproductive success of a plant, selecting for plant traits, and in some cases influencing reproductive isolation (van der Niet et al. 2014). However, we know that most plants are visited by a diverse community of pollinators (Waser et al. 1996), and that the diverse community of pollinators can be highly variable geographically (Herrera et al. 2006). Yet the vast majority of studies of plant-pollinator interactions are conducted in one geographic location; Herrera et al. (2006) calculates that 88.4% of plant-pollinator studies look at only one site. These local snapshots of the plant-pollinator interaction story comprise our current understanding of plant-pollinator systems and the role of pollinators in floral adaptation and
diversification. Broadening the geographical extent can only improve our understanding of plant-pollinator interactions. Moreover, knowing how pollinator communities vary across landscapes can help biologists understand local processes of pollinator-mediated plant adaptation.

The diversity and abundance of pollinators visiting a plant population in any given location and time form the “pollinator climate” (Grant and Grant 1965). A number of studies have documented variation in the pollinator climate in different plant populations within the same plant species. Most studies focus on a small number of sites (Miller 1981, Robertson and Wyatt 1990, Arroyo and Dafni 1995, Johnson and Steiner 1997, Price et al. 2005), and many are confined to a relatively small geographic area (Miller 1981, Robertson and Wyatt 1990, Arroyo and Dafni 1995, Johnson and Steiner 1997, Price et al. 2005, Gomez et al. 2008). These studies have contributed to the understanding that pollinator communities are variable. However, in the majority of studies there is no obvious pattern or process that explains the documented variation in pollinator communities, so the conclusions to these studies are limited to the specific populations studied. In order to better understand variation in pollinator communities, biologists should look at the pollinator climate across the entire plant range and attempt to link that variation to larger geographic patterns. Finding large-scale, consistent patterns in pollinator communities – such as latitudinal gradients – will provide testable hypotheses about the importance of the pollinator climate in plant adaptation and diversification.

Linking pollinator communities to established biological or geographical gradients can help expose the mechanisms that may be causing changes in pollinator communities. Large-scale geographical gradients such as altitude and latitude often represent a number of abiotic gradients, including climate and day length. Large-scale geographical gradients also represent a number of biotic variables and patterns in interspecific interactions. The latitudinal diversity gradient, for example, is well established and well studied in ecology (MacArthur 1984, Willig et al. 2003, Hillebrand 2004, Mittelbach et al. 2007); by documenting latitudinal gradients in plant-pollinator interactions, ecologists can expand on this already rich theoretical framework.

Linking pollinator communities to established biological and geographical gradients can also help predict plants’ response to changes in pollinator climate. Many studies have documented dramatic changes to plant traits that correspond with changes in the pollinator climate (Miller 1981, Armbuster 1985, Arroyo and Dafni 1995, Inoue et al. 1996, Boyd 2002, Malo and Baonza 2002, Galen 1996, 1999, Pérez-Barrales et al. 2007, Newman et al. 2014, Van der Niet 2014). These “pollination ecotypes” are a more specific case of plant “ecotypes”, plant populations that are morphologically and genetically distinct from other plant populations in the same species because of local adaptation to environmental conditions; they are plant populations that appear to be adapted to local pollinator climates (Johnson 1997). Many studies document dramatic changes in the floral phenotype, such as changes in tube or spur length (Miller 1981, Robertson and Wyatt 1990, Johnson and Steiner 1997, Boyd 2004). These studies provide convincing evidence that floral traits respond to variation in pollinator communities, and that
there may be pollination ecotypes in other systems with variation in the pollinator climate. These pollination ecotypes may be less obvious; there may be slight variation in floral color and shape, pollen grain characteristics and number, or any other floral trait. By linking changes in the pollinator climate to large-scale geographic gradients, we can begin to look carefully for corresponding gradients in pollination ecotypes.

Looking for and documenting large-scale, consistent patterns in pollinator communities requires a great deal of observational data. Studies are often limited to just one or a few plant populations due to limited resources and time (Herrera et al. 2006). Some limitations are specific to plant-pollinator studies; often the flowering season of a study species will limit the time available for traveling throughout the plant range, and pollinator identification can be very difficult for novice research assistants (Lye et al. 2011, Kremen et al. 2001). Recently, biologists have begun to employ the efforts of amateur naturalists and volunteers to increase the amount of data collected in their research efforts (Dickinson et al. 2010, Silvertown 2009). Citizen scientists can benefit research in many ways, including expanding the amount of data collected, reducing costs, and providing an opportunity for outreach and public education (Dickinson et al. 2010, Lye et al. 2011, Cooper et al. 2007). In ecology and evolution especially, there is great potential for harnessing the enthusiasm of amateur naturalists to support large-scale data collection and monitoring efforts (Dickinson et al. 2010). Citizen scientists are a great resource for pollinator monitoring; often, amateur naturalists have practice in plant and insect identification, are eager to spend time outdoors to contribute to environmental monitoring, and are already located throughout the range of the plant and pollinator species of interest (Kremen et al. 2011). Kremen et al. (2011) compared data sets of pollination observations collected by citizen scientists to data sets collected by experts, and found that although the citizen scientists missed some taxonomic diversity, the qualitative results were the same. Citizen science data can help identify how the pollinator climate varies in space and time, and help document large-scale geographic patterns in a plant species’ pollinator climate (Dickinson et al. 2010).

To better understand patterns in pollinator climates, we employed citizen scientists to do pollinator observations across the range of two plant species, *Claytonia virginica* and *Claytonia caroliniana*. Specifically, we ask: 1) Do pollinator climates vary along large-scale patterns like latitude, longitude, and altitude? 2) By conducting observations over an entire species’ range, can we uncover subtle but consistent variation in pollinator diversity and abundance? Anecdotal observations caused us to predict that the pollinator climate of *C. virginica* and *C. caroliniana* would vary latitudinally, with higher fly visitation in Southern populations. The resulting expansion in research effort through the addition of citizen scientists can lead to testable hypothesis about the role of pollinator variation in the ecology and evolution of plant species, especially the formation of pollination ecotypes through pollinator-mediated selection.
3.2. Methods

*Claytonia virginica* and *Claytonia caroliniana* (Portulacaceae), collectively known as “spring beauty”, are spring ephemeral wildflowers native to North American eastern woodlands, where they are visited by a variety of insects, among them the oligolectic solitary bee *Andrena erigeniae*, which collects pollen exclusively from these two species. Female bees may eat some pollen, but most pollen is used to provision *A. erigeniae* larvae. These two species of *Claytonia* and *A. erigeniae* have overlapping geographic ranges, and are phenologically matched (Davis and LaBerge 1975). A number of generalist species also visit *C. virginica* and *C. caroliniana*, collecting pollen and/or nectar from these plants and other sources (Table 2). The generalist bee species are from many genera and include both pollen-foragers and social parasites that do not amass pollen provisions. The other most frequent visitor, the hover-fly *Bombylius major*, is focused on nectar-collecting and mostly ignores pollen. *Bombylius major* is a parasite of solitary bee species, probably including *Claytonia’s* oligolectic *A. erigeniae*; therefore, the abundance of these insects may be linked. The distribution of these *Claytonia* species ranges from Georgia to Ontario, and from the East Coast west to Kansas and Nebraska. These species are protandrous; pollen and nectar are offered on the first day, in the male phase, while only nectar is produced in the succeeding female phase.

To recruit volunteers, we advertised the project on established email listserv, including Native Plant Societies and Master Gardener lists. The instructions and learning materials for participating in the project were compiled onto a website (Appendix C, www.springbeauties.wordpress.com). Before participating, we asked volunteers to visit the website, familiarize themselves with the project protocol, and study the identification of the bees and flies that they were likely to see in the field. Volunteers were also responsible for locating a patch of *C. virginica* or *C. caroliniana* in their local area; these patches could be in any habitat type.

Volunteers throughout the range of the plants located a patch of *C. virginica* or *C. caroliniana* in their area, recorded general information about the site and patch, and conducted observations a few times throughout the season. We asked volunteers to conduct observations three times throughout the season, but many volunteers were only able to do two, and some conducted many more than three. Observations were six five-minute observation periods, each focusing on a defined number of focal flowers. During observations, volunteers recorded the identity of visiting insects and the number of visits that each insect made to male- and female-phase flowers. To facilitate identification, we organized the floral visitors into groups according to taxon, morphology, and behavior. We provided volunteers with an information sheet with photographs and distinguishing characteristics of these pollinator groups, including size, color, and body shape; we asked that they refer to this sheet during observations (Appendix C). We encouraged volunteers to use these groups but also allowed identifications at any level or descriptions of the visitor (e.g., “unknown”, “unknown bee”, “small black bee with yellow stripes”). After conducting observations, volunteers submitted their data by mailing in their original data sheets, by entering and emailing data on a spreadsheet that we provided, or by entering and emailing data on a fillable PDF of the data sheets.
We encouraged volunteers to conduct observations on three different days throughout the season, and as much as possible on sunny days between 10am and 12:30pm. On each day of observations, volunteers recorded information on the date, site, and plants, including the location, the plant species observed, and the phenology of the plant individuals. Before each set of observation periods, volunteers recorded the temperature and provided a general rating of the amount of wind and cloud cover. Before each observation period, volunteers defined an observation area that included a number of flowers; volunteers chose the number of flowers that they observed during each observation period. Volunteers observed different flowers in each observation period, though in small patches the flowers may have been very close to one another. They defined their area of observation by using a hula hoop or other square or circular perimeter. Using a provided information sheet to help with classification (Appendix C2 and C3), volunteers identified how many of their focal flowers were male-phase and how many were female-phase. Before beginning their observation period, volunteers set a stopwatch for five minutes.

During each five-minute observation period, volunteers observed their focal flowers. When an insect visited, volunteers recorded the identity of that visitor to the best of their ability, and recorded the number of male- and female-phase visits that visitor made. Volunteers counted the number of total visits regardless of whether they were made by the same pollinator individual or different individuals. When identifying insects, we encouraged volunteers to use the functional groups that we provided; however, if the volunteer was not sure of the identification, the volunteer identified the insect as “unknown”.

All statistical analyses were done using generalized linear mixed models (GLMMs) in R 3.0.1 (R Core Team 2013). The function glmmADMB in the R library glmmADMB (Fournier et al. 2012) allowed us to account for highly variable observation times, include random effects, and account for overdispersion in the response variables. We analyzed the following in separate models: 1) the visitation rate of each visitor (i.e., the total number of visits within a visitor group, including re-visits), 2) the total visitation rate (i.e., the total number of visits across all groups), and 3) floral visitor diversity, or the number of visitor groups represented in an observation period.

To analyze (1) the visitation rate of each visitor, the response variable is the number of visits by any individual in a particular floral visitor group in 30 minutes; groups of floral visitors included A. erigeniae, B. major, small dark bees, parasitic bees, other bees, other flies, all bees, and all flies. At the start, we included the following predictor variables in each model: latitude; longitude; the interaction between latitude and longitude; elevation; whether observations were conducted in a designated natural area/park or a residential area; in natural areas, the approximate size of the park where observations were conducted; the plant species observed (C. virginica or C. caroliniana); the temperature; the approximate level of wind (windy, light breeze, or still); the approximate degree of cloud cover (sunny, partly cloudy, or overcast); and the proportion of flowers observed that were male-phase flowers. When one of these factors did not improve the model fit, as indicated by log likelihood ratios, we removed it from the final model. Of these response variables, all final models included only latitude, longitude, and whether
observations were conducted in a designated natural area/park or a residential area, because the
remaining response variables did not improve model fit. To account for the high variation in the number
of flowers observed and number of observation periods conducted, we included the total number of
flowers observed as an offset. Because visits observed by a volunteer on a particular day are not
independent, we included this as a random effect; as such, each day of observations for a particular
volunteer represents the level of replication in each model. To account for overdispersed data, we used a
negative binomial error distribution. Because weather influences pollinator visitation, we also ran each of
the models with significant results on a subset of the data that included only those observations that
occurred when the temperature was above 15°C. These models produced qualitatively similar results to
those presented here.

To analyze (2) the visitation rate of visitors as a whole, the response variable is the number of total visits
in 30 minutes. We followed the same procedures for determining predictor variables as for (1), and
included the same random factor and offset, and again used a negative binomial error distribution.

To analyze (3), floral visitor diversity, we counted the number of floral visitor groups represented during
all observations in one day of observations by a particular volunteer; this was our response variable. We
followed the same procedures for determining predictor variables as for (1), with the exception of the
weather data. Because the response variable already included all visits in one day, we did not need a
random factor for this model. Again, each day of observations for a particular volunteer represents the
level of replication.

3.3. Results

We received usable data from 27 volunteers over three years, who together conducted 655 observation
periods and observed a total of 14,159 flowers (Fig. 3.1).

The number of visits by the pollen specialist bee *A. erigeniae* in 30 minutes varied significantly with
longitude, with more visits in the Western part of the range than the Eastern (Table 3.1, Fig. 3.1c, *Z* =
-2.62, *P*=0.00088). The number of visits by the bombyliid fly *B. major* in 30 minutes varied significantly
with latitude, with more visits in the Southern part of the range than the Northern (Table 3.1, Fig. 3.1b,
*Z* = -3.25, *P*=0.0011). There was no significant effect of latitude on *A. erigeniae* visitation (Table 3.1, Fig.
3.1d, *Z*=0.40, *P*=0.687, and no significant effect of longitude on *B. major* visitation (Table 3.1, Fig. 3.1a,
*Z*= 0.29, *P*=0.772. The type of land use in the local area of the observations (whether the observations
were done in a residential area or in a natural area) had a significant effect on the model fit, with higher
*A. erigeniae* visitation in natural areas than residential areas.

The number of visits by small dark bees, parasitic bees, other bees, other flies, all bees, and all flies did
not vary significantly with latitude or longitude, and for the most part none of the other predictor
variables included in the model had a significant effect on visitation by these insects. The exception is that
there were more visits by other flies when there was a higher proportion of male-phase flowers observed ($Z=2.67$, $P=0.0076$).

The number of total visits by floral visitors also did not vary significantly with latitude or longitude, and none of the predictor variables included in the model had a significant effect on overall visitation. The diversity of visitor groups that visited in one day of observations also did not vary significantly with latitude or longitude, and none of the predictor variables included in the model had a significant effect on the diversity of visitor groups.

### 3.4. Discussion

To the best of our knowledge, this is the first study to use citizen science data to contribute to a foundation of knowledge in the biogeography of pollination systems. The use of citizen science data in this project has both advantages and disadvantages. Because of the data collected by volunteers, we were able to collect more data – and more importantly, with more coverage of *Claytonia*’s range – than we could have collected ourselves during *Claytonia*’s limited flowering period. The collection of these data came at a minimal cost to us and our institutions. However, citizen science data, and these data in particular, have a number of limitations. Volunteers received no formal training, and many had no experience in identifying the pollinators of *C. virginica*. However, the majority of volunteers had prior experience in insect and plant identification. Aspects of this project made it especially conducive to citizen scientist participation; the two pollinators of greatest interest in this study (*A. erigeniae* and *B. major*) are very different morphologically and behaviorally and are very easy to distinguish from one another. *Claytonia* is abundant in many areas across its geographic range, and many participants already had a connection to local *Claytonia* populations from local parks or even their own property. Because of these factors, volunteers were eager to participate and we can be more certain of the accuracy of their identifications.

Data collected by citizen scientists revealed a significant effect of large-scale geographic parameters on the visitation rate of two important pollinators of *C. virginica* and *C. caroliniana*. In other words, the pollinator climate – i.e., the diversity and abundance of *A. erigeniae* and *B. major* specifically – changes fairly predictably along both a latitudinal gradient and a longitudinal one. In lower latitudes, *Claytonia* populations are visited by more bombyliid flies; in western populations, *Claytonia* populations are visited by more *A. erigeniae* bees. We cannot explain the more specific factors that may be impacting these patterns. They may result from variation in the abundance of insect populations. Despite its importance, we do not understand the factors that underlie bee and fly population dynamics very well (Bischoff 2003, Franzen and Nilsson 2013). Interestingly, the bombyliid fly *B. major* is a parasite of solitary bee species, so our results conflict with the expectation that the abundance of *B. major* should vary with the abundance of solitary bees, if these bees are acting as *B. major* hosts. Instead, the patterns in our data may result from changes in relative visitation rates due to variation in floral attractiveness, the composition of plant
communities, or some other factor. In general, our results indicate that large-scale geographic gradients – those patterns that change with latitude and longitude – are likely to be important. Climate, day length, and the range limits of interacting species are some intriguing possibilities that deserve attention in future research.

Our analysis also revealed an association between particular land uses and visitation rate for the pollen specialist *A. erigeniae*. Similarly, Williams and Winfree (2013) found a modest effect of woodland area on *A. erigeniae* visitation of *C. virginica* in suburban Philadelphia. The effects of land development on pollinator populations is complex, with many studies finding positive, neutral, and negative effects using different methods and at different scales (as reviewed by Winfree et al. 2011). However, studies at the local scale that compare pollinator communities in two or more habitat types, like ours, more often show a positive effect of human land use on pollinator populations (Winfree et al. 2011); as such, our results are counter to expectations. Land development is likely to impact pollinator populations by affecting the availability of floral resources and the availability of nesting sites, and it is likely that the residential areas in our study have fewer floral resources and nesting sites than the parks, arboretums, trails, nature centers, and reserves where observations were conducted. However, we did not collect data on floral resources or nesting sites, and we have only limited information on the characteristics of the study sites. This topic deserves more investigation.

Surprisingly, we did not detect large-scale geographic patterns in visitation by the other pollinator groups included in this study, or links between visitation by any pollinator group and characteristics of the local site and weather, which have been shown to affect pollinator visitation (Willmer 1983, Stone et al. 1988, Stanton and Galen 1989, Herrera 1995). Because pollinator visitation is highly variable (Price et al. 2005, Richards et al. 2009), it can be difficult to detect patterns in observational data; perhaps with more volunteers and more years of study, additional patterns would emerge.

The identification of latitudinal and longitudinal gradients of pollinator climates is more useful than documenting differences in the pollinator climate with no underlying pattern. Because we documented gradients in the visitation rates of two significant pollinators of *Claytonia*, we have a better idea of the pollinator climate experienced by plant populations in different parts of the species’ range; western populations are visited more often by the oligolege *A. erigeniae*, while northern populations see *B. major* more often. We can now make predictions about how plant populations may respond these differences. The pollinators *A. erigeniae* and *B. major* are very different; the pollen specialist *A. erigeniae* is an adept and systematic forager that collects a great deal of pollen, while the bombyliid fly *B. major* is a haphazard forager that collects and deposits pollen only passively. While the two pollinators deliver similar numbers of grains, *A. erigeniae* removes substantially more pollen during visitation than *B. major* (Chapter 2 of this thesis). Where visitation by the bombyliid fly *B. major* is higher in the southern latitudes, there may be selection to increase *B. major* visitation. Where visitation by the pollen specialist *A. erigeniae* is higher in the northern latitudes, there may be selection to decrease the amount of pollen that the bee can remove in a
single visit (i.e., through delayed dehiscence of anthers) (Harder and Thomson 1989b). These results help identify traits that vary geographically that otherwise would be overlooked (Chapter 4 of this thesis).
3.5. Tables

Table 3.1. Results of generalized linear mixed models.

*P < 0.05, **P < 0.01, ***P < 0.001.

<table>
<thead>
<tr>
<th>Andrena erigeniae</th>
<th>Estimate</th>
<th>Standard Error</th>
<th>Z value</th>
<th>P value</th>
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<td>0.00088**</td>
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<td>Residential or Natural Area</td>
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<td>0.449</td>
<td>-3.40</td>
<td>0.00066***</td>
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</tbody>
</table>

<table>
<thead>
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<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
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<td>0.0011**</td>
</tr>
<tr>
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<td>0.4415</td>
<td>0.15</td>
<td>0.8842</td>
</tr>
</tbody>
</table>

3.6. Figures
Figure 3.1. Patterns in *A. erigeniae* and *B. major* visitation to *C. virginica* and *C. caroliniana*.

(a) The relationship between longitude and the number of visits by *B. major* per observation period. Curves were fit using locally weighted least squares (LOWESS). (b) The relationship between latitude and the number of visits by *B. major* per observation period. Curves were fit using locally weighted least squares (LOWESS). (c) The relationship between longitude and the number of visits by *A. erigeniae* per observation period. Curves were fit using locally weighted least squares (LOWESS). (d) The relationship between latitude and the number of visits by *B. major* per observation period. Curves were fit using locally weighted least squares (LOWESS). (e) Ratio of pollinator visits at each site of pollinator observations. Ratios are calculated as the number of *A. erigeniae* visits over the number of *A. erigeniae* and *B. major* visits. The color represents the ratio of pollinator visits, with more blue circles representing higher ratios of *A. erigeniae* visits, and more red circles representing lower ratios of *A. erigeniae* visits, which corresponds with higher ratios of *B. major* visits. The size of the circles represents the number of observation periods conducted at each site.
4. Abstract

Variation in the assemblage of pollinators visiting a plant can result in the formation of pollination ecotypes, or plant populations adapted to local pollinator environments. Most examples of pollination ecotypes document changes in floral morphology which correspond to the morphology of a locally-abundant pollinator; however, plant traits may also respond to pollinator behavior, specifically in their interactions with pollen grains. We documented variation in the pollinator assemblage for the spring ephemeral wildflower *Claytonia virginica*: the oligolectic bee *Andrena erigeniae* predominantly visited the Northern populations in Pennsylvania and Maryland, and the bee-fly, *Bombylius major*, visited more heavily in Southern populations in North Carolina. As a result, plants in the bee-heavy Northern populations experience faster pollen depletion than Southern populations. We also measured variation in plant traits; populations in the bee-heavy North had relatively higher pollen production and more delayed pollen presentation than the fly-heavy South. These plant traits may be the result of selection to increase pollen delivery via these pollen vectors. Because bees groom pollen grains and actively collect pollen to feed offspring, high pollen production and delayed pollen presentation may increase pollen delivery by bees, while fast presentation of pollen may increase pollen delivery by flies. These results are evidence that pollinator interactions with pollen grains are an especially important part of plant-pollinator interactions, and that pollination ecotypes may be more common than previously thought.

4.1. Introduction

It is widely agreed that floral traits are, to a certain extent, the result of selection by pollinators; plants can entice helpful animals to visit through color and scent, or improve the accuracy of pollen placement by adjusting the position or size of morphological features (as reviewed by Kay and Sargent 2009, Fenster et al. 2004). A rich library of examples supports the idea that pollinators mediate selection on floral traits such that plants morphologically match their pollination partner (e.g. Muchhala 2007, Aigner 2004, Cresswell 2000). The textbook example describes the coevolution of one plant and one pollinator taxa (e.g. Campbell and Reece 2002). However, modern workers recognize that biotically-pollinated flowers
are much more commonly visited by many different animals with diverse morphologies and behaviors (Waser et al. 1996), and that the diversity and abundance of these animals, and even their effectiveness as pollinators, can be highly variable in space and time (Herrera et al. 2006, Richards et al. 2009).

The recognition of pollinator assemblages and their highly variable nature has led to research on how pollinator diversity and pollinator variation influence selection on floral traits. Rather than requiring a specialized plant-pollination relationship, plant traits are often the result of selection by multiple pollinator species collectively. The diversity and abundance of floral visitors of a plant species in a given time and location—an assemblage of animals that contribute in some way to pollen transfer—make up the “pollinator climate” (Grant and Grant 1965). “Pollination ecotypes”, a specific type of plant ecotype, identify plant populations that are morphologically distinct from other conspecific populations due to local adaptation to pollinators (Johnson 1997). Plant ecotypes result from persistent differences in the abiotic or biotic environment that could select for phenotypic change; pollination ecotypes are the result of consistent differences in the pollinator climate (Johnson 1997). A few studies identify a highly variable pollinator assemblage, in which consistent differences in the abundance of two or more pollinators correlate with dramatic changes in the floral phenotype; although these studies are mainly observational, they provide evidence that selection by geographically-variable pollinator climate can result in pollination ecotypes (Miller 1981, Robertson and Wyatt 1990, Johnson and Steiner 1997, Boyd 2004).

Pollination ecotypes are demonstrated by variation in a floral trait which increases plant fitness via pollinators. Floral traits can increase pollinator attraction and visitation through floral features and rewards, such as size, color, scent, and nectar guides (Bell 1985, Stanton et al. 1986, Dobson 1987, Galen et al. 1987). Floral traits can also enhance the efficiency of pollen transfer by decreasing the amount of pollen lost during transport (wasted pollen, from the plant perspective) and increasing the number of grains that reach conspecific stigmas. Changes in the depth of flowers (the length of the corolla, floral tube, or spur) can improve pollen transfer; when floral length matches the visiting pollinator’s proboscis length, pollen is transferred precisely to a location with more surface area on a particular pollinator’s body, such as the animal’s face (Darwin 1877, Nilsson 1988, Johnson 1997). This precise pollen placement can increase the number of pollen grains deposited by pollinators and increase fruit set (Johnson and Steiner 1997). Most studies of pollination ecotypes identify variation in the depth of flowers that correlates with the abundance of a matching pollinator. Selection on plants to increase siring success can also vary other floral traits that increase pollen transfer efficiency; variation in the pollinator climate may affect investment in pollen production or the schedule of pollen presentation.

Aspects of a pollinator’s biology affect how a pollinator interacts with pollen grains, and therefore will affect that animal’s role as a pollinator. Primarily nectar-collecting animals, such as flies, butterflies, and hummingbirds, interact minimally with pollen grains after the pollen grains are removed. In contrast, female bees (usually) actively collect pollen in addition to foraging for nectar, and interact with pollen a great deal. Female bees often carefully and thoroughly groom pollen from their bodies and sequester it in
pollen transport structures ("corbiculae" or "scopae"). This sequestered pollen is primarily provisioned for young bees; pollen is the primary protein resource of developing larvae, and there is evidence that bee reproduction is limited by pollen (Strickler 1982, Müller et al. 2006, Ricketts et al. 2008, Roulston and Goodell 2011). As a result, adult female bees are motivated to deliver pollen back to the nest, rather than from plant to plant. They are successful in this effort; in one study, bumble bees delivered only 0.6% of removed grains to conspecific stigmas (Harder and Thomson 1989b), and much of the rest is groomed during flight to the bee's corbicula or scopa and fated for bee nests. This pollen is "lost from the system" and will not contribute to plant pollination (Thomson 1986, de Jong and Klinkhamer 1994).

Interactions with pollen grains affect an animal's role as a pollinator. Plant investment in pollen production can have diminishing returns for plant male fitness, e.g. when increased investment in male fitness (via pollen production) decelerates gains to male fitness (pollen delivery) (Harder and Thomson 1989, Feinsinger and III 1991, Galen 1992, Richards et al. 2009). All pollinators can cause diminishing returns of pollen production because of space limitations on a pollinator body (Lloyd 1984) or pollen competition (Lloyd 1984, Charnov 1982). However, these effects likely vary between pollinator groups. Female bees likely diminish returns on plant male fitness more so than passive pollen-collecting pollinators because large amounts of pollen trigger bee grooming (Harder and Thomson 1989, Castellanos et al. 2006, Richards et al. 2009). While these variables have not been examined in solitary bee species, Harder and Thomson (1989) demonstrated diminishing returns in pollen production for *Erythronium grandiflorum* visited by *Bombus occidentalis*; they showed that higher pollen availability in plant anthers increased pollen removal, increased grooming, and ultimately decreased the proportion of pollen that bumble bees delivered to stigmas.

Plants can manipulate the number of grains available to pollinators through: 1) investment in male function (specifically, resources allocated to pollen production), 2) manipulation of the schedule of pollen presentation (i.e., the timing of pollen availability) (Thomson and Barrett 1981, Lloyd and Yates 1982, Harder and Thomson 1989), or both. Plants can shift resource allocation towards male function (e.g., the number of pollen grains produced) at the expense of female function (e.g., the number of ovules produced) (Lloyd 1984) or other plant processes (LeBuhn and Holsinger 1998). Plants can also manipulate the schedule of pollen presentation through different pollen packaging and dispensing mechanisms. Packaging mechanisms divide a plant's pollen into units that are gradually made available through dispensing mechanisms (Lloyd and Yates 1982). Changes in the pollen presentation schedule can affect plant siring success without affecting resource allocation in other plant functions, and can allow plants to exert control over the number of visitors that remove pollen from a flower. For example, a flower with five anthers that dehisce at the same time would allow potentially only the first visitor to remove pollen grains, if that visitor removes all of the available pollen. However, a flower that dehisces those five anthers one at a time would provide at least five visitors with grains to export and deliver. If the five visitors combined exported and delivered more grains than the single visitor alone, then pollen
packaging increases pollen export and delivery. Consequently, both pollen production and the pollen presentation schedule affect the number of pollen grains available to pollinators.

Variation in the pollinator climate may exert different selection pressures on plants. Plants with either rare visitation overall or frequent visitation by passive pollen-collectors are expected to be selected for high pollen production because pollen delivery should increase proportionally with pollen production. However, selection may strongly favor increased pollen production for plants frequently visited by bees, because much of the pollen produced will be wasted. For these plants, higher pollen production may increase the probability that pollen grains will contribute to male fitness. Alternatively, plants visited by many bees may experience more relaxed selection for pollen production, if higher investment results in decelerating gains. Selection on the schedule of pollen presentation may also vary with the pollinator climate. Plants that are visited only rarely, or visited often by passive pollen-collectors, should experience selection to increase the amount of pollen available at any one time through fewer pollen packages. However, plants visited by many bees may experience selection for an increase in pollen packaging or more delayed pollen dispensing. Models show that when pollinators cause diminishing returns to male fitness, selection pressure to package and dispense pollen is high (LeBuhn and Holsinger 1998). In a comparative study of pollen presentation schedules in Penstemon species, Castellanos et al. (2006) found bee-pollinated plant species restricted pollen presentation more than hummingbird-pollinated plants; the authors linked this pattern to differences in the grooming behavior of the most abundant pollinator.

The number of pollen grains produced and the schedule of pollen presentation may affect pollen delivery. Just as the depth of flowers varies according to the proboscis length of the pollinator assemblage, variation in the pollinator climate may result in pollination ecotypes that differ in pollen production and the pollen presentation schedule. Here, we present the results of a preliminary investigation of geographic variation in floral traits that may be the result of differential selection by an active pollen collector (an oligolectic bee) and a primarily nectar-collecting fly. We hypothesize plant populations will exhibit geographic variation in pollen production. Relatively higher pollen production in populations with high visitation by the oligolectic bee may indicate that increases in pollen production increase pollen delivery. Alternatively, relatively lower pollen production in these populations may indicate relaxed selection on pollen production due to diminishing returns caused by bee pollination. We also hypothesize plant populations with high visitation by the fly will have a pollination ecotype with fewer pollen packages or faster pollen dispensing, while plant populations with high visitation by the oligolectic bee will have a pollination ecotype with relatively fewer pollen packages or slower pollen dispensing.

4.2. Methods

4.2.1. Study species

Claytonia virginica L. (Portulacaceae) is a self-compatible, perennial wildflower common in the woodland understory of the eastern United States. C. virginica flowers in the early spring and continues flowering for
2-4 weeks, with the flowers on a raceme opening one or two at a time during the flowering period. Flowers are protandrous. Anthers dehisce to offer pollen on the first day, and the stigma opens and becomes receptive on the second day. Flowers produce nectar in both the male- and female-phase. Flowers are regular, open, and bowl-shaped, contain five anthers with two locules per anther, and produce six ovules. The corolla is usually white or light pink, with pink or red nectar guides. Flowers open early or mid-morning and close in the early or late afternoon, depending on temperature and humidity (A. Parker and N.M. Williams, unpublished data). Flowers close during inclement weather.

A variety of insect species visit C. virginica flowers. Predominant among them is the oligolectic bee Andrena erigeniae, which collects pollen primarily from C. virginica and the closely-related C. caroliniana (Davis and LaBerge 1975). Andrena erigeniae females collect both nectar and pollen from C. virginica, but the males of the species collect only nectar. A second common visitor is the generalist bee fly Bombylius major, which hovers over flowers sipping nectar with its long proboscis. A diversity of other floral visitors also visit less frequently, including many other (primarily solitary) bees, a few flies, and the occasional butterfly (Schemske 1977, Motten et al. 1981).

4.2.2. Study sites

We conducted this study at sites in Pennsylvania (5 sites), Maryland (1 site), and North Carolina (3 sites) in 2009 and 2010. In 2009, we worked in Pennsylvania at sites in suburban Philadelphia within 50 km of each other, including the grounds of Lankanau General Hospital, Andorra Woods in Fairmount Park, Ridley Creek State Park, and two private woodlots. We collected these data between April 10 and April 29, 2009. In 2010, we worked in Maryland and North Carolina, primarily at sites in Patuxent National Wildlife Refuge in Laurel, Maryland and the Mason Farm Biological Reserve in Chapel Hill, NC. We also collected data at two private woodlots in North Carolina within 15 km from the Mason Farm Biological Reserve. We collected North Carolina data between March 25 and April 12, 2010 and Maryland data between April 14 and April 23, 2010. The majority of fieldwork was done in closed-canopy deciduous forested areas in large parks and reserves (Andorra Woods in Fairmount Park, Ridley Creek State Park, Patuxent NWR, and Mason Farm Biological Reserve, of 37.23 km², 10.55 km², 51.97 km², and 3.64 km² respectively) but some sites in Pennsylvania and North Carolina were in small woodlots on private property located close to residential and commercial areas.

We collected data only in good weather, i.e., on clear or lightly overcast days with a temperature of at least 15°C. We recorded temperature and wind speed every five minutes using a Kestrel 4500 weather meter (Loftopia LLC, Birmingham, Michigan).

4.2.3. Visitation

To compare the visitation rates of flower visitors to C. virginica, we observed flowers at each site at various times throughout the day (mid-morning until early or late afternoon). We conducted six sets of 5-
minute observations in each set of observations. Prior to each 5-minute observation period, we selected a new set of flowers that we could observe simultaneously and recorded the number of male- and female-phase flowers. We observed these flowers for 5 minutes and recorded the identity of all visitors to visitor group.

4.2.4. Pollen depletion

To compare the rates of pollen depletion among *C. virginica* populations, we collected data on the amount of pollen available in individual flowers (an estimate of the pollen standing crop) during the period of insect activity, from mid-morning until early or late afternoon. Each hour, we haphazardly chose eight flowers from throughout the local plant population and collected the anthers from each flower into a microcentrifuge tube containing 1 mL of 70% ethanol. We chose flowers regardless of the number of dehisced anthers. Each morning, we began anther collection when anthers first began to dehisce; this usually occurred between 8:45 and 10:00, but occasionally anther dehiscence began as late as 13:15 because of cool or wet weather. We concluded anther collection when insect visitation ceased or flowers began to close, usually between 13:00 and 15:00.

We counted the number of pollen grains available in each of the eight flowers collected each hour using a Coulter Multisizer 3 particle counter (As in Chapter 2, Beckman Coulter Inc., Brea, California). Prior to counting, we prepared samples by adding 20 mL 0.9% saline. To obtain a representative count of the number of pollen grains in each flower, we took the average of four 1mL subsamples and multiplied the mean by the total volume of the sample. These counts include the number of pollen grains in undehisced anthers because undehisced anthers opened and emptied during the counting process.

4.2.5. Pollen production

During each day of data collection, we covered a sample of male-phase flowers to prevent insect visitation; these flowers provided an estimate of pollen production. Flowers were covered with cages made from milk jugs with windows screened with mesh small enough to prevent insect entry but allow for air exchange. After insect visitation ended, we collected 5-15 samples of male-phase unvisited flowers to estimate the total number of pollen grains available to visiting insects in each flower. Because we collected these flowers at the end of each day, these counts do not include pollen that may have passively fallen from anthers while the flower was open.

4.2.6. Pollen presentation

To estimate the pollen presentation schedule in each population, we tracked anther dehiscence in ten male-phase flowers on each day of data collection. Prior to first anther dehiscence, we haphazardly chose ten fresh male-phase flowers and marked them with dental floss and a label. Every half hour, we recorded the temperature and then returned to these flowers and recorded the number of locules.
dehisced (two locules per anther for a total of ten locules). We concluded tracking each flower when all ten locules had dehisced or, if the ten locules did not dehisce completely, when insect activity ceased.

4.2.7. Data analysis

Analyses include data from six populations in Pennsylvania, one population in Maryland, and three in North Carolina; however, the majority of data come from Lankanau Hospital in PA, Patuxent National Wildlife Refuge in MD, and Mason Farm Biological Reserve in NC. Because of the unequal sampling effort and the relative proximity of the sites, we converted geographic location into a factor with two levels, “North” and “South”. The explanatory variable of interest in each of the models is this proxy (Fig. 3.1). We performed all statistical analyses with generalized linear mixed models in R 3.0.1 (R Core Team 2013).

4.2.7.1. Pollen depletion

To determine if the geographic location was a significant predictor of the rate of pollen depletion, we used a negative binomial model (function glm.nb in the library MASS). The response variable was the number of pollen grains in a flower. The predictor variables were time since anther dehiscence, geographic location, and their interaction. We used log likelihood ratios to determine if the interaction term improved model fit.

4.2.7.2. Pollen production

We compared the numbers of grains in an unvisited flower between Northern and Southern populations using a generalized linear mixed model. The model included the number of grains in an unvisited flower as the response variable and the geographic location as an explanatory variable. Because pollen production might vary through the flowering period, we included the date of collection as a random effect. In order to include both random and fixed effects and account for overdispersion through a negative binomial distribution, we used the R function glmmADMB in the library glmmADMB (Fournier et al. 2012).

4.2.7.3. Pollen presentation

We constructed a mixed effects logistic regression to describe the relationship between the number of locules dehisced on an individual flower over time using the glmer function in the lme4 library (Bates et al. 2013). We included the time since the first anther dehisced on that flower, the temperature at the time of data collection, and the geographic location as fixed effects and the individual flower as a random effect.
4.3. Results

4.3.1. Visitation

There was higher visitation by the specialist bee *A. erigeniae* to *C. virginica* male-phase flowers in Northern populations (PA and MD) than in Southern populations (NC) (Fig. 4.1). Northern *C. virginica* male-phase flowers received 3.61±6.52 (mean±SD) visits per hour by *A. erigeniae* females and 0.06±0.42 visits by *B. major*, but Southern male-phase flowers received 0.41±1.46 visits per hour by *A. erigeniae* females and 0.94±3.37 visits per hour by *B. major* (Fig. 4.1).

4.3.2. Pollen depletion

The slope of the pollen depletion line was steeper in bee-heavy Northern populations than in fly-heavy Southern populations; flowers in the North were depleted by 626 grains per hour and flowers in the South were depleted by 373 grains per hour. Pollen depletion depended on the rate of dehiscence which differed by geographic location (significant geographic location*time since dehiscence interaction; \(P<0.00001\)). We observed no autocorrelation structure in the average of the residuals over time in each time series (North and South).

4.3.3. Pollen production

An unvisited flower in the bee-heavy North had 2941±916 (mean±SD) pollen grains, which is significantly higher than the number of pollen grains in an unvisited flower in the fly-heavy South (2330±861, Fig. 4.3, GLMM, \(Z=-2.31, P=0.021\)).

4.3.4. Pollen presentation

Anthers dehisced more slowly in bee-heavy Northern populations than fly-heavy Southern populations. In Northern populations, anthers dehisced at a rate of approximately 2.35 locules per hour, while in Southern populations, anthers dehisced at a rate of approximately 3.79 locules per hour. In the model describing *C. virginica* anther dehiscence, there was a significant effect of the time since the first anther dehisced (Fig. 4.4, GLMM, \(Z=9.072, P<0.0001\)), the geographic location (North or South) (Fig. 4.4, \(Z=2.754, GLMM, P=0.0059\)), and the temperature (Fig. 4.4, GLMM, \(Z=7.123, P<0.0001\)) on the proportion of locules dehisced. There was also a significant interaction between the time since anther dehiscence and the temperature (Fig. 4.4, GLMM, \(Z=-4.873, P<0.0001\)), the temperature and the geographic location (Fig. 4.4, GLMM, \(Z=-3.603, P=0.0003\)) and the time since anther dehiscence and the geographic location (North or South) (Fig. 4.4, GLMM, \(Z=7.804, P<0.0001\)). We observed no autocorrelation structure in the average of the residuals over time in each time series (North and South).
4.4. Discussion

The two main pollinators in our study differ greatly in their interactions with pollen. *A. erigeniae* females are active pollen collectors and proficient groomers; as for bumble bees visiting *Erythronium* (Harder and Thomson 1989), we expect that increased pollen availability in a plant would cause *A. erigeniae* females to collect more pollen, groom more, and deliver a lower proportion to receptive stigmas. As a result, pollen delivery by *A. erigeniae* may be higher when pollen is divided into more packages or packages that dehisce more slowly (Harder and Thomson 1989), but increases in pollen production are unlikely to increase pollen delivery proportionally. The bombyliid fly *B. major* is primarily occupied by collecting nectar, and transports pollen only passively. Therefore, we expect increased pollen availability in a plant would cause *B. major* to accrue more pollen and deliver more to subsequent stigmas. Pollen delivery by *B. major* may be higher when pollen is divided into fewer packages or packages that dehisce more quickly.

The populations we sampled differed in the visitation rates of the two major pollinators, *A. erigeniae* and *B. major*, forming very different pollinator climates. These climates fell into two distinct groups: a group of sites in Pennsylvania and Maryland had high visitation by the oligolectic bee *A. erigeniae* and low visitation by *B. major*, while Southern populations in North Carolina had low visitation by the oligolectic *A. erigeniae* and high visitation by *B. major*, as well as lower visitation overall. There is evidence that this difference in the pollinator climates is part of a consistent latitudinal trend in pollinator abundance. Motten et al. (1981) documented high visitation of *B. major* to *C. virginica* in North Carolina 28 years before our data were collected; he estimated that *B. major* visited each *C. virginica* flower 2.4 times in four hours, a rate that is similar to our rate of .84 visits per hour. Moreover, Chapter 3 of this thesis documents a latitudinal gradient in *B. major* visitation of *C. virginica*, with higher visitation in Southern populations.

As we expected, we documented a difference in the rate of pollen depletion in these sites, with populations in the bee-heavy North depleting pollen faster than populations in the fly-heavy South (Fig. 4.2). It follows that rates of pollen depletion are linked to pollen collection by *A. erigeniae*.

Counts of the number of pollen grains in unvisited male-phase flowers suggest higher pollen production in the bee-heavy North (Fig. 4.3). This indicates that selection pressure exerted by *A. erigeniae* may favor increased pollen production to mitigate the loss of pollen to bee nests, i.e. increased investment to pollen production could result in at least minimal increases in pollen delivery, even if those increases are subject to diminishing returns. Alternatively, diminishing returns on pollen production for bee-visited plants may not be as pronounced in this system because of the way that these bees interact with pollen grains. Harder and Thomson (1989) found diminishing returns in pollen production for *Erythronium grandiflorum* visited by *Bombus occidentalis*, a corbiculate bee. However, these results may not apply to *A. erigeniae*, a non-corbiculate bee; diminishing returns may be more pronounced with corbiculate bees, because pollen grains groomed by this group of bees are highly manipulated, packed tightly into a pellet, and
functionally compromised, while pollen grains groomed and packed loosely by non-corbiculate bees (like *A. erigeniae*) retain function (Chapter 5 of this thesis). Alternatively, decreased investment in pollen production in the fly-heavy South may allow for increased investment in floral attraction or nectar production, which could increase *B. major* visitation and provide more benefit to pollen transfer than additional pollen grains.

In these populations, pollen packaging does not vary. All flowers (with only a few exceptions) had ten packages of five anthers with two locules each. However, the schedule of package dehiscence varies (Fig. 4.4); there is a significant difference in the pollen presentation schedule between the bee-heavy North and the fly-heavy South. In the North, *C. virginica* populations appear to dehisce anthers more slowly, parsing out the presentation of grains to pollinators; locules dehisce approximately once every 26 minutes. Here, male-phase flowers are visited by *A. erigeniae* pollinators approximately once every sixteen minutes, so anthers are probably stripped of pollen before the next dehisces. In Southern populations, *C. virginica* populations appear to dehisce anthers more quickly, increasing the rate of pollen presentation; locules dehisce approximately once every sixteen minutes. Male-phase flowers are visited by *A. erigeniae* only once every 2.45 hours and *B. major* approximately every hour, so anthers probably accumulate pollen from more than one anther before the next pollinator visit. These data matched our expectations; higher pressure by pollen collectors in the North may select for more delayed anther dehiscence to increase the proportion of those grains that are delivered to stigmas rather than bee nests (Harder and Thomson 1989). In the fly-heavy South, the increased presence of the bombyliid fly *B. major* may select for faster anther dehiscence, because more pollen available to these pollinators is likely to increase pollen delivery without the complication of active pollen grooming.

These data on pollen presentation schedules correspond with theory, for the most part. Harder and Thomson (1989) showed that pollen packaging can be adaptive for pollen dispersal when pollinators cause diminishing returns in pollen production, as they likely do in the bee-heavy North. Likewise, LeBuhn and Holsinger (1998) measured strong selection pressure for pollen packaging when pollinators exhibited diminishing returns and the number of visits was high. However, they also concluded that selection on the schedule of pollen dispensing would be weak, which is not supported by our data. Although Harder and Thomson (1989) investigated a situation in which all pollinators groomed, causing consistently diminishing returns of pollen production to plant fitness, Chapter 6 of this thesis compares simulation models with simultaneous and sequential anther dehiscence in a pollinator climate with high visitation by both an active pollen collector and a passive pollen collector. In these models, pollen delivery was higher when anthers dehisced simultaneously rather than sequentially (i.e., without and with pollen packaging) because sequential dehiscence lowered the amount of pollen delivered by the passive pollen collector. This study supports the idea that selection is acting to increase *B. major* pollen transfer in Southern populations through faster anther dehiscence.
4.5. Conclusions

The two pollinators in our study are very different biologically, and are likely to exert very different selection pressures on plants. In more Northern populations, we documented high visitation by the oligolege *A. erigeniae* and low visitation by the fly *B. major*, and the converse pollinator climate in Southern populations. We hypothesized that populations of *C. virginica* in the bee-heavy North should maximize pollen delivery by dehiscing anthers slowly enough that *A. erigeniae* visitors are presented with a smaller amount of pollen in each visit. We also reasoned that Southern populations of *C. virginica* should dehisce anthers more quickly, so that *B. major* visitors are presented with more pollen in each visit. Our data on pollen presentation schedules match these predictions. We also documented higher counts of pollen in unvisited male-phase flowers in the bee-heavy North, where the extra grains are more likely to be wasted.

This study is an initial investigation into a new kind of pollination ecotype, in which pollen production and pollen presentation schedules vary with geographic variation in the pollinator climate. Each of the plant traits measured is likely to also be affected by factors not measured here, such as plant or flower size, so the results of this study are preliminary. However, this study is the first to consider pollination ecotypes of pollen production and pollen presentation, and these results present testable hypotheses and new ideas for future work. In the future, experimental work manipulating pollinator visitation and common garden experiments would help establish a link between the pollinator climate and pollination ecotypes of pollen production or pollen presentation schedules.
4.6. Figures

**Figure 4.1.** Visitation rate of the specialist bee *A. erigeniae* and the fly *B. major* to male-phase flowers.

Each circle represents one observation period; the circles are offset around the observation site so that they may be viewed independently. The size of the circles represents the visitation rate of *A. erigeniae* (blue) or *B. major* (gray) in one observation period.
Figure 4.2. Pollen depletion curves in Northern and Southern *C. virginica* populations.

Each point represents the number of pollen grains remaining in one sample male-phase flower. Points represent real data, and the curves display fitted lines for each geographic location from the final negative binomial generalized linear model. Populations in the bee-heavy North had steeper depletion curves than populations in the fly-heavy South. There was a highly significant interaction between the hour after first dehiscence and the geographic location (*P* < 0.0001).
Figure 4.3. Pollen production in Northern and Southern C. virginica populations.

Points are means of the number of pollen grains in unvisited Northern and Southern C. virginica flowers. Error bars on the x-axis represent 95% CI of the A. erigeniae visitation rate to male-phase flowers. Error bars on the y-axis represent 95% CI of pollen production.
**Figure 4.4.** Rate of anther dehiscence in Northern and Southern *C. virginica* populations.

Each point represents the proportion of locules dehisced out of ten locules. Points represent real data and curves display a logistic regression fit from the data; dark curves (gray and blue) are anther dehiscence by population.
Grooming reduces pollen quality on corbiculate but not non-corbiculate bees

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5. Abstract

Female bees gather scattered pollen grains from their bodies by different modes of grooming. Species in the corbiculate Apidae, the “corbiculate” bees (such as honey and bumble bees), compress grains into dense pellets borne on the hind tibiae. Other species not in the corbiculate Apidae (“non-corbiculate” bees) sweep grains into local concentrations of hairs (scopae), typically around the legs (in Halictidae and Andrenidae) or the ventral abdomen (Megachilidae), in which grains remain loose. Do these modes of grooming affect pollen function? We transferred grains from the bodies of four groups of bees—the corbiculate bees *Bombus impatiens* and *Apis mellifera*, and the non-corbiculate bees *Megachile rotundata* and *Halictus* spp.—onto previously unvisited stigmas of *Brassica rapa*. We wiped groomed pollen from the corbula or scopa and ungroomed pollen from the rest of each bee’s body separately and measured the resulting seed set. Groomed pollen from corbiculate bees set significantly fewer seeds than ungroomed pollen, but there was no difference in seed set between groomed and ungroomed pollen from non-corbiculate bees. Therefore, loose scopal pollen is fully functional, but corbicular pollen is impaired. In some situations, non-corbiculate bees may be more valuable pollinators than corbiculate species because their treatment of pollen leaves its capabilities intact.

5.1. Introduction

Bees are a diverse group of flower-feeders that pollinate numerous crop and native plants. After acquiring grains at flowers, bees typically move those grains through grooming behavior, gathering loose pollen from their bodies and concentrating it into pollen transport structures. Because bees handle and manipulate pollen grains during grooming, this behavior may affect the quality of pollen grains delivered during pollination (Thorp 1979, Michener 2000). To better understand the role of bees as pollinators, it is important to understand how bees interact with pollen during grooming, and how interactions between bees and pollen grains may ultimately affect plant reproductive success.
Bees are a monophyletic group comprised of seven recognized families, including the diverse and economically valuable family Apidae (Michener 2000). One categorization of bees is based on structural adaptations on bee bodies for transporting pollen grains. “Corbiculate” bees are so named for their pollen transport structure, the corbicula or “pollen basket”, which is a concave plate with long, curved hairs on the hind tibia that securely contains pollen in a nectar-moistened pellet (Michener 1979). The corbiculate Apidae compose part of the current family Apidae. Non-corbiculate bees – the vast majority of bee species – transport pollen using a scopa, or “brush”, a dense group of elongated hairs (Thorp 1979). These non-corbiculate bees make up the remaining six families of bees and the remaining genera in the family Apidae. Within the non-corbiculate bees, the location of the scopa varies. Here, I refer to bees within corbiculate Apidae as “corbiculate” bees, as they are widely known (Michener 1998). I refer to other bees not in the corbiculate Apidae as “non-corbiculate” bees. Some “non-corbiculate” bees may in fact have a small corbiculae, but the primary mode of pollen transportation is scopal hairs (Michener 1998).

Corbiculate and non-corbiculate bees likely differ in their grooming behavior as a result of these well-defined differences in pollen storage structure. Non-corbiculate bees are generally “messier” and hold pollen more loosely (Thomson and Plowright 1980), and corbiculate bees (and some other bees) moisten pollen with nectar during grooming while most non-corbiculate bees transport pollen dry (Thorp 1979). Although much of the available information is anecdotal and focused on one or a few bee groups, Thorp (1979) generalizes that corbiculate bees perform more systematic and thorough grooming behaviors and manipulate pollen more than non-corbiculate bees; this difference in grooming may affect the pollen’s ability to pollinate (Kendall 1973).

Pollen grains vary in their ability to pollinate, i.e. to adhere to floral stigmas, fertilize plant ovules, and cause seed set. Specifically, pollen varies in its viability, or the capacity of the pollen grain to fertilize plant ovules when conditions are ideal. Pollen also varies in its propensity to adhere to stigmas during pollination; for example, pollination may be facilitated by electrostatic forces attracting the pollen to the stigma (Vaknin et al. 2000) or it may be deterred by nectar adhering the grains to the bee body and to one another, forming clumps (Thorp 1979). Both of these factors will ultimately affect whether or not pollen grains have the ability to pollinate. Researchers measure components of pollination in two ways: through in-vitro methods, which measure pollen germination in a medium such as water, agar, or gelatin (Stanley and Linskens 1974), or through in-vivo methods, which look at the siring ability of pollen by measuring pollen tube growth or seed set.

Passive contact with bee bodies or active grooming may affect the ability of pollen to pollinate. Ants produce cuticular antibiotic secretions that disable pollen through passive contact with ant bodies (Beattie et al. 1984, Dutton and Frederickson 2012). Reductions in viability through passive contact have also been reported in the moth Helicoverpa armigera (Richards et al. 2005), and flies in the families Tachinidae and Bibionidae (Rader et al. 2011). Bees also produce antibiotic secretions (Stow et al. 2007, 2010), but
bees do not affect pollen in the same way that ants do, either because they do not secrete the same antibiotics, or perhaps because the bee hairs separate pollen grains from bee cuticles (Harriss and Beattie 1991, Dutton and Frederickson 2012). However, other studies document conflicting effects of bee contact with pollen: one found pollen on *A. mellifera* bodies to be inviable after 12 hours (Kraai 1962) and another found that pollen from bee bodies exhibited decreased viability in one year and no difference in viability in another (Kendall 1973). However, Kraai (1962) did not control for decreasing pollen viability over time, and both of these studies did not distinguish between groomed and ungroomed grains. Therefore, the reduced viability could be a result of groomed grains rather than passive contact.

Active grooming by bees might affect pollen function more than passive contact. While grooming, honey bees mix collected pollen with nectar and secretions from the mouth before packing pollen into the corbicula (Seddick et al. 2012). Honey bees also secrete antibiotic compounds in their cuticles or in their venom that may influence pollen viability (Stow et al. 2007, 2010, Hoggard et al. 2011). Keularts and Linskens (1968, as cited in Stanley and Linskens 1974) showed that an acid toxic to pollen was present in the mandibular glands of *A. mellifera* and *Bombus hyponorum*. Although they also showed that this acid was inactivated by *Petunia* stigmas upon pollen deposition, this acid might affect other pollen differently or be an indication of the presence of other chemicals in bee mouth and mandible secretions. Inside colonies, honey bees intensively manipulate pollen for storage using enzymes, acids, and other bacteriocidal and phytocidal substances (Stanley and Linskens 1974, Winston 1991, Stanciu et al. 2009). Although this processing is probably occurring mostly within the hive, the process may be initiated during grooming. Additionally, groomed pollen may pollinate less often than ungroomed grains due to manipulations by the bee such as the addition of nectar; added moisture clumps pollen, making it less likely to flake off during future flower visits.

Groomed pollen from *A. mellifera* is less viable both *in-vitro* (Mesquida and Renard 1989) and *in-vivo* (Alspach et al. 1992). However, both of these studies focus on the corbiculate bee *A. mellifera*; there is no evidence that the same is true for non-corbiculate species. Thomson et al. (2000) and Thorp (2000) state that corbicular pollen is not available for pollination, but that scopal pollen may be; however, the pollination ability of these types of pollen has not been empirically tested. Apple pollen from insect bodies was as viable as pollen from unvisited flowers, in 12 out of 14 species of bees and flies (Kendall 1973). Body-borne pollen declined in viability only on males of the bee *Andrena wilkella* and the syrphid fly *Rhingia campestris*. However, Kendall did not distinguish between ungroomed grains on the bee body and groomed grains in the corbicula or scopa, leaving the role of grooming unsettled.

To better understand the effect of bee grooming on pollen function, we conducted siring tests *in-vivo* on groomed and ungroomed pollen separately on both corbiculate and non-corbiculate bee species. We can therefore isolate the specific effects of grooming, and further ask whether those effects depend on whether the grooming is into corbiculae or scopae.
5.2. Materials and methods

We conducted this study in two parts; the first in the spring and summer of 2012 in the greenhouse at the University of Toronto using commercially reared Bombus impatiens, and the second in the summer of 2012 at the University of Toronto’s Koffler Scientific Reserve in King City, ON (44.01.48 N, 79.32.01 W) using the naturally occurring pollinators Apis mellifera, Megachile rotundata, and two species in the genus Halictus, Halictus ligatus and Halictus confusus. The bees B. impatiens and A. mellifera are corbiculate. The scopa of M. rotundata is located on the underside of the abdomen, while the scopa of the Halictus spp. is located on the tibia and femur of the hind legs in approximately the same location as the corbicula of A. mellifera and B. impatiens. Both parts of the study examined pollination of the self-incompatible annual herb Brassica rapa L. (Brassicaceae). Naturalized populations of B. rapa are visited by a diverse group of insects, including but not limited to corbiculate and non-corbiculate bees, and has many ovules per flower. We grew our plants from seed harvested from populations of B. rapa in Quebec, Canada.

We collected foraging pollinators and wiped their bodies onto virgin stigmas of Brassica rapa, transferring pollen directly from the bee body onto the stigma using a method similar to an experiment by Kendall (1973). To determine the effect of grooming by bees on collected pollen, we wiped groomed pollen (pollen from an individual bee’s corbicula or scopa) on one flower and ungroomed pollen on another. We captured each bee directly into a clean vial, and then placed the insect into a cooler for ten minutes to slow its movement. Then we grasped the hind leg of a bee with forceps and brought the bee into controlled contact with the stigma, ensuring that only the area of interest on the bee body made contact. We wiped the entire surface of the area of interest twice to transfer as much pollen as possible from the bee to the stigma. We returned the bee to the vial and froze it for future identification; specimens are deposited at the University of Toronto. We then labeled each flower, collected the fruit when it was mature but undehisced, and counted the resulting seeds. In order to reduce our manipulation of the plants, we did not count the number of grains that were transferred in each wipe, but in most cases, we were able to see that the number of pollen grains transferred greatly exceeded the number of ovules. One exception was ungroomed grains on A. mellifera, where grains were sometimes not abundant enough to be visible. When groomed grains did not appear to transfer because of clumping in the bee corbiculae or scopae, we removed a chunk of pollen from the corbicula or scopa and hand pollinated the recipient stigma. For all bee species, there were noticeably more groomed grains than ungroomed grains in each wipe or hand pollination.

5.2.1. Greenhouse experiment

We grew a population of B. rapa in the greenhouse with a 12-12 hour day-night cycle; this population numbered 200-300 and was used only as a source of pollen. We also grew plants from a line of male-sterile B. rapa plants previously developed by Ison and Weis through a series of crosses to integrate the autosomal loci for rapid cycling and male sterility from the Wisconsin Fast Plants ® lineage into the
Quebec naturalized genetic background; the use of male-sterile *B. rapa* plants as pollen recipients prevented contamination by self-pollen and incidental pollen transfer by contact among plants on the greenhouse bench. Recipient plants were kept in pollinator exclusion structures before and after pollinator wiping to ensure that all open flowers used for pollinations were previously unvisited. Pollinator exclusion structures were wooden structures approximately 3x3x2m covered in wire mesh. In this experiment we used captive-reared colonies of *Bombus impatiens*, a common local native, obtained from Biobest Canada Ltd. (Leamington, Ontario, Canada). We released 3-5 individuals from a colony of *B. impatiens* allowing them to forage until a mass of pollen was groomed into the corbicula; this occurred quickly, but each bee used in the study foraged for at least two minutes and visited at least two flowers. To ensure that enough pollen remained ungroomed for the ungroomed treatment, we restricted the individual's leg movement in the vial by using a smaller vial to gently “squish” the bee against the bottom. In this experiment, we had three treatments – flowers wiped with groomed pollen, ungroomed pollen, and a mix of the two – and we used each bee for only one wipe.

5.2.2. Field experiment

In this experiment, we grew a population of over 1000 plants in an open area at the Koffler Scientific Reserve. These were freely visited by the naturally occurring *Apis mellifera*, *Megachile rotundata*, and *Halictus* spp as well as other insects. When male-sterile recipient plants were unavailable for part of the experiment, we used emasculated hermaphrodites; these plants were kept in a pollination exclusion structure with the male-sterile recipient plants. We observed bees visiting at least two of our population plants before we collected them for wiping. Unlike the greenhouse experiment, in this experiment we wiped the groomed pollen from an individual bee on one flower and ungroomed pollen from that same bee on another flower of the same plant (i.e., the data for the field experiment are paired), and we did not include a third treatment with a mix of groomed and ungroomed pollen.

5.2.3. Data analysis

All statistical analyses were done using R 3.0.1 (R Core Team 2013). We compared numbers of seeds per fruit produced by groomed and ungroomed pollen on the different bee taxa using generalized linear mixed models (GLMMs). Many flowers did not set seed. To account for zero-inflation, we used zero-inflated generalized linear mixed models using the function *glmmADMB* in the library *glmmADMB* (Fournier et al. 2012). We used a negative binomial error distribution because of high variance. Multiple comparisons were conducted using Tukey-corrected Wald Z-tests, using the *glht* function in the library *MULTCOMP* (Hothorn et al. 2008)

For the greenhouse experiment, we included the date as a random effect because environmental factors inside the greenhouse were highly variable over time. For the field experiment, we accounted for the paired design by including the bee individual as a random effect (Hall 2004, Min and Agresti 2005). To determine if the type of recipient plant (whether it was a male-sterile or a wild type emasculated...
hermaphrodite) had an effect on the results, we also included the plant type as a random effect. In addition, we conducted the analysis including only the male-sterile plants, and obtained qualitatively similar results. Within the genus *Halictus* we included both *Halictus ligatus* (n=30) and *Halictus confusus* (n=5), but the same analysis including only *Halictus ligatus* individuals resulted in qualitatively similar results.

5.3. Results

5.3.1. Greenhouse experiment

Groomed (corbicular) grains on *B. impatiens* sired significantly fewer seeds per fruit than did ungroomed grains on the *B. impatiens* body, with means of 5.15 and 10.05 seeds respectively (Fig. 5.1, Z=-2.421, \( P=0.035 \): Fig. 5.1). Double wipes of both groomed and ungroomed grains from *B. impatiens* produced seed sets that were intermediate between single wipes of either type, with a mean of 7.48 seeds (Fig. 5.1, double versus groomed, Z=-1.01, \( P=0.535 \); double versus ungroomed, Z=-0.999, \( P=0.542 \)).

5.3.2. Field experiment

Groomed (corbicular) grains on *A. mellifera* sired significantly fewer seeds per fruit than ungroomed grains on the *A. mellifera* body, with means of 6.78 and 13.95 seeds respectively (Fig. 5.1, Z=5.005, \( P<0.001 \)). Groomed and ungroomed grains sired similar numbers of grains for both *M. rotundata* (Fig. 5.1, means of 13.36 seeds and 13.44 seeds, Z=0.625, \( P=0.985 \)) and *Halictus* spp. (Fig. 5.1, means of 17.05 and 15.66 seeds, Z=0.367, \( P=0.998 \)). All analyses included both male-sterile and hermaphrodite plants; when we conducted analyses using only male-sterile plants we obtained qualitatively similar results.

5.4. Discussion

Fruits from pollinations with groomed pollen from *A. mellifera* and *B. impatiens* resulted in 49% and 55% fewer seeds than those with ungroomed pollen. This result supports the hypothesis that grooming affects the ability of pollen grains to pollinate. This result is consistent with the only other study that distinguished groomed and ungroomed pollen on bee bodies, which found decreased viability in grains from *Apis mellifera* corbiculae (Mesquida and Renard 1989). Given the number of studies that document manipulations by *A. mellifera* during pollen handling, such as toxic acids in mandibular glands and intensive manipulation before pollen storage (Stanley and Linskens 1974, Winston 1991, Stanciu et al. 2009, Seddick et al. 2012), it is not surprising that there is some effect of grooming on the ability of grains to pollinate. Lower seed set by groomed pollen could help explain the inconsistency in those studies examining viability on *A. mellifera* bodies, in which some pollen from bee bodies affected pollen viability (Kraai 1962, Kendall 1973), but some did not (Harriss and Beattie 1991, Dutton and Frederickson 2012). Perhaps those studies that documented lower viability in bee-collected pollen
sampled a higher proportion of groomed pollen, while those that documented no effect were based on a higher number of ungroomed grains.

In the non-corbiculate *M. rotundata* and *Halictus* spp., groomed pollen sired as many seeds per fruit as did ungroomed pollen. This result is consistent with differences in grooming behavior between corbiculate and non-corbiculate bees, in particular, that non-corbiculate bees do not moisten pollen with nectar for transport (Thorp 1979) and observations in the field that scopal pollen is generally held more loosely than corbicular pollen (Thorp 1979, Thomson et al. 2000). When transferred to stigmas, these grains may more easily adhere to the surface of the stigmas and more individual grains may form pollen tubes, which in turn may increase the number of seeds produced.

Pollen groomed by the two corbiculate bees in our study set fewer seeds than pollen groomed by the two non-corbiculate bees, so the effects of grooming on pollen appear to depend on the type of grooming. The mechanisms behind these differences require further study. Known differences between corbiculate and non-corbiculate grooming are likely to be important, such as the corbiculate bee behavior of moistening of pollen and packing pollen tightly into a pellet. Moistening may cause physiological changes in the pollen grain or it may increase pollen clumping, reducing adherence to stigmas, or both. Tight packing may result in fewer grains being transferred or fewer grains adhering to stigmas. The age of pollen grains may be important, as well; pollen grains lose viability rapidly (Thomson et al. 1992), and groomed pollen may be older, on average, than ungroomed pollen. The location of pollen on the bee body does not seem to be a factor, because *Halictus* spp. and *M. rotundata* store pollen in different locations while the scopae and corbiculae of *Halictus* spp. and *A. mellifera* are in essentially the same place. More research on the physiochemical consequences of corbiculate and non-corbiculate grooming would help resolve this issue.

We chose to examine the ability of pollen to pollinate on two corbiculate bees and two non-corbiculate bees, but other divisions between the bees may be important. Differences in the pollen transport structures of bees are phylogenetically structured (Michener 2000), and therefore these differences covary with other differences, such as sociality. Corbiculate bees are mainly eusocial, while non-corbiculate bees are mainly solitary, with some exceptions (e.g., orchid bees are solitary and corbiculate, and some species in the Halictidae are primitively eusocial and non-corbiculate) (Michener 2000). The strength of antimicrobial defense is correlated with sociality in bees (Stow et al. 2007), so the differences found in our study could be the result of sociality rather than grooming. However, both *Halictus* spp. in our study are in the minority as primitively eusocial non-corbiculate bees, but groomed and ungroomed pollen from both *Halictus* spp. showed similar seed set. This may be an indication that the type of grooming behaviors performed is more important than the level of antibacterial defense, that *Halictus* spp. has lower antibacterial defense than more complex eusocial bees, or that some other factor is involved. Future research should examine more bee groups with higher diversity in grooming behavior in order to provide further insight on the importance of each of these factors in pollination.
Corbiculate and non-corbiculate bees are likely to differ in other relevant behaviors not measured here; for example, bees may differ in the average number of groomed and ungroomed grains on their body, where the ungroomed grains are located on the bee body, and how often those areas with ungroomed grains contact plant stigmas (Harder and Wilson 1998). These variables have only been examined in some bee species in relation to a few plant species, but this research demonstrates that pollen is not distributed uniformly on bee bodies (Beattie 1971, Bosch 1992, Vallejo-Marín et al. 2009). Pollen may be concentrated on “safe” areas of the bee body that are inefficiently groomed (Beattie 1971, Harder and Wilson 1998) or strategically placed on areas that are likely to contact conspecific stigmas, as demonstrated for mirror-image flowers and heteranthery (Darwin 1864, Jesson and Barrett 2005, Vallejo-Marín et al. 2009). However, *B. rapa* is a radially symmetric, open flower, making precise pollen placement on bee bodies unlikely (Harder and Wilson 1998). Pollinators visiting these flowers seemed to move haphazardly across open flowers; from our observations, it seems likely that the contacts with both anthers and stigmas would be distributed widely across bees’ bodies.

The use of the bee wipe technique does not simulate a pollinator visit, as it removes the variability in the behavior of bees on flowers. Instead, it provides an estimate of the maximum success of these grains in each treatment. The relevance of these results depends on the extent to which bee corbiculae or scopae contact floral stigmas during pollinator foraging; if bee corbiculae and scopae never contact stigmas during foraging, then the differences between bee groups that we document will not be important for plant reproductive success. However, very little research specifically reports contact between corbiculae or scopae and plant stigmas (but see Bosch 1992), and the extent of contact will be specific to the plants and pollinators observed. During this study, we observed stigmatic contact of the corbiculae of *A. mellifera* and *B. impatiens*, as well as the scopae of *M. rotundata* and *Halictus* spp., but we did not quantify the frequency of such contacts. Similarly, using the bee wipe technique included grains in pollination that may not ordinarily have been included; for example, we wiped dorsal, lateral, and ventral sides of the bees equally, although Jesson and Barrett (2005) showed that pollen on the lateral and dorsal sides of *B. impatiens* is more likely to contact *S. rostratum* stigmas than pollen on the ventral surface. Without further study on the differences between grains on different parts of the bee body, we have no reason to believe that ungroomed grains on different parts of the bee body would affect seed set differently.

To minimize our manipulation of the flowers, we chose not to collect data on the number of grains deposited in each wipe; therefore, it is possible that the number of grains transferred varies between treatments. However, for the most part, we had no trouble observing that many grains were transferred – a number that very likely exceeded the number of ovules. One exception was ungroomed grains on the *A. mellifera* body, for which we were sometimes unable to see pollen on the stigma. Despite the low pollen load, this treatment showed high seed set, even when no pollen could be seen (Fig. 5.1). When groomed grains on any bee species did not readily transfer, we supplemented our bee wipes by removing a chunk of grains from the corbicula or scopa and hand pollinating the recipient stigma. This supplemental
pollination ensured that our results are due to the ability of these grains to pollinate, rather than the number of grains that were transferred.

How do these differences between bee groups affect these floral visitors’ values as pollinators? Our results show that scopal pollen is fully intact, but that corbicular pollen is impaired. Bees’ interests are served when as much pollen as possible is groomed into the corbicula or scopa for transportation back to the nest; for corbiculate bees, grooming may decrease pollination success, while there is no effect for non-corbiculate bees. As such, selection on bees to increase pollen collection through grooming is likely to decrease the value of corbiculate bees, but not non-corbiculate bees, as pollinators. In addition, there may be situations in which there are not enough ungroomed pollen grains on corbiculate bees to accomplish sufficient pollination. In these situations, visits by non-corbiculate bees may deliver more healthy pollen grains and result in higher plant reproductive success. Moreover, Harder and Wilson (1998) predict that the heterogeneous structure of pollen on bee bodies caused by grooming and layering will increase the distance that pollen is carried. However, this result assumes that groomed and layered pollen is still able to participate in pollination; our results indicate that this may not be true for corbiculate bees.

The maintenance of pollen quality in the groomed pollen of non-corbiculate bees may also be important for other dimensions of pollen quality, such as the distance that pollen moves between the pollen donor and recipient. As bees forage, they continuously collect and groom pollen, constantly moving grains from flowers and the rest of their bodies into the corbicula or scopa. These accumulated grains may form layers, the bottom layer with the oldest pollen and the youngest at the top; as the bee continues foraging, the youngest pollen will be deposited first, and the oldest pollen may be exposed later (Lertzman 1981, Thomson 1986, Harder and Wilson 1998). This layering is more likely to occur in areas where groomed grains are transported such as the corbicula or scopa (Lertzman 1981, Thomson 1986, Harder and Wilson 1998). Therefore, groomed grains are likely to be older and to have traveled farther from the pollen donor than ungroomed grains. If the groomed grains on non-corbiculate bees maintain viability and the ability to adhere to stigmas longer than the groomed grains on corbiculate bees, then the seeds set from non-corbiculate bees may be more often sired by pollen from farther away. If there is a spatial component to genetic diversity (i.e. if pollen from farther away is more likely to be genetically diverse), non-corbiculate bees may maintain genetic diversity more than corbiculate bees. This same mechanism may also apply to other spatially determined aspects of pollen quality, such as geitonogomy and outcrossing rates in self-compatible species.

By conducting tests in-vivo on groomed and ungroomed grains we were able to isolate the effect of grooming by corbiculate and non-corbiculate bees on pollen’s ability to pollinate, as measured through the number of seeds produced. This is the first study to examine both corbicular and scopal pollen in this way. Our results indicate that non-corbiculate bees have a unique role in plant pollination, and that in some situations may be particularly valuable to plant pollination when they are abundant. Given that
native bees are mainly non-corbiculate, supporting native bee populations in native and agricultural areas will help ensure adequate pollination service of natural and agricultural plants (Winfree et al. 2008).
5.5. Figures

(a) 

(b) 

Bombus impatiens

Apis mellifera Halictus spp. Megachile rotundata
Figure 5.1. Boxplot of the number of seeds set by a B. rapa flower following a wipe of groomed or ungroomed pollen from bee bodies.

Within a box, treatments sharing a letter are not significantly different at $P < 0.05$. (a) The number of seeds set resulting from groomed grains (white boxes, n=30), a combination of groomed and ungroomed grains (grey boxes, n=25), and ungroomed grains (black boxes, n=31) on the corbiculate bee B. impatiens. (b) The number of seeds set resulting from groomed grains (white boxes) and ungroomed grains (black boxes) on the corbiculate bee A. mellifera (groomed n=87, ungroomed n=87), the non-corbiculate bee Halictus spp. (groomed n=35, ungroomed n=35), and the non-corbiculate bee M. rotundata (groomed n=25, ungroomed n=25).

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6. Abstract

Pollinators contribute to plant reproduction by exporting and delivering pollen grains between conspecific individuals; however, they also negatively affect plant reproduction by removing pollen grains that they do not subsequently deliver. In some situations, a pollinator can be conditionally parasitic if some visits result in a net decrease in total pollen delivery. We investigated the negative contribution of pollinator wastefulness using a simulation model, which we constructed and parameterized based on the oligolectic bee *Andrena erigeniae* and the fly *Bombylius major* visiting the spring ephemeral *Claytonia virginica*. We compared the effect of variation in plant and pollinator characteristics on the value of a pollinator visit. Specifically, we compared the effect of different removal and deposition rates, sex-phase visitation bias, the schedule of pollen presentation, and grooming behavior on total pollen delivery. In our models, visits by the oligolectic bee *Andrena erigeniae* lowered overall pollen delivery when bee and fly visitation rates were very high, removal rates were higher than empirical values, deposition rates were lower than empirical values, and pollen was presented simultaneously. These results indicate that conditional parasitism is likely to occur in natural plant-pollinator systems with these characteristics; moreover, our results emphasize the importance of context in the determination of pollinator value.

6.1. Introduction

The interaction between a plant and a pollinator is often portrayed as a classic example of mutualism: a visiting animal contributes to a plant individual’s fitness through the delivery and export of pollen grains in return for a reward. Pollination involves the removal of pollen grains from a flower, the transportation of those grains some distance, and the deposition of those grains on the same or another flower. The process is inherently wasteful, in that only a fraction of pollen grains will fertilize ovules; a great deal of pollen is lost to the environment, actively sequestered by animals, or both (Harder and Thomson 1989, Harder and Johnson 2008). From the plant perspective, the grains that are removed but not delivered to conspecific stigmas are wasted, because they cannot fertilize ovules. Intrinsic characteristics of pollinators cause these animals to remove and deliver pollen at different rates (Motten et al. 1981, Kearns 2001, Sahli and Conner 2007, Muchhala and Thomson 2010) and therefore contribute differentially to the male and female fitness of plants (Johnson et al. 1998, Galen and Cuba 2001, Fenster et al. 2004); however,
intrinsic characteristics of pollinators will also result in differences in the amount of pollen that is 
removed but not delivered in pollinator visits (“pollinator wastefulness”). (Thomson 2003, Castellanos et 
al. 2003).

Most pollinators visit flowers as part of a generalist assemblage of multiple foraging species interacting 
within an environment of fluctuating conditions and rewards (Waser et al. 1996, Thomson 2003). When 
multiple floral visitor species visit flowers, their different removal and delivery rates interact with the 
supply of available pollen, producing complicated interactions that affect the amount of pollen that is 
delivered to stigmas. For example, a pollinator may monopolize the pollen supply in early visits, leaving 
little for future floral visitors to accumulate and distribute. Wasteful visits divert grains that may have 
otherwise been delivered by a less wasteful pollinator; therefore, a single visit by a pollinator can deliver 
some pollen grains but prevent the delivery of other grains by removing them from access to other 
visitors. In a possibly extreme case, a wasteful pollinator that visits flowers in the company of less 
wasteful visitors may even decrease total pollen delivery – the number of pollen grains that are exported 
and delivered over the course of many pollinator visits – more than it increases it (Thomson 2003, 
Castellanos et al. 2003). Therefore, wasteful pollinators that appear to be increasing plant fitness may 
sometimes decrease it, and pollinators that seem to be in a mutualistic relationship with plants may 
sometimes be parasitic. These “conditional parasites” increase total pollen delivery within one pollinator 
assemblage and decrease it in another; these pollinators may therefore increase or decrease plant fitness 
depending on the ecological context.

The opportunity cost of undelivered grains and the effect of a pollinator visit on total pollen delivery is 
not well understood due to the difficulty of tracking pollen grains in plant populations. We are interested 
in evaluating whether a pollinator visit has a positive or negative net result on total pollen delivery, or how 
total pollen delivery ($G$) in a specified situation would change as a result of one additional pollinator visit 
($\Delta G$). $\Delta G$ values would frequently be positive, but they can be negative, in which case those visitors are 
acting as pollen parasites. To make these measurements empirically would require meticulous 
manipulation of pollinator visits in a controlled environment. Instead, we can predict the consequences 
of different combinations of visits using simulation models.

Previous pollen depletion models have shown that visits with a negative $\Delta G$ are possible within certain 
parameters and in some contexts (Harder and Thomson 1989, Thomson and Thomson 1992, Thomson 
2003). However, we can make pollen depletion models more realistic by incorporating more parameters 
from real systems. Unexplored characteristics of plants and pollinators, such as foraging biased by plant 
sex, are likely to affect the value of a pollinator visit to total pollen delivery. Here, we extend pollen 
depletion models to explore different patterns of removal and deposition, and incorporate additional 
characteristics of plants and pollinators. To maintain the connection to natural systems, we explore these 
parameters only within realistic visitation rates of ten visits or less per flower per pollinator group; 
Motten et al (1981) estimated that individual $C. virginica$ flowers were visited 4.1 times by bees and 2.4
times by flies over the lifetime of a flower; therefore, in *C. virginica*, some flowers probably receive up to ten visits by each visitor group. In other study systems with similar visitation rates, the total number of visits will be higher because the lifetime of flowers is usually more than one day. Our goal is to determine whether conditional parasitism and visits with a negative Δ*G* are likely to be an important part of plant-pollinator interactions in nature, or simply a seldom attained theoretical possibility.

### 6.2. The ecological situation

Here, we seek to build more specific models that reflect a natural situation. *Claytonia virginica* is a spring ephemeral wildflower visited by a variety of insects in eastern North America, including the oligolectic bee *Andrena erigeniae* and the bee fly *Bombylius major*. Several aspects of this pollination system make it interesting for the study of context-dependence in pollination; many of these characteristics are common in other plant-pollinator relationships and may be generalizable across systems. In particular, we modeled the following four aspects, using parameters derived from the *C. virginica* system.

#### 6.2.1. Active and passive pollen collection

As in many generalized pollination systems, the visitors of *C. virginica* include both passive and active pollen collectors: flies and butterflies are typically passive, whereas female bees are typically active. While most pollinator foraging is motivated primarily by nectar rewards, female bees actively forage for pollen – in addition to nectar – in order to provision or feed larvae. Female bees are proficient at pollen collection; they have been shown to remove 70-90% of pollen in a single visit (Strickler 1979, Larsson 2005), and they sequester that pollen on their body through “grooming”, stereotypical movements and manipulations that concentrate pollen in certain structures for transport to the bee nest (Thorp 1979).

Because “active” pollen collectors are motivated to transport pollen to their offspring rather than to floral stigmas, they are more likely to be wasteful floral visitors than their more passive co-foragers. When visiting *C. virginica*, *A. erigeniae* remove an average of 61% of available grains and *B. major* remove an average of 24%. The high removal rate of *A. erigeniae* is due to active pollen collection, while the removal rate of *B. major* is probably a result of passive pollen removal. When active and passive pollen collectors visit concurrently, is it possible for the active pollen collector to reduce pollen delivery (have a negative Δ*G*)? What combination of visits by active and passive pollen collectors could result in conditional parasitism?

#### 6.2.2. Sex-phase visitation bias

Herkogamy and dichogamy are common in plants (Hargreaves et al. 2009), and biotically-pollinated plants are vulnerable to a bias in pollinator foraging towards one sex over another due to differential pollen and nectar production. Sex-biased foraging may be a cost of gender separation in plants, since plants visited by sex-biased foragers will experience fewer of the benefits of pollinator visits (pollen export and delivery) and potentially higher costs (pollen wastefulness) (Bierzychudek 1987, Charlesworth
1993, Ashman 2000, Lau and Galloway 2004). For example, there are many examples of female bees biasing their visitation towards male or male-phase plants in order to collect more pollen (Ågren et al. 1986, Bierzychudek 1987, Eckhart 1991, Ashman and Stanton 1991, Wilson and Thomson 1991, Delph and Lively 1992, Eckhart 1992b, Ashman 2000). *Claytonia virginica* is protandrous, functioning as male-phase flowers before transitioning to the female-phase; because the sexes are separate, pollen-collecting *A. erigeniae* females preferentially visit pollen-donating male-phase flowers over female-phase flowers that offer only nectar. *A. erigeniae* females make 80% of visits to male-phase flowers (A. Parker, unpublished data). In addition, although both male- and female-phases provide nectar, the female-phase provides more . As a result, nectar-collecting *Bombylius major* preferentially visits the female-phase, making only 35% of visits to male-phase flowers. Does sex-phase biased foraging lower total pollen delivery or make conditional parasitism more likely?

6.2.3. The pollen presentation schedule

The packaging of pollen into anthers and the dispensing of pollen can decrease the amount of pollen available to be removed in initial visits and increase the amount of pollen available for later visits. In this way, packaging and dispensing can reduce pollen wastefulness and increase pollen export and delivery (Thomson and Harder 1989). Manipulating the schedule of pollen presentation (i.e., the timing of pollen availability) may be an adaptive strategy employed by plants to increase the number of pollinators that export pollen grains without increasing investment in pollen production (Thomson and Harder 1989). *Claytonia virginica* has five anthers with two locules each; these locules dehisce sequentially throughout the first few hours of pollinator visitation. Can the sequential dehiscence of anthers mitigate pollen wastefulness? Does sequential dehiscence make conditional parasitism less likely?

6.2.4. Grooming behavior

Grooming behavior may affect pollen wastefulness, as well. When female bees groom, the pollen that they sequester into pollen baskets (“corbiculae” or “scopae”) may no longer be in the active pool of grains that can participate in plant pollination, and are therefore more likely to be wasted. Corbiculate bees (such as honey bees and bumble bees) pack moistened grains tightly into corbiculae, reducing or eliminating their ability to pollinate stigmas (Chapter 5 of this thesis). Large amounts of pollen trigger bee grooming (Harder and Thomson 1989), meaning that higher pollen availability in a flower will increase bee grooming and therefore increase pollen wastefulness by corbiculate bees, while lower pollen availability will decrease pollen wastefulness; this pattern of “decelerating deposition” has been shown for *Bombus occidentalis* individuals visiting *Erythronium grandiflorum* (Harder and Thomson 1989). However, non-corbiculate bees (such as *A. erigeniae*) hold dry pollen more loosely in hairy scopal regions, and these grains are more likely to remain a part of the active pollen pool. Therefore, although higher pollen availability may still stimulate bees to groom, these grains are not so strongly fated to be wasted, but may remain an active part of the pollen pool with the ability to fertilize ovules. Since pollen grains in bee
scopae are less likely to be wasted than those in bee corbiculae, are corbiculate bees more likely to be conditional parasites than non-corbiculate bees?

6.3. Model structure

Our pollen depletion model calculates total pollen delivery for various combinations of numbers of bee and fly visits that remove and deposit pollen according to pollen removal and deposition functions (Table 6.1, Fig. 6.2). The model simulates a set of flowers which are associated with a quantity of pollen available for removal by visitors. The plant population contains 2000 individual plants of separate sexes; for simplicity, each plant has only one flower, for a total of 1000 male-phase flowers and 1000 female-phase flowers. Each male-phase flower begins with a number of pollen grains that increases with anther dehiscence and reduce with pollen removal, and each female-phase flower begins with clean stigmas that accumulate pollen grains over successive visits. The model tracks the number of pollen grains carried by 100 be visitors and 100 fly visitors; each bee and fly body begins with no pollen, but pollen is accumulated over successive visits as pollen is removed from male-phase flowers, and concurrently reduced as pollen is lost to the environment and deposited on floral stigmas of female-phase flowers (Fig. 6.2). The number of pollen grains deposited is a proportion of pollen grains on the bee or fly body.

In each individual simulation, each flower receives a combination of visits by these bee and fly pollinators, ranging from 1-10 visits by a bee and 1-10 visits by a fly per female-phase flower, for a total of 1,000-10,000 visits by bees and 1000-10,000 visits by flies in the population consisting of 1,000 female-phase flowers. We conducted these individual simulations for each combination of 1,000 visits by bees or flies (for example, 1000 visits by bees and 1000 visits by flies, then 1000 visits by bees and 2000 visits by flies, then 1000 visits by bees and 3000 visits by flies, etc.). We then constructed the corresponding pollen delivery surface (Figs. 6.3 and 6.4).

Pollinators are chosen to make a floral visit randomly from those available, and visit a flower also chosen at random. There is no spatial structure. The total quantity of pollen delivered to stigmas in the plant population (consisting of 1000 female-phase flowers) is reported at the end of each simulation. To simplify presentation, we divided the total number of pollen grains delivered in the plant population by the total number of female-phase plants (1000) to estimate the average quantity of pollen delivered per female-phase flower. The conclusions we draw are based on average pollen delivery when different combinations of visits occur; total pollen delivery to individual plants varied above and below that average value.

6.3.1. Interpretation of simulation results

The response variable in each simulation is total pollen delivery ($G$). We can compare pollen delivery for various combinations of visits by multiple pollinator species through the use of a multidimensional response surface, or “delivery surface” (a three-dimensional representation is shown in Fig. 6.1). Each of
the horizontal axes represents the average number of visits by one species of floral visitor to an individual plant, while the vertical axis records the average number of grains successfully delivered to a female-phase flower given the combination of visits. On the delivery surface, the slope of the line between two adjacent points indicates the sign of the interaction; when the slope is positive, the difference in the visitation regimes causes a net increase in pollen delivery (a positive $\Delta G$), but when the slope is negative, the difference in the visitation regimes causes a net decrease in pollen delivery (a negative $\Delta G$). When increases in the number of visits by a pollinator (on a horizontal axis) is associated with increases in pollen delivery (on the vertical axis) in one section of the delivery surface and decreases in pollen delivery in another section, then that pollinator is considered a conditional parasite.

6.3.2. Model parameters

6.3.2.1. The *C. virginica* representative ecological context

To compare between ecological contexts, we used as much empirical data as possible to construct a representative ecological context, a specific ecological situation defined by the characteristics of *C. virginica* and its pollinators *A. erigeniae* and *B. major*, including visitation by one active and one passive pollen collector, removal and delivery coefficients that are derived from empirical values, sequential dehiscence, and a sex-phase visitation bias by both bees and flies. We call this delivery surface the *C. virginica* “representative delivery surface” (Table 6.1).

The *C. virginica* representative delivery surface is parameterized, as much as possible, using estimates derived from common, easily collected empirical data. (For details of empirical data collection, see Chapter 2 of this thesis). We estimated total pollen production per male-phase flower from empirical measurements of the number of pollen grains in unvisited male-phase flowers with completely dehisced anthers, with a mean of 2764 grains. For models with sequential pollen dehiscence, we estimated initial pollen availability ($A_0$) to be 10% of the total number of pollen grains in an unvisited male flower ($A_0 = 276.4$), the approximate number of pollen grains in one locule of one anther. We assumed that locules would dehisce at a standard rate during the period of pollinator visitation (one locule after each 10% of pollinator visits), and that the number of pollen grains that become available as each locule dehisced would be 10% of total pollen production ($s = 276.4$) (Table 6.1).

We parameterized removal and deposition functions using single visit measurements of removal and deposition, measurements that are commonly used in studies of pollinator value to plants (e.g. Sahli and Conner 2007, Larsson 2005, Thomson and Goodell 2001). Single visit removal data usually consist of measurements of the number of pollen grains removed by a pollinator in a single visit ($R$), or a comparison of the number of pollen grains on an unvisited flower ($A_0$) and the number of pollen grains remaining after a single visit by a pollinator ($A_3$). Single visit deposition data usually consist of the
number of pollen grains deposited on the stigma of a previously unvisited female-phase flower (D) (Table 6.1).

We used empirical data on the number of pollen grains on an unvisited flower ($A_0$) and the number of pollen grains remaining after a single visit by $A. erigeniae$ ($A_b$) or $B. major$ ($A_f$) to estimate removal coefficients, or the proportion of pollen removed by a pollinator in a single visit (Table 6.1, Fig. 6.2, $k_b$ and $k_f$). All removal coefficients are based on data on first visits.

Equations 6.1

$$k_b = \frac{A_0 - A_b}{A_0} \quad k_f = \frac{A_0 - A_f}{A_0}$$

We used empirical data on the number of pollen grains deposited on the stigma of a previously unvisited female-phase flower (D) to estimate deposition coefficients for $A. erigeniae$ and $B. major$ (Table 6.1, Fig. 6.2, $d_b$ and $d_f$) and an attenuation coefficient for deposition for $A. erigeniae$ (Table 6.1, Fig. 6.2, $g$). The mean number of pollen grains deposited by $A. erigeniae$ and $B. major$ in a single visit is 39 grains and 30 grains, respectively. We estimated values for the deposition and attenuation coefficients $d_b$, $d_f$, and $g$ that would result in the deposition of 30 and 39 grains in one particular single visit, chosen arbitrarily (the second visit by $B. major$ depositing 30 grains and the third visit by $A. erigeniae$ depositing 39 grains).

We used empirical measurements on the rate of visitation to male-phase and female-phase flowers to determine the ratio of visitation in the model; $A. erigeniae$ make 80% of visits to male-phase flowers while $B. major$ make 35% of visits to male-phase flowers. We also estimated that 14% of the pollen grains on a bee body would be lost to the environment because, in the only study to measure this value, three bee species visiting Erythronium grandiflorum lost 14% ± 8.31% of removed pollen to the environment (Harder and Thomson 1989) (Table 6.1, Fig. 6.2).

6.3.2.2. Deviations from the $C. virginica$ representative ecological context

The other simulations represent purposeful deviations from this basic reference surface; in each one, we examine the effect of variation in one of the following parameter sets: removal and deposition coefficients, sex-phase visitation biases, and the pollen presentation schedule. For simplicity, we examined the effect of variation in removal and deposition functions and the type of grooming for only one pollinator; we kept the fly parameters the same and changed only the parameters for the bee (Table 6.1, Fig. 6.2).

6.3.3. Grooming behavior

To examine the effect of grooming behavior, we constructed a second reference surface that uses a different deposition function to better represent decelerating deposition (Table 6.1, Fig. 6.2); in this
deposition function, the number of pollen grains deposited in each visit is a function of the number of
grains on the bee body to the exponent $g$, the attenuation coefficient. We constructed this decelerating
deposition representative surface in the same way as the basic $C.\ virginica$ reference surface, and all other
parameters remained the same. We then varied the bee’s removal coefficient ($k_b$), the bee attenuation
coefficient ($g$), the sex-visitation bias, and the pollen presentation schedule, and compared each of the
resulting delivery surfaces to the decelerating deposition representative surface.

6.4. Results

6.4.1. Conditional parasitism in $Claytonia\ virginica$

In all simulations, the diversity and abundance of pollinator species affects the shape of the delivery
surface and whether adding another pollinator visit contributes positively or negatively to pollen delivery
(a positive or negative $\Delta G$). When there are low numbers of visits by bees and flies, additional visits by
any pollinator always contribute substantially to increased pollen delivery. However, when there are many
bee visits, bee visits are not additive; some visits contribute substantially while others contribute little to
total pollen delivery because there is no pollen available left to remove and deliver.

One simulation best represents the $Claytonia\ virginica$ ecological situation because it includes active and
passive pollen collection, a sex-phase bias for each pollinator, sequential dehiscence, and a proportional
deposition function for bee pollen delivery, which is probably more applicable for non-corbiculate bees
like $A.\ erigeniae$ (Fig. 6.3a). In this simulation, bee visits always increase pollen delivery ($\Delta G$ is positive for
all visits), and bees do not act as conditional parasites.

6.4.2. Active and passive pollen collection

The amount of pollen removed and deposited changes the shape of the delivery surface and affects the
contribution of bee visits (Figs. 6.3a, 6.3b, 6.3c). Increases in the pollen removal coefficient of the bee
pollinator above empirical values decreases pollen delivery slightly for all combinations of visits that
include bees (compare Fig. 6.3a and 6.3b). With high visitation by both bees and flies, increased numbers
of visits by bees decreases total pollen delivery; therefore, when the removal coefficient is greater than .7
and fly visitation is high, bees act as conditional parasites (Fig. 6.3b).

Similarly, decreases in the pollen deposition coefficient of the bee pollinator above empirical values
decreases pollen delivery slightly for all combinations of visits that include bees. This makes sense
because pollen is being removed at the same rates as in the empirical delivery surface, but a lower
proportion of grains are being delivered. With high visitation by both bees and flies, increased numbers
of visits by bees decrease pollen delivery slightly for all combinations of visits that include bees (compare
Fig. 6.3a and 6.3c). With high visitation by both bees and flies, increased numbers of visits by bees
decreases total pollen delivery; therefore, when the deposition coefficient is less than .01 and fly visitation is high, bees act as conditional parasites (Fig. 6.3c).

6.4.3. Sex-phase visitation bias

Sex-phase visitation bias changes the shape of the delivery surface only slightly and does not affect whether bee visits contribute positively or negatively to pollen delivery (a positive or negative $\Delta G$) (Figs. 6.3a and 6.3d). Including sex-phase visitation bias at empirical levels decreases pollen delivery slightly for all combinations of visits (compare Figs. 6.3a and 6.3d). With high visitation by both bees and flies, increased numbers of visits by bees continue to increase total pollen delivery; therefore, a sex-phase visitation bias does not cause bees to act as conditional parasites (Fig. 6.3d).

6.4.4. Pollen presentation schedule

Changes in the pollen presentation schedule change the shape of the delivery surface and affect whether bee visits contribute positively or negatively to pollen delivery (a positive or negative $\Delta G$) (Figs. 6.3a and 6.3e). Simultaneous anther dehiscence (as opposed to empirical anther dehiscence, which is sequential) increases pollen delivery for all combinations of visits (compare Figs. 6.3a and 6.3e). With high visitation by both bees and flies and simultaneous anther dehiscence, increased numbers of visits by bees decrease total pollen delivery; therefore, bees act as conditional parasites (Fig. 6.3e).

6.4.5. Grooming behavior

The function determining pollen deposition does not substantially change the shape of the delivery surface or whether bee visits contribute positively or negatively to total pollen delivery (a positive or negative $\Delta G$) (compare Fig. 6.3a and 6.4a). For both representative delivery surfaces (Fig. 6.3a and 6.4a), pollen delivery increases with number of visits for both bees and flies, and the contribution of bee visits is positive. Bee visits contribute positively in all visits even when the removal coefficient is increased (compare Fig. 6.4a and 6.4b) and when sex-phase visitation bias is removed (compare Fig. 6.4a and 6.4d). Some bee visits have a negative contribution to total pollen delivery (a negative $\Delta G$) when the attenuation coefficient is decreased (compare Fig. 6.4a and 6.4c). As in the representative delivery surface with proportional deposition, sequential pollen dehiscence results in a lower total pollen delivery ($G$) than simultaneous pollen dehiscence for all combinations of visits. Some bee visits contribute negatively to total pollen delivery (a negative $\Delta G$) when anthers dehisce simultaneously, but all bee visits have a positive contribution (a positive $\Delta G$) when anthers dehisce sequentially.

6.5. Discussion

The value of a pollinator visit depends on the ecological context, especially the diversity and abundance of other members of the pollinator assemblage. When multiple species visit alongside one another, the
amount of pollen available affects how each individual removes and deposits grains; by removing some grains from the available pool, each individual in turn affects removal and deposition by future floral visitors. The simulation models presented here demonstrate the variety of ways in which pollinator species can interact in interesting ways within a context of changing pollen availability and variable plant and pollinator characteristics. Wasteful pollinator visits may actually depress pollen delivery because the wasteful species intercepts pollen that would otherwise have been delivered; our simulations predict that this extreme case is possible, and even likely, in particular realistic ecological contexts.

These simulations document changing rates of pollen delivery as pollinators interact with a supply of available pollen; observing these fluctuations through simulations should affect the way that we think about pollinator value to plants. Within the literature, an assortment of terms and measurements describe a consistent and characteristic value of a pollinator species to a plant species. Researchers usually describe the value of a pollinator to a plant as a species-specific constant (as reviewed by Ne’eman et al. 2010). Studies document a floral visitor species’ visitation rate, its “effectiveness” – its contribution to plant reproductive success independent of its visitation rate and its “efficiency” – its contribution to plant reproductive success in relation to the resources spent by the plant (as reviewed by Ne’eman et al. 2010). In the context of the delivery surfaces presented here, these terms are not relevant. We cannot describe *A. erigeniae* as more effective than *B. major*, or *B. major* as more efficient than *A. erigeniae*, because their value can only be calculated in the context of the particular visit. The value of a pollinator to a plant is not a single parameter but instead a complex, context-specific function of numerous parameters (Thomson 2003). Therefore, we need to conceptualize plant-pollinator relationships as context-dependent, and work to improve measurements of pollinator value to plants to reflect the importance of the ecological context.

### 6.5.1. Conditional parasitism in *Claytonia virginica*

The *C. virginica* representative delivery surface (Fig. 6.3a) predicts that in *Claytonia virginica* natural systems, *A. erigeniae* visits always increase pollen delivery and do not act as conditional parasites for all visitor combinations of less than ten visits by each pollinator. However, small changes in the characteristics of plants and pollinators would potentially result in conditional parasitism (Table 6.2). In our simulations, bees act as conditional parasites in three ecological contexts: when the number of visits by bees and flies is high and the removal coefficient is higher than the empirical value (Fig. 6.3b), when the number of visits by bees and flies is high and the deposition coefficient is lower than the empirical value (Fig. 6.3c, Fig. 6.4c), and when the number of visits by bees and flies is high and anthers dehisce simultaneously (Fig. 6.3e, Fig. 6.4e).

Models using a deposition function with an attenuation coefficient (Fig. 6.4), that simulates the loss of pollen function that occurs because of bee grooming, behaved very similarly to the models that used a proportional deposition function (Fig. 6.3). This similarity indicates that grooming behavior may not have
an important effect on pollen delivery or conditional parasitism, and that the distinction between active and inactive grains may not be especially meaningful. Though surprising, this result is good news for researchers, especially considering that we know very little about the grains that end up in each category and the role of pollinator biology in determining pollen function. Because corbiculate bees inactivate grains through grooming and provisioning (Chapter 5 of this thesis), this result blurs one distinction between corbiculate and non-corbiculate bees.

Overall, the pollen delivery surfaces emphasize the importance of visitation rate. In all cases of conditional parasitism, these bee visits were accompanied by high visitation by flies. Therefore, we should look closely at the contribution of bee visits in systems with pollinator communities that have high visitation rates by flies or other passive pollen-collectors.

6.5.2. Sex-phase bias

Simulations with sex-phase biases by *A. erigeniae* and *B. major* did have lower total pollen delivery, but the decrease was not substantial; total pollen delivery by ten bees and ten flies was only 24 grains lower when they foraged in a biased manner (compare Fig. 6.3a and 6.3d, and 6.4a and 6.4d). While sex-phase visitation bias may affect the number of visits to male and female-phase flowers, it does not appear to have an important affect on total pollen delivery independent of the visitation rate.

6.5.3. Pollen presentation schedule

The pollen presentation schedule had a substantial effect on the pollen delivery surface (compare Fig. 6.3a and 6.3e, and Fig. 6.4a and 6.4e). With sequential dehiscence of anthers, the contribution of a bee visit to total pollen delivery was always positive (a positive $\Delta G$), but with simultaneous dehiscence, some bee visits actually lowered total pollen delivery (a negative $\Delta G$) when fly visitation was high. When all pollen was available simultaneously, bees removed more pollen in initial visits, which lowered the availability of pollen and decreased pollen transfer by future bees and flies. In the context of high visitation by flies, bee visits decreased total pollen delivery, acting as conditional parasites. These results correspond with Harder and Thomson’s (1989) result that pollen packaging reduces pollen wastefulness and increases the number of pollinators that export and deliver pollen grains. In our models, pollen packaging does increase pollen delivery for a few bee visits.

However, initial bee visits and all fly visits delivered a higher quantity of pollen when pollen was available simultaneously rather than sequentially (compare Fig. 6.3a and 6.3e, and Fig. 6.4a and 6.4e). In our simulations, this higher delivery was enough to outweigh the negative effect of the conditionally parasitic bees; when anthers dehisced simultaneously, total pollen delivery was higher for all combinations of bee and fly visits. For example, total pollen delivery by ten flies was 172 grains higher with simultaneous dehiscence than with sequential dehiscence, and total pollen delivery by ten bees and ten flies was 56 grains higher. This result contrasts with the conclusions of a pollen depletion model by Harder and
Thomson (1989), who found that pollen packaging increased pollen delivery by the corbiculate bee Bombus occidentalis. This difference in results is partially explained by the inclusion of the fly B. major in our models, a less wasteful pollinator that deposits more grains when more are available; we expected pollen delivery by passive pollen collectors to be higher with simultaneous dehiscence. However, even when we compare pollen delivery by only bees between simultaneous and sequential dehiscence (the curve along the right side of the pollen delivery surfaces in Fig. 6.3a and 6.3e, and Fig. 6.4a and 6.4e), we see that total pollen delivery with simultaneous dehiscence is also higher for all numbers of bee visits. Initial bee visits delivered a higher quantity of grains when anthers dehisced simultaneously, and this quantity was enough to outweigh the negative effect of these visits. This is true regardless of the function determining pollen deposition; total pollen delivery by bees was higher overall with simultaneous dehiscence when the function determining bee deposition was proportional and decelerating. The results of our model do not support the idea that sequential dehiscence is an adaptive strategy employed by plants to increase pollen delivery.

6.5.4. Applicability to other systems

Small changes in the functions and coefficients of the C. virginica model result in bee visits having a negative $\Delta G$ when fly visitation is high (Fig. 6.3b and 6.3c, Fig. 6.4b and 6.4c); this result suggests that there are other systems in which bee visits are lowering total pollen delivery. In the C. virginica empirical data, bees remove an average of 61% of pollen available. Our models show that if that average was greater than 70%, bee visits would lower total pollen delivery when fly visitation is high. There are many empirical examples of bee removal rates exceeding 70%: Harder and Thomson (1989), Larsson (2005), and Patton (1993) calculated that bees remove 87.9%, 62 ± 34%, and 87% of available pollen in their respective systems. The simultaneous dehiscence of anthers is probably also fairly common. Floral pollen packaging is not well-studied, so it is unclear how many plants dehisce their anthers sequentially or employ other pollen packaging strategies, but Percival (1955) found that 29 of the 81 Welsh plants that she surveyed exhibited simultaneous anther dehiscence.

6.5.5. Future directions

The conclusions drawn from the models presented here are best followed up with more empirical data in different ecological contexts. These simulations consider only two different pollinators; many pollinator communities, including that of C. virginica, contain many more. Extending our delivery surfaces to include all of the visiting pollinators will increase the complexity of the conclusions, but also increase our understanding of how pollinators interact in real systems. Moreover, the simulations presented here only address quantitative aspects of pollen grains, and only in the context of a single-flowered plant. The identity and quality of pollen grains delivered are very important aspects of plant reproduction; spatial factors of pollen transfer will be especially important in multi-flowered plants, because pollen transferred within the same plant individual is likely to reduce that plant's female and male fitness.
These models have identified factors that are likely to be especially important in determining pollen delivery in plants. To move forward, we can identify systems in which bee visits may have a negative contribution to total pollen delivery, use the model to make predictions, and then look at pollen delivery experimentally. Systems that are likely to experience a conditional parasitism by bees will have 1) high visitation rates by an actively pollen-collecting bee and an alternative – likely nectar-collecting – pollinator, 2) a high removal coefficient by the bee, 3) a low removal coefficient by an alternative pollinator, and 4) simultaneous dehiscence.

6.6. Tables
Table 6.1. Description of model parameters and variables.

### a) The representative *C. virginica* delivery surface

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_0 = ) 276.4</td>
<td>The number of pollen grains initially available in a male-phase flower</td>
</tr>
<tr>
<td>( s = ) 276.4</td>
<td>The number of pollen grains added to pollen availability during pollinator visitation</td>
</tr>
<tr>
<td>( k_b = ) 0.61</td>
<td>The proportion of pollen grains removed by a bee in a single visit to a male-phase flower</td>
</tr>
<tr>
<td>( k_f = ) 0.24</td>
<td>The proportion of pollen grains removed by a fly in a single visit to a male-phase flower</td>
</tr>
<tr>
<td>( d_s = ) 0.016</td>
<td>The proportion of pollen grains on the bee body that are deposited in a single visit to a female-phase flower</td>
</tr>
<tr>
<td>( g = ) 0.47</td>
<td>The exponent determining the number of pollen grains on the bee body that are deposited in a single visit</td>
</tr>
<tr>
<td>( d_f = ) 0.045</td>
<td>The proportion of pollen grains on the fly body that are deposited in a single visit</td>
</tr>
<tr>
<td>( l = ) 0.15</td>
<td>The proportion of pollen grains that are lost to the environment after each visit to a male-phase flower</td>
</tr>
</tbody>
</table>

### b) Variations on the representative delivery surface

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_0 = ) 2764</td>
<td>The number of pollen grains initially available in a male-phase flower</td>
</tr>
<tr>
<td>( k_b = ) 0.8</td>
<td>The proportion of pollen grains removed by a bee in a single visit to a male-phase flower</td>
</tr>
<tr>
<td>( d_s = ) 0.01</td>
<td>The proportion of pollen grains on the bee body that are deposited in a single visit to a female-phase flower</td>
</tr>
</tbody>
</table>

### c) Variables for all models

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A )</td>
<td>The number of pollen grains available in a male-phase flower</td>
</tr>
<tr>
<td>( B_b )</td>
<td>The number of pollen grains on a bee body</td>
</tr>
<tr>
<td>( B_f )</td>
<td>The number of pollen grains on a fly body</td>
</tr>
<tr>
<td>( R )</td>
<td>The number of pollen grains removed in a single visit to a male-phase flower</td>
</tr>
<tr>
<td>( D )</td>
<td>The number of pollen grains deposited in a single visit to a female-phase flower</td>
</tr>
<tr>
<td>( G )</td>
<td>Total pollen delivery</td>
</tr>
<tr>
<td>Topic</td>
<td>Comparison</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------------------------------------------------------</td>
</tr>
<tr>
<td>Pollen collection</td>
<td>Empirical pollen removal coefficient (.5)</td>
</tr>
<tr>
<td></td>
<td>Increased pollen removal coefficient (.7)</td>
</tr>
<tr>
<td>Pollen deposition</td>
<td>Empirical pollen deposition coefficient (0.027)</td>
</tr>
<tr>
<td></td>
<td>Decreased pollen deposition coefficient (0.01)</td>
</tr>
<tr>
<td>Sex-phase bias</td>
<td>Empirical sex-phase visitation bias</td>
</tr>
<tr>
<td></td>
<td>No sex-phase visitation bias</td>
</tr>
<tr>
<td>Pollen presentation schedule</td>
<td>Empirical pollen presentation schedule (sequential)</td>
</tr>
<tr>
<td></td>
<td>Simultaneous pollen presentation</td>
</tr>
</tbody>
</table>
6.7. Figures

![Figure 6.1](image-url)

**Figure 6.1.** A delivery surface representing the contribution of pollinator visits to total pollen delivery.

Each of the horizontal axes represents the number of visits by one species of floral visitor, while the vertical axis records the expected number of grains exported from a focal flower receiving that combination of visits. The slope of the line between two points on the delivery surface indicates the sign of the interaction.

Visits by pollinator B contribute negatively to overall pollen delivery ($\Delta G$ is negative). As the number of visits by pollinator B increases, the number of pollen grains delivered decreases.

Visits by pollinator B contribute positively to pollen delivery ($\Delta G$ is negative). As the number of visits by pollinator B increases, the number of pollen grains delivered increases.
Pollen availability in male-phase flower

\[ A_n \]

Simultaneous dehiscence
Number of grains available is what is left after previous visits

\[ A_n = A_{n-1} + s \]

Sequential dehiscence
Number of grains available is what is left after previous visits. Additional pollen becomes available periodically during the visitation period

Visitor visits male-phase flower
Visitor removes pollen. Number of grains removed is a proportion of the available grains.

\[ R = k_b \cdot A \]

\[ B_{b1} = B_{b1} + R \]
Number of grains removed is added to the number of grains already on the visitor's body

Simultaneous pollen delivery

\[ A_{n+1} = A_n - R \]
Number of grains removed is subtracted from the number of available grains

Visitor visits female-phase flower
Visitor deposits pollen. Number of grains deposited is a proportion of the pollen on the visitor's body.

\[ D = B_{b3} g \]

Proportional deposition
Visitor deposits pollen. Number of grains deposited is the number of grains on the visitor's body raised to the exponent g

\[ B_{b4} = B_{b3} - D \]
Number of grains deposited is subtracted from the number of grains already on the visitor's body

Sex-phase visitation bias
Multiple male-phase visits in between female-phase visits

\[ G_n = G_{n-1} + D \]
Deposited grains are added to grains already deposited on the flower during previous visits

Decelerating deposition
Visitor deposits pollen. Number of grains deposited is the number of grains on the visitor's body raised to the exponent g
Figure 6.2. Schematic outlining the structure of simulation models.

A floral visitor visits male- and female-phase flowers and removes and deposits pollen according to removal and deposition functions. Pollen grains on male-phase flowers are replenished by anther dehiscence and depleted by pollinator visitation, while pollen grains accumulate on female-phase flowers.
Figure 6.3. Delivery surfaces with proportional deposition.

Factors of the *Claytonia virginica* interaction affect the contribution of bee visits to total pollen transfer and change the shape of the delivery surface. a) The *Claytonia virginica* representative delivery surface, including removal and deposition coefficients derived from empirical values, sex-visitation bias, and sequential dehiscence, b) The delivery surface, as in (a) but with a removal coefficient of .8, c) The delivery surface as in (a) but with a deposition coefficient of .01, d) The delivery surface as in (a) but with no sex visitation bias, e) The delivery surface as in (a) but with simultaneous dehiscence.
Figure 6.4. Delivery surfaces with decelerating deposition.

Factors of the *Claytonia virginica* interaction affect the contribution of bee visits to total pollen transfer and change the shape of the delivery surface. a) The *Claytonia virginica* representative delivery surface, including removal and deposition coefficients derived from empirical values, sex-visitation bias, and sequential dehiscence, b) The delivery surface as in (a) but with a removal coefficient of .8, c) The delivery surface as in (a) with an attenuation coefficient of .4., d) The delivery surface as in (a) but with no sex-phase visitation bias, e) The delivery surface as in (a) but with simultaneous dehiscence.
7. Concluding Discussion

This thesis compares pollinator groups in their importance in pollen delivery and plant male fitness. I show that some rarely-examined details of pollinator biology – such as the specifics of grooming behavior – can have very important implications for pollinator value to plants. In each study, I highlight the contribution of fly pollination and bolster the reputation of non-corbiculate bee pollinators. Above all, this thesis emphasizes the importance of context-dependence in pollinator value. These studies indicate that it is possible for a pollinator to have a negative contribution to pollen delivery, and that conditional parasitism is likely to occur in some natural plant-pollinator systems.

7.1. Major findings

7.1.1. The **Claytonia virginica** system

The collection of studies here explores the implications of pollination variation in the spring ephemeral wildflower *Claytonia virginica*. The oligolectic bee *Andrena erigeniae* and the bombyliid fly *Bombylius major* exhibit extensive variation in their visitation rate across the geographic range, which may translate into extensive variation in their value as *C. virginica* pollinators.

Overall, it seems as though *A. erigeniae* acts as a mutualistic partner, contributing positively to pollen delivery in most ecological contexts. In Chapter 2, it appears that despite causing substantial pollen depletion, *A. erigeniae* is important for *C. virginica* pollen export and delivery because visitation rates of the other floral visitors are not nearly as high. In Chapter 6, I explore those ecological contexts that will result in conditional parasitism: I discover that while many of these ecological contexts are probably realistic for plant-pollinator systems in general, they are not realistic for *C. virginica* populations. For example, conditional parasitism is made more likely by simultaneous dehiscence of anthers, and in my experience, all *C. virginica* populations present pollen sequentially. Most strikingly, conditional parasitism appears to occur only with high visitation by flies: at least 6 visits per flower, and often 8 to 10, over the lifetime of that flower. In Chapter 3 and 4, I document extensive variation in pollinator communities, including high visitation rates by *B. major*; however, these visitation rates are not high enough in any location to render *A. erigeniae* consistently parasitic.

7.1.2. Selection to mitigate pollinator wastefulness

While *A. erigeniae* may not be conditionally parasitic in the populations that I studied, there could still be selection on plants to mitigate *A. erigeniae* pollen wastefulness and increase pollen export and delivery; selection will occur when pollen delivery affects plant male or female fitness. Some floral traits will affect pollen delivery in all pollinator climates; for example, floral traits that increase *B. major* visitation should increase pollen delivery for all *C. virginica* individuals. In Chapter 2, I document removal and deposition rates by *B. major*, the less wasteful pollinator, and conclude that increased visitation by *B. major* should
always increase pollen delivery with minimal cost. However, throughout my time studying *C. virginica* populations in nature, I did not observe any floral traits that would attract *B. major* but not *A. erigeniae*.

Other floral traits may affect pollen delivery depending on the pollinator climate. If floral traits affect pollen delivery by only one member of the pollinator assemblage – or, if floral traits affect pollen delivery differently for each member of the pollinator assemblage – the abundance of that pollinator will determine the strength or the direction of selection. Increased pollen production may increase pollen delivery when a wasteful pollinator is abundant; in Chapter 4, I document higher pollen production in Northern populations of *C. virginica*, where *A. erigeniae* is the dominant pollinator. Rapid pollen presentation may increase pollen delivery when higher availability leads directly to higher delivery, such as when a passive pollen collector is abundant; in Chapter 4, I document more rapid pollen presentation in Southern populations of *C. virginica*, where there is low visitation overall and *B. major* is more abundant. These traits are certainly not the only ones that may be under selection to increase pollen delivery in each of these pollinator climates; future research should explore additional possibilities.

### 7.1.3. Grooming behavior

Grooming by bees affects the relationship between pollen that is removed and pollen that is subsequently delivered, which has important implications for conditional parasitism and pollinator value to plants. The chapters in this thesis provide some information on the relationship between removal and delivery for *A. erigeniae*, though ultimately we do not know how this species’ interactions with pollen grains affect its value to *C. virginica*. Two possibilities are the most likely: (a) delivery by *A. erigeniae* is an increasing but decelerating function of pollen removal, or (b) delivery by *A. erigeniae* is a proportional function of pollen removal. Previous work supports option (a), and Chapter 5 supports option (b).

We assume flies (and other passive pollen collectors) have a proportional relationship between removal and deposition because they do not actively groom pollen; the higher the number of grains removed, the higher the number that are delivered. We share this assumption with Castellanos et al. (2006). Previous work (Harder and Thomson 1989) documented that the bumble bee *Bombus occidentalis* visiting *Erythronium grandiflorum* is an increasing but decelerating function of pollen removal because high pollen removal triggers bee grooming. The *Bombus*-*Erythronium* relationship with pollen is exponential; the higher the number of grains on the bee body, the larger the proportion that is distinctly “wasted” and the lower the proportion that is delivered. If *A. erigeniae* and *B. occidentalis* interact with pollen in a similar way, then *A. erigeniae*’s interactions with pollen likely contrast with *B. major*’s. This hypothesized difference between *A. erigeniae* and *B. major* would further support the results of Chapter 4, where I document significant variation in floral traits that would increase pollen delivery according to differences between the pollinator groups.

In Chapter 5, I document impaired pollen function for groomed *B. rapa* pollen on corbiculate bee bodies (*Bombus impatiens* and *Apis mellifera*), and intact pollen function for groomed pollen on non-corbiculate bee...
bodies (*Megachile rotundata* and *Halictus* spp.). In corbiculate bees, pollen appears to be more discretely classified as functional (ungroomed pollen) or not functional (groomed pollen), while the pollen on non-corbiculate bees appears to retain functionality regardless of grooming. The results of Chapter 5 support option (b) for non-corbiculate bees, because while high removal may still trigger bee grooming, these grains retain function and therefore are not so strongly fated to be “wasted.”

7.1.4. The pollen presentation schedule

In Chapter 4, I document evidence that the schedule of pollen presentation varies geographically according to the pollinator climate; pollen is presented more slowly in the bee-heavy North than in the fly-heavy South. However, in Chapter 6, when we compare the pollen delivery surface between simultaneous and sequential dehiscence, pollen delivery is higher for all combinations of bee and fly visits when pollen is presented simultaneously, rather than sequentially (Chapter 6, compare Figures 3a and 3d). Chapter 4 supports the hypothesis that more pollen packaging may be an adaptive response to pollinator wastefulness, but Chapter 6 supports the opposite hypothesis, that less pollen packaging will increase pollen export and delivery. More research is needed to investigate these hypotheses.

7.2. Implications and contributions

7.2.1. Solitary bee and fly pollination

This thesis bolsters the reputation of two groups of under-appreciated pollinators, solitary bees and flies. The results of Chapter 4 provide evidence that while corbiculate, mainly eusocial bees impair pollen function by grooming pollen into corbiculate, non-corbiculate bees leave pollen grains intact. This demonstrates the value of non-corbiculate bees, which are mainly solitary, because pollen grains remain available for pollination and are less likely to be wasted.

The results of Chapter 2 demonstrate that in the *C. virginica* system, bombyliid flies deposit almost as many grains in a single visit as bees; moreover, these flies do not remove nearly as many grains. Therefore, fly pollinators contribute positively to *C. virginica* reproductive success with minimal cost. In Chapter 6, I describe some situations in which oligolectic bees may contribute negatively to pollen delivery, yet visits by flies always contribute positively. These findings supplement a growing literature on the importance of fly pollination (Kearns 2001, Ssymank et al. 2010, Woodcock et al. 2014). The benefits of fly pollination extend beyond quantitative pollen delivery; *Brassica rapa* pollen on fly bodies is more diverse and travels farther than *B. rapa* pollen on bee bodies (J. Ison, A. Parker, and A. Weis unpublished manuscript).

These chapters are valuable contributions to the current consensus that native pollinators – including the many solitary native bees and a variety of native flies – play a very important role in both natural and

### 7.2.2. The context-dependence of pollinator value

The classic view of a plant-pollinator relationship involves one pollinator species contributing to the reproduction of one plant species by transferring pollen between plant individuals in return for a reward. Our current understanding of pollinator value to plants reflects this view. Within the literature, an assortment of terms and measurements describe a consistent and characteristic value of a pollinator species to a plant species; the value of a pollinator to a plant is often viewed as a species-specific constant (as reviewed by Ne’eman et al. 2010). Studies document a floral visitor species’ visitation rate, its “effectiveness” – its contribution to plant reproductive success independent of its visitation rate, its “efficiency” – its contribution to plant reproductive success in relation to the resources spent by the plant, and its “importance” – the overall effect of a pollinator species on plant reproduction (as reviewed by Ne'eman et al. 2010).

This thesis supports the contrasting viewpoint, that most pollinators are visiting flowers as members of a multi-species assemblage of multiple foraging species interacting within an environment of fluctuating conditions and rewards (Waser et al. 1996, Thomson 2003). In single visit measurements of pollen delivery, ecological factors are controlled such that the ecological milieu does not interfere in pollen transfer. However, in nature, pollinator visitation usually occurs within a pollinator climate with a number of visits by individuals of more than one species. These chapters emphasize the difference between these ideal and constrained scenarios; the amount of pollen a pollinator *can* deliver is much higher than the amount of pollen that a pollinator *does* deliver when outside factors interfere. In Chapter 6 especially, the value of a pollinator to a plant is not a single parameter but instead a complex, context-specific function of numerous parameters, as proposed by Thomson (2003). For example, a pollinator that forages in a pollen-rich environment is more likely to act as a valuable pollinator by accumulating pollen loads on its body and inadvertently distributing that pollen to other flowers; a pollinator that encounters flowers with depleted pollen will not contribute as much to pollen transfer.

Figure 7.1 of this discussion is an example of how treating removal and deposition as a species-specific constant or as a function can drastically change total pollen delivery. Fig. 6.1a shows a hypothetical pollen delivery surface when pollen deposition is a species-specific constant and increases linearly with visit number. Fig. 6.1b shows a hypothetical pollen delivery surface that demonstrates pollen delivery when pollen deposition is a function of pollen availability (such as those presented in Chapter 6; in this case, the number of grains deposited in each visit is a proportion of pollen availability). When pollen delivery is a function of availability, pollen delivery may increase with visit number - but not linearly - or may actually decrease it.
Pollen delivery is context-specific, and depends on more factors than just species-specific pollinator characteristics.

### 7.3. Directions for future research

The results of this thesis could be evaluated and further developed with a better understanding of how pollinator interactions with pollen varies among pollinator groups. Harder and Thomson’s (1989) work documenting the relationship between removal and deposition for *Bombus occidentalis* visiting *Erythronium grandiflorum* should be repeated in other pollinator groups such as primarily nectar-collecting flies and butterflies and non-corbiculate bees, especially oligolectic bees. The range of options certainly extends beyond those considered here, and the nature of this relationship has interesting and important implications for pollinator value to plants.

A logical next step is experimental work. Experimenters can manipulate pollinator visitation and plant and pollinator characteristics to recreate the ecological contexts modeled in Chapter 6. This would be best accomplished with a highly rewarding plant species that would encourage visitation by both active and passive pollen collectors; this plant species should have a large stigma to accommodate high variation in pollen delivery. Pollinator visitation may be manipulated using flight cages or by encouraging or discouraging pollinator visitation in the field, and plant traits may be manipulated by gluing anthers or by supplementing or removing pollen in anthers. Pollinator traits are more difficult to manipulate and will require some experimental ingenuity. Hargreaves and Harder (2010) compared pollen delivery in large flight cages of sunbirds visiting *Aloe maculata* with and without *Apis mellifera*; they found that *A. mellifera* decreased total pollen delivery. Including more variation in this kind of experimental setup will greatly add to our understanding of how the ecological context affects pollinator value to plants.

This thesis provides information on those plant and pollinator characteristics that may make conditional parasitism more likely. The next step is to look for plant-pollinator relationships that exhibit these characteristics, model pollen delivery using removal and deposition functions, and then perform experiments to solidify the nature of their relationship. Conditional parasitism is most likely when there is high visitation by at least one active and a passive pollen collector; I would look for systems with at least 8 visits by each pollinator over the course of a flower's lifetime. The pollen presentation schedule of these flowers will be simultaneous or relatively rapid, and the active pollen collector will remove more than 70% of pollen grains in each visit.

### 7.4. Conclusion

The studies presented here form a significant contribution to the field of pollinator comparisons; they contribute to a growing view that the value of pollinator species are affected not only by characteristics of the pollinator species, but also by the ecological context in which they visit. These studies provide a starting point for future observations of conditional parasitism in plant-pollinator relationships.
Moreover, they emphasize the importance of basic research in bee biology, including the specifics of bee behavior and their interactions with pollen grains. Finally, I demonstrate some under-appreciated benefits of fly and non-corbicate bee pollination.
Figure 7.1. Pollen delivery surfaces: additive and proportional.

Treating removal and deposition as functions of pollen availability changes the shape of the delivery surface. a) Deposition is a constant. When bees and flies deposit the same number of grains in each visit, delivery in each visit is equivalent and additive. b) Deposition is a function of pollen availability. When the number of grains removed and deposited changes with availability, the contribution of an individual visit decreases with increasing visits.
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Appendices

Appendix A. *Claytonia virginica* floral visitors

**Table A.1.** The makeup of *C. virginica* floral visitors, arranged in groups.

*Bolded genera and species are common visitors.*

<table>
<thead>
<tr>
<th>Group</th>
<th>Species included</th>
<th>Reward collected</th>
<th>Frequency of visitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombyliid flies</td>
<td><em>Bombylus major</em></td>
<td>Nectar</td>
<td>frequent</td>
</tr>
<tr>
<td>Small solitary bees</td>
<td><em>Ceratina spp., Lasioglossum spp., Hylaeus spp.</em></td>
<td>pollen and nectar</td>
<td>frequent</td>
</tr>
<tr>
<td>Medium solitary bees</td>
<td><em>Halictus spp., Andrena spp., Osmia spp.</em></td>
<td>pollen and nectar</td>
<td>infrequent</td>
</tr>
<tr>
<td></td>
<td><em>Augoclora spp., Augochlorella spp.</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Syrphid and Tachinid flies</td>
<td><em>Toxomerus marginatus, Melanostoma sp., Gonia sp.</em></td>
<td>pollen and nectar</td>
<td>rare</td>
</tr>
<tr>
<td>Cleptoarasitic* bees</td>
<td><em>Nomada spp., Sphecodes spp.</em></td>
<td>Nectar</td>
<td>frequent</td>
</tr>
<tr>
<td>Oligolectic females</td>
<td><em>Andrena erigeniae</em></td>
<td>pollen and nectar</td>
<td>frequent</td>
</tr>
<tr>
<td>Males of Oligolectic species</td>
<td><em>Andrena erigeniae</em></td>
<td>nectar</td>
<td>infrequent</td>
</tr>
</tbody>
</table>

*Cleptoparasitic bees lay their eggs on pollen masses collected by other bees, and therefore do not collect pollen of their own.*
Appendix B. Does the order of pollinator visitation affect pollinator value to plants?

We investigated pollinator value to plants by measuring pollen delivery in two different pollinator climates: 1) in the traditional way, by measuring “single visit pollen delivery” – the number of grains on a stigma after a single visit by a pollinator to an unvisited flower, and 2) within a context of high visitation by multiple pollinators, by measuring overall pollen transfer by individuals of two pollinator species visiting groups of plant individuals. We experimentally manipulated the context of visitation by comparing two treatments of high visitation by multiple pollinators, one in which pollen-collecting bees visit prior to nectar-collecting butterflies and another in which nectar-collecting butterflies visit prior to pollen-collecting bees.

B.1. Methods

We conducted this study in the summer of 2012 at the University of Toronto’s Koffler Scientific Reserve in King City, ON (44.01.48 N, 79.32.01 W). Using flight cages, we controlled visitation by pollinators to groups of plants. We compared pollen delivery for single flowers visited once by an individual pollinator (single visits) and pollen delivery for multiple flowers by two pollinator species and experimentally varied the order of visitation.

*Brassica rapa* (Brassicaceae) is a weedy, self-incompatible, annual herb. Native to Eurasia, *B. rapa* is a naturalized weed common in the US and Canada. *Brassica rapa* is hermaphroditic, and has many ovules per flower. We grew our plants from seed harvested from populations of *B. rapa* in Quebec, Canada. We chose *B. rapa* because it is a highly generalist plant that is visited by many pollinators; of those pollinators, we chose two common pollinators, the bumble bee *Bombus impatiens* and the butterfly *Pieris rapae*. Both pollinators occur locally at KSR and are known to visit naturalized *B. rapa* populations. We obtained captive-reared colonies of the bumble bee *B. impatiens* from Biobest Canada Ltd. (Leamington, Ontario, Canada) and captive-reared larvae of the cabbage white butterfly *Pieris rapae* from Carolina Biological Supply Company (Burlington, North Carolina, USA). We reared butterfly larvae on leaves from *B. rapa* and organic cabbage. We supplemented butterflies with individuals caught at Koffler Scientific Reserve during the study period.

For both single visit and multiple visit treatments, we prepared plants two days before each trial. To ensure that all flowers were previously unvisited, we cut off all open flowers and placed the plants in a pollinator-exclusion cage overnight. On the day of the trial, when flowers had opened and anthers were completely dehisced, we cut off all open flowers except for one to maintain consistency between the plants. After each trial, we marked all open flowers with dental floss and a label and returned plants to the pollinator exclusion cage to allow pollen to adhere to stigmas. We collected stigmas from the flowers 24...
hours later into microcentrifuge tubes containing 1.00 mL of 70% ethanol, and removed plants from the pollinator exclusion structure.

Before each trial, we prepared twenty plants and moved them into a flight cage. We then released individuals of one of the two pollinators (bumble bees or butterflies) into the cage. We introduced one more plant to the cage, allowed a single visit to the flower on that plant, and then immediately removed that plant from the cage and returned it to the pollinator exclusion structure.

We conducted ten experimental trials with two treatments each. One treatment allowed visitation by the bumble bee *B. impatiens* first and then the butterfly *P. rapae* (“bumble bee first”), and the other allowed visitation by the butterfly *P. rapae* and then the bumblebee *B. impatiens* (“butterfly first”). Before each trial, we prepared twenty plants and moved them into a flight cage. We then released individuals of one of the two pollinator species into the cage, counted the total number of visits, and then collected the pollinators. We then repeated the protocol with the other pollinator species. We initially released 3 individuals of each pollinator for each trial; however, often one or more of the individuals failed to visit flowers, so we released an additional one or two individuals to allow the trial to continue. For each trial, we allowed a total 85 visits by each pollinator group. Because collecting the pollinators at the end of the trial took some time, we were sometimes unable to prevent a few extra visits; however, each trial had a total of between 85 and 90 visits by each pollinator.

All statistical analyses were done using R 3.0.1 (R Core Team 2013). We used generalized linear mixed models (GLMMs) to compare pollen delivery between treatments. We used the function `glmmADMB` in the library `glmmADMB` (Fournier et al. 2012) because it allowed us to include random effects and account for overdispersion using a negative binomial distribution. To compare pollen delivery between multiple visit treatments, the predictor variable was the treatment (bumble bee first or butterfly first) and the response variable was the number of pollen grains delivered, at the level of the individual stigma. We accounted for multiple stigmas per trial by including the trial as a random effect. Because weather and pollinator behavior can be variable across days, we also included date as a random effect. In order to determine if the number of bumble bee visits, the number of butterfly visits, or the number of total visits had an effect on pollen delivery, we compared models that included each of these as fixed effects; none had a significant effect on the model fit, so we removed them from the final model. To ensure that extra visits beyond 85 did not confound our results, we fit a linear model to the relationship between the number of visits and the number of pollen grains delivered. For these analyses, the number of visits (butterfly, bumble bee, and total) was the predictor variable and the number of pollen grains in an open flower was the response variable. For linear models on individual days we used the `lm` function in the library `stats` (R Core Team 2013).
B.2. Results

In a single visit, bumble bee individuals visiting *B. rapa* delivered significantly more pollen (390.5±236 grains; mean ± SD) pollen grains than butterfly individuals (143±215 grains, Fig. B.1a, GLMM, Z = -3.56, P= 0.00037).

Within the multiple visit treatment, there was no significant affect of visitation order on pollen delivery (Fig B.1b). In total, we were able to include 12 replicates of the bumble bee first treatment (for a total of 120 stigmas), and 14 replicates of the butterfly first treatment (for a total of 141 stigmas). The number of pollen grains delivered per stigma in the bumble bee first treatment (426.2±281.48, mean±SD) was higher than the number of pollen grains delivered per stigma in the butterfly first treatment (378.86±189.87), but not significantly so (GLMM, Z=-1.47, P=0.14). When we combined 9 stigmas from each trial to estimate an effect of the treatment on total pollen delivery per trial, pollen delivery in the bumble bee first treatment (3826.5±997.41) was only slightly higher than pollen delivery in the butterfly first treatment (3514.93±483.98). The variance in total pollen delivery in the bumble bee first trials was much higher than the variance in the total pollen delivery in the butterfly first trials.

We found no significant relationship between the number of visits and pollen delivery to each stigma. The number of bumble bee visits had a very slightly positive relationship to the number of grains delivered per stigma, but not significantly so ($r^2= -0.003$, P= 0.755). The number of butterfly visits had a negative relationship to the number of grains delivered per stigma, but not significantly so ($r^2= 0.006$, P= 0.11). The number of total visits also had a negative relationship to the number of grains delivered per stigma, but not significantly so ($r^2= -0.001$, P= 0.41).
Figure B.1 Boxplots of pollen delivery per stigma after a single visit from a single pollinator individual.

The solid line indicates the median value, boxes indicate quartiles, solid black circles indicate mean values, and small black dots represent individual samples. Bumble bee individuals deliver significantly more grains than butterfly individuals.
Figure B.2 Boxplots of pollen delivery per stigma after multiple visits by two pollinator species.

The solid line indicates the median value, boxes indicate quartiles, solid black circles indicate mean values, and small black dots represent individual samples. The order of visitation by these pollinators does not affect pollen delivery; bumble bee and butterfly first treatments deliver similar numbers of grains.
Appendix C. Citizen Science materials

C.1. Website

www.springbeauties.wordpress.com
C.2. Bee and fly identification guide

<table>
<thead>
<tr>
<th></th>
<th><strong>Bees</strong></th>
<th><strong>Flies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Wings</strong></td>
<td><strong>Wings</strong></td>
</tr>
<tr>
<td></td>
<td>When at rest, bee wings are folded into their bodies</td>
<td>When at rest, fly wings are out or resting at an angle</td>
</tr>
<tr>
<td></td>
<td>Bees have two sets of wings</td>
<td>Flies have one set of wings</td>
</tr>
<tr>
<td></td>
<td><strong>Eyes</strong></td>
<td><strong>Eyes</strong></td>
</tr>
<tr>
<td></td>
<td>Bees’ eyes are more on the side of the head</td>
<td>Flies’ eyes are more on the front of the head, and generally larger</td>
</tr>
<tr>
<td></td>
<td><strong>Pollen</strong></td>
<td><strong>Pollen</strong></td>
</tr>
<tr>
<td></td>
<td>Bees (females) are often (but not always) carrying a lot of pollen</td>
<td>Flies carry only the pollen that sticks to their bodies</td>
</tr>
<tr>
<td></td>
<td><strong>Antennae</strong></td>
<td><strong>Antennae</strong></td>
</tr>
<tr>
<td></td>
<td>Antennae tend to be longer</td>
<td>Antennae tend to be short and stubby, and hard to see</td>
</tr>
</tbody>
</table>
C.3. Pollinator identification guide