Humming along or buzzing off?
The elusive consequences of plant-pollinator mismatches and factors limiting seed set in the Coast Range of British Columbia

by

Jason Ryan Straka
B.Sc., Trent University, 2009

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the School of Environmental Studies

University of Victoria

Supervisory Committee

Humming along or buzzing off?
The elusive consequences of plant-pollinator mismatches and climate change and factors limiting seed set in the Coast Range of British Columbia

by

Jason Straka
B.Sc., Trent University, 2009

Supervisory Committee

Dr. Brian Starzomski (School of Environmental Studies)
Supervisor

Dr. John Volpe (School of Environmental Studies)
Departmental Member

Abstract

Supervisory Committee
Dr. Brian Starzomski (School of Environmental Studies)
Supervisor
Dr. John Volpe (School of Environmental Studies)
Departmental Member

There is concern that climate change may cause mismatches between timing of flowering and activity of pollinators (phenology). However, concluding that mismatches will occur, and have serious consequences for pollination services, requires assumptions that have not yet been tested. I begin by discussing a set of these assumptions, bringing past research into the context of mismatch. Briefly, the assumptions are that 1) dates of first-flowering or emergence (DFFE) correctly describe phenology (and therefore mismatch); 2) differences in DFFE represent the magnitude of mismatch; 3) advancement of DFFE will be the primary phenological change; 4) shifts will be random and independent for each species; 5) populations of plants and pollinators are "bottom-up" regulated by their mutualistic interactions; 6) all interactions are of similar strength and importance; 7) dispersal, and the spatial context of phenological mismatches can be ignored; and ecological processes including 8) phenotypic plasticity and adaptive evolution of phenology, 9) competition and facilitation, and 10) emergence of novel interactions, will not affect mismatches. I then describe novel experiments, which could help to account for some of these assumptions, clarifying the existence and impacts of mismatches.

Next, I present an original field experiment on factors affecting seed set in an alpine meadow in the Coast Range of British Columbia, Canada. I found evidence contradicting the assumption that seed set is primarily limited by pollination. My data highlight the roles of phenology, temperature (degree-days above 15°C, and frost hours), and interactions with pollinators (mutualists) and seed-predators (floral antagonists) in driving patterns of seed set. Seed set of early and late-flowering species responded differently to a 400m elevation gradient, which might be explained by phenology of bumble bees. My data suggest that the consequences of mismatch may be smallest for plants that are fly-pollinated and self-fertile. Non-selfing, bee-pollinated species might be more prone to reproductive limitation through mismatch (affected by snowmelt and cumulative degree-days). Plants that are limited by seed-predators might be negatively affected by warming temperatures with fewer frost hours, and extreme events such as late-season frosts and hail storms can prevent plants from setting seed entirely. Overall, my work emphasizes the importance of complementing theory, data-driven simulations, and meta-analyses with experiments carried out in the field.

Table of Contents

List of Tables

List of Figures

Acknowledgments

I would like to thank my supervisor, Brian Starzomski, and fellow members of the Starzomski Lab, Kimberly Carlson and Katharine Baldwin-Corriveau, for support throughout the process of completing this thesis. Kim Carlson's skill at keying out difficult plants was particularly appreciated. John Volpe, my second reader, provided helpful comments as did Luise Hermanutz, my external examiner. I am grateful to Jessica Forrest, David Inouye, Rebecca Irwin, and one anonymous reviewer for extensive comments on Chapter 2, in manuscript form. Terri Lacourse, Ashley Park, and members of the R Ecology mailing list provided helpful advice on ways of approaching my statistics. Field work was accomplished with the help of my assistant, Erika Dort, and on the strong backs of Andrew Sherriff, Guthrie Gloag, Kim Carlson, and Katharine Baldwin-Corriveau. Gabrielle Lee, Kath Oddleif, Jesse Howardson, Nicholas Mundy, Jacob Earnshaw, Julie Mundy, and Hilary Decker all helped with counting thousands of *Anemone* seeds in the lab. Elaine Hopkins, Lori Erb, and Alina Fisher patiently and skillfully guided me safely through the shoals paper work. This project was funded by the Natural Sciences and Engineering Council of Canada, Pacific Institute for Climate Solutions, University of Victoria, Mackenzie King Open Scholarship, Ian McTaggart-Cowan Scholarship, Dairyland Scholarship for Environmental Studies, and Toyota Earth Day Scholarship. I received training in pollination ecology and pollinator identification courtesy of the Canadian Pollination Initiative (CANPOLIN). Finally, I would like to thank my parents, Richard and Emily Straka, and extended family for exposing me to plant-pollinator interactions and alpine environments from an early age. While writing this thesis, Douglas Adams and P.G. Wodehouse allowed me to look on the world with dangerous (and wonderful) flippancy, while Lindsay Monk taught me that there was a time and a place for it (or at least made a valiant effort to do so).

Dedication

This thesis is dedicated to Aunt Kitty (Katharina Matter), and Jolana “Babka” Gdovin whose generosity has supported me through so many years of school. Babka passed away in December 2011 at the age of 90 and still fondly recalled the time when, as a child barely able to walk, I had told her not to pick flowers so that they would be there for future generations.

Chapter 1
General Introduction

To the chagrin of many, the 14th annual version of the staggeringly popular Salt Spring Island Organic Apple Festival was cancelled in 2012 "due not only to that cold, wet, long spring (poor pollination), but also the invasion of tent caterpillars that completely stripped leaves off most apple trees in May and June" (Salt Spring Island Publishing 2012). Was lack of pollination and attack by herbivores the cause of the weak crop, or did something else (like weather or temperature) affect fruit set more directly? Why was this year so bad? Could this be a result of climate change, expected to occur more and more frequently, or simply a rare temperature anomaly? Were the pollination deficits the result of mismatches between emergence of pollinators and flowering time, or the results of generally poor pollination? Was only fruit affected, or were bees (and honey-production) affected by lack of pollen and nectar resources as well?

All of these questions relate to the causes and consequences of phenological mismatches between plants and pollinators. The study of these mismatches is characterized by an urgency driven by a purportedly high risk of imminent declines in pollination services (Kearns et al. 1998; Memmott et al. 2004, 2007; Steffan-Dewenter et al. 2005; Potts et al. 2010), yet empirical evidence for such declines remains controversial and scant (Hegland et al. 2009; Miller-Rushing et al. 2010; Willmer 2012). The first source of controversy is that it is difficult to demonstrate phenological mismatches (Visser & Both 2005), particularly between plants and pollinators. Phenological shifts are one of the best-documented responses to recent climate change (Parmesan et al. 2003; Cook et al. 2012; Diez et al. 2012), but long-term phenological data are typically available for only plants (reviewed by Parmesan 2006; and e.g., Inouye 2008; Miller-Rushing & Primack 2008; Rafferty & Ives 2011; Molnár et al. 2012), much less commonly for non-pollinating insects (Gordo & Sanz 2005; Altermatt 2010; Ellwood et al. 2012), rarely for pollinators (Gordo & Sanz 2006), and almost never for both plants and pollinators (Gordo & Sanz 2006; Bartomeus et al. 2011). The second reason for controversy is that, even once mismatches are carefully defined and demonstrated, the consequences of mismatches are not clear because of a lack of empirical studies with sufficient controls of confounding factors (Hegland et al. 2009; Miller-Rushing et al. 2010).

In the past few years, there has been an explosion of studies on the topic of plant-pollinator mismatches, but concluding that mismatches will occur, and have serious consequences, requires a number of assumptions that have yet to be tested. In Chapter 2, I describe novel experimental designs, which could help to account for a specific set of these assumptions, in hopes of inspiring important research that can clarify the existence and impacts of plant-pollinator mismatches. Not all of the assumptions are openly expressed in the literature. Some of them, in fact, were proven to be incorrect many years ago, but they are either being frequently used as “straw man” arguments, justifying further studies, or permeating the literature through uncritical citation of several papers. The most notable of these papers is by Memmott et al. (2007), which was cited over 200 times as of September 2012, likely in ways that were not intended by the authors, who have openly addressed their early assumptions with follow-up studies (Memmott et al. 2004, 2007, followed by Kaiser-Bunbury et al. 2010). Yet the explosion of papers on this topic and research programs in pollination ecology, combined with ever more stringent restrictions on page length and numbers of references, means that few studies are able to give due credit to historical literature and consider the deeper context of their studies in relation to the enormous amount that we know (and don’t know) about plant-pollinator interactions.

My review of previous work on plant-pollinator mismatch and climate change is presented as a series of questions intended to bring past research in pollination ecology into the context of mismatch and identify goals for future research. Briefly, the questions are 1) Do dates of first flowering or emergence provide reliable estimates of phenology for whole populations? 2) Will advancement of flowering or emergence be the only response to climate change? 3) Are plants pollen limited, and can pollen limitation be driven by phenology? 4) Are responses of species to climate change random, and independent? 5) Are all pollinators functionally equivalent? 6) Will changes to co-flowering or co-flight have negative demographic consequences? 7) Can plasticity or adaptive evolution ameliorate the effects of changes to phenology? 8) Can “sub-optimal” phenological matching be the result of more complex adaptive strategies? 9) Will new interactions arise with changes to phenology? and 10) Are plant-pollinator interactions comparable across gradients in latitude and elevation? Questions 1,2,8, and 9 have been the focus of recent work but are only partially answered. Few data are available to address questions 6, 7, and 9; to date they have been approached through simulation studies, the predictions of which would benefit from empirical testing. Questions 3-5 and 10 have been partly addressed by historical work, but are rarely considered in the context of climate change.

In Chapter 3, I attempt to answer one of the above questions by presenting an original field experiment on factors affecting seed set of alpine plants at the scale of a flowering plant community. In doing so, I find evidence contradicting the assumption that seed set is always limited by pollination and interpret my results in terms of predicted consequences of climate change for reproduction of alpine plants in British Columbia. This highlights the important role of phenology in driving patterns of seed set, as well as variables such as temperature (degree-days above 15°C, and frost hours), and interactions with pollinators (mutualists) and seed-predators (floral antagonists). I also find evidence for different effects between early and late-flowering species, between flowers that were pollinated by bees versus flies, and between plants that are obligatory outcrossers versus those that are self-fertilizing. In general, these new data suggest that the consequences of climate change for reproduction will be least severe for plants that are fly-pollinated and self-fertile, which have high seed set regardless of when they flower. Plants that are non-selfing and pollinated by bees might be more prone to reproductive limitation through mismatch (affected by a combination of snowmelt and cumulative degree-days). Plants that are limited by seed-predators might be negatively affected by warming temperatures with fewer frost hours, although extreme events such as late-season frosts and hail storms can prevent plants from setting seed entirely.

The process of writing this thesis (and particularly the review) has emphasized how quickly the field of pollination ecology has been developing in the past few years. The Canadian Pollination Initiative (CANPOLIN) is nearing the end of its four-year funding cycle, with many intensive and collaborative projects nearing publication (Vamosi et al. 2012). In the United States, the National Science Foundation, in partnership with the Xerces Society for Invertebrate Conservation, has recently established funding programs to promote preservation of habitat for native pollinators and pollination services within agricultural landscapes (Xerces Society 2011). One of the challenges of doing good science is to ask the right question, and devise the right test to provide a useful answer, at exactly the right time. I would assert that this thesis has fallen quite near that mark. By September 2011, I had written the first draft of Chapter 2 and in April 2012, I submitted it for publication. At the same time, Jessica Forrest, then a PhD student at the University of Toronto, and Nicole Rafferty, at the University of Madison, Wisconsin, both began to publish a series of outstanding articles on the topic of plant-pollinator phenological mismatches, some of which included experiments that I had independently conceived, which were highlighted in an article in *Current Biology* (Willmer 2012). At the Ecological Society of America 2012 meeting, where I presented the results of my original study (Chapter 3), it was clear that many researchers are thinking about similar topics to those highlighted in my review, and actively studying them, but these ideas have not yet been fully developed in writing. I hope this thesis will be a partial remedy for these gaps, and lay down a foundation for future developments in pollination ecology. Finally, having been trained as a biologist, but preparing a thesis for an M.Sc. in the University of Victoria's School of Environmental Studies, I often found myself combining what I had learned from a number of different fields (and subfields) of ecology. I therefore hope that this thesis will resonate with general readers in environmental studies and conservation biology, but also with experts in botany, entomology, pollination ecology, community and population ecology, and evolutionary ecology.

Chapter 2:
The elusive consequences of plant-pollinator mismatches

Abstract:

Spatial and temporal mismatches between plants and pollinators, driven by climate change, are considered a potential cause of worldwide declines in populations of these taxa, yet field studies demonstrating such declines are uncommon. Here I revisit the predicted consequences of climate-driven phenological mismatch in plant-pollinator systems by identifying 10 assumptions that are violated in real systems, or insufficiently understood. Briefly, the assumptions are that 1) dates of first-flowering or emergence (DFFE) correctly describe phenology (and therefore mismatch); 2) differences in DFFE represent the magnitude of mismatch; 3) advancement of DFFE will be the primary phenological change; 4) shifts will be random and independent for each species; 5) populations of plants and pollinators are “bottom-up” regulated by their mutualistic interactions; 6) all interactions are of similar strength and importance; 7) dispersal, and the spatial context of phenological mismatches can be ignored; and ecological processes including 8) phenotypic plasticity and adaptive evolution of phenology, 9) competition and facilitation, and 10) emergence of novel interactions, will not affect the outcomes. Ignoring these assumptions has implications for the direction, extent, and accuracy of predicted consequences, but they can be addressed through carefully-designed experiments that elucidate the nature of mutualisms and assign treatments using natural gradients.

Introduction:

Many authors have suggested that there is an impending “pollination crisis” that will have grave consequences for diversity of plants and pollinators, and widespread economic effects on human systems such as agriculture (Steffan-Dewenter et al. 2005). Many types of threats are now being intensively investigated as possible causes of declines in pollinators (Table 2.1).

Table 2.1 - Major global threats to pollination services.

Threat	Explanations/examples of potential effects	References
Changing land use (including loss and fragmentation of habitat)	Increase in large-scale intensive agriculture (decreased heterogeneity of resources and habitats) and urbanization (fragmentation of suitable habitats), importing of non-native species for agricultural pollination, and loss of historical disturbance regimes maintaining plant diversity in communities.	Kearns & Inouye 1997, Aguilar et al. 2006, Hendrickx et al. 2007, Potts et al. 2010 (and references therein)
Pesticide use, and disruption of biogeochemical cycles	Direct mortality, or other deleterious effects (signal-disruption or loss of reproductive potential) of pesticides and agrochemicals. Loss of pollen from “weed” species. Increased severity of attacks by parasites and disease.	Johansen 1977; Kearns et al. 1998; Morandin et al. 2005; Brittain & Potts 2011
Invasive and exotic species	Introduction of parasites/parasitoids, genetically modified organisms with no co-evolutionary history, competition between native and non-native pollinators, and poor adaptation of imported pollinators to new environments	Kearns & Inouye 1997; Thomson 2006; Morales & Traveset 2009; Potts et al. 2010
Climate change	Spatial mismatch (via changes in range) or **phenological mismatch** (via changes in timing of seasonal events) between plants and pollinators. Includes reductions to ranges or overlap in ranges of mutualists due to physiological constraints, or inability to disperse.	Parmesan et al. 1999; Parmesan 2006; Schweiger et al. 2008
Cumulative effects: Interactions among threats listed above	Largely speculative at this time. Difficult to study, but highly likely to occur. May drive non-linear and synergistic responses.	Didham et al. 2007; Tylianakis et al. 2008; Schweiger et al. 2010

The most recently identified threat, that of climate change, is one that particularly warrants further exploration. Despite being identified as a critical area for investigation for a number of years (e.g., Bazzaz 1990; Parmesan 2006; Hegland et al. 2009), the subjects of factors influencing phenological synchrony, the extent to which plants and pollinators synchronize their life cycles, and the extent to which plants are reliant on synchrony with pollinators were recently re-listed as key topics on the research agenda for ecology by Willmer (2012) and Miller-Rushing et al. (2010), and for pollination ecology in particular by Mayer et al. (2011).

I begin by providing a brief evaluation of the current state of evidence for climate-driven phenological mismatch in plant-pollinator systems. I then proceed by examining major assumptions made in recent studies seeking to demonstrate the consequences of mismatch, and suggest ways to address these assumptions using various investigative methods, some of which are under-used in this area of research. Several recent reviews (Hegland et al. 2009; Miller-Rushing et al. 2010; Willmer 2012) provide excellent summaries of past work on plant-pollinator mismatch, so case-studies presenting evidence for phenological mismatch will only be treated briefly. Demonstrating demographic impacts of mismatches resulting from climate change is more difficult, and requires “that a change in interaction strength or frequency has occurred, that this change is the result of climate change and that the change has altered the vital rates of one or more of the species involved ” (Forrest & Miller-Rushing 2010). This can be particularly challenging to achieve for long-lived plants, but it is not impossible. I conclude my discussion with some directions for future work, and emphasize the need to address the assumptions or limitations that have arisen in recently-published studies from this area of research.

Phenological mismatches: The match/mismatch hypothesis

While the match/mismatch hypothesis has its origin in marine food webs and predator-prey interactions (Table 2.2), this review focuses on the prediction that rapid climate change may cause mismatches in the timing of seasonal interactions (phenology) between pollinators and their host plants (Bazzaz 1990), and that those mismatches will have severe demographic consequences for both plants and pollinators.

Table 2.2 - A brief and generalized history of developments in the match/mismatch hypothesis. Inter-trophic mismatches have been shown to occur in many systems (see Donnelly et al. 2011 for a thorough review).

Context	Value	Reference
General hypothesis: "combined effects of elevated CO_2 and other aspects of climate change, such as rising temperature, may cause large shifts in phenology such that the activities of the plants and their pollinators become decoupled"	First proposed that climate change could lead to the occurrence of phenological mismatches between plants and their pollinators	Bazzaz 1990
Marine: recruitment success of juvenile herring was linked to the degree of temporal coupling between larval fish and cycles of abundance in copepods as a limiting food source	Proposed that mismatches in phenology among interacting trophic levels, driven by climatic events, could have a limiting effect on populations that were directly dependent on a food source belonging to a lower trophic level	Cushing 1990
Europe: recruitment of Great Tits, *Parus major*, depended on availability of insects for food in the spring, specifically on their breeding grounds	Highlighted the importance of environmental cues in determining to what degree synchrony would be possible between breeding schedules and food availability for offspring	Visser et al. 1998
Europe: larval recruitment of moths, *Operophtera brumata*, depended on timing of bud-burst in host oak tree, *Quercus robur*	Demonstrated potential for disruption of phenological cues under climate-warming	Visser & Holleman 2001
Marine: members of open-water plankton communities responded differently through time to changes to climate, and these emerging differences in phenology could affect higher trophic levels through changes in the abundance of prey	Linked occurrence of match/mismatch to long-term changes to climate	Edwards & Richardson 2004
Europe: Pied Flycatchers, *Ficedula hypoleuca*, and their caterpillar prey responded to different cues, causing dramatic declines in Dutch populations of these birds	Attributed population declines to inter-trophic mismatch	Both et al. 2006

In their simplest form, phenological mismatches are likely to occur if plants and pollinators respond at different rates to changes in climatic drivers through time (Figure 2.1). Investigations have established that inter-trophic mismatches have occurred (reviewed by Donnelly et al. 2011; Diez et al. 2012), but few of these have focused on plant-pollinator interactions. A recent survey of non-pollinating insects in Japan concluded that it was unclear whether mismatches should be expected because recent phenological shifts have varied among 14 species and could not be separated from other confounding factors – particularly demographic changes that affected observations

(Ellwood et al. 2012). Over the past 40 years, the dates of first emergence for Japanese insects was generally negatively correlated with temperature, but positively correlated with temperature when precipitation was considered (Ellwood et al. 2012). It was unreasonable to draw conclusions about mismatches in relation to plants beyond stating that they could not be ruled out (Ellwood et al. 2012). A recent examination of 10 species of bees found that their emergence times had shifted at a similar rate to advancement in flowering times of plants over a 130-year period (Bartomeus et al. 2011). Corresponding shifts in phenology of plants and pollinators might suggest that some species could be resilient to the effects of climate-driven phenological mismatch by altering phenology in adaptive ways (Willmer 2012), but no clear mechanistic link has been made between historical shifts in phenology for one species and adaptive evolution in another. Hungarian orchids may be responding adaptively to climate change in terms of their phenology, since pollination mechanisms (selfing, deceptive, or nectar-producing) are good predictors of phenological changes over 50 years (Molnár et al. 2012). However, no direct link has been made to changes in phenology of pollinators (i.e., the agents of selection) with which these orchids interact (Molnár et al. 2012). Self-pollinating species were more likely to advance their flowering time than insect-pollinated species, which might be predicted if advancement in flowering time of insect-pollinated species was selected against (or constrained) by consistently later flight times of insects while selfing species were unconstrained (Molnár et al. 2012). Progress has also been made in constructing models for inferring phenological mismatches based on long-term data sets from Lepidoptera (Altermatt 2010a). While there has been debate over the suitability of butterflies as “indicator species” for terrestrial invertebrates in general (Lawton et al. 1998; Lovell et al. 2007), they continue to provide the highest-resolution data sets available for insects (Thomas 2005). However, the ecological and economic importance of pollinators such as bees and flies means that further attention should be paid to these taxa. They also provide a variation on the “classic” match/mismatch hypothesis (Table 2.1) because of the (typically, although not exclusively) mutualistic nature of their relationship: pollinators rely on plants for pollen and nectar, while plants rely directly on pollinators for reproduction. This provides a more direct link between floral visitation and demography for plants. Plants and insects also access resources differently; pollinators are mobile foragers, but plants must acquire resources from their immediate environment (McNickle et al. 2009). The functions performed by the partners in the mutualism therefore relate to the organisms' life histories in different ways because successful reproduction depends on a number of additional processes for both the plant and the pollinator. For pollinators, the ability to collect pollen and nectar has consequences for reproductive fitness by affecting their ability to mate, the size and number of their offspring (or related offspring) and their chances of survival (Eickwort & Ginsberg 1980). Plants may achieve fitness through both (or only one of) male function (pollen) or female function (seed). Male function is relatively low-cost, but relies on pollinators for dispersing pollen to available females (i.e., unfertilized ovules), which must then develop viable seeds. Female function can be costly, and availability of resources (e.g. nutrients, water) can affect quantity and quality of seeds produced (Galen 1985; Zimmerman & Pyke 1988).

Typically mismatches are demonstrated by using long-term and large-scale datasets to show historical differences in the "reaction norms" of paired mutualists to climate change (Gordo & Sanz 2005; Parmesan 2006; Miller-Rushing et al. 2010). While recent papers have begun to bridge the gap between these long-term trends in phenology and demographic consequences (Thomson 2010; Rafferty & Ives 2011), most studies to date have dealt primarily with the trends (Parmesan 2006, Cleland et al. 2012). This leaves many unanswered questions on the *consequences* of mismatches (Hegland et al. 2009; Miller-Rushing et al. 2010), particularly when they involve plant-pollinator interactions.

While my main focus is on these *phenological* mismatches, a closely-related issue that has been considered but rarely examined is the effect of emerging *spatial* mismatches between plants and pollinators (Box 2.1). As I move on to the discussion of phenological mismatch, and particularly the consequences thereof, it is important to remember that temporal interactions are always occurring in a spatial context.

Modelling approaches and a suite of assumptions:

Theoretical models provide useful tools for generating predictions about the potential demographic consequences of plant-pollinator mismatches. The generality of these models' results is what makes them so useful for generating predictions, but it is important to review and re-examine their assumptions. The main purpose of this chapter is therefore to provide a list of these assumptions and discuss them in depth. My intention is to be explicit about what assumptions have been used, which have been empirically examined, and which could benefit from further empirical investigation. Memmott et al. (2007) produced a widely-cited model that, by tacitly making a number of assumptions (Table 2.3), predicted reductions in the phenological overlap between plants and pollinators that would eventually lead to extinction or decline of 17-50% of their pollinator species due to lack of floral resources.

Table 2.3 - Key assumptions (explicit, or implicit) on the consequences of climate-driven phenological mismatches for plant-pollinator interactions.

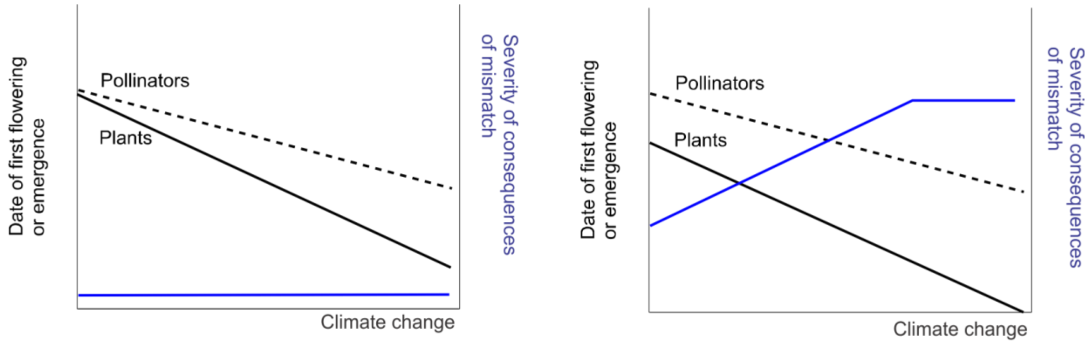
Assumption	Examples	Challenges/new concepts	Effect	Methods to address
1) Dates of first flowering (plants) or activity (pollinators) provide useful estimates of phenology at the population level	Gordo & Sanz 2005; 2006, Memmott et al. 2007; Rafferty & Ives 2011; Bartomeus et al. 2011.	Selective pressures or effectiveness of pollinators may differ between early and late individuals or flowers and vary inter-annually (Forrest & Thomson 2011; Thomson 2010; Rafferty & Ives 2011). Early-flowering individuals may actually have severely reduced fitness due to damage by frost (Inouye 2000; 2008). Pollination itself can affect the duration of flowering (Fründ et al. 2011).	Underestimates severity of mismatch in cases of mid or late season deficits in pollen or pollinators. Overestimates severity if flowering and flight times are long and abundance or mobility is high. Overestimates severity by ignoring cases where unvisited flowers stay open longer.	Establishment of detailed monitoring programs in which phenological milestones are assessed as frequently as possible *throughout* the year or growing season, and linked to population trends (Thomas et al. 2010). Working in degree-days instead of calendar days (Lindsey & Newman 1956; Schemske et al. 1978) .

2) Advancing phenology will be the only response of plants and pollinators to climate change	Gordo & Sanz 2005; 2006, Memmott et al. 2007; Bartomeus et al. 2011	Many subtle changes are likely to occur. Early flowers may advance; late-season flowers may be delayed. This might be mediated by variables besides temperature (Dunne et al. 2003; Kudo & Hirao 2006). Changes to voltinism may occur in insects (Altermatt 2010b).	Underestimates severity of mismatch in case of mid-season or late-season deficits. Overestimates severity if flowering times and flight times are long.	Understanding of important phenological cues and testing of phenological models for particular species using field studies and experimental manipulation of cues. Simultaneous assessment of phenological shifts in multiple species and entire communities.
3) Plant reproduction is always pollen-limited, and pollinators are limited by availability of hosts	Kudo et al. 2004; Memmott et al. 2007; Kaiser-Bunbury et al. 2010; Rafferty & Ives 2011	Considerable variation in severity of pollen limitation exists among populations (e.g., Ashman et al. 2004). Plants often have alternative methods of reproductive assurance (Knight et al. 2005), or *other* factors may be limiting such as ovules, or the abiotic resources and conditions needed to set seed (Harder & Aizen 2010).	Overestimates the demographic impacts of mismatch.	Community-wide studies on context-dependence of pollen limitation, and use of controls (e.g., pollen-supplementation experiments) to verify that plants are pollen-limited during field studies on mismatch; use of self-incompatible or dioecious species.
4) Responses of species (plants and pollinators) to climate change will be random, and independent for each species	Memmott et al. 2004; 2007; Kaiser-Bunbury et al. 2010	Responses to cues will likely be correlated among taxa (Willis et al. 2008), and vary according to evolutionary history or life-history (Altermatt 2010a), but will not be random (Miller-Rushing & Primack 2008; Molnár et al. 2012). Some species appear to respond adaptively while others do not (Rafferty & Ives 2010; Bartomeus et al. 2011).	Underestimates impacts of mismatch if highly diverse/important groups are disproportionately affected. Overestimates impacts if adaptive responses are common, particularly within diverse/important groups.	Community-wide studies on responses of species to climate change that are phylogenetically controlled and analyzed by guilds, functional groups, or other important life-history criteria.

5) All pollinators are similarly effective (as measured by pollen transferred per-visit) and effectiveness is consistent throughout the season; all plants are similarly useful to pollinators.	Gordo & Sanz 2005; Vázquez et al. 2005; Memmott et al. 2007; Kaiser-Bunbury et al. 2010; Rafferty & Ives 2012	Evolution or adaptation may be important. Some taxa (e.g., *Bombus*) are more abundant, or effective pollinators than others (Wall et al. 2003; Thomson 2010), and effectiveness can vary *within* years (Sánchez-Lafuente et al. 2011; Rafferty & Ives 2012). Climate change might make flowers more attractive (e.g., larger displays), yet provide fewer resources to pollinators (Hoover et al. 2012)	Ignoring obligate specialization could underestimate negative impacts. Overestimating specialization overestimates negative impacts. Counting all visits as *effective* visits can underestimate negative impacts by overestimating pollination services.	Use of highly-specialized study systems (single-pair mutualisms), high taxonomic resolution when monitoring visitation; experiments in which only single visits are allowed; use of proper controls (comparing experimental plants to plants with “open” pollination).
6) Changes to patterns of co-flowering or co-flight will fail to mitigate (or will exacerbate) the effects of mismatch.	Few studies to date, but identified as key questions: Miller-Rushing et al. 2010, Rafferty and Ives 2012	Evidence for facilitation exists in co-flowering displays (Rathcke 1983), or provision of supplemental pollen to maintain populations of pollinators, even by some invasive plants (Moeller 2004; Sargent et al. 2011) and bees (Goulson 2003).	When flowers compete, if co-flowering *increases*, negative impacts of mismatch might be exacerbated. This would be reversed in cases of facilitation.	Community-wide studies on the context-dependence of the competitive vs. facilitative relationship between plants and pollinators.
7) Phenotypic plasticity or adaptive evolution cannot mitigate the consequences of phenological mismatches	Harrison 2000; Kudo et al. 2004; Memmott et al. 2004, 2007	Synchronized phenology may be maintained by rapid evolution, plastic responses to changing cues, or novel interactions (Kaiser-Bunbury et al. 2010; Singer & Parmesan 2010; Rafferty & Ives 2011). *Little direct evidence to date is available to address this question.	Overestimates negative consequences of mismatch	Experimental forcing of mismatches under field conditions (for assessing plasticity of phenological responses to environmental cues). Long-term studies on heritability of responses to cues, and strength of selection on the cues under varying conditions.

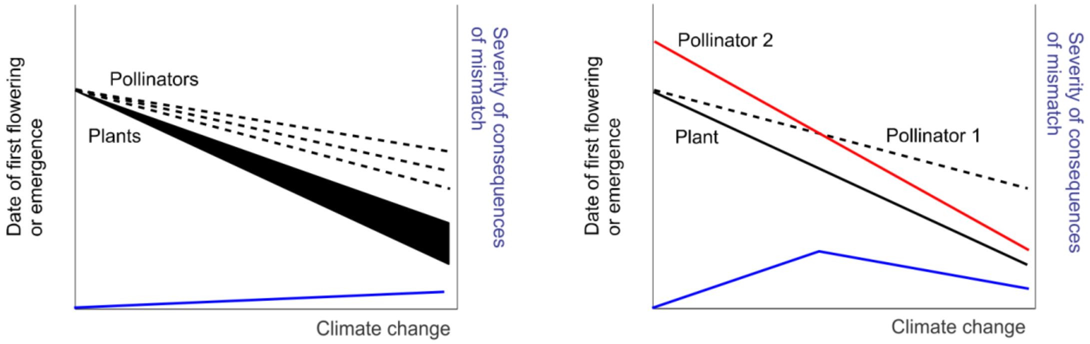
8) We are correctly measuring the "optimal" match; perceived mismatches are not simply the short-term result of longer-term adaptive strategies	Many papers, e.g., Wall et al. 2003; van Asch & Visser 2007; Bartomeus et al. 2011	Precise synchrony may not be the "natural" (or baseline) state in some systems, and may not be as widespread as we assume. Poor synchrony could be driven by other important tradeoffs relating to life-histories (Singer & Parmesan 2010; McNamara et al. 2011).	Overestimates severity of mismatch if precise synchrony is not the norm and other factors are more important, but disruption of systems with poor baseline synchrony could have severe impacts if synchrony is still important.	Multi-year studies of plant-pollinator interactions, using repeated measures on long-lived plants, if possible; consideration of trade-offs and multiple, interacting aspects of life-history, including *lifetime* fitness, and male and female fitness (plants).
9) New mutualisms will not arise (or ancient mutualisms will not be restored); parasitism will remain constant	Harrison 2000, Wall et al. 2003, Kudo et al. 2004, Memmott et al. 2007, Kaiser-Bunbury et al. 2010	Plants with shifts in phenology may have many options for pollination (Rafferty & Ives 2011). New interactions may arise that preclude pollen limitation for plants (Kaiser-Bunbury et al. 2010; Olesen et al. 2011) or resource-limitation for pollinators. Plants or pollinators may be released from parasitic interactions.	Overestimates negative consequences of mismatch	Experimental forcing of mismatches under field conditions. Choice/no-choice experiments with high taxonomic resolution. Manipulation or control of density in addition to identity of resources.
10) Plant-pollinator interactions are comparable across ranges of latitude and elevation; there is no spatial element to mismatch	Wall et al. 2003, Kudo et al. 2004, Memmott et al. 2007, Bartomeus et al. 2011	Importance of flies vs. bees varies with latitude and moisture regime (Elberling & Olesen 1999). Nature of intra-specific interactions may also vary from competitive to facilitative. Pollen limitation and seasonality also vary across space, but these patterns require additional investigation and synthesis	Might underestimate severity of mismatch at high elevations and latitudes where pollen is limited, seasonality is high, and availability of resources (e.g., moisture, snowfall) is limited.	Comparative studies along latitudinal and altitudinal gradients; field studies in environments with high spatial heterogeneity; increased emphasis on poorly-studied areas (tropical forests and arctic/alpine tundra)

Implicit in predictions from these types of models is that the evidence of threats to mutualistic populations is indirect: demographic consequences are only inferred, often subject to many assumptions. The list provided in Table 2.3 is far from exhaustive, but focuses on assumptions that are most likely to lead to problems in interpretation of results (Figure 2.2) that will need to be addressed with future work (Figures 2.3 and 2.4).



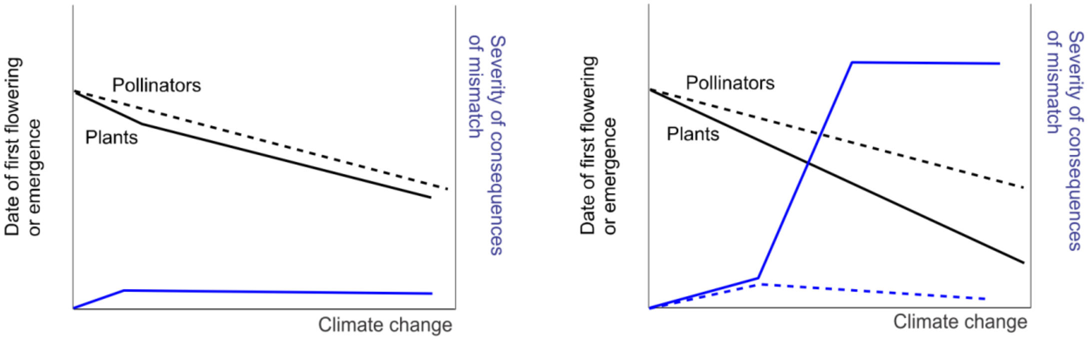
1) Dates of first flowering and emergence do not represent mismatch or 3) Vital rates of plants or pollinators are not affected by mismatch (plants are not pollen-limited, and/or pollinators are not limited by pollen or nectar)

8) Perceived mismatch is actually the “baseline” state, or result of other adaptive strategies. Increasing mismatch still has severe consequences until complete disruption of the interaction, or extinction.



2) Delay of flowering or emergence is not the only response to climate change (e.g., plants have longer flowering period, or pollinators have multiple generations per season).

9) Novel mutualisms arise, historical mutualisms are restored, and/or parasitic interactions are lost, compensating for mismatch.



Responses of plants and pollinators to climate change are coupled (4), phenotypically plastic, or subject to strong selection, driving adaptation (7)

6) Disruption of facilitative (blue) or competitive (blue, broken) effects among plants or pollinators (e.g. due to extinction or invasion).

Figure 2.1 - Some possible outcomes in terms of plant-pollinator mismatch under scenarios where common assumptions about mismatch are violated. Numbers correspond to assumptions discussed in text, summarized in Table 2.3.

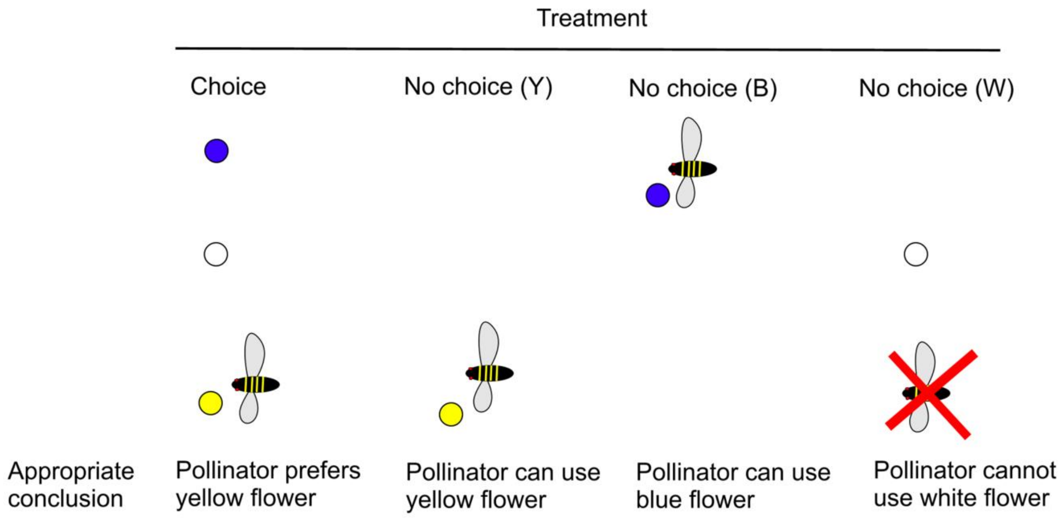


Figure 2.2 - Simple choice/no choice experiment, in which a pollinator is offered blue (B), yellow (Y), and white (W) flowers. From this we may conclude that the pollinator could persist in the absence of yellow flowers IF blue flowers are available, but not if white flowers alone are available. Note that the “No choice (Y)” treatment is not strictly necessary unless there is reason to believe that the use of yellow flowers is reliant on the presence of blue and/or white flowers.

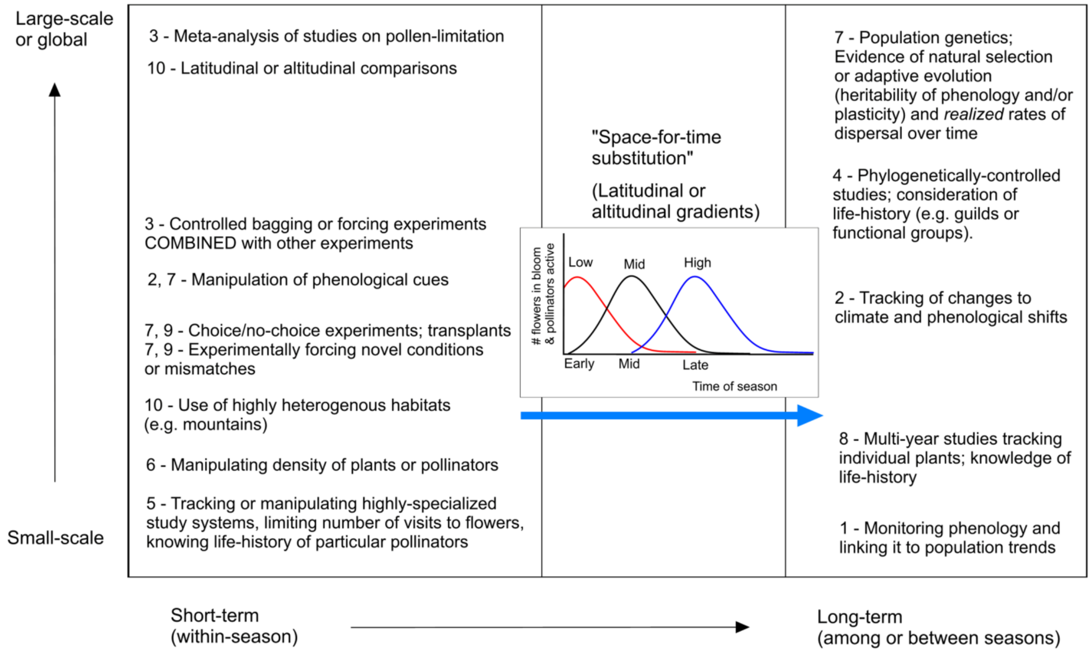


Figure 2.3 - Some approaches to addressing important questions about the future impacts of climate-driven plant-pollinator mismatch, covering a range of scales in space and time. Numbers correspond to assumptions discussed in the text, and summarized in Table 2.3. Work among seasons may cover many years, decades, or longer.

Indeed, recent work on plant-pollinator mismatch emphasizes the importance of testing models through meta-analyses, and observational or experimental field studies. The sections below will discuss each of the 10 assumptions in turn, with reference to the types of work that will be necessary to address unanswered questions.

1) Do dates of first flowering or emergence provide reliable estimates of phenology for whole populations?

Because they are available from long-term or historical data made up of many “incidental” observations, dates of first flowering or emergence (DFFE) are often used as a measurement of phenology in plants and insects, respectively (e.g., Gordo & Sanz 2005; Willis et al. 2008; Rafferty & Ives 2011). In contrast, the strict definition of phenological synchrony (and thus the concept of mismatch) involves the concept of a “peak” in both the requirement for pollination from the perspective of the plants, and in the availability of pollen, nectar, and other resources from the perspective of the pollinators (Singer & Parmesan 2010; Figure 2.1).

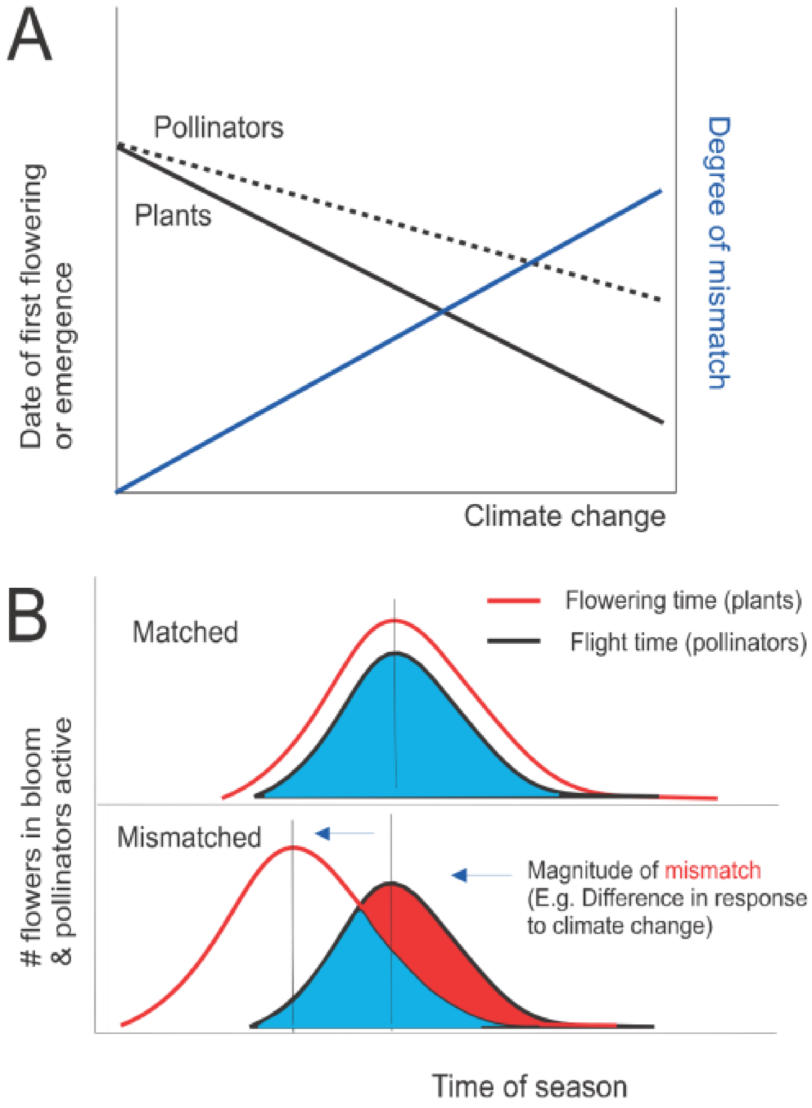


Figure 2.4 - Simplified representations of A) increasing phenological mismatches (space between lines) between plants and pollinators occurring when their changes in timing respond differently to climatic drivers through time and B) the predicted consequences in terms of historical plant-pollinator overlap (blue) that can no longer occur (red). In this example, plants have advanced their flowering times more than pollinators have shifted their flight times. Assumptions of these simplified models are discussed in the text.

Assessments of the degree of synchrony (and thus, asynchrony) involve quantifying the amount of overlap of the area under the curves for plants and pollinators. This assumes that complete overlap represents perfect synchrony, and that the true shapes of the curves can be approximated by DFFE. Thomson (2010) recently tested the validity of this assumption by recording the number of open flowers every few days over an entire field season. While considerable variation in flowering time did exist among individuals in populations of *Erythronium grandiflorum*, there was evidence for a positive skew towards early flowering, which provides some support for the current focus on DFFE on *Erythronium* and other early-flowering plants (Forrest & Thomson 2010). Forrest & Miller-Rushing (2010) have noted that phenological patterns in all populations are expected to follow statistical distributions that can be viewed as variation around a population mean, constrained by the first and last dates of flowering, but these properties are rarely measured in practice.

The shapes of phenological curves might also differ between pollinators and flowers due to differences in their physiology and life-history (Figure 2.5).

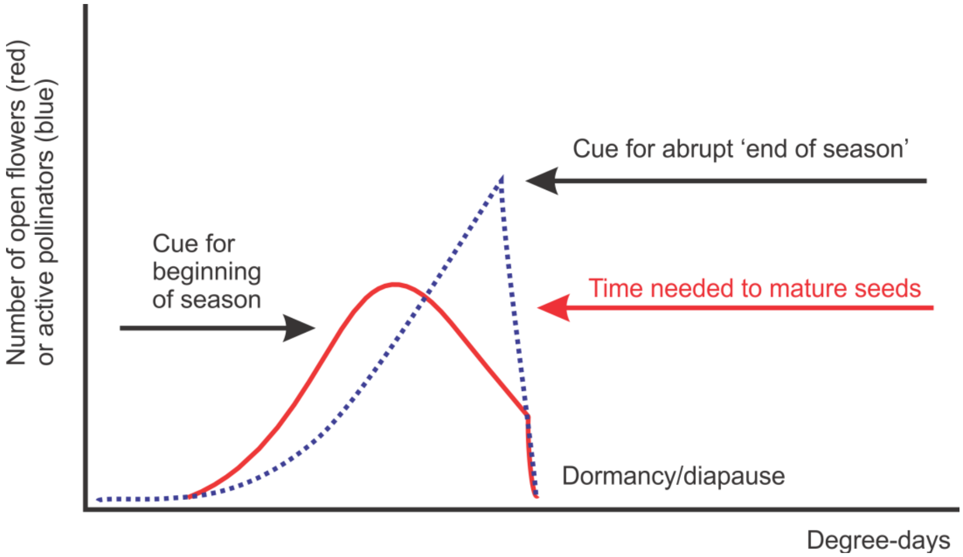


Figure 2.5 - Hypothetical alternative shapes of phenological curves for flowers (red) and pollinators such as bees (blue, dashed) in seasonal environments. Both are constrained by inappropriate conditions (e.g. snow, low temperatures, frost) at the beginning and end of the curve, but plants are additionally constrained by time needed for seeds to mature. Many insects, in contrast, can continue to reproduce until an abrupt die-off forces a switch to the dormant state.

Flowering times are expected to follow a curved distribution, subject to stabilizing selection acting on physiological cues constraining early-season development (e.g. snow-melt, growing degree-days, vernalization requirements) (Dunne et al. 2003, Cook et al. 2012), and constrained by time needed after pollination for seeds to mature (Galen &

Stanton 1993). In contrast, pollinators in temperate environments such as bumble bees can continue reproducing until the end of the season. Rather than declining gradually, abundance of worker bees can increase exponentially until an abrupt die-off of all but the overwintering queens. At best, monitoring phenology for randomly selected plants or plots can be representative of the wider population, providing higher quality data than ‘incidental’ observations, and enabling researchers to avoid drawing false conclusions about changes to phenology over time (Miller-Rushing et al. 2008).

Prevalent concepts of phenology also rarely consider that the duration of flowering time for whole communities of flowers can be directly dependent on whether or not those flowers are pollinated (e.g., Doorn 1997; Fründ et al. 2011). Flowering time can therefore be strongly linked to the behaviour of pollinators (and vice versa) rather than simply to abiotic factors (Doorn 1997; Fründ et al. 2011). Furthermore, calendar days may be less relevant than accumulated degree-days above a relevant threshold for growth or development (e.g., Lindsey & Newman 1956; Schemske et al. 1978). This suggests that DFFE are not always meaningful measurements of phenology, and calls into question the accuracy of models that make this assumption. For example, plants that remain open longer when they are not pollinated might be naturally buffered against phenological mismatch because their date of last flowering (and thus their flowering period) is partly dependent on *pollination* rather than date of first flowering. Predictions based on dates of first flowering would often ignore this buffering capacity. In contrast, lack of floral resources early in the season could have severe consequences for pollinator populations later in the season (Bowers 1985, 1986). An example would be the life-history of bumble-bees, where the success of early-emerging queen bees directly affects the quality and quantity of successive broods of workers (Bowers 1985, 1986), or the carpenter bee *Megachile*, which produces more and larger offspring after periods of abundant floral resources (Kim & Thorp 2001).

Viewing phenological schedules as a phenomenon with considerable variation means that field experiments on phenological synchrony between plants and pollinators should encompass a range of dates stretching from early to late in the flowering season. This approach involves repeating experiments on the same species throughout the season (e.g., Gross & Werner 1983; Ackerman 1989; Widén 1991), or doing multiple experiments on species with different flowering times so that flowering time is an explanatory variable, and pollen limitation is the response. The results of such longitudinal, within-site studies have not yet been fully considered in the context of plant-pollinator mismatch. In general, they seem to indicate that pollen-limitation is context-specific and often driven by abiotic factors (such as weather, or availability of resources) rather than mismatch (Gross & Werner 1983; Ackerman 1989). This is a situation in which researchers can take advantage of altitudinal gradients or multiple aspects within sites (Dunne et al. 2003). For example, at a given field site (e.g., on a mountain slope) late in the field season, early individuals for high populations might be blooming at high elevations or north-facing slopes while late individuals for low populations would be blooming at low elevations and south-facing slopes (Figure 4, inset). By spending an entire field season at a relatively small site, multiple replicates of early, peak, and late flowering “cohorts” could be followed in ways that would be impossible without the gradient in elevation (Kameyama & Kudo 2009). In locations where phenology of plants is known to be closely linked to an easily-manipulated cue such as snowmelt, temperature, or sunlight, treatments such as snow-addition or removal, warming chambers, or shading may be applied along existing gradients (e.g., Dunne et al. 2003) to simultaneously examine the effects of abiotic cues on plants and pollinators. This approach can provide simulations of different climate-change scenarios for shifts in phenological overlap, in a space-for-time substitution (Figure 2.4). However, space-for-time substitutions rely on the assumption that there is no spatial variation in relevant factors other than timing. It is therefore important to consider the scale at which these manipulations are done (e.g., Sargent et al. 2011), because in places where pollinators are highly mobile, these treatments would simulate changes of flowering time for patches of habitat within a wider ecosystem, rather than phenological shifts for entire ecosystems (Kudo & Hirao 2006; Kameyama & Kudo 2009). The result might be a study of pollinators’ ability to find or avoid patches of resources in heterogenous landscapes, rather than representing a response to phenological mismatch. An alternative (and also under-used) way of addressing these questions might be through the use of latitudinal experiments, transplanting plants into locations with different phenological conditions (Waser 1979). Variation in density of individuals or effective population size would have to be controlled with this type of experiment (Hegland et al. 2009), but this has been accomplished by working with artificial arrays of flowers, set apart from natural habitat (Rafferty & Ives 2011, 2012).

2) Will advancement of phenology be the only response to climate change?

Evidence is accumulating that while advancement is often the most obvious and easily measurable effect of warming on phenology, it is not the only response that is occurring or is likely to occur. Sherry et al. (2007) found that one year of experimental warming caused community-level advancement of early flowers (nine species), as well as a delay of late flowers (three species) in a tall grass prairie. Cook et al. (2012) attributed such divergent patterns to failure of some plants to achieve their vernalization requirements due to warming, causing delayed phenology. Similar patterns have been found for a wider community of plants at Rocky Mountain Biological Laboratory between 1974 and 2009, with an emerging shift from a single flowering peak to multiple peaks, driven by differences among habitat types (Aldridge et al. 2011). This implied that the consequences of mismatch might be greatest where there is a mid-season deficiency in pollen availability (Aldridge et al. 2011), and is an example of divergent responses to climate warming among members of a single species at a single location, as well as divergent responses within flowering communities. In addition to changes in timing of single generations, the number of flight periods per year (voltinism) can change in some Lepidoptera (e.g., Altermatt 2010b). Although I was unable to find any papers describing evidence for this occurring in pollinators such as bees and flies, it is likely that a similar effect might occur for the genus *Megachile*, which is multivoltine (Kim & Thorp 2001), or for *Bombus* (Hymenoptera: Apidae), which produces multiple broods of workers throughout the summer and is a highly abundant and effective pollinator – particularly in Arctic and alpine environments (Goulson 2010). Increases in the number of flights per year are of interest because they would increase the period of resource-requirements from the perspective of pollinators (e.g., Cartar & Dill 1990), but could also mitigate pollen-deficiency of plants by ensuring that pollinators are always present – a phenomenon that might already have begun to occur during mild winters in Europe (David Inouye, personal communication). In contrast, decreasing the number of flights per year could create “gaps” during which pollinators are not present, and the fitness of plants is reduced due to lack of pollination.

Here, I re-emphasize the importance of carrying out manipulations of pollen-availability (pollen-supplementation or pollinator-exclusion) and density of pollinators (removal) throughout the entire flowering season (Figure 2.4), while noting the identities and abundances of pollinators and flowers to constantly monitor the composition of the ‘background’ community. With these data, one could first ask *when* the consequences of mismatch would be most severe, and then ask *why*. For example, one could test the hypothesis that pollen-limitation is driven by abundance of bumble bees in early-flowering plants (e.g., Thomson 2010) but driven by competition for pollinators in late-flowering plants. One might predict that early in the season, excluding or removing bumble bees would lower seed set, pollen-supplementation would increase seed set, and removal of competing flowers would have little effect on seed set. Later in the season, one might expect to find lower visitation rates for individual flowers, but a stable number of *total* visits for all flowers in the population, distributed among higher densities of competing flowers and stable abundance of pollinators. In this case, late-season removal of competing flowers would have a strong, positive effect on seed set. Alternatively, visitation rates per flower could remain stable throughout the season despite differing abundance. Identities of visitors or temporary pollen limitation (e.g., in the middle of the season) could be linked to fluctuating abundance of specific pollinators and/or competing flowers. Monitoring or manipulation of abundance of flowers and pollinators in the community, could help to predict consequences of mismatches by moving beyond the assumption that differing rates of advancement in DFFE for insects and plants will cause mismatches, with negative demographic impacts.

3) Are plants pollen limited, and can pollen limitation be driven by phenology?

Demographic consequences of phenological mismatch in plant-pollinator interactions have been predicted based on the assumption of widespread pollen limitation in plants (Miller-Rushing et al. 2010). While extensive literature exists on the causes and consequences of pollen limitation for flowering plants in general (Burd 1994; Ashman et al. 2004; Knight et al. 2005), there has been almost no discussion with specific reference to spatial or temporal mismatch with pollinators (Harder & Aizen 2010). This may be because it is difficult to manipulate communities of pollinators in ways beyond exclusion experiments (Kearns & Inouye 1993), although Fründ et al. (2012) recently presented such a manipulation, building eight-metre squared flight cages in a field to investigate the relationship between diversity of a bee community and pollination services. There is evidence to suggest that pollen limitation (or lack thereof) can be related to the synchrony between flowering time and the spring emergence of important pollinators (Thomson, 2010), the ability of plant species to adjust the length of flowering period to allow for sufficient visitation (Doorn 1997; Fründ et al. 2011), and the number of pollinator visits need to ensure seed-set (e.g., Harder & Thomson 1989; Kawai & Kudo 2008). Rates of pollination are known to vary throughout the season, affecting seed set in alpine habitats where bumblebees are the main pollinators (Thomson 2010), but the temporal elements to pollen limitation and abiotic conditions that drive them requires further investigation (Hegland & Totland 2008; Forrest & Thomson 2010). Although the number of ovules (female function) is typically smaller than the number of pollen grains (male function), pollination interactions only transfer small amounts (< 20%) of carried pollen per interaction (e.g., Harder & Thomson 1989; Kawai & Kudo 2008). This has been presumed to be an adaptation to promote outcrossing (Kawai & Kudo 2008). It also relates to the discussion of plant-pollinator mismatch because it suggests that pollen limitation (and thus, reduced recruitment) could occur in their study system in situations where pollinator populations are unusually low.

Variation in pollen limitation among sites is important for estimating the consequences of phenological mismatch. Assuming plants are pollen limited overestimates negative impacts of mismatch on seed set. In a recent review, Schemske et al. (2009) called for more studies to be conducted on latitudinal variation in pollination interactions. They noted that pollen limitation tends to be greater in communities with more species, apparently because of increased inter-specific competition for pollinators (Bell et al. 2005, but see discussion of facilitation, below). Vamosi et al. (2006) used a large-scale meta-analysis to show that the most pollen-limited communities often tend to be the most species-rich communities (in terms of both plants and pollinators), located at lower latitudes. This implies that there is a species-richness x latitude interaction in pollination communities, and that species richness could contribute to pollen limitation by increasing competition among plants for access to pollinators. To our knowledge, this idea has not been tested experimentally, but it could be done by monitoring visitation rates to model flowers, placed into similar pollination contexts with varying levels of floral species richness. A recent observational test examined pollen limitation of *Lasthenia fremontii* in vernal pools with varying levels of floral species richness (Sargent et al. 2011). It showed that facilitation can outweigh the effects of competition as plant communities increased in richness of closely-related species (Sargent et al. 2011). This was thought to be because the presence of related species can promote higher abundance of shared pollinators (Sargent et al. 2011), but the study lacked statistical power and did not provide any data on the pollinator community. Martén-Rodríguez & Fenster (2010) also indicated that the effects of pollen limitation might be different for plants that are specialists versus generalists with regard to their community of potential pollinators. Specialists might theoretically be more pollen-limited, but many lineages have also evolved self-compatibility, or various forms of asexual reproduction as techniques for reproductive assurance (e.g., Silvertown 2008; Martén-Rodríguez & Fenster 2010).

Given that multiple factors likely influence the severity of pollen limitation at a site, it often remains unclear whether pollen limitation, when it exists, is primarily caused by phenological mismatch, or other peculiarities of the site (competition versus facilitation among co-occurring plants for pollinators, specialization of interactions, or identity of the pollinators (Hegland & Totland 2008). One way to answer this question would be by measuring pollen limitation across both an altitudinal and temporal gradient at the same field site, and combining this with information on how the pollinator community varies over space and time (Figure 4). Again, I emphasize the importance of conducting experiments covering a diverse range of sites and abiotic conditions, and the importance of gathering information about the identities of pollinators. This information can then be used to assess a) whether the impacts of mismatch can be accurately predicted by models relying on the assumption that plants are always pollen-limited and b) how the accuracy of these models may be affected by the “context” of a community.

4) Are responses of species to climate change random, and independent?

Experimentally-induced mismatches between flowering time of individual plants and their populations are known to be detrimental to seed set through the effects of reduced visitation by pollinators and increased impacts of seed predators (Augspurger 1981), but ways in which these interactions may be affected by climate change, and mediated by taxonomic affiliations, require further investigation. Some taxa are known to be disparately affected by climate change in terms of shifting phenology and declining abundance (Willis et al. 2008, Cook et al. 2012), but it is not known how these patterns affect plant-pollinator interactions. Rafferty & Ives (2011) recently did an experimental test of the consequences of phenological mismatches between plants and pollinators at a community scale. They explicitly addressed the assumption that responses to climate change would be random with respect to species identity by looking for differences between plants that had historically advanced their dates of first flowering, versus those that had not (Rafferty & Ives 2011). By addressing this assumption, Rafferty & Ives (2011) found that the predicted severity of mismatches caused by climate change may have been overestimated. Plants that had historically advanced did not suffer from significant reductions in visitation rates by pollinators when compared to plants that had not advanced their phenology (Rafferty & Ives 2011). Unfortunately, it was not clear that mismatches could explain this pattern, since no data were available on whether or how pollinator phenologies have changed (see Molnár et al. 2012 for similar problems with historical data).

Although he did not address plant-pollinator interactions, Altermatt (2010a) found that European Lepidoptera that were herbivores on deciduous woody vegetation responded to 150 years of climate warming with larger advancements in their flight periods and smaller increases in the number of flights per year than Lepidoptera specializing on evergreen herbaceous vegetation. He also predicted that there would be a stronger selective pressure for high synchrony with hosts among insects that specialize on woody vegetation (“burst” resources). A possibility that follows from this is that there might be a similar effect related to patterns of flowering. Forrest & Thomson (2010) found that their populations of *Mertensia* in Colorado, USA, were skewed towards early-flowering, with a long tail of rare, late-season flowers. Here we can make the distinction between "pulse" resources – those that are highly abundant for short periods of time, and "press" resources, which are available with similar abundance for an extended period of time (Bender et al. 1984). The question of whether selection on phenological schedules (and thus, the likelihood of mismatch) applies differently to pollinators that rely on different types of resources (early-spring "pulses," versus "presses" that last for an entire season) remains to be answered.

One way of testing this might be to compare the likelihood and consequences of mismatch between temperate and tropical species, or compare the life-history strategies of the same or closely-related species in temperate versus tropical regions. In general, resources in temperate locations tend to be more spatially predictable (continuous meadows or large patches of resources with relatively low species turnover), but temporally unpredictable (a narrower "peak" in which all flowers of one species are blooming, or all pollinators are active) (e.g., Janzen 1971). Tropical species, in contrast, can often release their resources continuously throughout the year or once every few years (although seasonal changes in moisture can also be an important cue for release of resources (e.g., Tauber et al. 1998), and the resources are less abundant and more spatially unpredictable (i.e. there is higher species turnover across space; Janzen 1971). Here we might predict that temperate pollinators and plants would respond strongly, and in similar ways, to environmental cues and have fairly restricted ranges for foraging. Tropical pollinators, in contrast, might be predicted to have weaker responses to cyclical environmental cues (with the exception of moisture), but might compensate for the low abundance of homogeneous resources by traveling farther while foraging. Another relevant consideration for tropical species is biotic attrition (Colwell et al. 2008); while species in the tropics may shift their ranges up-slope over relatively steep environmental gradients and small spatial scales (Box 2.1), they are less likely to shift their ranges latitudinally because of the more gradual temperature gradient compared to temperate locations. Attrition could occur when species in lowlands go extinct or migrate up-slope, but there may be no species that are physiologically capable of replacing them in the lowlands as there is nowhere for new species to have migrated *from*.

As was introduced in the context of spatial mismatch (Box 2.1), the degree of specialization of the pollinator and its host plant could be important. Memmott et al. (2007) put forward several hypotheses to this effect, predicting that (1) specialized pollinators should be least *susceptible* to mismatches because they would be predicted to shift their phenology most closely with that of their hosts via shared cues, and (2) specialized pollinators should be most severely *affected* by mismatches because of their lack of alternative food sources. Theory indicates that the division between "specialists" and "generalists" may not even tell the entire story. Pollination networks suggest that mutualisms in general tend to be *asymmetrical*, meaning that when one partner is heavily reliant on another species, that other species tends not to be heavily reliant on the partner (Bascompte et al. 2003; Petanidou et al. 2008). Specialist pollinators often visit generalist plants, while generalist pollinators visit specialist plants (Bascompte et al. 2003). The properties of pollination networks are predicted to increase their resilience, enabling them to avoid worst-case scenarios such as lack of food for pollinators or lack of sexual reproduction for plants (e.g., Memmott et al. 2007; Kaiser-Bunbury et al. 2010). Long-term phenotypic plasticity (for long-lived species in particular) and adaptation through microevolution (for short-lived species) must be considered (Visser 2008; Heard et al. 2011; Box 2.2). The severity of the impact of climate change will be contingent on the ability of particular species to respond adaptively to shifts in their own phenology, as well as shifts in the phenology of species with which they interact (Visser 2008).

5) Are all pollinators functionally equivalent?

Past approaches to studying plant-pollinator mismatches have often suffered from "phyto-centrism." This is understandable given that the economic impacts of a pollination crisis would be felt primarily through effects on agricultural plants (Steffan-Dewenter et al. 2005), but warning about a pollination crisis while focusing on plants is analogous to studying a response variable without understanding the key predictor. As sessile organisms, plants are easy to track and manipulate. Seed set (or potential contribution of offspring to the next generation) can be measured directly, and reproductive fitness can be estimated from seed set (females) and pollen transfer (males), or estimated through visitation by pollinators (Kearns & Inouye 1993). But greater knowledge of demographic drivers for pollinators is needed to adequately answer questions such as: What will be the demographic consequences of climate change for plants and pollinators? How will pollination services be affected by climate change? Where will changes be most severe, or which relationships will be resilient? and What steps can we take to conserve these services? The challenge of achieving higher resolution, and a more equitable representation from the perspectives of both plants and pollinators is significant. It necessitates a knowledge of life-history, including abiotic and biotic interactions at the level of the individuals, populations, and communities; and consideration of both historical and contemporary data. These types of data have been easier to access for plants. Few studies are available that consider differences in life histories among pollinators in a community beyond a superficial level. Different types of pollinators are known to vary widely in the efficacy of pollen-transfer they provide for plants (e.g. Motten et al. 1981; Kearns & Inouye 1994; Vázquez et al. 2012), but consideration of how nectar and pollen contributes to reproductive success of bees (e.g. Cartar & Dill 1990; Hoover et al. 2012), and of activities that bees perform besides visiting flowers (e.g. mating, nesting, provisioning young, being consumed by other organisms), is relatively scarce. The complexity of pollinators' life histories are rarely considered with respect to the consequences of mismatch, beyond focusing only on taxa that are deemed to be most effective at pollinating plants, such as *Bombus* (e.g., Thomson 2010). An exception to this was a recent study by Rafferty & Ives (2012), in which focal flowers were limited to single visits by pollinators, which indicated that the effectiveness of the same pollinators (from the plant's perspective) varied throughout the season. Another exception was a study that again used deposition of pollen per visit to estimate the importance of pollinators for reproduction of plants, but simultaneously used trap-nests to estimate the importance of pollen for reproduction of larval bees (Vázquez et al. 2012). This study found that frequency of interactions (visitation, or pollen collected) was strongly and positively correlated with their estimated importance (seed set, or larvae produced), supporting the link between interaction frequency and demographic consequences. It was unclear, however, whether these findings could be generalized to most pollination networks, and concluded that the strength of interactions (rather than frequency alone) was necessary to describe the impacts of interactions as positive or negative (Vázquez et al. 2012). Ideally, we should measure variation in both the effectiveness of pollinators of plants, and their likelihood of being affected by changes to phenology. Again, studies on Lepidoptera have made progress because of data available in Europe. Altermatt (2010a) recently found that shifts in flight periods of European Lepidoptera were best predicted by their overwintering stage, composition of larval diet, and European range. The largest shifts were observed in species that overwintered as eggs and specialized on woody (rather than herbaceous) plants. If phenology of pollinators is affected by physiological constraints on development, properties of pollinators' life-history, and properties of host plants, species are not functionally equivalent. This suggests that a greater diversity of responses to climate change may be observed than have been predicted from current models. By experimentally forcing mismatches to occur, observing whether or not novel interactions between plants and pollinators emerge, and evaluating the results of those interactions in terms of their demographic consequences for plants (by measuring rates of visitation and seed set) as well as pollinators (by measuring reproductive success or survival), it may be possible to determine whether novel interactions might be equivalent to historical mutualisms (Starzomski, in press).

6) Will changes to co-flowering or co-flight have negative consequences?

In addition to the direct effects of phenological mismatches between insects and their pollinators, climate change may alter patterns of co-flowering or co-flight, which could increase competition among plants for pollinators (Mitchell et al. 2009) or competition among pollinators for floral resources (Potts et al. 2010; Schweiger et al. 2010). Increased competition is predicted to have negative demographic consequences for plants or pollinators, but relationships between co-flowering plants can also be facilitative (Thomson 1981; Rathcke 1983). While many studies have focused on competition among plants for pollinators (reviewed by Mitchell et al. 2009), it remains unclear to what extent pollen limitation is important in many natural systems as a direct result of such competition. In fact, competition as the primary interaction among co-flowering plants has been challenged theoretically (Baker 1963; Heinrich & Raven 1972), and by empirical studies showing that co-flowering can be associated with greater local populations of pollinators by attracting shared pollinators (Waser & Real 1979; Thomson 1981), and providing supplemental pollen during ‘off-peak’ flowering (Moeller 2004; Sheffield et al. 2008). Unfortunately, most studies on facilitation have been conducted using no more than two or three species over small study areas (< 2 km^2), and focus on the effects of competition from invasive plants (Kearns et al. 1998) rather than co-flowering driven by phenological changes. This may select for good competitors rather than focusing on species that are likely shift their phenology. There is also a bias toward studies conducted at temperate latitudes and low elevations. Meta-analysis might be useful tool for answering the questions: Will the effects of changing co-flowering patterns on seed set be negative (driven by competition) or positive (driven by facilitation)? and Are mismatches expected to have equally severe consequences at different latitudes, or in different ecosystems?

To our knowledge, no studies have examined variation in the relationship between competition and facilitation for co-flowering plants with respect to availability of pollinators at the community level. There are theoretical reasons to predict that this relationship might vary over gradients in latitude or elevation. For example, relationships within plant communities are known to change from competitive to facilitative with movement to higher elevations (Callaway et al. 2002). Although the mechanisms are unclear, it has been suggested that the difference might have been due to the “harsher” environment (wind, cold, and water stress) experienced by plants at high-elevations (Callaway et al. 2002). An important area of future research is the effect of changes to flowering time at the scale of communities, including simultaneous manipulation of multiple species. Rafferty & Ives (2011) described the effects of artificially advancing the dates of first flowering (DFF) of 14 species of perennials in terms of their subsequent interactions with pollinators. They showed that plants that had historically advanced their DFF were more likely to receive higher rates of visitation in the “advanced” treatment, while the opposite was true of plants that showed no historical advancement in flowering time (Rafferty & Ives 2011). This suggested that plants were shifting their DFF over time in a way that improved their reproductive output (i.e., responding adaptively), but the mechanisms behind this pattern were unclear (Rafferty & Ives 2011), and additional studies of this kind are needed (Figure 4). By varying not only the identity, but the number of plants that flower simultaneously, we could attempt to isolate the relative importance of competition for pollinators, facilitation, and phenological mismatch.

7) Can plasticity or adaptive evolution ameliorate the effects of changes to phenology?

While projections suggest that ecosystems (particularly in the Arctic and alpine) will experience rapid changes to climate (IPCC 2007), it is unclear to what degree evolution might mitigate the effects of those changes on interactions among species (Parmesan 2006; Visser 2008). In particular, it is the *rapidity* and *variability* of the changes that are of concern, since many species present today have coped with climate change at some point in their evolutionary history. Species or populations faced with changes to climate may:

1) Move through **dispersal** to locations with appropriate conditions,
2) Undergo adaptive **evolution** (or have sufficient phenotypic plasticity) to cope with new conditions,
3) Suffer **extinction.**

These scenarios are all relevant to the discussion of the demographic consequences of phenological mismatches driven by climate change, and must be considered before assuming that the results of mismatch will always be extinction (Bond 1994). Unfortunately, evaluating which of these three scenarios are applicable to various species must be done on a case-by-case basis, or through broad-scale attempts to discern patterns among functional groups or feeding guilds (Altermatt 2010a). Only recently have researchers attempted to answer this question experimentally for plant-pollinator communities (Rafferty & Ives 2011). Despite this lack of empirical data, consideration of the factors at play suggests some lines of investigation.

Dispersal may be most relevant in terms of spatial mismatch (Box 2.1), and possibly temporal mismatch in areas with high habitat heterogeneity, such as alpine environments (Kameyama & Kudo 2009). Interactions in which both (or all) interacting species have the ability to disperse over long distances could be less likely to suffer from spatial mismatches, where mismatches are driven by changes in species ranges (Thomas et al. 2004). At the same time, dispersal would not be helpful in the event that the species in question differ in their ecological amplitudes or physiological constraints, and one of the interacting species is unable to expand its range *despite* its ability to arrive in a new area through dispersal.

Adaptive **evolution** or phenotypic plasticity relating to physiological traits, including phenology, is also important. In the context of spatial mismatches, phenotypic plasticity, or high phenotypic variation would determine the ability of the interacting species to expand their ranges through dispersal (which itself is subject to natural selection), or to establish themselves in new areas through competitive ability and tolerances of abiotic conditions. Alternatively, heritable variation and/or phenotypic plasticity for the trait of flowering (e.g., Widén 1991), or emergence times could enable species to deal with changing conditions *in situ*. Adaptation *in situ* is expected to be important for long-lived, perennial species. Short-lived or annual species with potential for rapid evolution (e.g., Franks et al. 2007), in contrast, might be more likely to shift their phenological schedules in response to changes in phenology of species with which they interact via incorporation of more reliable or novel cues, enabling them to maintain synchrony under novel conditions (Visser 2008). This has been predicted because of the strong selective pressures on synchrony (Augspurger 1981; Widén 1991), and is supported by observational data on historical shifts in phenology for some species (Bartomeus et al. 2011; Rafferty & Ives 2011; Cleland et al. 2012). Species could also be behaviorally flexible in their ability to form and benefit from novel interactions, or rapidly evolve the ability to do so. Rafferty & Ives (2011) recently demonstrated that species of perennials that had shown historical advancement in flowering times (i.e. apparently had high adaptive capacity or plasticity for the trait of flowering time), were also able to take advantage of a community of pollinators with earlier flight-times while suffering no apparent reduction in reproductive fitness (although fitness was only inferred from visitation rates).

The last option, that of **extinction**, assumes limited ability to adapt, evolve, or move in response to changing conditions. It often forms the basic assumption of models that predict deleterious outcomes of climate change (e.g., Memmott et al. 2007). Again, the main concern tends to be the rate at which microevolution or range-shifts might occur, rather than the capacity for it to occur. Visser (2008), for example, theorized that 'reaction norms' encompassed by phenotypic plasticity should no longer be adaptive as phenology shifts, making it necessary for natural selection to drive further change. Rates of microevolution can be affected by existing genetic diversity (particularly for the traits of interest), effective population size, life-history characteristics such as generation time and reproductive output, and by the strength of selection (Yang & Rudolf 2010). Although long-distance dispersal is notoriously difficult to estimate (particularly for insects), it is predicted that range-expansions in plants will be more limited by their own rates of dispersal than by presence of pollinators (Thomas et al. 2004, but see Chacoff et al. 2012). Warmer temperatures can also allow some butterflies to expand their range by using new hosts (e.g., Pateman et al. 2012).

Finally, the data needed to address these questions will require a lot of time, effort, and creativity to collect, but several basic steps are important. These include collection of information on reproductive fitness (Yang & Rudolf 2010), tracking of individual plants over multiple years to determine plasticity of phenological responses to abiotic conditions (Forrest & Thomson 2010, Thomson 2010), and population-genetic studies that determine heritability of traits that could facilitate rapid evolution of phenology (Figure 2.4).

8) Can "sub-optimal" phenological matching be the result of more complex adaptive strategies?

This question deals with the assumptions inherent in the commonly-adopted definition of phenological synchrony (Figure 2.1). Typically, synchrony could be said to occur "when the peak timing of pollinator feeding coincides with the peak requirement by the plant for pollinator visits" (Singer & Parmesan 2010). While this concept seems simple enough, in reality it may be difficult to establish whether or not plants and pollinators are naturally synchronized. Working in a plant-herbivore system, Singer & Parmesan (2010) recently demonstrated that the emergence of caterpillars of *Euphydryas editha* has historically been poorly synchronized with the seasonal availability of its host plants, *Plantago* and *Castilleja*. They suggested that this was actually the result of a long-term adaptive strategy for living in fluctuating climates that involved a complex trade-off between the ability of females to grow large during the feeding season and produce many offspring on one hand, and risk of death due to incomplete development on the other. Consideration of trade-offs complicates the task of assessing whether shifts in plant/pollinator synchrony might represent truly maladaptive mismatches. One of the problems with use of long-term data to infer evolutionary relationships is that it remains difficult to establish baselines for matching of phenology (Visser & Both 2005, Parmesan 2006). This could be addressed by raising pollinators and their hosts in a series of growth chambers simulating variability in climatic conditions (e.g. temperature) to examine how it affects their degree of matching and reproductive success (i.e., the hypotheses outlined in Figure 2.1). There would be logistical challenges to running such experiments, keeping pollinators alive for multiple generations on limited floral resources, but supplementing pollinators' diets with a fixed amount of nectar, provided in feeders, could ensure survival while preserving biologically significant differences in success between pollinators in matched versus mismatched treatments. Again, a thorough understanding of the life histories of the organisms concerned is critical for drawing accurate conclusions regarding the likelihood and consequences of phenological mismatch.

9) Will new interactions arise with changes to phenology?

Predicting the demographic consequences of phenological mismatch is inhibited by the fact that we don't know how much change in interactions among plants, pollinators, and parasites is possible. In response to climate change, new mutualisms might arise, and parasitisms (thieving/robbing of floral resources, or seed-predation) might be either lost or gained. It is possible that there will be no extinctions (see above), single-species extinctions (resulting from asymmetrical interactions), co-extinctions of paired mutualists (Memmott et al. 2004), or cascading co-extinctions (Koh et al. 2004). Kaiser-Bunbury et al. (2010) recently presented a simulation study based on pollination networks from Mauritius, and found fewer extinctions were predicted when pollinators were allowed to switch food sources. Dietary flexibility or 're-wiring' is predicted to increase stability of pollination networks, with the caveat that a baseline level of species diversity and abundance is necessary to avoid total collapse of the network (Kaiser-Bunbury et al. 2010). The threshold for collapse of the Mauritian networks was extinction of '50–60% of the 24 most connected animals (not plants)' (Kaiser-Bunbury et al. 2010), but it is not known how this number might translate to other pollination networks.

Flexibility of interactions is relevant to the broader debate about whether specialists or generalists will be more affected by climate change. While specialists have a higher risk of population declines than generalists when considering broad-scale patterns for birds, invertebrates, and vertebrates, there are many exceptions to this rule (Colles et al. 2009). Exceptions occur mostly because of methodological problems with distinguishing specialists from generalists in the long term (Colles et al. 2009). When specialization is more rigorously defined (for example, by examining multiple axes to describe niche breadth, controlling for abundance and range, and requiring direct observations of resource-use), the link between specialization and risk of extinction becomes less clear (Colles et al. 2009). Memmott et al. (2007) predicted that obligate specialists would have more tightly-coupled cues, and therefore show similar shifts in response to climate change. However, they also acknowledged the relative rarity of specialist relationships (e.g., Petanidou et al. 2008). Schweiger et al. (2010) recently took a different theoretical stance, and (implicitly assuming the absence of tight coupling) posited that generalists would have the advantage because of their ability to occupy wider fundamental niche space, and thus increase their potential for forming novel interactions in the future, either with other generalist species, or with invasive, non-native species. Rafferty & Ives (2011) found that, among 14 perennials, generalist species were no more likely to have advanced their DFF (while still experiencing similar rates of visitation by pollinators) than specialist species during a 70-year period. These results are difficult to interpret due to lack of data on phenology of pollinators for the same period (and thus, inability to establish whether mismatches were occurring). If DFE of pollinators was known to have shifted at a similar rate during the same time period (e.g., Bartomeus et al. 2011), this might allow more meaningful inferences to be made about the relationship between specialization and long-term tracking of phenology between plants and pollinators.

A conceptual gap in contemporary studies of the consequences of phenological mismatch in plant-pollinator interactions has been lack of consideration for either flexibility or unexpected rigidity in plant-pollinator interactions (Colles et al. 2009; Kaiser-Bunbury et al. 2010), as well as the interactions between plants and floral antagonists. Even in cases where pollination webs are thought to be well-understood, evidence from long-term studies of pollination networks has shown that plant-pollinator interactions fluctuate naturally (and sometimes unexpectedly) between years (Olesen et al. 2008). There is inter-annual variation in the phenology and abundance of both plants and pollinators, and the nature of relationships among particular species may vary from year to year (Petanidou et al. 2008). It has been suggested that climate change may lead to “bizarre” phenology (Memmott et al. 2007), but the consequences of this are only now being considered experimentally in realistic communities (Raffery & Ives 2011). Emphasis has been on negative impacts predicted by loss of mutualistic interactions (pollination, or foraging) (Memmott et al. 2004, 2007; Kaiser-Bunbury et al. 2010), but it is equally possible that there will be positive impacts due to loss of parasitic or antagonistic interactions. Shifts in phenology may release plants from floral antagonists such as robbers of nectar or pollen, florivores, or seed-predators, and/or may release pollinators from deceptive plants that offer no rewards. Conversely, new antagonistic interactions may arise, which could have negative impacts on demography of the losing partner. The effects of newly-arising mututalistic or antagonistic interactions on consequences of phenological mismatch have received little attention. A strategy for studying these consequences empirically might be through the use of choice/no-choice experiments (Box 2.2). The key concept of choice/no choice experiments (similar to ‘network rewiring’, above) is that if pollinators are not exposed to situations in which the community of available plants is carefully manipulated, we can only know what plants they *prefer*, but have no information on which plants they could use if forced to do so (i.e. through real or experimentally-induced mismatch).

10) Are plant-pollinator interactions comparable across gradients in latitude and elevation?

In general, pollination by animals is relatively more common than pollination by wind at lower latitudes with birds and bats being more important closer to the equator (Regal 1982). Elberling & Olesen (1999) have also noted that Diptera tend to be the main pollinators in field sites at higher latitudes, but this general pattern is qualified and often confounded by the apparent increase in importance of Diptera relative to Hymenoptera in areas with higher moisture (Devoto et al. 2005). While these patterns have been known to exist for a relatively long time, there is little reference to them in the recent literature on phenological mismatches. Despite the lack of attention, information on identities of pollinators may be important because pollinators are *not* all functionally equivalent (see discussion above). Flies and bees are different in a number of life-history traits, all of which may interact in relevant ways with the plant-communities that they pollinate. Plants have long been known to exhibit "pollination syndromes" in which the morphology of flowers is subject to strong selective pressures from the community of pollinators (Fenster et al. 2004). However, seed-predators are also known to influence evolution of floral traits, including phenology, and the validity of the "pollination syndrome" concept has been questioned (e.g., Brody 1997).

Another example of ways in which pollinator taxa are not equivalent is sociality. Sociality can allow different pollinators to exploit resources that are presented as presses versus pulses, because social taxa can combine efforts to store food (Eickwort et al. 1996). This can interact with gradients of latitude or elevation via strength and manifestation of seasonality (Eickwort et al. 1996). Pollinators that are social and generalized are predicted to have advantages over species that are solitary and specialized (Schweiger et al. 2010). *Bombus,* for example, tends to have large variation in body sizes of workers, and multiple flight-periods per year (Goulson 2010) . Specialized social castes allow the entire colony to forage more effectively by locating productive feeding patches on longer flights, and by accumulating sufficient resources to allow for survival during transient periods of low resource-availability (Schweiger et al. 2010). These traits are predicted to increase the likelihood of *Bombus* adapting well to changes in phenology of plants because of flexibility in food-sources and flight-times. An alternative hypothesis is that sociality increases the baseline amount of resources necessary to support an entire colony (as opposed to a few individuals), in an area, throughout an entire season, increasing the risk of a population collapsing. Flies, in contrast to some bees, are not social, and the predominance of Diptera as pollinators at higher latitudes (Elberling & Olesen 1999) suggests that consequences of climate-change relating to phenological mismatches could differ at higher latitudes. Again, knowledge of large-scale patterns such as latitudinal gradients in pollen limitation might be important in answering this question. Indirect lines of evidence, such as the reduced importance of sexual (versus clonal) reproduction at higher latitudes (Dorken & Eckert 2001) might suggest the opposite – that mismatches between plants and pollinators at higher latitudes may have less severe consequences for demographic trends of plants (at least initially). Clonality could buffer individual plants from negative demographic consequences of mismatch by increasing the number of flowers per individual, which increases the probability of visitation, and thus seed set. Clonal reproduction could also provide a demographic buffer by ensuring recruitment that does not rely on seed-production or pollination.

Meanwhile, many of the factors mentioned above (prevalence of flies versus bees, pollen limitation, generalization, allocation of resources to sexual versus asexual reproduction) likely vary with respect to altitude, but to our knowledge have not been investigated in depth. Körner (2007) noted that there is a lack of studies that make use of gradients in elevation in general, which is the case in studying the consequences of phenological mismatches (Miller-Rushing et al. 2010). For example, precipitation tends to increase with altitude (Galen & Stanton 1991, Sevruk 1997), which could provide useful conditions for natural experiments on the interaction of moisture and phenological change in plant-pollinator interactions. This might also provide a way to investigate the impact of late-season moisture deficits on plant-pollinator interactions (e.g., visitation rates) and seed set. It has been predicted that there will be increased frequency and severity of late-summer droughts as a result of climate change, particularly in mountainous regions (IPCC 2007). Late-season droughts are already known to reduce survival and recruitment of seedlings (Galen & Stanton 1991), but might also have negative impacts on flowering and seed-production as a consequence of physiological stress (Jamieson et al. 2012). As mentioned previously, altitudinal gradients also provide natural but predictable variation in flowering times over small geographical areas that could lend themselves to experiments on plant-pollinator mismatch.

Conclusions:

In light of the broken assumptions and complex considerations discussed above, the current state of evidence for demographic consequences of mismatched plant-pollinator interactions appears sparse, but this is a new and fast-moving area of research. There is already evidence that plant-pollinator mismatch is not fully or accurately described by DFFE alone (Forrest & Thomson 2010), that the range of species and community responses to climate change is poorly understood (Diez et al. 2012), and that these responses tend not to be random or independent among species (Rafferty & Ives 2011). Furthermore, populations of plants and pollinators can be regulated by many processes besides the frequency of their interactions (e.g., Rafferty & Ives 2012), particularly where opportunities for sexual reproduction are irregular and species tend to be long-lived; plant populations may not be limited by seed set, and pollinators might not be limited by food. Pollen limitation of reproduction due to competition and facilitation appears to be highly context-specific (e.g., Harder & Aizen 2010), and not all interactions are equally beneficial (Rathcke 1983; Wall et al. 2003; Mitchell et al. 2009). While negative demographic consequences of mismatch might be exacerbated by non-linear responses of plant and pollinator populations to climate change, they might also be mitigated by a combination of phenotypic plasticity and adaptive evolution of phenology, generalization, properties of pollination networks (specifically, their nested, asymmetrical structure), habitat heterogeneity, dispersal, and the emergence of novel (or restoration of ancient) interactions. Pollination ecologists are poised to make important discoveries as long as we are able to continue to address explicitly the assumptions discussed. In particular, we must consider the spatial and evolutionary context of our study systems, and include as much relevant detail as possible regarding the life histories of study organisms before we can begin to look for larger-scale patterns. While the potential consequences of plant-pollinator mismatches may be enormous (Steffan-Dewenter et al. 2005), it is encouraging to note that this discussion has highlighted many ways in which the negative effects of mismatch might be mitigated. Because these topics in particular have not yet been studied in sufficient detail, there may be plenty of room for good news to keep us humming along.

Acknowledgements:

Funding was provided by the Natural Sciences and Engineering Research Council of Canada, the Pacific Institute for Climate Solutions, and the University of Victoria. I wish to thank Jessica Forrest, David Inouye, Kimberly Carlson, and Katharine Baldwin-Corriveau for comments on the manuscript.

Box 2.1: Spatial mismatches

Spatial mismatches refer to situations where changes to climate might cause divergence in the ranges of interacting species whose ranges have historically overlapped (Schweiger et al. 2008). Spatial mismatches might occur when differences exist in the ability of mutualistic partners to disperse to and colonize new locations, establish populations, and adapt or evolve for long-term persistence, although they are typically modelled simply as changes in the area a species can occupy after changes to conditions such as average air temperature (e.g., Thomas et al. 2004). Examples have been documented in Malaysia, where pollinating fig wasps were extirpated from a region because of a drought, despite the persistence of their host trees (Harrison 2000), and predicted for a butterfly and its larval host plant in Europe, based on projected changes in niche-overlap (Schweiger et al. 2008).

Both spatial and temporal mismatch could occur simultaneously, which would have effects that cannot be easily predicted (Schweiger et al. 2010). For example, species that can adapt to changes in climate, disperse and establish widely, or form new mutualistic interactions might be less affected than species that have low rates of dispersal and high host-specificity (e.g. Harrison 2000, Schweiger et al. 2008). Indeed, European butterflies have not expanded their ranges at the same rate as shifting climatic envelopes from 1990-2008 (Devictor et al. 2012), suggesting that adaptation *in situ* might be more important than escaping changing conditions through range-expansion. The lack of range expansion might be because butterflies' host plants had not yet changed in range, but no data were available to address this hypothesis (Devictor et al. 2012). The exclusivity of a mutualism (i.e. degree of specialization of the pollinator and its host plant) could be important because high specialization might restrict the ability of either partner to expand its range (Chalcoff et al. 2012, Schweiger et al. 2012). This is because the potential range of an obligate mutualist would be restricted to the range of its partner, while a more generalized species could theoretically change hosts to expand its range (Schweiger et al. 2012). Pollination is vital in this respect, because 1) pollen-movement could be equated to gene-flow (Schmitt 1980), defining the effective size of a population and 2) long-distance dispersal of plants is typically undertaken through the production of seed via outcrossing (Silvertown 2008). The genetic variability associated with sexual reproduction is notably advantageous when coping with uncertain futures (reviewed by Silvertown 2008), or at the limits of a species' range (Starzomski, *in press*).

An element of spatial mismatches that has not been studied in detail is the effect of high microclimatic complexity characteristic of mountainous environments, due to the influence of aspect, slope, and elevation. Accounting for topographic complexity is important because it could affect (and likely restrict) the spatial scale at which both phenological and spatial mismatches occur, reducing the geographical distances that need to be spanned to mitigate the mismatch (Heikkinen et al. 2010). Spatial mismatches are typically modelled in two dimensions based on constructions of "bioclimatic envelopes" that describe *potential* ranges for species, combined with known rates of dispersal, ideally in response to historical environmental change (e.g. Thomas et al. 2004, Schweiger et al. 2008). However, these approaches are unlikely to describe accurately situations in which species occupy a much smaller area of their theoretical range (i.e., the realized niche). Furthermore, fine-scale variation in microclimate might exist at scales that can be traversed by larger pollinators, particularly when resources are scarce and the pollinators are "searching" rather than foraging within smaller patches (Thomson et al. 1982). In mountainous environments with high topographic complexity, the large variation in abiotic conditions (moisture, insolation, temperature, precipitation, and wind), coupled with high dispersal ability or large foraging ranges of some pollinators might provide some flexibility to prevent deleterious spatial or phenological mismatches from occurring (Schweiger et al. 2012). Based on calculations from Colwell et al. (2008), in the neotropics there is about 1°C drop in temperature for every 170 m gain in elevation, while there is about a 1°C drop in temperature per 145 km of increased latitude. Thus, the distance travelled to mitigate the mismatch is theoretically about an order of magnitude greater if travelling across latitude rather than elevation. In this case, one could also predict that pollinators with larger foraging ranges (e.g. larger-bodied bees and social bees such as *Bombus*; Greenleaf et al. 2007; Zurbuchen et al. 2010) would be less likely to suffer negative effects of mismatch because their host plants, despite having shifted their geographical ranges in tandem with a shifting climactic envelope, might still be *spatially* close.

Box 2.2: Choice/no-choice experiments

Relatively simple choice/no-choice experiments have been a classic tool employed by behavioural ecologists for decades, and are now understood to be fundamental in clarifying the concept of specialization with respect to trophic interactions (Johnson 1980). Unfortunately, this tool has not yet made widespread appearances in the literature on plant phenology. Figure 2.3 shows an example of a simple choice/no choice experiment using pollinators and flowers. The key concept behind choice/no-choice experiments is that the full range of possibility for trophic interactions cannot be reliably assessed simply by observing interactions in the field. This is because such observations merely represent the realized interactions, which may be the result of complex decision-making processes or trade-offs that have not been considered, and represent foraging preferences under specific conditions. A number of long-term, high-resolution studies of pollination networks have been published and provide the basis for constructing models of plant-pollinator interactions (e.g. Bascompte et al. 2003, Memmott et al. 2004, 2007, Olesen et al. 2008, 2011), but the predictions generated from these models are reliant on the assumption that *all* possible interactions are being considered. Distinguishing obligate relationships from facultative relationships is fundamental to addressing the potential consequences of climate-driven mismatch between plants and pollinators; treating relationships as obligate when they are actually facultative overestimates the negative consequences of mismatches.

While experiments like the one depicted in Figure 3 may at first appear to be logistically difficult for non-domesticated species, they are not beyond reach. Manipulating the presence or absence of specific members of the community of flowering plants could be done by strategically placing plants from greenhouses into the field (e.g., Rafferty & Ives 2011), by transplanting flowers into “novel” situations and observing whether or not they still receive visits from pollinators under mismatched conditions, or by deliberately removing a species or set of species from a community of flowering plants.

Moving a single plant species at a time from a greenhouse to another location (a greenhouse with pollinators, or a field that is not yet in bloom) where it is the only species blooming would represent a no-choice experiment for the resident pollinators, while moving an array of species to the same setting would provide a choice. Using potted plants, arranged in arrays, can also control for the effects of density and provide a local source of pollen. Arrays of potted plants have recently been used to manipulate pollination conditions for perennials as well as annuals (e.g. Rafferty & Ives 2010), and could be followed for many years to evaluate long-term fitness and variability among years. Here, the choice scenario (an array of species) would represent the phenological shift of an entire community of flowering plants, or a control scenario in which no shifts occurred. The no-choice scenarios would indicate the consequences of phenological shifts of a single species (to a time when all other plants were not blooming), or a shift of all other species resulting in availability of only one species for the pollinators. Species could also effectively be removed or reduced in density for a period of time by selectively pruning flowering heads over a given study area, which could simulate a no-choice scenario for the single remaining species. This could be compared with scenarios presenting the full range of choices for pollinators in sites (or plots) where no species (or only some) were pruned. By measuring the relative rates at which pollinators visit plants under various scenarios, the consequences of changes to communities of flowering plants could be inferred. This would provide a more direct indication of the consequences of phenological mismatch for pollinators and plants.

While it is more difficult to manipulate pollinator communities than it is to move around plants, capturing and removing bumblebees to reduce their local abundance can have significant effects on the foraging behaviour of other species (e.g., Inouye 1978), and bees introduced into flight cages of eight square metres appear to forage and behave normally (Fründ et al. 2012). Forrest & Thomson (2011) recently “transplanted” some pollinating bees by moving nesting-boxes to different locations along an altitudinal gradient. Future experiments could use nesting-boxes, warmed by incubators to alter emergence times. This could simulate an altered phenological response by the insects, and the consequences of mismatch could be measured by following the success of individuals using the nesting boxes (as suggested by Forrest & Thomson 2011). Individual bees have never been followed for long periods of time because of logistical constraints, but this might be done using chemically marked (or individually genotyped) bees, which could be encouraged to re-nest in nest-boxes if their ranges for foraging or dispersal are small, or if they are able to complete their life cycles in flight cages that contain boxes. While the nuances of multiple-choice feeding experiments are beyond the scope of this paper, methods have also been developed for quantifyin*g* an animal’s preference for one resource over others (e.g., Roa 1992; Manly 1993), and these types of approaches could be used to make predictions about the resilience of pollination networks to changes in synchrony.

Chapter 3:
Factors limiting seed set in alpine meadows

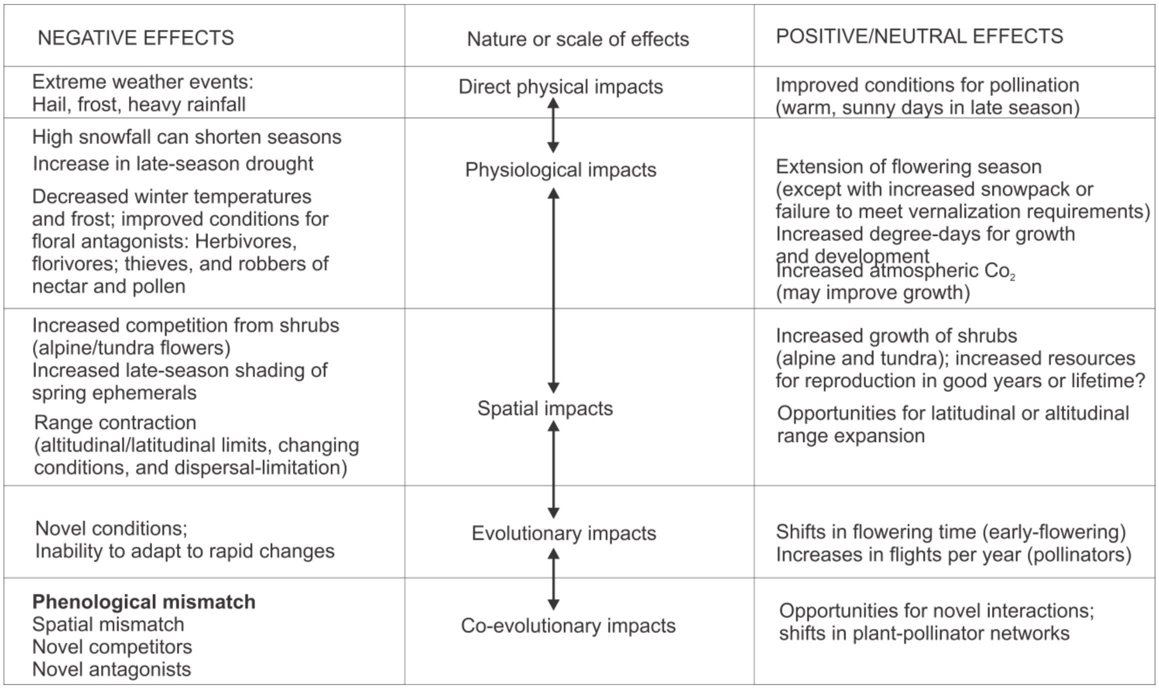
Abstract:

Predicting demographic consequences of climate change for flowering plants requires understanding which factors influence seed set, and how climate change may alter those factors. To determine what affects seed set in the alpine, I used pollen-manipulation experiments combined with measurements of natural variation in temperature and abundance of pollinators along a 400 m elevation gradient. I did this for a community of seven species of flowering plants in the Coast Range of British Columbia. The number of viable seeds set by plants was influenced by pollen limitation (quantity of pollen received), mate limitation (quality of pollen received), temperature, relative abundance of pollinators, seed predation, and combinations of these factors. Three of the four early-flowering species had higher seed set at high elevations while two of three three late-flowering species had higher seed set at low elevations - a pattern that might be explained by synchrony with the timing of emergence and inter-generational gaps of bumble bees, or variation in pollinator preference through time. Degree-days above 15°C were good predictors of seed set, particularly in bee-pollinated species, further suggesting a relationship with phenology. The number of viable seeds in *Arnica latifolia* (Asteraceae), a late-flowering, primarily fly-pollinated, outcrossing species, was strongly influenced by seed predators (Tephritidae) at middle elevations, where there were fewer hours of frost during the flowering season. *Anemone occidentalis* (a fly-pollinated, self-compatible species) had high seed set at all elevations, likely due to extraordinarily high rates of visitation at all locations. This chapter highlights the importance of studying multiple factors contributing to seed set as opposed to focusing on single causes. I end with a brief discussion of ways in which climate change may influence the factors determining seed set to make alpine plants either vulnerable or resilient to climate change, and provide suggestions for future research.

Introduction:

Predicting the demographic consequences of climate change for flowering plants is one of the most difficult but important questions faced by ecologists today (Hegland et al. 2009; Forrest 2011). These predictions require thorough understanding of which factors influence seed set, and how conditions may be altered by climate change. Many factors have already been identified, but the ways in which they could be affected by rapid climate change are largely unknown (Table 3.1).

Table 3.1 - Factors influencing seed set and predicted (hypothetical) positive or negative demographic impacts related to climate change (references provided in text).



NEGATIVE EFFECTS	Nature or scale of effects	POSITIVE/NEUTRAL EFFECTS
Extreme weather events: Hail, frost, heavy rainfall	Direct physical impacts	Improved conditions for pollination (warm, sunny days in late season)
High snowfall can shorten seasons Increase in late-season drought Decreased winter temperatures and frost; improved conditions for floral antagonists: Herbivores, florivores; thieves, and robbers of nectar and pollen	Physiological impacts	Extension of flowering season (except with increased snowpack or failure to meet vernalization requirements) Increased degree-days for growth and development Increased atmospheric Co$_2$ (may improve growth)
Increased competition from shrubs (alpine/tundra flowers) Increased late-season shading of spring ephemerals Range contraction (altitudinal/latitudinal limits, changing conditions, and dispersal-limitation)	Spatial impacts	Increased growth of shrubs (alpine and tundra); increased resources for reproduction in good years or lifetime? Opportunities for latitudinal or altitudinal range expansion
Novel conditions; Inability to adapt to rapid changes	Evolutionary impacts	Shifts in flowering time (early-flowering) Increases in flights per year (pollinators)
Phenological mismatch Spatial mismatch Novel competitors Novel antagonists	Co-evolutionary impacts	Opportunities for novel interactions; shifts in plant-pollinator networks

A way in which many of these factors are mediated, and a place where they can be studied, is through the processes affecting pollination, and pollen limitation in particular. The question of why, (and whether) sexual reproduction of flowering plants is generally limited by availability of pollen has been the subject of much interest and debate (reviewed by Burd 1994; Larson & Barrett 2000; Knight et al. 2005), and most of the field work on this topic has been done in temperate or alpine locations. Large-scale meta-analyses of pollen limitation have shown that most flowering plants are pollen limited (e.g., Larson & Barrett 2000; Knight et al. 2005). This runs contrary to the basic prediction that the number of ovules produced by plants should be optimized to match availability of pollination services and resources (Haig & Westoby 1988), but there is a great deal of variation among different ecological contexts, and many studies do not find evidence for pollen limitation (Burd 1994; Totland & Schulte-Herbrüggen 2003; García-Camacho & Totland 2009).

The factors that drive pollen limitation are varied in space and time (Ehrlén 1992; Totland 2001; García-Camacho & Totland 2009), frequently confounded with other forms of reproductive limitation (Knight et al. 2005), and not well understood in terms of overarching patterns (Mayer et al. 2011; Gulías & Traveset 2012). Self-compatible species, for example, benefit from outcrossing (Darwin 1862), but tend not to be pollen limited (Totland & Schulte-Herbrüggen 2003). The number and quality of seeds that plants produce may be limited by lack of resources, unpredictability of resource-availability, or inadequate distribution of resources (Lee & Bazzaz 1982; Ehrlén 1992; Totland 1997), failure to attract pollinators (Burd 1994), and spatial or temporal variation in preferences of pollinators (Totland 2001; Forrest & Thomson 2009) or effectiveness of pollinators (Rafferty & Ives 2012). Even when fertilization occurs, seeds are often lost to seed-predators (e.g., Lee & Bazzaz 1982), or unable to reach maturity during short seasons (e.g., Thórhallsdóttir 1998; Cooper et al. 2011).

Climate change could act as an indirect driver of seed set by altering weather and temperature. Seasonal weather conditions including sunlight, snow pack, and atmospheric degree-days can affect seed set indirectly by influencing patterns of pollinator activity or abundance (Schemske 1977; McCall & Primack 1992; Totland 1994a, 1994b) or by inducing plant-pollinator mismatch (described in Chapter 2) through altered phenology (e.g., Bazzaz 1990, Inouye et al. 2000, 2002; Kudo et al. 2004). Temperature conditions interact with plant physiology to negatively affect reproductive output in cases where temperatures are not warm enough or seasons are not long enough for maturation of seeds (Galen & Stanton 1993; Burd 1994), with frost in particular leading to aborted ovules before or after fertilization (Inouye 2000, 2008; Kudo & Hirao 2006). Plants can also have highly plastic responses to variables linked with climate change. Experimental warming of alpine plants using open-topped chambers can lead to increased leaf area and seed weight, allowing plants to better acquire resources and allocate them to higher-quality seeds with higher germination success (Totland 1999; Totland & Alatalo 2002; Klady et al. 2011). Overhead heaters have been shown to increase seed set when averaged over a community of ten subalpine species, but without having any significant effects on individual species (Price & Waser 1998). Conversely, heat stress can cause flowers and seed pods to be aborted (e.g., Monterroso & Wien 1990) and winter warming events can severely disrupt annual phenology and negatively impact reproductive potential (Bokhorst et al. 2008). Resource limitation or physiology can also be confounded with pollen limitation (Totland 1997, 2001), which makes it difficult to understand the mechanisms behind increased seed set and seed quality and make predictions about the effects of climate change based on warming experiments. While elevated CO_2 is expected to increase growth of entire plants, it has been found to delay flowering time while decreasing the number, size, and nectar concentration of flowers (Hoover et al. 2012). Temperature can counter some of these effects by advancing flowering time, and increasing the number of flowers and volume of nectar produced, but it can also have a negative effect on the size of flowers (Hoover et al. 2012). Where plants and their pollinators are well-adapted to large inter-annual variation in flowering time, climate-warming has been predicted to increase seed set due to lengthening of the snow-free flowering season (Price & Waser 1998; Thórhallsdóttir 1998). Rather than driving mismatches between plants and pollinators, climate change may actually reduce existing mismatches (Singer & Parmesan 2010), or cause a shift in networks of plant-pollinator interactions with neutral or positive impacts on seed set (Kaiser-Bunbury et al. 2010; Forrest 2011; Rafferty & Ives 2011).

While pollen manipulation experiments have been a tool used to study breeding systems and pollen limitation for many years (Kearns & Inouye 1993; reviewed by Knight et al. 2005), much of what we know about the factors driving pollen limitation comes from meta-analyses of existing data sets collected in various years and locations (e.g., Ashman et al. 2004; Knight et al. 2005), or from studies focusing on a single species in a single ecological context (e.g., Schemske 1977; Totland 1997; Kudo & Hirao 2006). Many studies rely on assumptions about variation in abundance of pollinators without measuring it (e.g., Fabbro & Körner 2004), or use temperature data from no more than a few weather stations, which are sometimes poorly representative of conditions at the field site or within different areas of the field site (Forrest 2011). Few studies have simultaneously manipulated the availability of pollen for multiple species (i.e., a community; e.g., Motten 1986) in an alpine environment, across a measured gradient in abundance of pollinators and temperature.

To address some of these issues, I performed community-level pollen limitation experiments in a series of alpine meadows (Marriott Basin) in the Coast Range mountains, British Columbia, Canada. Pollen manipulation experiments can be used to provide information on both pollen limitation and breeding systems of flowering plants (Kearns & Inouye 1993), both of which can have implications for the resilience of flowering plant populations to climate change (Thórhallsdóttir 1998; Fabbro & Körner 2004). In this study, *pollen limitation* refers to limitation of seed set because of failure of ovules to receive sufficient quantities of pollen for fertilization, and is differentiated from situations where the quality of available pollen limits fertilization. Plants that are not pollen limited receive sufficient quantities of pollen to fertilize all mature ovules. In the context of pollen manipulation experiments, pollen limitation can be estimated by comparisons between pollen-supplemented plants and “open” (or control) plants experiencing natural levels of pollination; larger effects of pollen-supplementation indicate that plants are probably pollen limited (Kearns & Inouye 1993). *Breeding system* refers to whether plants are able to set seed through self-fertilization, or whether they require pollen from other sources (outcrossing) (Richards 1997). While outcrossing is important in promoting sexual selection and adaptation in the long-term, self-fertilization can also provide means of reproductive assurance against pollen limitation (Darwin 1859, 1862), particularly where pollinators are scarce (Stebbins 1950).

The objectives of this experiment were to 1) determine the breeding systems of the most abundant flowering plants at Marriott Basin, 2) test the assumption that reproduction of alpine plants is primarily limited by access to pollinators, 3) test whether pollen limitation or patterns of seed set varies with elevation and 4) determine the major drivers of variation in reproductive limitation (seed set) at Marriott Basin. If access to pollinators limits the reproduction of plants, I predicted that pollen-supplemented flowers (saturated with pollen) would have the highest rates of seed set, while open flowers with natural levels of pollination would have lower seed set, and bagged flowers (where pollinators are excluded) would have little or no seed set, except as a result of selfing. Based on the common assumption that pollinators are more scarce at high elevations due to harsher conditions (Fabbro & Körner 2004), I expected that plants exposed to 'ambient' levels of pollination at high elevation would experience more severe pollen limitation than conspecifics at low elevation. I therefore predicted that pollen-supplementation would cause the greatest increase in seed set for plants at high elevation. Finally, I tested a series of alternative hypotheses regarding drivers of reproductive limitation in alpine plants (Table 3.2). The question I asked was: *what limits seed set of plants at Marriott Basin*? To answer this question, I evaluated evidence for six non-mutually exclusive hypotheses: pollen limitation (pollen quantity), abiotic limitation, biotic limitation, combined abiotic and biotic limitation, mate limitation (pollen quality) and limitation by seed-predation (Table 3.2).

Table 3.2 –Non mutually exclusive hypotheses, and their predictions, to answer the question of what limits seed set of alpine plants. (B = bagged, PS = pollen-supplemented, O = control or "open").

Hypothesis	Explanation/Statement	Predictions
Pollen limitation	Seed set is limited by availability (quantity) of pollen	Pollen-supplementation should always result in increased seed set, except for species with high selfing rates. (PS > O, PS > B; in selfing species, PS ≈ O ≈ B).
Abiotic Limitation	Seed set is limited by physiological responses to abiotic factors such as temperature, wind, and snowmelt.	There should be variation in seed set among elevations (different temperature regimes), even when similar quantities of pollen are received (B and PS plants should show variation with elevation). Temperatures should be a good predictor of variation in seed set among elevations. (Low ≠ mid ≠ high for B and PS).
Biotic Limitation	Seed set is limited by biotic factors such as relative abundance of insects (pollinators, or seed-predators) or competition among co-flowering plants for access to pollinators.	There should be variation in seed set among elevations (different temperature regimes) only for plants with natural levels of pollination. "Open" plants should show variation with elevation, but seed set of bagged (B) and pollen-supplemented (PS) plants should not vary with elevation. Relative abundance of insects from pan traps should be a good predictor of variation in seed set among elevations.
Combined biotic and abiotic limitation	Seed set is limited by both abiotic and biotic factors (see above).	There should be variation in seed set along an elevation gradient among plants that receive constant (B, PS) and naturally varying (O) levels of pollination. Both temperatures and relative abundance of insects should be good predictors of variation in seed set among elevations.
Mate-limitation (pollen-quality)	Seed set is limited by the quality of outcrossed pollen received; pollinators provide pollen from multiple pollen donors, assuring that some pollen will be viable.	"Open" plants should set more seeds than either bagged or pollen-supplemented plants, assuming that pollinators provide pollen from > 3 donors. (O ≥ PS > B). Abundance of pollinators should be positively associated with seed set for open plants.
Limitation by seed-predation	Number of viable seeds should be limited by the number of seed predators.	Seeds or flowers should show evidence of seed-predation. Seed predators should negatively affect seed set; plants with signs of seed-predation should have significantly less viable seed than plants with no evidence of seed-predation.

To test the predictions from this set of hypotheses, I combined an experimental approach (pollen-manipulation) with measurements of natural variation in abiotic and biotic factors that could limit seed set, across an elevation gradient.

Methods:

Field Site:

Marriott Basin (50.4119 N, 122.3525 W), is located in the Coast Range mountains of British Columbia. The field site included a series of alpine meadows ranging in elevation from approximately 1700-2140 m (Figure 3.1).

Figure 3.1 - The study site at Marriott Basin, August 2011. Light green patches are alpine meadows, spanning a 400 m gradient of elevation from the lake (approx. 1650 m) to the ridge-line (approx. 2200 m).

The most abundant flowering species at Marriott Basin can be separated into early and late ‘flushes’ (Pojar 1974). Early-flowering species include *Erythronium grandiflorum*, *Anemone occidentalis*, *Claytonia lanceolata*, and *Caltha leptosepala*; late-flowering species are *Valeriana sitchensis*, *Arnica latifolia*, *Lupinus arcticus*, *Sorbus sitchensis*, and *Vaccinium membranaceum* (Pojar 1974). *Erigeron alpinus*, *Salix* sp., *Thalictrum alpinum*, *Chamerion angustifolium* are also abundant, but with patchier distributions than the first set of species, and *C. angustifolium* did not bloom during the summer of 2011.

The most abundant visitors of flowers at Marriott Basin in 2011 were *Bombus* sp., a diverse assemblage of Syrphidae, sawflies in the family Tenthredinidae, and many species of Muscidae, Anthomyiidae, and Sarcophagidae. A list of insect taxa observed visiting the more common species of flowering plants is provided in Appendix A.

Study species:

I initially targeted nine species of plants for pollen-manipulation experiments, and analyzed data from the seven species with seeds that I could reliably collect and count. I selected focal species using a series of traits including taxonomy, structure of flowers and seeds (ease of manipulation), abundance, range, pollination syndrome, growth form, and economic or cultural importance (Table 3.3).

Table 3.3– Species used for pollen limitation experiments in alpine meadows at Marriott Basin, BC, and some of their traits. All species are long-lived perennials, identified in the field or lab using Pojar et al. (1994).

Species & abbrev.	Traits as study sp.	Pollination	Size, habit	Flowers/ inflor.	Fl. display, resources	References
Lupinus arcticus S. Watson (Fabaceae). Arctic lupine Abbrev. *L. arcticus*	Late-flowering. Abundant, widespread. N-fixer, related to many crops. Robust; easy to manipulate	Bees. Self-compatible but usually non-selfing.	Large perennial, woody at base but herbaceous stems. ~ 20-60 cm tall.	Racemes, usually with many (30-100) flowers/ per raceme.	Purple. Scent, nectar. Usually up to four racemes per plant (can have more).	Pojar 1974, Pojar et al. 1994, personal obs.
Erythronium grandiflorum Pursh (Liliaceae). Yellow glacier lily Abbrev. *E. grandiflorum*	Early-flowering. Abundant, widespread Countable seeds Robust; easy to manipulate	Bees, humming-birds, usually non-selfing. Sometimes pollen-limited.	Short, herbaceous perennial 'geophyte' Usually 15-20 cm tall.	Single flowers (rarely two or three).	Showy, drooping, yellow flowers. Scented. Abundant nectar.	Pojar 1974, Thomson et al. 1986; Thomson 2010
Caltha leptosepala D.C. (Ranunculaceae). White mountain marsh marigold Abbrev. *Calt. leptosepala*	Early-flowering. Abundant. Countable seeds Robust plant; easy to manipulate	Bees, flies, butterflies; often pollen-limited	Short, herbaceous perennial; 5-15 cm tall.	≤ 4 flowers per cyme (typically 1-2).	Small white flowers with blue tinge. Obvious nectaries.	Pojar 1974, Thomson 1980; Bell et al. 2005
Arnica latifolia Bong. (Asteraceae). Broad-leaved arnica.	Late-flowering. Abundant Countable seeds Robust plant; easy to manipulate	Syrphidae (flower flies), butterflies, muscid flies, some bees.	Perennial, herb, 20-60 cm tall.	~50-100 fertile disc flowers per inflor.; 1-3 heads per plant.	Large, showy, composite. Abundant nectar, pollen.	Pojar 1974, Thomson 1980, 1982
Sorbus sitchensis M. Roem. (Rosaceae). Sitka mountain ash.	Late-flowering. Abundant Provides fruit for birds, mammals	Insect-pollinated (primarily bees, flies)	Tall, woody, perennial shrub; 1-4 m tall.	Large, showy inflor. with ~50-80 flowers.	Scented. Nectar. Many inflor. per plant.	Pojar 1974, Pojar et al. 1994, personal obs.

Vaccinium membranaceum Douglas ex Torr. (Ericaceae). Black huckleberry. Abbrev. *V. memb.*	Late-flowering. Abundant, with large geographic range Culturally, ecologically important fruit	Non-selfing, specialized for pollination by *Bombus*.	Woody, perennial shrub. ≤ 1.5 m tall.	Single flowers in leaf axils. Max. 50 per plant (usually fewer)	Small flowers. Scented. Abundant nectar., may be ‘buzz-pollinated’	Pojar 1974, Vander Kloet 1988
Valeriana sitchensis Bong. (Valerianaceae). Sitka valerian.	Late-flowering. Abundant Easily manipulated	Mostly flies, some bees and butterflies.	Perennial, herbaceous plant. Stems 40-120 cm tall	Cluster of ~50-200 flowers.	Large, white inflor. Strong scent, abundant nectar.	Pojar 1974, Pojar & et al. 1994, personal obs.
Claytonia lanceolata Pall. ex Pursh (Portulacaceae). Western spring beauty Abbrev. *Clay. lanceolata*	Early-flowering. Abundant Seeds easy to collect and count	Bees and flies (muscids, Syrphidae)	Small, delicate herbaceous perennial. Stems 5-15 cm tall.	Raceme with 3-15 flowers (usually only 1-3)	Small, delicate, pinkish-white flowers. Scent and abundant nectar.	Pojar 1974, Schemske 1977, personal obs.
Anemone occidentalis S. Watson (Ranunculaceae). Western pasqueflower. Abbrev. *Anem. occidentalis*	Early-flowering. Abundant. Seeds easy to collect.	Self-compatible. Flies (muscids, syrphids), more rarely by bees.	Perennial, herbaceous Woody base. Stems ~10-60 cm tall	Single flowers.	Probably heat rewards. Abundant pollen. No scent or nectar.	Pojar 1974, personal obs.

1) Estimating pollen limitation:

In July 2011, I divided the slope at Marriott Basin (Figure 3.1) into nine sites, stratified into three low-elevation sites (~1706-1772 m), three mid-elevation sites (~1841-1870 m), and three high-elevation sites (~2010-2090 m) (Table 3.4).

Table 3.4 - GPS locations (WGS-84 datum) of the centre points of sites for pollen-limitation experiments, temperature recording, and insect-trapping at Marriott Basin, B.C.

Site No.	Easting	Northing	Elevation (m)	Elevation category
1	W122 28.025	N50 25.711	1727	Low
2	W122 27.720	N50 25.823	1867	Mid
3	W122 27.779	N50 25.992	2062	High
4	W122 27.809	N50 25.674	1747	Low
5	W122 27.606	N50 25.785	1870	Mid
6	W122 27.466	N50 25.932	2090	High
7	W122 27.506	N50 25.488	1710	Low
8	W122 27.347	N50 25.591	1847	Mid
9	W122 27.101	N50 25.708	2088	High

At each of the nine sites, I opportunistically located and marked 36 flowers of each of the nine study species prior to anthesis. For each species, I randomly assigned individuals at each site into three treatments: pollen-supplemented (n=12), bagged (n=12) or control (open) (n=12). I labelled open flowers individual plant numbers and treatments. Plants designated as “pollen-supplemented” or “bagged” were covered with 10 cm x 12 cm transparent cloth bags, which have little or no effect on floral development (Kearns & Inouye 1993), and the bags were closed around the stems with drawstrings. The treatments and controls were thus randomly assigned to individual flowers, and dispersed across the extent of the alpine meadows at Marriott Basin (an area of approximately 1.2 km^2), with 36 plants of each treatment, at each elevation.

After the plants were marked and assigned to treatments or controls, I returned to each site every two days to watch for the onset of flowering. Approximately 10% of the plants that were originally marked and assigned to treatments were destroyed as a result of weather (hail, heavy rainfall), or destroyed by animals (hoary marmots, American pikas, and Sooty Grouse). Plants from all three treatments were destroyed in approximately equal proportion, and were replaced haphazardly by labelling the nearest possible closed flower at the same stage as the previously-labeled flowers, and assigning it to the appropriate treatment. At worst, this would be expected to reduce the size of any effects detected by increasing “noise” in my results due to differences in the amount of time that treatments (bags) were applied. Replacement plants flowered at about the same time as the original plants because I replaced them with plants in the same stage, and each of the treatments had similar proportions of flowers destroyed. Final sample sizes are reported in the *Results* section.

As flowers opened, I applied the pollen-supplementation treatments. Open (control) flowers were left untouched, in order to represent “ambient” conditions for pollination. I removed the cloth bags from bagged flowers, being careful not to brush the anthers or stigmas or damage the flowers, and replaced them. The purpose of removing the bags was to control for the effect of damage or self-pollination that might have been inadvertently caused by manipulating the bags, at least for the comparison between bagged and pollen-supplemented flowers. Finally, I removed the bags from the pollen-supplemented flowers and brushed their stigmas with pollen taken directly from the anthers of at least three separate and haphazardly-selected “donor” individuals that were blooming within the same site (usually 10-15 m away from the treated plant, to avoid accidental geitonogamy – or transfer of pollen among flowers or clones of the same plant). Whenever I removed bags, I prevented insects from landing on flowers by waving my hands. This prevented unwanted pollen from being transferred to the stigmas; all bees and Syrphidae seemed to be deterred simply by my presence. I then allowed the plants to set seed, and re-visited them every 2-4 days to ensure that the bags were not removed and plants were not destroyed. Once the seeds were sufficiently developed to count (but before they dispersed), I collected the seed heads from all marked plants, and counted the number of viable and non-viable seeds per seed head or flower. I differentiated between viable and non-viable seeds based on size, shape, and colour. Viable seeds were generally large, thick, firm, and dark, while non-viable seeds were small, thin, soft, and pale. For plants with only one flower (e.g., *E. grandiflorum*, *Anem. occidentalis*, *Arnica latifolia*), I expressed seed set as the number of viable seeds *per plant*. For plants with large inflorescences and more than one flower contained within a bag (e.g., *V. memb.*, *L. arcticus*, *Clay. lanceolata*, *Calt. leptosepala*), I treated flowers or seed heads as sub-replicates, averaging the number of viable seeds for flowers within the same bag or treatment, and expressed this as the mean number of viable seeds *per flower*.

2) Measuring temperature

At the beginning of the growing season (25-Jul-2011), I installed four wooden stakes per site, at least 5 m apart, to which I taped Thermochron iButton temperature loggers (Maxim Integrated Products, Inc., Sunnyvale, CA, USA) 10 cm above ground level. I programmed the iButtons to record temperature at hourly intervals. I collected the loggers at the end of the season and calculated the mean temperature values of the four temperature loggers, which I then used to calculate variables based on temperature for each site, which included degree-days (see below), average temperatures, minimum and maximum temperatures, and hours below freezing throughout the recording period. After returning from the field, I discovered that the iButtons at mid-elevation sites had only recorded a three-day period of useful temperature data. To obtain a full set of temperature data for mid elevations, I extrapolated missing values from known temperatures during the recorded period, based on a strong ($R^2 \approx 0.95$) polynomial fitted relationship between values at high elevations and those at mid elevations for the period where they were recorded. I also recorded daytime weather data (important for determining the activity patterns of insects and short-term phenology of flowering plants) and visual evidence of phenological stages of various species using time-lapse PlantCams, which were set to take photographs at low elevations every three hours between 8:00 am and 8:00 pm, every day between July 29 and August 29, 2011. These videos are available online at: http://www.youtube.com/watch?v=wnDQcweDsjE , and: http://www.youtube.com/watch?v=7WoB2DXNZaE&feature=relmfu, as well as a series of still photos showing observations of factors that can directly affect seed set, at http://www.youtube.com/watch?v=0MiADreir8g.

3) Trapping insects over elevation gradients:

The objective of trapping insects was to determine whether there was significant variation in the relative abundance of potential pollinators and herbivores/seed-predators across the 400 m elevation gradient used for my pollen manipulation experiments. I therefore used the same site for the trapping study as I did for the pollen manipulation experiment, but the traps were set up a minimum of 20 m from the study sites to minimize interference with flowers from my experiment (either by attracting pollinators to traps instead of flowers, or depressing local abundance of pollinators by removing them from the population). To capture insects, I set out 45 traps perpendicular to the slope, spaced at three-metre intervals, at each of the three elevations (high, medium, and low). The 45 traps included 15 traps of each of three colours: yellow, blue, and white (Figure 3.2A). The colours were set in alternating order, but systematically permutated among elevations to avoid bias from changes across the slope (Figure 3.2B).

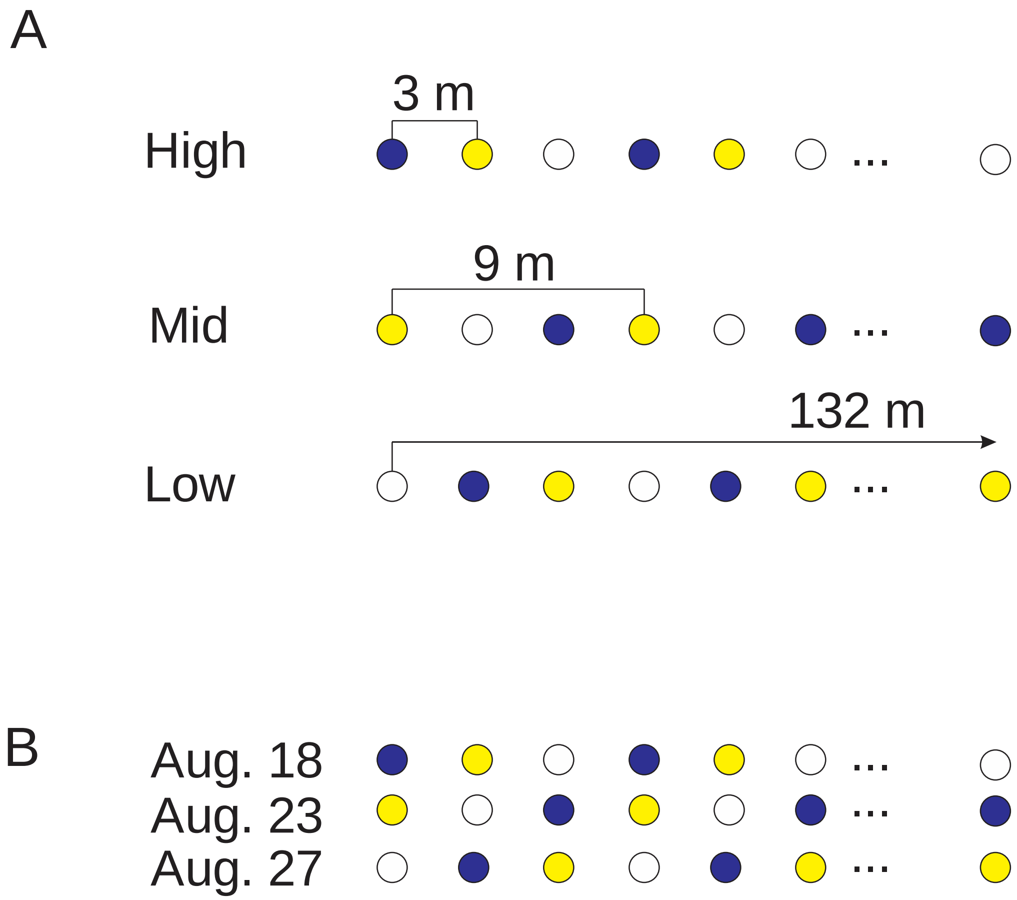


Figure 3.2 - Layout of coloured pan traps per elevation (A) and permutation of traps between sampling intervals (B) used for capturing insects at Marriott Basin in 2011.

The colours I used were from standard protocols followed by the Canadian Pollination Initiative (CANPOLIN) and the United States Department of Agriculture (USDA) (Droege et al. 2010), and were designed to maximize the number of insects trapped, while representing a variety of different-coloured flowers from the natural community (Table 3.5).

Table 3.5 - Correspondence between colours of abundant flowers and colours of traps used for collecting insects at Marriott Basin, summer 2011. Genera listed in parentheses ( ) were present at the site but not used in this study.

Trap colour	Genera of flowers
Blue	*Lupinus*, (*Veronica)*
White	*Anemone, Valeriana*, *Caltha, Vaccinium, Claytonia, Sorbus*
Yellow	*Arnica, Erythronium, (Potentilla, Ranunculus)*

The traps were Solo brand 6 oz plastic cups (Solo Cup Company, USA) painted with outdoor acrylic paint. Traps were set up and filled with water before the slope received full sunlight (approximately 8 am, before most pollinators were active), and collected around dusk (approximately 8 pm), well after the peak in daily pollinator activity, minimizing the difference in the time for which they were set up. To account for differences among sampling dates due to weather, and shifts in the insect community through time, we carried out trapping on three separate days: 18, 23, and 27-Aug-2011. As shown in Figure 3.2, the locations in which traps of each colour were set were permutated between different sampling dates to prevent bias resulting from placement of certain colours in certain microclimates; the entire data set therefore included each of the three colours at each possible location along the slope, and colours were pooled for analysis.

Statistical Methods:

1) Pollen-limitation and breeding system

Because of the large number of zero values (flowers with no viable seeds), the data could not be transformed, nor analyzed with statistics assuming a normal distribution. I therefore used Kruskal-Wallis and Mann-Whitney U Tests with Dunn’s Multiple Comparison Test in GraphPad Prism v. 5.01 (GraphPad Software, San Diego, U.S.A., 2007) to determine whether the number of viable seeds among bagged, pollen-supplemented, and open plants were significantly different. I chose a critical significance level of α = 0.05 for all tests. I also calculated effect sizes as log response ratios of seed set to pollen-manipulation (following Hedges et al. 1999), pooled across the elevation gradient (provided in Appendix B).

2) Factors limiting seed set

I performed multiple regressions in R, version 2.15.0 (R Development Core Team, 2012), using the **zeroinfl( )** function in the **pscl** package (Zeileis et al. 2008). I assessed the significance of the models (including predictors) in relation to a null model (without predictors) using likelihood ratio tests implemented by the **lrtest()** function in the package **lmtest** (Hothorn et al. 2012).

i) Temperature:

I recorded hourly temperatures between July 31st and August 28th, 2011, using 12 iButtons placed at each elevation (four per site). Two iButtons failed, so my final number of replicates was 10. I tested for differences in temperature between high and low elevations (the two extremes, for which there was a complete temperature record with 10 spatial replicates) using repeated-measures Analysis of Variance (ANOVA) in GraphPad Prism, with each hourly temperature record as a repeated measure. For the purpose of the analysis, I converted temperatures recorded from the iButtons to degree-days by counting each hour during the recording period for which the temperature recorded exceeded a given temperature threshold. The temperature thresholds I used for degree days were DD0 (degree days above 0 °C), DD05 (above 5 °C), DD10 (above 10 °C), DD15 (above 15 °C), and DD20 (above 20 °C). I calculated a series of temperature variables based on the recorded temperatures, averaged within each elevation. The variables included average temperature; degree days (for the thresholds mentioned above); maximum temperature; minimum temperature; frost hours (total number of hours with temperatures below 0°C); and maximum number of continuous frost hours.

Previous studies indicate that developmental thresholds are generally higher for insects than for plants, but also that different degree day thresholds can be most important for particular species (e.g., Campbell et al. 1974; Nealis et al. 1984; Larl & Wagner 2006;

Hülber et al. 2010; Forrest & Thomson 2011). To estimate which temperature variables were most useful as predictors to be included in the regression models, I constructed a series of models that varied only in the temperature variable used as a predictor. I then compared these models using log-likelihoods and Akaike's Information Criteria (AIC) (Akaike 1973), which provide measures of how well the model describes the data observed, with penalties for using higher numbers of parameters (Johnson & Omland 2004). I then used the temperature variable that provided the best-supported model as a predictor in the regression model.

ii) Diversity and abundance of invertebrates from pan traps:

To test whether there were significant differences in the average number of pollinators each elevation, I used Repeated Measures ANOVA with elevation as a three-level factor and each of the three sampling dates treated as a repeated measure. Treating date as a repeated measure ensured that my results were conservative by controlling for the effect of re-sampling the same locations, which could have included similar environmental conditions or carry-over of insect populations between dates. I then characterized differences in the diversity, abundance, and community composition of invertebrates among the three elevations and three sampling dates based on Bray-Curtis Dissimilarity (Bray & Curtis 1957), calculated from a square-root transformed, normalized matrix of abundance for 170 'morphospecies' (see Appendix C; original morphospecies keys are available upon request). To test for differences in the composition of the insect community at low, mid, and high elevations I used Analysis of Similarity (ANOSIM) and Similarity Percentages (SIMPER) in PRIMER-E, version 6.1.13 (Primer-E Ltd, Lutton, UK) (Clarke 1993). I ran separate analyses based on three taxonomic levels: morphospecies, families, and functional groups (see Appendix C for details). SIMPER revealed that trends at all taxonomic levels were driven by the trends for the "black fly" morphospecies which was by far the most abundant flower-visitor at Marriott Basin (Figure 3.6A), and included a mixture of Muscidae, Anthomyiidae, and Sarcophagidae. Patterns of abundance of known pollinating taxa (bees, Tenthredinidae, and Syrphidae) across the elevation gradient were similar to those of black flies. I therefore used the total number of invertebrates trapped (N) as a predictor in my multiple regression models. For a more conservative estimate of pollinator abundance, I repeated the analyses using a subset of morphospecies that fit strictly into the “pollinator” category, which included Syrphidae, Tenthredinidae, and all bee genera (*Andrena* and *Bombus*). Using the full set of potential pollinators including black flies did not substantially change the results of the model-selection, so I have only presented the results using the full suite of potential pollinating taxa (i.e., flower visitors). I used the same log-likelihood-based procedure (described for the temperature variables above) to assess the quality of different predictors based on abundance of invertebrates, which included the total abundance of insects collected at each site (as a proxy for abundance of pollinators), the total abundance of pollinating taxa collected at each site, and the average number of pollinators collected by each colour of trap at each site.

iii) Seed predators:

After collecting seeds, I carefully inspected all seed pods or flower heads for signs of seed-predation and recorded the number of seed-predators per flower when they were present. Seed-predation was rare for most species, and did not seem to vary in frequency between bagged versus unbagged plants. In *E. grandiflorum* and *Calt. leptopsepala* it tended to involve the entire seed head (the entire head being removed and eaten), so plants that were affected by seed-predation were not included in the analysis. In *L. arcticus*, seed-predation was rare, and usually caused by larval Syphyta or Coleoptera. In the case of *Arnica latifolia*, I discovered that the plants had been bagged once eggs or larvae of one species of Tephritidae (fruit fly) were already present on the flower head. These larvae therefore developed on the flower head throughout the season, and remained there when I collected the seeds. When the seeds were collected, I placed the entire flower heads in cloth bags, and the adult flies then emerged and were contained in the bags. I counted the number of emerged adult flies per flower, and inspected the flower heads for pupal cases to confirm the number of fly larvae per flower. I tested the effect of seed-predators on the number of viable seeds using Mann-Whitney U Tests to compare flowers with predators to those without predators. I tested for differences in the number of seed predators among the three elevations using Kruskal-Wallis Tests with Dunn’s Multiple Comparisons. For *Arnica*, I also used the number of seed-predators as a predictor for the zero-inflated negative binomial regression with the number of viable seeds per flower as the response, and the number of insect larvae per flower as a single predictor.

Predicting limitations to seed set:

To evaluate temperature and abundance of insects as predictors of seed set, I used multiple regression with the number of viable seeds per flower as a response variable, and a combination of predictors based on temperature and abundance of insects. Having examined the effects of pollen-manipulation experiments separately, I only used “open” (control) plants for the multiple regression because abundance of insects should not be a useful predictor of seed set for bagged or pollen-supplemented plants (from which insects were excluded).

Before performing regression analyses, I centred and scaled all variables using the **scale( )** function in R. For each model, I chose one temperature variable as a predictor and one measurement of insect abundance as a second predictor. I chose temperature variables based on developmental thresholds that were found to be important for plants and insects at the Rocky Mountain Biological Laboratory, Colorado (Forrest & Thomson 2011). While the data from Colorado (the best available to date) were useful for predicting phenology, it was unclear whether the same thresholds might apply for predicting seed set, which is likely to be reliant on thresholds for pollinator activity and seed-development rather than development and emergence of pollinators and flowers. For *Arnica latifolia* (the only species with evidence of frequent seed predation), I incorporated the number of seed predators per flower as a third predictor. Finally, I performed a sensitivity analysis by creating a series of models using each of the predictors in turn, which I then compared using log-likelihoods and Akaike’s Information Criterion (AIC) scores.

To account for non-normality, overdispersion, and a large number of zeros in my response variable, I used zero-inflated count models based on a negative binomial distribution. I verified the validity of this approach using Vuong tests (Vuong 1989), comparing zero-inflated models to generalized linear models based on the negative binomial or Poisson distribution. Zero-inflated, negative binomial models (ZINB) can account for unequal variances across samples, and model the zeros in a data set as part of either a zero-inflated component (a process generating the large number of zeroes) or a count component (a process generating values equal to or greater than zero) (Zeileis et al. 2008). I verified my results by running the same model structures using hurdle models (ZANB) (Zeileis et al. 2008; Zuur et al. 2009), which account for the presence of zeroes by breaking the model into a count component and a zero-inflation component where the count component does not contain any zeroes (i.e., includes only values greater than zero) (Zeileis et al. 2008). In contrast, zero-inflation models assume that zeroes can be part of either the count component or a separate zero-inflation component (Zeileis et al. 2008; Zuur et al. 2009). ZINB and ZANB models performed similarly (i.e., had nearly identical log-likelihoods) for these data, so I have presented results from only the ZINB models. This decision was based on biology of the system, since some small number of flowers with zero seed set could result from unknown biological processes apart from the factors of interest, which were pollination and temperature (e.g., failure to develop due to somatic mutations, shading, physical damage).

I began by using full (‘saturated’) models with both temperature and insect abundance as predictors, then simplified the models using a backward-forward model selection procedure implemented by the **stepAIC ( )** function in R. I verified the results of the model-selection by directly comparing AIC scores, dropping predictors to simplify the model when removing them from the models did not reduce the AIC score by more than 2. Only the best-supported models are reported in the Results section.

Power analysis:

Once my data were collected, I performed a *post hoc* power analysis using the statistical software program G*Power v.3.1.2 (Faul et al. 2009). I calculated effect sizes using mean values and standard deviations, and calculated statistical power ($1 - \beta$) using my total sample size, at an α-error probability of 0.05. For multiple regression, I calculated statistical power using the linear multiple regression: fixed model protocol, for 2 predictors with a “medium” effect size (0.15), α-error probability of 0.05, and total applicable sample sizes for each species; the calculations for *Arnica latifolia* were done for 3 predictors because of the inclusion of seed-predators as a third predictor. The results of my power analysis are presented in Appendix D. Despite using non-parametric tests, statistical power was high for treatment effects ($1 – \beta = 0.83$-0.99), but more variable and generally lower for comparisons of seed set among elevations, within treatments ($1 – \beta = 0.06$-0.83), mainly because of large variance in seed set among individual plants. Statistical power for regression analyses was high ($1 – \beta = 0.64$-0.96).

Results:

Pollen-limitation and breeding system:

Rates of self-fertilization were generally low for all species. Pollen-supplementation increased seed set over bagged flowers, but flowers left open to natural rates of pollination set the most seeds (Figures 3.3-3.4). Bagging significantly reduced seed set in *L. arcticus* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), *V. memb.* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), and *Clay. lanceolata* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$) (Figure 3.3), and there was little or no self-fertilization in these three species, indicated by the large effect size of bagging (log response ratios for bagging were between -1.9 and -3.0) (Figure 3.3). Bagging also significantly reduced seed set for *Arnica latifolia* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$), and *Calt. leptosepala* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$) (Figure 3.3), but there were enough viable seeds in bagged plants to suggest that self-fertilization occurred in these species (log response ratios for bagging were -0.3 for *Arnica latifolia* and -1.3 for *Calt. leptosepala*) (Figure 3.3). *Anem. occidentalis* and *E. grandiflorum* showed evidence of self-fertilization (log response ratios for bagging were -0.5 for *Anem. occidentalis* and -1.0 for *E. grandiflorum*) (Figure 3.4) but bagging still reduced seed set for both *Anem. occidentalis* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$) and *E. grandiflorum* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$) (Figure 3.3).

For most species, open flowers set significantly more seeds than pollen-supplemented flowers (Figure 3.3B), indicating an advantage to insect pollination over hand pollination. This was the case for *Anem. occidentalis* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), *E. grandiflorum* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$), *L. arcticus* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), and *Clay. lanceolata* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.01$). There were two exceptions to this pattern: pollen-supplementation significantly increased the number of viable seed per flower in *Arnica latifolia* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$) (Figure 3.3), and *Calt. leptosepala*, but the increase for *Calt. leptosepala* was not statistically significant (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P > 0.05$) (Figure 3.3).

Most species had higher seed set in pollen-supplemented flowers than in bagged flowers, indicating that the pollen-supplementation treatments were usually effective at increasing seed set beyond levels achievable through self-fertilization (Figures 3.3A,3.4). This was the case for *Arnica latifolia* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), *L. arcticus* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$), *Calt. leptosepala* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.01$), *Clay. lanceolata* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.001$), and *V. memb.* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.01$) but not for *E. grandiflorum* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P > 0.05$) or *Anem. occidentalis* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P > 0.05$). Both *E. grandiflorum* and *Anem. occidentalis* had high rates of selfing in bagged plants, resulting in small effect sizes (log response ratios for pollen-supplementation were 0.14 for *Anem. occidentalis* and 0.38 for *E. grandiflorum*) (Figure 3.4).

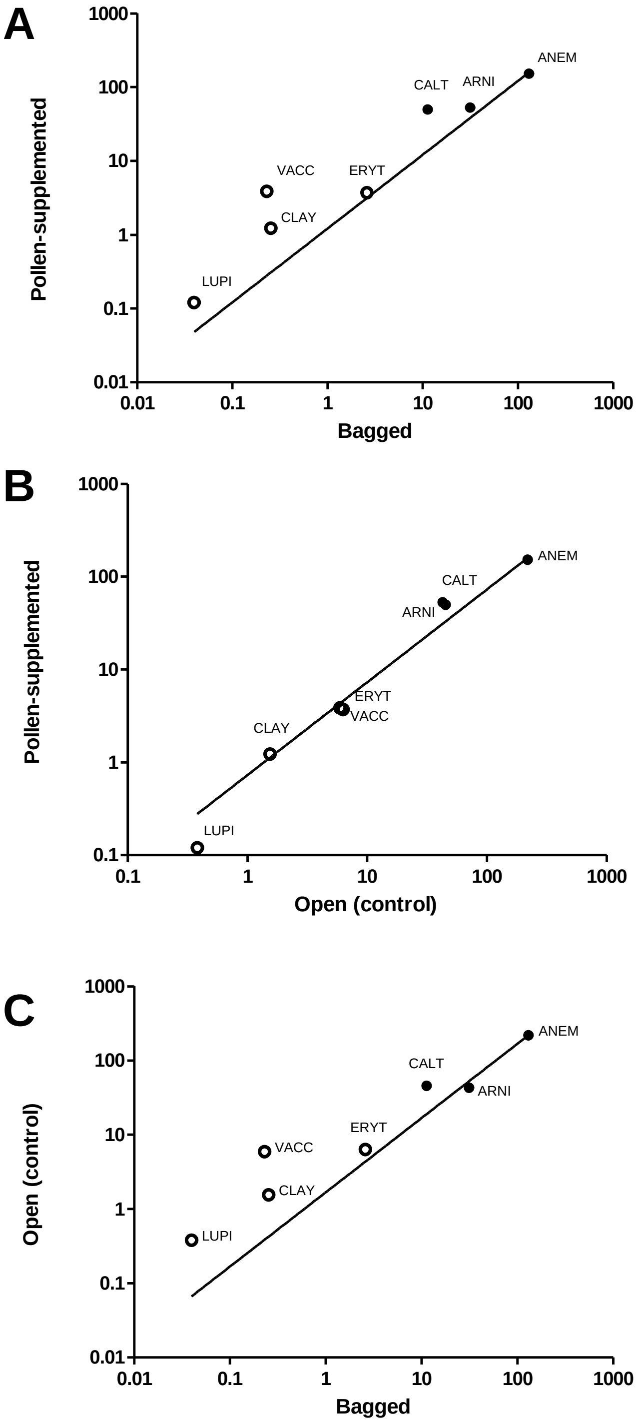


Figure 3.3 - Comparisons of seed set (logged viable seeds per flower) for two treatments (bagged, pollen-supplemented) and one control (open) used in pollen-manipulation experiments. Open circles represent primarily bee-pollinated species while filled circles are fly-pollinated. Straight lines represent a 1:1 relationship on log-linear axes so that deviations from the line represent significant effect sizes

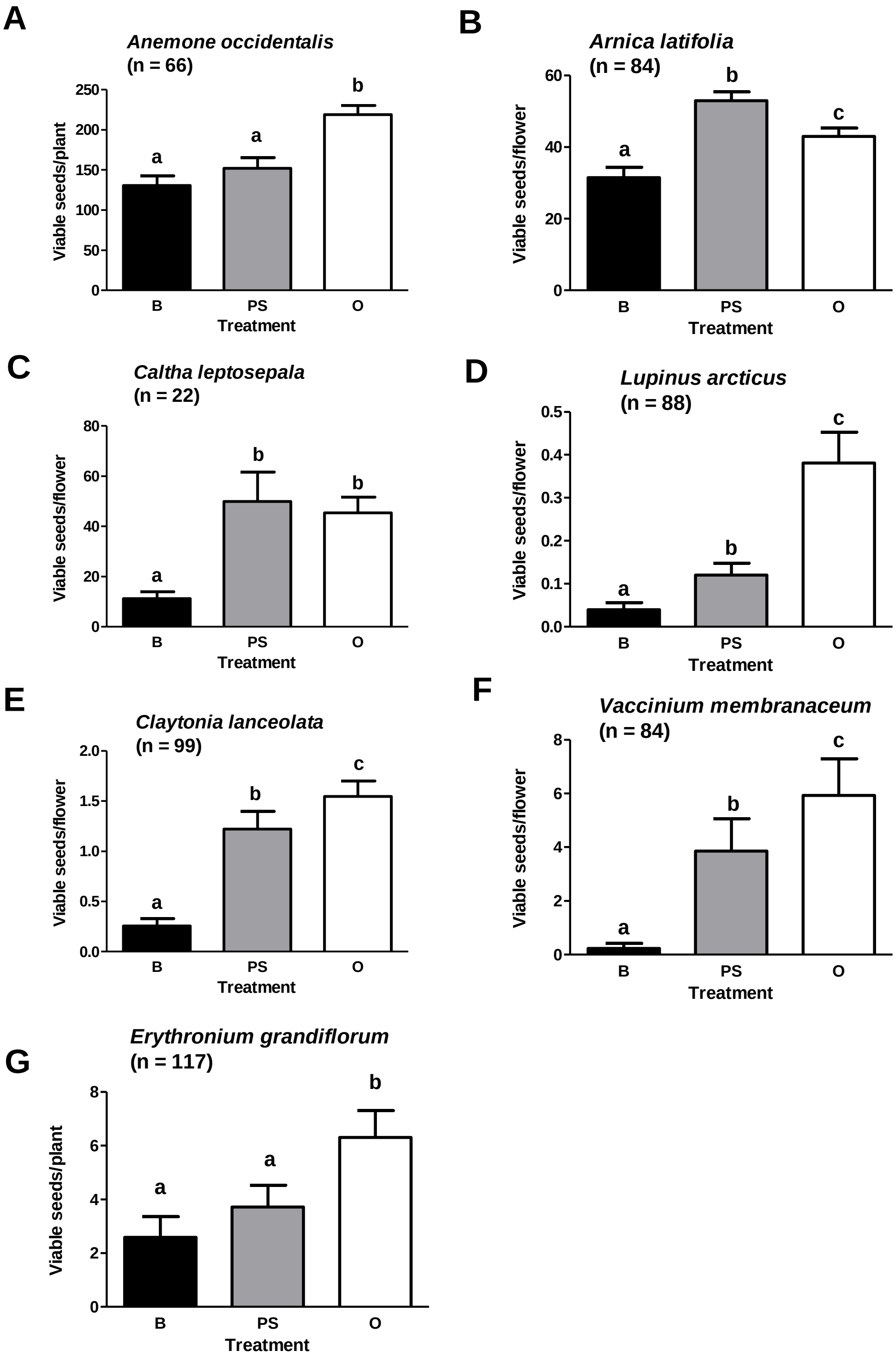


Figure 3.4 - Seed set among pollen-manipulation treatments (B = bagged, PS = pollen-supplemented, and O = open or control) in seven species of alpine flowering plants pooled across all elevations; n = total number of replicate plants per treatment. Bars are means +/- SE. Different letters above bars represent statistically significant differences (Kruskal-Wallis tests, $P < 0.05$).

Differences among elevations:

In general, seed set did not differ among plants from the three elevations (Figure 3.5). This was mainly due to small effect sizes and large variances (i.e., the lack of biologically significant differences), rather than inability to detect statistically significant differences due to small sample sizes (Appendix D). For all seven species, seed set of bagged flowers did not vary significantly among low, mid, and high elevation (Kruskal-Wallis and Mann-Whitney U Tests: $P > 0.05$) (Figure 3.5). Seed set of bagged, open, or pollen-supplemented flowers did not differ significantly among the three elevations for *Anem. occidentalis* or *L. arcticus* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P > 0.05$) (Figure 5B,E). Seed set of open flowers had widely differing mean values among elevations for *Lupinus* and was higher at lower elevations, but the large variance in seed set per flower meant that differences were not statistically significant (Figure 3.5), perhaps reflecting an "all-or-nothing" style of pollination. Variance was smaller for *Anem. occidentalis* (which was largely selfing or fly-pollinated), but the mean number of viable seeds per open flower was nearly identical across the elevation gradient (Figure 3.5).

When species were separated into early-flowering versus late-flowering, there was evidence of a weak pattern, with early-flowering species having higher seed set at high elevations, and late-flowering species having higher seed set at low elevations (Figure 3.5). Within the pollen-supplemented treatment, plants at high elevations set significantly more seed than those at low elevations for the early-flowering species *Erythronium* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.01$) and *Claytonia* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$) (Figure 3.5A,F). Open (control) flowers at high elevations also set more seed than those at low elevations for *Claytonia* (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$) and the early-flowering *Caltha* (Mann-Whitney U Test: $U = 27.00$, $P_{[\text{two-tailed}]} = 0.0302$) (Figure 3.5D,F).

Vaccinium, a late-flowering species, showed the opposite trend: seed set was greater at low elevations than at high elevations for both pollen-supplemented (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$) and open flowers (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P < 0.05$; Figure 3.5C). *Arnica*, a late-flowering species, was an exception to this pattern because low and high elevation sites did not have significant differences in seed set (Kruskal-Wallis Test, Dunn's Multiple Comparison: $P > 0.05$), but mid-elevation sites had significantly lower seed set than low and high sites for both pollen-supplemented (Kruskal-Wallis Test: K-W Statistic = 19.05, $P < 0.0001$) and open flowers (Kruskal-Wallis Test: K-W Statistic = 15.73, $P = 0.0004$; Figure 3.5G).

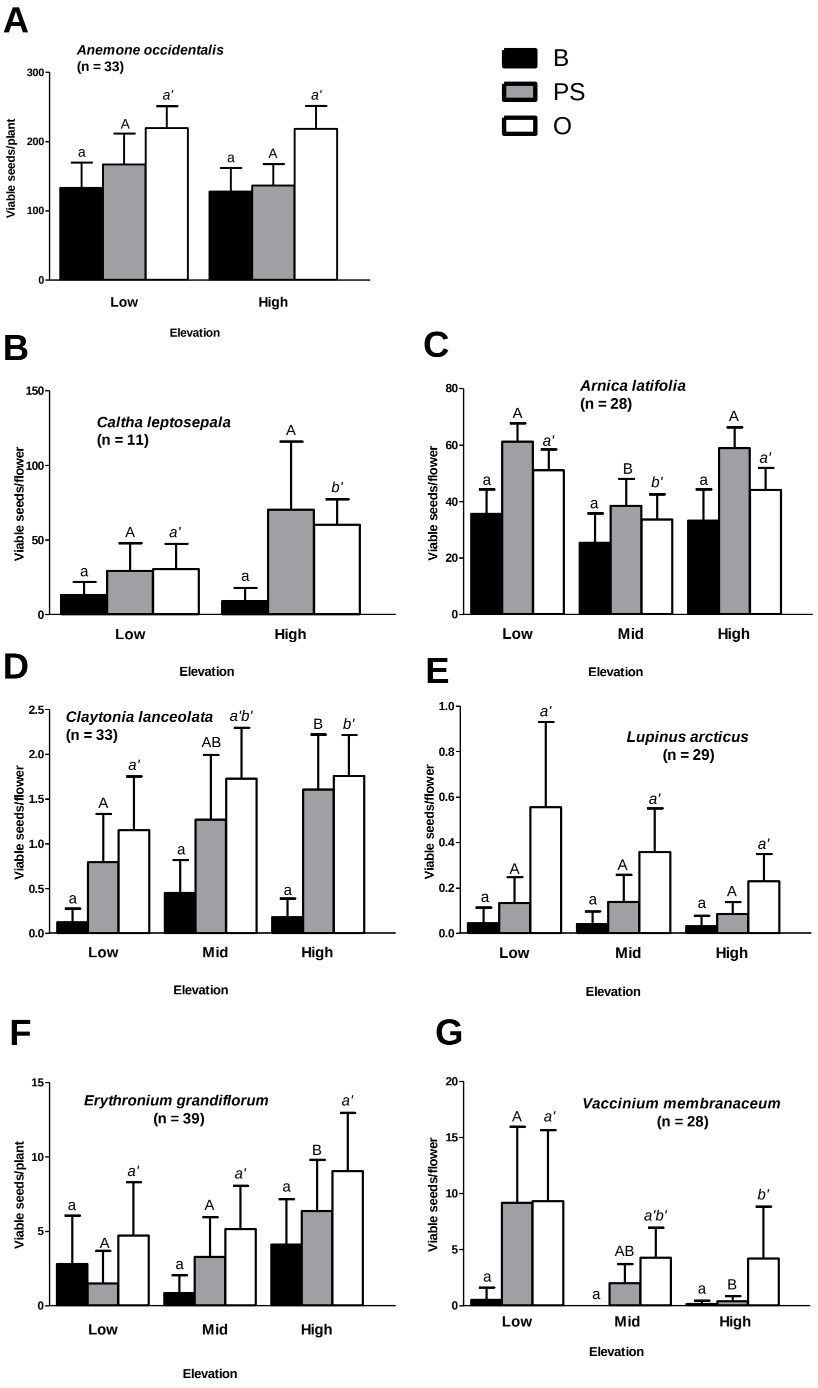


Figure 3.5 – Effect of pollen-manipulation treatments, applied across three elevations to seven species of alpine flowers at Marriott Basin, BC. Species on the left panel (A,B,D,F) are early-flowering, while those on the right (C,E,G) are late-flowering. Different letters indicate statistically significant differences as calculated using Kruskal-Wallis and Mann-Whitney U Tests ($P < 0.05$). Statistical tests were only done as planned comparisons between letters of the same case (e.g., a vs b, or a' vs b'). Bars are means +/- SE; n = number of replicate plants per treatment, per elevation.

Temperature:

Temperatures were significantly different among low and high elevations (Repeated Measures ANOVA: F = 6.960, P = 0.0167, df = 1), and there was a significant interaction between temperature and time when hourly temperatures were analyzed as repeated measures, indicating that temperature does not have the same relationship with elevation at all times (Repeated Measures ANOVA: P < 0.0001, F = 4.55, df =688). The ability to detect differences in temperature among elevations was limited by the small sample size that was necessary for avoiding pseudo-replication (10 sites per elevation), so these estimates should be considered conservative. Based on the interaction between temperature and elevation over time, summarizing temperature differences among the three elevations was not as straightforward as calculating mean values, so I proceeded by using several predictors based on temperature for multiple regressions (Table 3.6).

Table 3.6 –Temperature variables at three elevations in Marriott Basin, B.C. for July and August, 2011. DD10 and DD15 indicate degree-days above 10°C and 15°C, respectively. Frost hrs. are shown as the number of hours below 0°C (Frost hrs.) and the maximum number of continuous frost hours.

Elev.	Avg T (°C)	Max (°C)	DD10	DD15	DD20	Frost hrs.	Max # cont. frost hrs.
Low	10.79	32.88	11.29	8.04	5.25	12	7
Mid	11.42	36.13	11.42	7.94	5.08	7	5
High	10.58	35.63	11.04	7.63	4.79	14	10

Temperature was a significant predictor of seed set for *Erythronium*, *Lupinus*, *Caltha*, and *Vaccinium* when used in multiple regression models (Table 3.8). Several temperature variables were useful predictors of seed-set, and the variable with greatest predictive ability varied among species. For both *E. grandiflorum* and *V. membranaceum* DD15 were important in predicting the number of instances of zero seed set (i.e., zero-inflation). In *E. grandiflorum*, higher numbers of degree-days above 15°C were associated with higher probabilities of zero seed set, but in *V. membranaceum*, DD15 were negatively associated with instances of zero seed set. For *V. membranaceum, C. leptosepala*, and *L. arcticus*, DD15 was also a significant predictor of the number of seeds. Higher DD15 were associated with lower seed counts in *V. membranaceum* and

C. leptosepala, while higher DD15 were associated with higher seed counts in *L. arcticus*. Higher maximum temperatures were positively associated with seed set in *C. lanceolata*.

Relative abundance and diversity of invertebrates:

I identified a total of 170 morphospecies of invertebrates from Marriott Basin. Invertebrate communities were significantly different among the three elevations (ANOSIM: Global R = 0.173, P = 0.016), as were average abundances of pollinators in all three colours of traps combined (Repeated Measures ANOVA: F = 5.41, df = 2, P = 0.0127). Communities were significantly different between low and mid elevation sites (R = 0.261, P = 0.007), and between mid and high elevation sites (R = 0.204, P = 0.028), but the communities at low and high elevation sites were not significantly different (R = 0.062, P = 0.141) (Figure 3.6A). The taxonomic dissimilarities between sites were primarily driven by large (> 5 mm) and small (< 5 mm) black flies, including Muscidae, Anthomyiidae, and Sarcophagidae (Table 3.7).

Table 3.7 – Major drivers of taxonomic dissimilarity (%) between elevations. 'Black flies' included Muscidae, Anthomyiidae, and Sarcophagidae. Strictly pollinating taxa (third column) are the only strict pollinators (bees, syrphids, and sawflies) that were in the top 90% of contributors to between-elevation dissimilarities. In all cases, strictly pollinating taxa contributed less than 2% of dissimilarity between sites

Comparison	Primary driver	Secondary driver	Strictly pollinating taxa
Low vs Mid	'Black flies' (37.96%)	Sciaridae (6.65%)	Tenthredinidae: 2 spp. (0.75%)
Mid vs High	'Black flies' (35.32%)	Sciaridae (7.97 %)	Tenthredinidae: 1 sp. (0.93%)
Low vs. High	'Black flies'(44.70 %)	Sciaridae (7.59 %)	Tenthredinidae: 2 spp. (1.77 %)

Differences in abundance of Sciaridae contributed an additional ~ 7 % of dissimilarity, and the only strictly pollinating taxa (taxa that were confirmed pollinators, rather than floral visitors or generalists) appearing in the top 90% of contributors to dissimilarity were two species of Tenthredinidae (sawflies), which contributed less than 2% of dissimilarity between sites (Table 3.7).

There was a strong interaction between trapping date and abundance of invertebrates over the elevation gradient when the three trap colours were analysed separately (Figure 3.6B; Repeated Measures ANOVA: Blue: $P_{[\text{date x elevation}]} = 0.0064$, $F = 4.149$, $df = 4$; $P_{[\text{elevation}]} = 0.0127$, $F = 5.410$, $df = 2$; White: $P_{[\text{date x elevation}]} = 0.0408$, $F = 2.744$, $df = 4$; $P_{[\text{elevation}]} = 0.0634$, $F = 3.155$, $df = 2$; Yellow: $P_{[\text{date x elevation}]} = 0.0045$, $F = 4.425$, $df = 4$; $P_{[\text{elevation}]} = 0.0632$, $F = 3.158$, $df = 2$). Most striking was the peak in abundance of invertebrates at low elevations on 23-Aug-2011, when the abundance of invertebrates was far lower at mid and high elevations (Figure 3.6B).

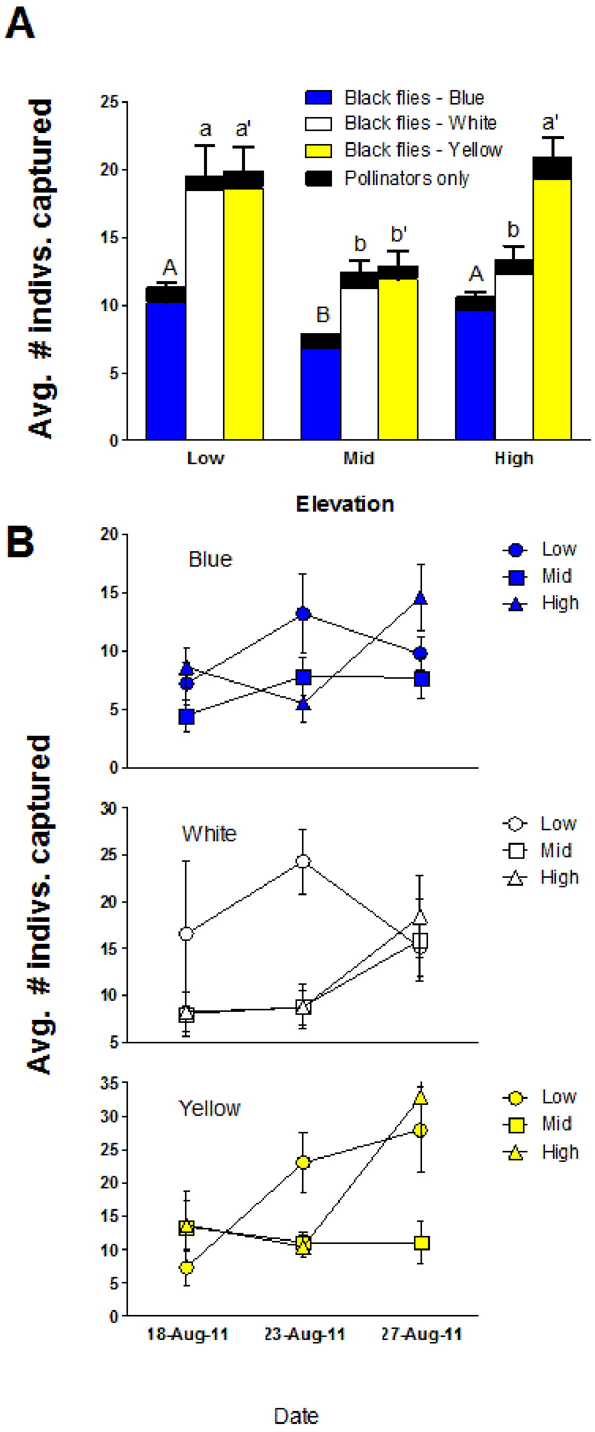


Figure 3.6 - A) Mean number of black flies (Muscidae, Anthomyiidae, and Sarcophagidae) and pollinators trapped in three colours of pan traps, combined for three sampling dates, across an elevation gradient at Marriott Basin, B.C.. B) Variation in the number of pollinators trapped among three sampling dates, at three elevations. Bars or points are means +/- SE. Different letters indicate statistically significant differences (Repeated Measures ANOVA, $P < 0.05$).

Images from time lapse cameras and recorded temperatures indicate that the weather on the 23-Aug-2011 trapping date was overcast with cooler but also more variable average temperatures (mean temperatures in °C: 6.89 ± 3.84 SD for August 18, 7.99 ± 8.24 SD for 23-Aug, and 12.75 ± 7.79 SD for 27-Aug). This may have accounted for the relative peak in activity at lower-elevation if less exposed sites mid and high elevation sites were windy or covered in low cloud.

Functional groups:

The three elevations had significantly different communities of invertebrate functional groups (ANOSIM: Global R = 0.146, P = 0.033), but this was again driven almost entirely by variation in abundance of a few morphospecies: the black flies (likely pollinators/flower-visitors) and Sciaridae (mycetovores). Important functional differences among the communities at the three elevations were therefore encompassed by analyses based on abundance of morphospecies, presented above.

Effect of seed-predators:

The only species for which I observed large numbers of seed predators was *Arnica latifolia*, in which the seed heads were attacked by one unidentified species of larval Tephritidae (fruit flies; likely *Tephritis* sp.). This occurred more frequently at mid elevations than it did at low or high elevations (Kruskal-Wallis Test: K-W Statistic = 49.81, $p < 0.001$) (Figure 3.7A). The number of seed predators was a highly significant predictor of the number of viable seeds (ZINB regression: $P < 0.0001$, df = 5). There were significantly fewer viable seeds in flowers with seed predators (36.70 ± 21.55 SD) than in flowers without seed predators (54.26 ± 27.51 SD; Figure 3.7B) (Mann-Whitney U Test: U = 5030, $P_{[\text{one-tailed}]} < 0.0001$).

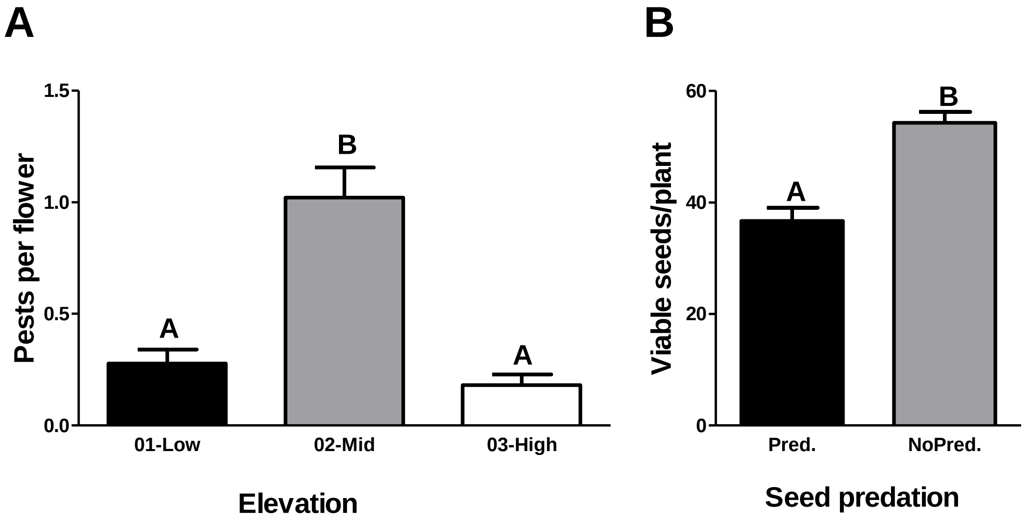


Figure 3.7 - A) Average number of seed predators per flower on *Arnica latifolia* at three elevations at Marriott Basin, BC. B) Effect of seed predators on the number of viable seeds per flower for *Arnica latifolia* at Marriott Basin, BC. Bars are means +/- SE. Different letters indicate statistically significant differences (Kruskal-Wallis and Mann-Whitney U Tests: P < 0.001).

Calt. leptosepala, *L. arcticus*, and *E. grandiflorum* also had some evidence of seed-predation, but it was far less frequent and sample sizes were not sufficiently large for statistical tests. Seed predation of *C. leptosepala* and *E. grandiflorum* appeared to be mostly caused by mammals and birds. Seed-predation by *L. arcticus* was more often caused by larvae of Symphyta or Coleoptera.

Factors limiting seed set:

Temperature, abundance of invertebrates, and abundance of seed-predators were all useful as predictors of seed set, but with varying effects among the species of flowering plants at Marriott Basin in 2011 (Table 3.8).

Table 3.8 - Zero-inflated multiple regression models describing the relationship between seed set (response) and predictors based on temperature (Temp.) and abundance of invertebrates (Invert.) across an elevation gradient, for species of alpine flowering plants at Marriott Basin, B.C. “ZI” indicates predictors that were significant only for the zero-inflated component of the model (seed set = 0); all other significant predictors refer to the count portion of the model (seed set ≥ 0). Coefficients indicate the expected log change in seed set for each unit increase in the predictor. DD15 = degree-days above 15°C, max = maximum temperature, N = number of invertebrates captured , Pred. = number of seed predators per flower, TotalPoll = number of pollinators captured. Species in **boldface** are primarily bee-pollinated.

Species (Sig)	Predictor/Sig. (Temp.)	Predictor/Sig. (Invert.)	Coefficient (Temp.)	Coefficient (Invert.)
E. grandiflorum (***)	DD15/* (ZI only)	N/***	0.4041 (ZI)	0.3133
L. arcticus[1] (NS)	DD15/*	NS	0.5960	NS
V. memb. (***)	DD15/***, **(ZI)	TotalPoll/***	-0.2543, -0.836 (ZI)	0.4377
Clay. lanceolata (NS)	Max/*	NS	0.2156	NS
Arnica latifolia (***)	Frost hours/***	TotalPoll/***	0.2334	NS
	-	Pred./***	-	-0.1794
Calt. leptosepala[1] (***)	DD15/***	-	-0.24590	-
Anem. occidentalis (NS)	-	-	-	-

*** P < 0.001, ** P = 0.001 - 0.01, * P = 0.01 - 0.05, “NS” P > 0.05

[1] Fit using the zero-inflated Poisson distribution

The best predictors of seed set were similar among species that rely on the same pollinators, suggesting a distinction between bee-pollinated and fly-pollinated species. A combination of temperature and abundance of invertebrates predicted seed set for the bee-pollinated species *E. grandiflorum* (Likelihood Ratio Test: P = 0.0002695) and *V. membranaceum* (Likelihood Ratio Test: P = 2.2 x 10^{-16}). For *E. grandiflorum*, the best predictors were DD15, which was positively associated with instances of zero seed set; and total number of invertebrates captured (N), which was positively associated with the number of seeds. For *V. membranaceum*, the best predictors were DD15, which was negatively associated with instances of zero seed and number of seeds; and the total number of pollinators captured, which had a positive relationship with the number of seeds. For *C. leptosepala*, which was pollinated by bees, flies, and butterflies, DD15 was a highly significant predictor of seed set (Likelihood Ratio Test: P = 4.625 x 10^{-13}), having a negative relationship with the number of viable seeds. For the bee-pollinated species *L. arcticus*, DD15 was a weakly significant predictor of seed set, but the overall model was not significant, indicating that neither temperature nor abundance of invertebrates were good predictors of seed set (Likelihood Ratio Test: P = 0.06358).

Maximum temperature was a weakly significant predictor of seed set for *C. lanceolata*, which is pollinated by both bees and flies, but the overall model was not significant, indicating that neither temperature nor abundance of invertebrates were good predictors of seed set in this species (Likelihood Ratio Test: $P = 0.148$). As noted above, there was little variation in seed set of the fly-pollinated, self-compatible species *A. occidentalis* between high and low elevations, and temperature or abundance of pollinators were not significant predictors of seed set (Likelihood Ratio Test: $P = 0.1414$). Seed set of the fly-pollinated species *Arnica latifolia* was best predicted by a combination of abundance of seed predators with frost hours (Likelihood Ratio Test: $P = 2.924 \times 10^{-9}$). The total number of frost hours had a positive relationship with seed set, while the number of seed predators had a negative relationship with seed set.

For most models, Log(theta) was significantly different from zero, indicating that the data were overdispersed, and best modelled using the zero-inflated negative binomial. The two exceptions to this were the models for *L. arcticus* and *C. leptosepala*, which were fit equally well by a zero-inflated Poisson distribution. Vuong tests indicated that zero-inflated models were always significant improvements on negative binomial or Poisson models without zero-inflation.

Evidence for main factors limiting seed set:

Seed set at Marriott Basin was limited by a combination of pollen availability (quantity) and quality, abiotic conditions (temperature), and biotic interactions (abundance of pollinating insects and seed-predators) (Table 3.9).

Table 3.9 - Hypotheses for limitation of seed set in alpine plants (introduced in Table 1), and species that fit into each, based on pollen manipulation experiments and natural variation over an elevation gradient at Marriott Basin, B.C.. Brackets ( ) indicate limited or inconclusive evidence for a particular hypothesis. PS = pollen-supplemented, O = open (control), B = bagged (pollinators excluded); L = low-elevation, M = mid-elevation, H = high-elevation.

Hypothesis	Species	Evidence (patterns of seed set and predictors)
Pollen limitation	*Arnica latifolia*	PS > B and PS > O
	(*C. leptosepala*)	(PS > B; PS > O, but small effect; not sig.)
Abiotic limitation	(*E. grandiflorum*)	(PS: L, M < H; Temp. is a useful predictor)
	(*V. memb.*)	(PS: L < H; Temp. is a useful predictor)
Biotic limitation	*C. leptosepala*	O: L < H; B and PS show no differences among elev.
	(*L. arcticus*)	(O: L < M < H, but large variation; not sig.) (B and PS show no differences among elevation, but insects are poor predictors of seed set)
Combined abiotic and biotic limitation	*V. memb.*	PS: L > H; O: L > H Temp, insects are both good predictors of seed set
	E. grandiflorum	PS: L, M < H; O: L, M < H, but small effect; not sig. Temp., insects are both good predictors of seed set
	(C. lanceolata?)	(PS: L < H; O: L < H, but temp, insects are poor predictors of seed set)
Mate-limitation or pollen quality	*V. memb.*	O > PS. Insects are a good predictor of seed set
	E. grandiflorum	O > PS. Insects are a good predictor of seed set
	A. occidentalis	O > PS; no variation in seed set across elevation
	(*L. arcticus*)	(O > PS, but insects are a poor predictor of seed set)
	(*C. lanceolata?*)	(O > PS, but insects are a poor predictor of seed set)
Seed-predation	*Arnica latifolia*	Remains of eaten seeds were found in some bags with flower heads that had few viable seeds. Seed-predators were good predictors of seed set.

The factors that are most important in limiting seed set vary among species, but the best-supported explanations are pollen limitation and seed-predation (for *Arnica latifolia*), biotic limitation (for *C. leptosepala*), combined abiotic and biotic limitation (for *V. membranaceum* and *E. grandiflorum*) and mate-limitation or pollen quality (for *V. membranaceum, E. grandiflorum*, and *A. occidentalis*). I revisit these hypotheses in detail below, and interpret them in terms of broader patterns that separate species based on pollination syndrome (bee versus fly pollination and self-fertilization), flowering time (early versus late flowering), and susceptibility to seed predators. I also discuss the implications of these patterns for the predicted resilience of alpine species to the effects of climate change.

Discussion:

The results of pollen manipulation experiments, combined with observations of natural variation in seed set, indicate that several processes are simultaneously driving reproductive limitation of flowering plants at Marriott Basin, British Columbia. These include a combination of biotic factors (indicated by limited availability or quality of pollen in most species, and seed-predation in one species), and abiotic climatic factors such as cumulative degree-days, maximum temperatures, and frost (Table 3.9). Most species had higher seed set in open (control) flowers than in bagged or pollen-supplemented flowers. This effect was not likely due to the bags alone, since previous studies indicate that the effect of loose, transparent, cloth bags on seed set is negligible, as long as the bags allow for air flow and light penetration (Kearns & Inouye 1993). Also, the pollen-supplementation treatment significantly increased seed set over that in bagged flowers for all species, except for *Anemone* and *Erythronium*. In *Anemone* and *Erythronium*, pollen-supplemented flowers did set more seeds on average but the effect was not statistically significant. This pattern suggests that there is a strong advantage to outcrossing over selfing in terms of the number of viable seeds produced, and that the effect of outcrossing was greater in plants that were pollinated by insects (control plants) than in those that were hand-pollinated (Motten 1986; Kearns & Inouye 1993). In environments where plants are not strongly pollen limited, this makes sense for several reasons. One of the notable disadvantages of pollen manipulation treatments is that the precise magnitude of pollen supplementation treatment (i.e., the amount and quality of pollen transferred during hand pollination) is not known (Knight et al. 2005). Although hand pollinations were done repeatedly, and always with a minimum of three pollen donors, it is possible that control plants received pollen from more than three donors (theoretically limited by the effective population size at the field site, foraging behaviour of pollinators, and spatial distribution of clones or potential mates) and the result was an increase in seed set (Rigney et al. 1993; Rafferty & Ives 2012). This effect could be particularly pronounced in cases where one or more of the three donors produced infertile or poor quality pollen, and when pollination services are high (Kearns & Inouye 1993). Lower seed set in hand-pollinated plants could also be the result of failure to pollinate the flower repeatedly throughout its period of receptivity, or clogging of stigmas with excess pollen, both of which are less likely to occur during pollination by insects (Kearns & Inouye 1993). I inspected plants with hand-lenses in the field to evaluate receptivity and ensure that pollen was being deposited, but could not evaluate the effects of stigma-clogging in hundreds of treated plants. These results lend support to the mate-limitation (or pollen quality) hypothesis (Table 3.1, 3.9), which is a favoured explanation for studies that find no evidence of pollen limitation, particularly in cases where resources are not limited (e.g., Motten 1986; Totland 1997; García-Camacho & Totland 2009).

Elevation, flowering time and seed set

There is evidence of a weak pattern that distinguishes early-flowering species from late-flowering species at Marriott Basin. The early-flowering species *E. grandiflorum*, *Calt. leptosepala*, and *Clay. lanceolata* all set more seed at high elevations than at low elevations, although the differences were not always statistically significant due to the large amount of variation (Figure 3.4: A, D, F). The early-flowering species *Anemome occidentalis* did not follow this pattern because it had no variation in seed set across the elevation gradient, some explanations for which are discussed in greater detail below.

For early-flowering species in the alpine, flowering times are closely tied to dates of snowmelt (Price & Waser 1998; Thórhallsdóttir 1998; Inouye et al. 2002; Kudo & Hirao 2006), which are themselves affected by late-winter precipitation (Inouye et al. 2002), cumulative degree-days (Dunne et al. 2003; Forrest & Thomson 2011), spring rainfall (Lambert et al. 2010), and local topographic heterogeneity (Kudo & Hirao 2006). Early flowers begin developing before snow melts (Kimball & Salisbury 1974), open several days after the date of first snowmelt, are receptive for only a short period of time (Price & Waser 1998; Thórhallsdóttir 1998), and tend to be vulnerable to late-season frosts (Inouye 2000, 2008; Kudo & Hirao 2006). In this study, seed set of *E. grandiflorum*, *Clay. lanceolata*, and *Calt. leptosepala* was linked to temperature, but with inconsistent patterns. Higher maximum temperatures during the same period (which were greatest at high elevations) were associated with higher seed set in *Clay. lanceolata* and *Calt. leptosepala*. The pattern in *Clay. lanceolata* and *Calt. leptosepala* could be explained either through the influence of maximum temperatures on snow melt, or by the dramatic increase in pollination activity associated with hot, sunny days in the alpine (McCall & Primack 1992; Totland 1994a, 1994b). Earlier flowering (promoted by higher maximum temperatures early in the season) has been linked to higher seed set in some alpine plant species because of higher pollination services gained from early-emerging bees, particularly queens of *Bombus* sp. (e.g., Motten 1986; Thomson 2010), but when flowering occurs *too* early (or too late), bee-pollinated species may suffer from severe pollen-limitation (e.g., Thomson 2010) while fly-pollinated species are not affected (Motten 1986; Kudo et al. 2004). Although bees are more effective pollinators *per visit*, flies can make up for pollination deficits through sheer abundance (Kearns & Inouye 1994), although experiments are needed separate floral visitors from legitimate pollinators (e.g., Hunter et al. 2000).

The link between seed set of *E. grandiflorum* and temperature is more difficult to explain. Cumulative DD15 during the period of flowering and seed-development were positively associated with cases of zero seed set for *E. grandiflorum*. It is possible that this pattern corresponds with some other variable that was not measured, or indicates something particular to *E. grandiflorum* but not other early-flowering species. One possibility is that DD15 were associated with growth or phenology of tall, late-blooming plants (e.g., *Chamerion angustifolium*, *Salix*, *Thalictrum*, *Valeriana sitchensis* and *Veratrum*), which shaded the shorter *E. grandiflorum* to a degree that prevented fruit set from occurring (e.g., Schemske 1977). Higher DD15 might also have been associated with the onset of late-season drought caused by melting snowpack and drying soil, which is thought to be a primary physiological stressor of plants in mountainous environments (Galen & Stanton 1991, Jamieson et al. 2012). This could have caused failure of seed set late in the season, even in cases where fertilization occurred in the spring.

If this were the case, we would expect to observe a similar pattern for the other early-blooming species which are also fairly small and equally prone to physiological stress, but this was not observed in *Clay. lanceolata* or *Calt. leptosepala*. Another possibility is that DD15 affected flowering time of *E. grandiflorum*, or emergence time of its main pollinators, in such a way that there was a mismatch between the timing of flowering and pollinator activity at low and mid elevations, causing failure of many flowers to set seed. This is perhaps the best explanation, since the most notable difference between *E. grandiflorum* and the other species is its pollination syndrome, which is associated with *Bombus* (mainly queens) and occasionally hummingbirds (Pojar 1974, Thomson et al. 1986; Lambert et al. 2010). While the other early-flowering species were occasionally visited by *Bombus*, they were most often pollinated by a diverse community of Diptera (mainly Syrphidae, Muscidae, and Anthomyiidae). If the emergence time of *Bombus* was strongly linked to DD15, the queens might have emerged before the date of first snowmelt, causing *E. grandiflorum* to bloom during the nesting period of queens (i.e., after their initial emergence) but before the emergence of workers, thus missing the peak in pollination services (e.g., Thomson 2010, Figure 3.8). Other studies have shown that the effect of flowering time on seed set and pollen limitation is most pronounced in early-flowering species that rely on synchrony with *Bombus* rather than flies (e.g., Motten 1986; Kudo et al. 2004; Thomson 2010).

V. membranaceum, and *L. arcticus*, both late-flowering species, showed the opposite pattern from the early-flowering species, with higher seed set at lower elevations for open and pollen-supplemented plants, although the differences were not statistically significant for *L. arcticus* due to large variation in seed set. The opposite trends in early versus late-flowering plants would be predicted if flowers were affected by differences in the pollination community across the elevation gradient. Early-flowering plants might be best pollinated by high-elevation pollinators, while late-flowering plants might be best pollinated by low-elevation pollinators. This explanation has little support in the current study however, because the pollination community (mainly made up of the ‘black fly’ morphospecies) was similar in abundance and composition between low and high elevation sites. A more plausible explanation is that late-flowering species such as *V. membranaceum* have different temperature thresholds for development than *E. grandiflorum*. This is reasonable, given that flowering time in late-flowering species tends to be driven primarily by cumulative degree-days or chilling requirements rather than patterns of snowmelt (Price & Waser 1998; Dunne et al. 2003; Dunnell & Travers

2011, Cook et al. 2012). The importance of temperature for some species is also anecdotally supported by the observation that *V. membranaceum* was often substantially shorter at higher elevations, which could be a sign that plants were physiologically stressed and growing near the limits of their range. In *V. membranaceum*, cumulative DD15 were negatively associated with both seed set, and the number of flowers that set zero seeds. Abundance of insects was also a significant, positive predictor of seed set. Despite the opposite trends in seed set with respect to elevation, the best predictors of seed set for *V. membranaceum* (DD15 and abundance of insects) followed a similar pattern to *E. grandiflorum*. A possible explanation for this is that like *E. grandiflorum*, *V. membranaceum* is pollinated almost exclusively by *Bombus*, and cannot generally be accessed by flies (Vander Kloet 1988). The existence of some relationship between temperature and seed set for these species may reflect the influence of temperature on the phenology of their shared pollinators. Although there has been much interest in phenological "cues", Forrest (2011) recently observed that this terminology still does not reflect an understanding of underlying physiological mechanisms, which may ultimately be more important for predicting responses of plant-pollinator interactions to climate change. The same temperature threshold (DD15) is also a good predictor of seed set for *L. arcticus*, which is another bee-pollinated flower, but the pattern of seed set with respect to elevation is weak for *L. arcticus* due to large amounts of variation in seed set. One factor that might have obscured predicted patterns for *L. arcticus* was frequent nectar-robbing where flowers were chewed through at the corolla (most often by sawflies in the family Tenthredinidae). This likely damaged reproductive structures, leading to a decrease in seed set and increase in variance, although incidental pollination during nectar-robbing can sometimes have a positive effect on seed set (Irwin et al. 2001).

Based on the important interaction between flowering time and timing of emergence of key pollinators, Figure 3.8 presents a hypothetical scenario to explain the differences in seed set for early and late-flowering species across the elevation gradient.

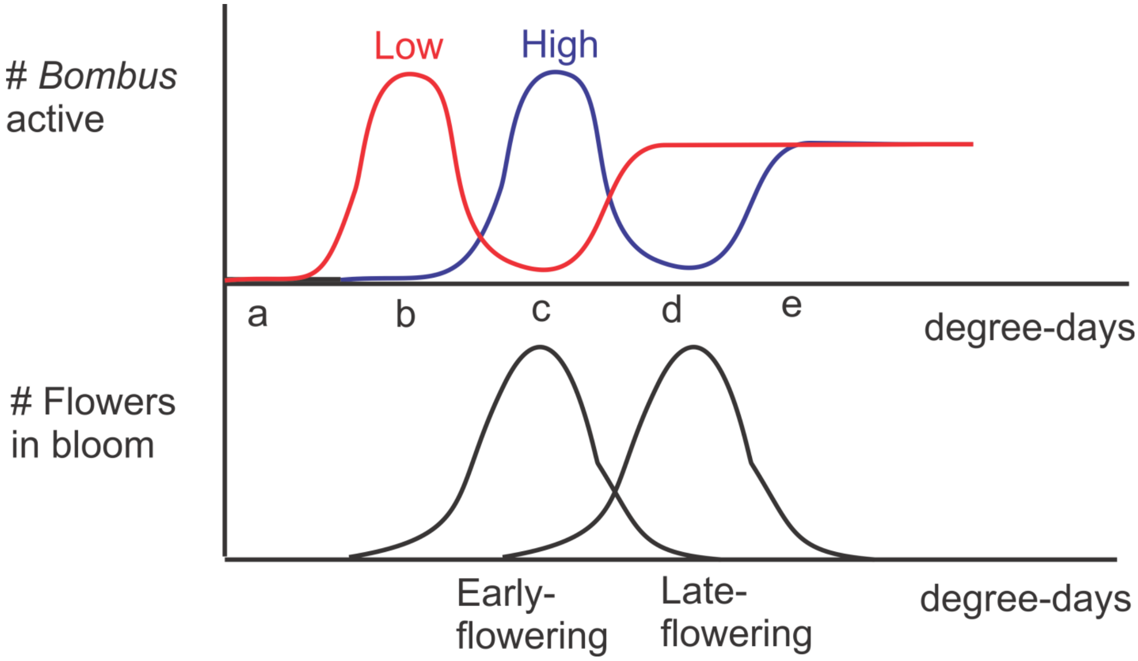


Figure 3.8 - Hypothetical emergence times of *Bombus* (top panel) at low (red line) and high (blue line) elevations, and flowering time of early and late-flowering plants (bottom panel). Letters denote the following stages: a) pre-flowering for all flowers and pre-emergence for bees, b) pre-flowering for all flowers, but queen bees have emerged at low elevations, c) queen bees emerge at high elevations as early-flowering plants are in bloom; queen bees are nesting at low elevations, d) worker bees emerge at low elevations as late-flowering plants are in bloom; queen bees are nesting at high elevations, and e) post-flowering at all elevations; abundance of worker bees is similar at all elevations.

This scenario is different from other recently-identified mismatch scenarios in that instead of flowers being too early for pollinators (e.g., Thomson 2010), the insects are emerging before the flowering season (Inouye et al. 2000). Forrest (2011) noted that this would be possible under the combination of heavy spring snowpack and unseasonably high temperatures. This is the scenario that occurred during 2011, with heavy spring snowpack (due to the regional effect of the La Niña portion of the ENSO cycle) at low elevations being compounded by the effect of avalanches (http://www.youtube.com/watch?v=0MiADreir8g). At Marriott Basin in 2011, higher elevations had higher maximum temperatures, but lower elevations had more rapid degree-day accumulation (Table 3.6). Since alpine plants have lower thresholds for development than insects (Forrest 2011; Forrest & Thomson 2011), insects would be expected to respond more strongly to warmer temperatures, and develop faster under conditions with more rapid degree-day accumulation (i.e., at lower elevations). Under this scenario, queen bees would emerge first at low elevations, and begin nesting before early-flowering plants begin flowering (Figure 3.8, a-b). The emergence of queen bees at high elevations (delayed because of the slower accumulation of degree-days) would then correspond with the flowering time for early-flowering plants (Figure 3.8, c), resulting in high seed set for early-flowering plants at high elevations. Finally, the emergence of worker bees at low elevations (and nesting of queen bees at high elevations) would correspond with the onset of flowering for late-flowering species (Figure 3.8, d). This would result in high seed set for late-flowering plants at low elevations.

Unfortunately, the insect trapping during this study was unable to gather sufficient information to determine whether gaps among generations of *Bombus* were actually occurring. The trapping took place after the peak flowering time for both early and late flowers (Figure 3.8, e), and caught few *Bombus* (5 in total, out of 135 traps), either because they were rare relative to other pollinators, or because they were less likely to be caught by traps. I have previously caught large numbers of *Bombus* from alpine sites in these same traps (J. Straka, *unpublished data*), so the explanation may be related to abundance. Proper tracking of emergence times for *Bombus* would have required regular trapping throughout the season, or frequent trapping or monitoring of visitation rates prior to and during flowering (which was unfortunately the time that I was most occupied with pollen manipulation experiments). Despite the lack of quantitative data, my daily field notes indicate that the first queens were seen on July 27 at low elevations, and July 29th at high elevations, while the first workers were seen on August 11 at low elevations and August 17th at high elevations (Table 3.10).

Table 3.10 - Incidental field observations of emergence times for different stages of *Bombus*, and flowering times for early-flowering and late-flowering plants in July and August at Marriott Basin, 2011.

Date (2011)	Observations (*Bombus*)	Observations (plants)
16 July	No bees (only flies)	Some *Erythronium*, *Anemone*, *Caltha*, *Claytonia* blooming where snow has melted (mid-elevation only)
27 July	Queens only (low elevation).	Some *Erythronium*, *Anemone*, *Caltha*, *Claytonia* blooming where snow has melted (mid-elevation only)
29 July	Queens only (all elevations)	*Erythronium*, *Anemone*, *Caltha*, and *Claytonia* blooming; *Arnica*, *Lupinus*, *Vaccinium*, *Sorbus*, and *Valeriana* not yet blooming.
5 August	Queens nesting (low elevations)	*Erythronium*, *Anemone*, *Caltha*, and *Claytonia* blooming; *Arnica*, *Lupinus*, *Vaccinium*, *Sorbus*, and *Valeriana* not yet blooming.
7-8 August	Queens mostly nested (low elevations)	*Erythronium*, *Anemone*, *Caltha*, and *Claytonia* finishing; *Arnica*, *Lupinus*, *Vaccinium*, *Sorbus*, and *Valeriana* not quite blooming.
11 August	First workers emerging (low elevations); few queens still present (high elevations)	*Erythronium*, *Anemone*, *Caltha*, and *Claytonia* finished; *Arnica*, *Lupinus*, *Vaccinium*, *Sorbus*, and *Valeriana* beginning to bloom.
14 August	All queens nested (all elevations)	Early-flowering species finished blooming Late-flowering blooming (*Vaccinium*, *Lupinus*, *Arnica*, *Sorbus*, *Valeriana*)
17-20 August	Workers emerged at all elevations (high and low)	Early-flowering species setting seed. Late-flowering species finishing. First snowfall ~ 20 Sept.

These data indicate the possibility of gaps in pollination by *Bombus* before July 27 (prior to activity), around August 8-11 (while queens are nesting) at low elevations, and August 14-17 (while queens are nesting) at high elevations. Future studies would benefit from systematic surveys of bee abundance in fixed plots with standardized effort spent hand-netting or observing bees, which could add quantitative data to these incidental observations. It should be noted that, while the "peak" flowering time varied by several days between low and high elevations, this study controlled for flowering time by only working with plants at a similar stage at all elevations. Differences in seed set might also have resulted from variation through time in the preferences of pollinators, measured during a limited experimental period (Figure 3.9).

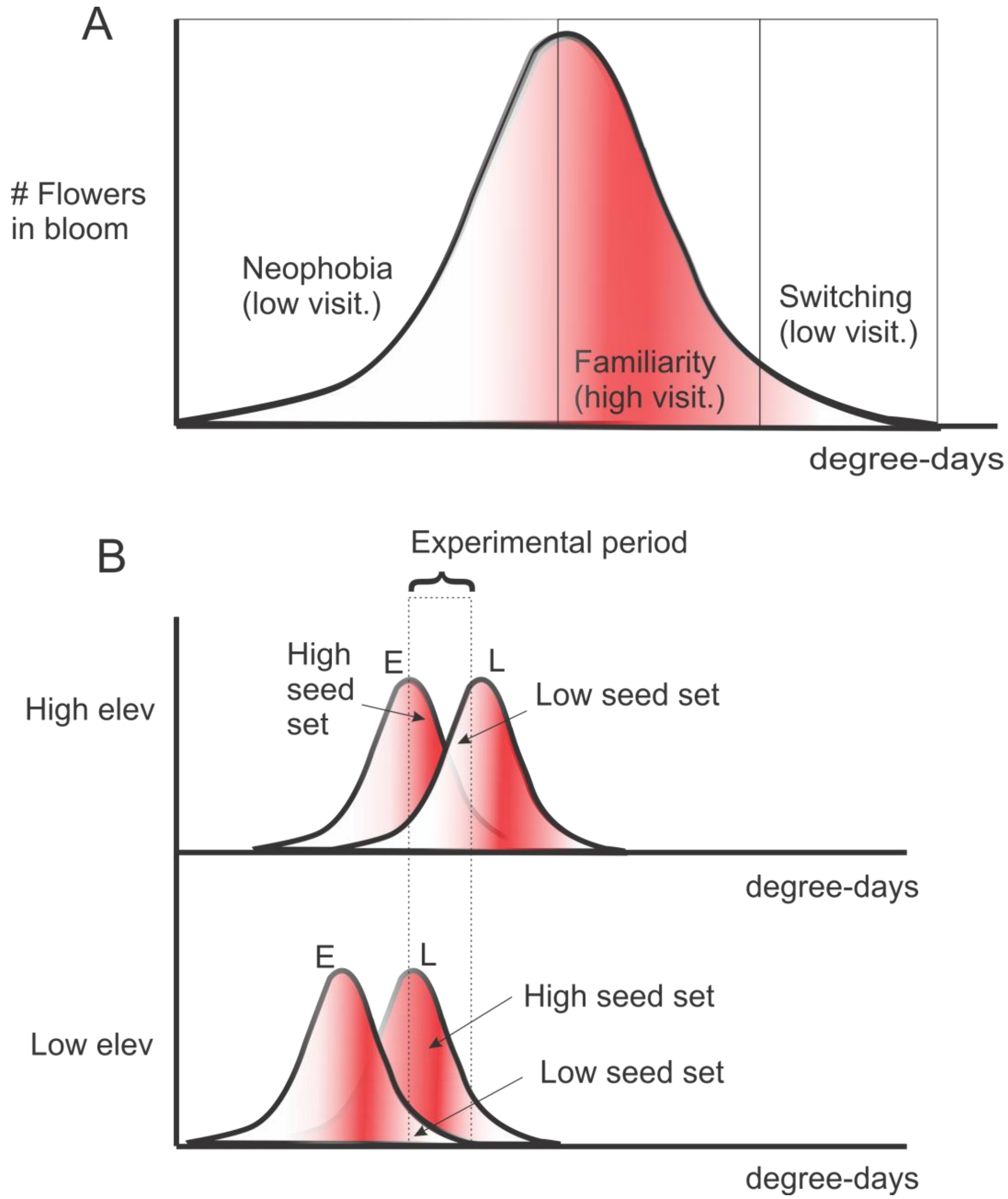


Figure 3.9 - A) Hypothetical variation through time in visitation by pollinators, based on abundance of flowers and pollinator neophobia. Pollinators avoid novel flowers even when they reach relatively high abundance, until a certain amount of time has passed, and switch to another resource when abundance becomes too low at the end of the flowering season. B) Temporal variation in pollinator preferences can create variation in seed set within a limited experimental period wherein early-flowering (E) species have high seed set at high elevations and low seed set at low elevations while late-flowering (L) species have low seed set at high elevations and high seed set at low elevations.

During the period of the experiment, individuals from the early-flowering species might have been late-flowering individuals relative to the rest of their population (i.e., just after the peak of the flowering curve), while late-flowering species might have been represented by relatively early-flowering individuals (i.e., before the peak of the flowering curve) (Figure 9). If pollinators discriminate based on the flowering time of an individual relative to the rest of its cohort, this could account for differences in visitation rates, and therefore the observed variation in seed set for early and late-flowering individuals at low and high elevations (Figure 3.9). Recent studies indicate that *Bombus* can be "neophobic," tending to avoid new flowers even when they are abundant (Forrest & Thomson 2009), and that pollinators can be less effective at transferring pollen earlier in the season (Rafferty & Ives 2012). This suggests an advantage to being a late-flowering individual (flowering after the peak), and the observed differences in seed set might have reflected a comparison between early-flowering and late-flowering individuals.

Species-specific responses: seed-predation and self-fertilization

Besides showing patterns related to flowering time and association with particular pollinators, seed set also responds to other aspects of species' life histories. The following section presents potential explanations for species-specific patterns that could not be explained by flowering time or pollination syndrome (discussed above), and are unique among species within this study.

Arnica latifolia was the only species strongly affected by seed-predators. It showed a different pattern from other late-blooming species, and had the highest seed set at both low and high elevations, but this was because patterns in number of viable seeds were driven by higher seed-predation at middle elevations (Figure 3.7A), which had a strong negative impact on seed set (Figure 3.7B). The genus *Arnica* is known to be a host to at least several species of specialist Tephritidae (White 1987), which can have a large negative impact on the number of viable seeds produced by their hosts (Scheidel et al. 2003). Studies on these Tephritidae have shown that populations can vary over small (400 m) elevation gradients, but the cause of this variation is uncertain, and might be attributable to differences in density of *Arnica* among sites rather than variation in temperature, or a host of other biotic and abiotic factors (Scheidel et al. 2003; Hodkinson 2005). Other Tephritidae are known to be limited in range by heavy frosts (Gutierrez et al. 2008). For *Arnica latifolia*, there was also a positive relationship between the number of frost hours and seed set, which might have reflected a negative relationship between frost hours and abundance of seed predators. There were fewer hours below freezing and a lower maximum number of hours of continuous frost at middle elevations (Table 3.6), where seed-predators were most abundant (Figure 3.7A). The lower seed set of *Arnica latifolia* at mid elevations could also be explained by the fact that seed-predators were more abundant, while pollinators were *less* abundant at mid elevations (Figure 3.7A). This unexpected pattern of variation in the insect community challenges the assumption that abundance of pollinators (and thus pollination services) should decline as elevation increases (Fabbro & Körner 2004), emphasizing the importance of testing this assumption when attempting to use elevation as a natural treatment of variation in pollination.

Unlike the other species used in this study, *Anem. occidentalis* showed little evidence of any sort of reproductive limitation at Marriott Basin (Table 3.9). It is self-compatible, and has high rates of self-fertilization, indicated by the high seed set in bagged flowers, which was not significantly different from seed set of pollen-supplemented flowers (Figure 3.4C). Open flowers with natural levels of pollination had significantly higher seed set than bagged or pollen-supplemented flowers, and there was no variation in seed set with elevation. This pattern of uniformly high seed set may have been caused by the ability of *Anem. occidentalis* to attract large numbers of pollinators, and therefore receive plenty of outcrossed pollen regardless of its environment. *Anem. occidentalis* flowers were frequently covered by black flies (Muscidae, Anthomyiidae, Sarcophagidae, Faniidae, and Phoridae) which were generalist flower-visitors, and the most abundant morphospecies of insect observed and trapped at the field site (Table 3.7). While the flowering time of *Anem. occidentalis* was closely tied to date of first snowmelt, dense patches were present between June and August as snowmelt was delayed in areas that were shaded or received larger snow accumulation.

Resilience to climate change

The distinct responses to climate related variables for early- and late-blooming species is consistent with other studies on the relationships between climate, phenology, and pollination. Flowering time of early-flowering species in the alpine is driven primarily by patterns of snowmelt (Price & Waser 1998; Inouye et al. 2002; Dunne et al. 2003; Lambert et al. 2010), and seed set in those species is often reliant on bumble bee queens for pollination (Inouye et al. 2000; Kudo et al. 2004; Kudo & Hirao 2006; Thomson 2010). In contrast, late-flowering species respond more to accumulation of atmospheric degree-days during the growing season (Price & Waser 1998; Dunne et al. 2003; Rafferty & Ives 2011), tend to have more stringent vernalization or chilling requirements during the winter period (Cook et al. 2012), and rely on pollination from worker bees or other insects (Totland 1994b; Forrest 2011). Rafferty & Ives (2011) found that early-flowering species were more likely to advance their flowering times in response to climate change over time, and that species responding adaptively in the past were less likely to suffer from pollen limitation when their flowering times were artificially advanced. Insect-pollinated species have historically been more likely to shift their dates of first flowering than wind-pollinated species, which might be the result of coupled responses between plants and pollinators (Fitter & Fitter 2002). Bartomeus et al. (2011) examined shifts in dates of emergence for 10 species of bees over the past 130 years and found that they were similar to rates of advancements in flowering time of plants over the same period. These recent studies suggest that some species might be resilient to the effects of climate-driven phenological mismatch by advancing phenology in adaptive ways. The present study illustrates a caveat to their conclusions: that shifts in flowering time can only be effective in cases where some degree of synchrony with pollinators is maintained, or when plants are able to take advantage of pollinators that are highly abundant at various locations and times. While maintenance of synchrony has been inferred from similar advancements in dates of first flowering and emergence (Bartomeus et al. 2011), the assumption that these ‘proxies’ can provide accurate assessments of phenological synchrony remains to be tested (see Chapter 2). Poor synchrony resulting in pollen limitation because of inter-generational gaps in *Bombus* (rather than initial synchrony of flowering time and emergence) could challenge the validity of this assumption. Other studies indicate that earlier flowering may be selected against because of neophobia in pollinators (Forrest & Thomson 2009), and lower pollinator effectiveness with earlier flowering (Rafferty & Ives 2012), which could restrict adaptive advancements in flowering time.

In this study, there was little direct evidence of pollen limitation in early *or* late-flowering species, mainly because any effect of pollen-supplementation was swamped by what appears to be high rates of outcrossing in open (control) flowers. However, there was evidence that seed set (and thus, reproduction) could be limited by seed-predators (in *Arnica latifolia*), and degree days during the flowering season could be a useful predictor of seed set (in *E. grandiflorum*, *L. arcticus*, and *V. membranaceum*). In *Arnica latifolia*, frost hours were an important positive predictor of seed set, perhaps because of a link to development or survival of seed-predators, which typically require higher temperatures to break dormancy and develop, and can be killed by late-season frosts (Hodkinson 2005; Gutierrez et al. 2008). This suggests that climate change in the form of warming could decrease the number of frost hours during the flowering season, increasing the abundance of seed-predators at all elevations, which would be predicted to have a negative effect on populations of *Arnica latifolia*. However, predicting future patterns of abundance of alpine herbivores is difficult given that existing variation across elevation gradients seems to be taxon-specific, and best explained by a combination of many factors such as local topography and aspect, and interactions with hosts, predators, and competitors (Hodkinson 2005).

In other species, degree-days above 15°C (DD15) were the most important predictor of seed set, but the relationship between DD15 and seed set was not straightforward. The species for which this temperature threshold was important are all bee-pollinated, suggesting that DD15 might be a useful predictor of phenology for bees. These results agree with studies finding different responses to seasonal or climactic drivers between bee-pollinated and fly-pollinated flowers (Motten 1986; Kudo et al. 2004).

Although higher temperatures can promote pollinator activity on a short-term basis (e.g., McCall & Primack 1992), the timing of emergence can be more important for seed set than the overall amount of pollinator activity (Forrest 2011). In this study, there is some evidence that bee-pollinated flowers (*E. grandiflorum*, *V. membranaceum*, *L. arcticus*, and *Clay. lanceolata*) might have relied on synchrony with either the early-season emergence of queens, or the emergence of workers later in the season (Figure 3.8). Seed set may have been limited in cases where flowering times, driven by DD15, fell into gaps in pollination services between these two generations of *Bombus* (Figure 3.8). This suggests that bee-pollinated species could be vulnerable to pollen-limitation, but in ways that are difficult to predict at this time, as predicting (a)synchrony would rely on a more complete knowledge of the drivers of phenology for both *Bombus* and the flowers they visit (Forrest 2011). Developing models to better describe plant-pollinator synchrony is important because such models might describe more than just seed set. For example, Figure 3.8 suggests that early-emerging queen bees at low elevations may not receive sufficient resources if flowering times are delayed (by high snow accumulation, for example), which could also affect subsequent broods of workers, thus having cascading effects on late-season pollination in addition to early-season pollination. It is difficult to predict responses to climate change in this case, since it is not clear to what degree pollen is a factor that limits populations of *Bombus* in natural conditions (Mayer et al. 2011).

Unlike bee-pollinated flowers, fly-pollinated species seem to show little evidence of reliance on particular flowering times for effective pollination (Motten 1986; Kudo et al. 2004). *Anemone occidentalis*, for example, had high seed set and high rates of visitation at all elevations, throughout the field season. Based on this evidence alone, fly-pollinated species would be predicted to respond neutrally or positively to climate change in the Coast Range of B.C.. In general, flies are associated with pollination at high elevations and latitudes (Kevan 1972; Warren et al. 1988; Elberling & Olesen 1999), emerge regularly, and are evenly abundant throughout the flowering season (e.g. Kudo et al. 2004). This might explain why it was easier to detect trends for reproductive limitation in bee-pollinated species. Furthermore, flies are generally associated with pollination in moist environments, while bees generally have higher water-use efficiency, and are associated with pollination in dry environments (Arroyo et al. 1982; Elberling & Olesen 1999).

Climate change at Marriott Basin is predicted to cause an increase in frost-free days, warmer winters, and hotter summers with a greater number of degree days above 0°C, 5°C, and 18°C both annually, and particularly during the growing season (Wang et al. 2012). Climate projections for Marriott Basin also predict increased snowfall by 2050 but decreased snowfall by 2080, with more rapid snowmelt and decreased summer precipitation resulting in late-season drought (Wang et al. 2012). The increased temperatures and decreased summer precipitation might accelerate the development of some insects, which generally rely on higher temperature thresholds than plants (Forrest 2011). Warmer, drier conditions might also improve physical conditions for pollination, which tends to occur mostly on hot, sunny days (e.g., McCall & Primack 1992). However, it is unclear how these climate variables might influence plant-pollinator mismatch (discussed in Chapter 2), particularly given the importance of date of first snowmelt as a development cue for early-flowering plants in the alpine (e.g., Dunne et al. 2003, Kudo & Hirao 2006), and whether conditions might likewise be improved for floral antagonists. As mentioned previously, seed-predators such as Tephritidae would likely benefit from the increased number of frost-free days, particularly during the growing season. Increased snowfall by 2050 could lead to higher moisture in areas around snowpack that persists late into the flowering season, which would be advantageous to flies. However, the predicted increase in summer temperatures causing late-season drought may negatively affect populations of flies in some parts of their range (IPCC 2007; Forrest 2011) and provide more favourable conditions for bees.

Unfortunately, populations of *Bombus* are in decline throughout North America and the United Kingdom (Cameron et al. 2011; Hoover et al. 2012). Populations of bee-pollinated flowers may therefore be at risk from a multitude of indirect threats to their pollinators (Williams et al. 2007; Hoover et al. 2012; see Chapter 2). The behaviour of bees may constrain their ability to respond to shifts in flowering time, which would affect selection for earlier flowering times in bee-pollinated flowers; experiments with *Bombus impatiens* conducted with artificial communities of flowering plants in a greenhouse indicated that bees were reluctant to visit unfamiliar flowers because of their relative rarity, but also because of neophobia (Forrest & Thomson 2009).

Finally, warmer temperatures and might interact with the physiology of species to increase seed set in some cases. There was evidence in this study of physiological limitations to seed set in *V. membranaceum*, *Clay. lanceolata*, and *E. grandiflorum*, where seed set varied with elevation despite flowers receiving similar amounts of pollen through hand-pollination. Warmer temperatures might ensure faster development of flowers and extension of the season where ripening of seeds is possible (Thórhallsdóttir 1998), and increased rates of early-season snowmelt might ensure a greater number of snow-free days (Price & Waser 1998), but increased late-spring snow accumulation could have the opposite effect, forcing back flowering times and leading to shorter flowering seasons (Inouye et al. 2002; Kudo & Hirao 2006), which can have negative effects on seed set (Cooper et al. 2011). As mentioned in Chapter 2, drought can potentially lead to physiological stress and failure of seed set (Jamieson et al. 2012). Extreme late-season weather events such as the dramatic hail storm on 10-Aug-2011 can also directly destroy flowers or seeds.

Conclusions, and future directions:

Overall, there was little evidence for strong pollen limitation of seed set in open flowers, with only two of seven species having lower seed set in open flowers than in hand-pollinated flowers. In contrast, five of seven species produced more viable seed when exposed to natural levels of pollination than they did through self-pollination or hand pollination. Although the 2011 flowering season at Marriott Basin was short due to high snow pack and cool weather, pollination services provided by insects seemed to be high. This study confirmed that, in general, biotic and abiotic factors both limit seed set in alpine environments, and the most important factors for predicting seed set vary among species, often in ways related to their life-history. While this makes it difficult to predict community-wide demographic responses to climate change at this time, several patterns emerged that can be used to guide future investigations on this topic:

1) Early-flowering species seemed to respond differently to elevation gradients than late-flowering species. Although the reason for this was unclear, two possible explanations are that seed set was affected by the degree of synchrony with emergence times of bumble bees, which varied along the elevation gradient, or that early-flowering

individuals set less seed because of neophobic behaviour of pollinators and/or increasing pollinator effectiveness later in the flowering season.

2) It is difficult to draw strong conclusions from a limited sample, but there are some indications that different factors are driving reproductive limitation in bee-pollinated versus fly-pollinated plants, suggesting that species with different pollinators may have divergent responses to climate change. Bee-pollinated flowers may risk pollen-limitation in cases where their flowering time is poorly synchronized with emerging queen bumble bees in the early spring, or workers later in the season. In contrast, fly-pollinated plants seem to be more successful at setting seed regardless of their flowering time, particularly when they are self-compatible, as was the case in *Anemone occidentalis*.

3) Several temperature variables were important, with degree-days above 15°C being most important for several bee-pollinated species, suggesting a possible link between this temperature threshold and phenology of *Bombus*. Frost hours were a good predictor of seed set in *Arnica latifolia*, which was strongly limited in seed set by the presence of seed predators (Tephritidae) at mid elevations. Seed-predators had a strong, negative effect on seed set, which overshadowed the weaker effects of pollination and interactions between physiology and abiotic conditions. Insects that rob pollen or nectar may have similar negative effects, but those could not be quantified from this study. While the phenological “cues” that are important for plants are fairly well known, little work has been done on this topic for pollinators, seed predators, or other floral antagonists (Forrest 2011). Improving our understanding of phenological drivers for insects and how these respond to climate change is vital for predicting the possibility or magnitude of mismatches between plants and pollinators due to divergent responses to changing temperatures.

4) This study emphasizes the importance of carefully considering life-history characteristics and multi-trophic interactions of study species, as these traits will inevitably affect experimental outcomes and implications of results (Miller-Rushing & Inouye 2009). In order to carry out experiments to test specific predictions and have meaningful results, it is sometimes desirable to carefully select study species to control for aspects of life-history such as breeding system, flowering time, or pollination syndrome. For example, studies on pollen limitation of *Anemone* should not expect large

effect sizes because of its high rates of self-fertilization, and would have to incorporate more specific treatments such as self-fertilization or emasculation (removal of anthers) to control for self-fertilization. Unfortunately, this information is rarely available or time-consuming to assemble. There would be great benefit to developing a shared, easily navigable online archive of relevant ecological information on breeding systems and pollination syndromes of different species, ideally integrated with existing databases such as BC's eFlora (http://www.geog.ubc.ca/biodiversity/eflora/) or the Encyclopedia of Life (http://eol.org/). In addition, longevity, seed banks, and clonality might be predicted to mitigate the demographic consequences of mismatch.

5) It has been noted that many of the plants in alpine environments are long-lived perennials, and years with no sexual reproduction or seed set can be offset by years of high productivity which can contribute to the standing seed bank (Thórhallsdóttir 1998). Most studies still do not consider lifetime reproductive success, and ignore the extent to which observed short-term patterns of reproduction in alpine plants (particularly when they appear to be sub-optimal) may be longer-term strategies or adaptations to uncertain conditions (Hodkinson 2005; Singer & Parmesan 2010). This is particularly important in the context of resilience to climate change, since these are the sorts of adaptations that will allow species to persist under rapidly changing conditions. An under-studied example is the important role of local topographic heterogeneity in mountainous environments, which is known to promote beta-diversity and predicted to support species under climate change (e.g., Anderson & Ferree 2010), but also creates small-scale variation in phenology (Hodkinson 2005; Kudo & Hirao 2006). Given the emphasis on short-term studies rather than lifetime fitness and population trends, the demographic consequences of plant-pollinator mismatches or, more generally, the demographic responses to climate change are still very much an open question (Forrest 2011; Rafferty & Ives 2011).

Chapter 4
General Conclusion

This thesis is a combination of two components: a review of recent and historical literature on plant-pollinator phenological mismatches (Chapter 2); and an original, data-driven, field experiment (Chapter 3). Both were challenging in very different ways, but are intended to complement each other in the final document.

In gathering material and writing the review, I was forced to think critically about the process of doing science, mainly how experts decide which questions to ask, how best to answer them, and what to make of the results. I delved not only into the foundational material on phenological and spatial mismatches, but hundreds of reviews and papers citing that material. This allowed me to assess original work on its own merits, but also take note of how this work had been received by the scientific community. In some cases, I discovered that high-impact, formative papers in the area of plant-pollinator mismatch were (and are) being cited frequently but uncritically, with higher emphasis on severe consequences and risks than on potential for adaptation and resilience (e.g., citations of Memmott et al. 2007 by Lawler et al. 2008; Tylianakis et al. 2008; Berg et al. 2010; Gilman et al. 2010). It is possible that this has been done to emphasize the uncertainty surrounding any future predictions, and the importance of policymakers adopting the precautionary principle. Perhaps there is also hope that warnings about “threats” or “crises” will promote investment in ecological research and greater concern over the impending consequences of climate change (Shellenberger & Nordhaus 2004). Reflecting on the ways that previous work on the potential impacts of climate change has been presented was an important step in considering the dissemination and promotion of my current (and perhaps future) work as an academic researcher.

Another aspect of writing a review was that it allowed me to engage in one of the most important steps in the scientific process, and certainly for any field ecologist: imagination. It is a highly recommended and worthwhile exercise to imagine how one would answer an important question with unlimited time and money to throw at it. This ‘thought experiment’ allows researchers to focus their ideas, arriving at (or hopefully near) the best possible design for a study or experiment before beginning to carry it out.

Results can have clear interpretations when an experiment fits the description of Popper's "critical experiments" (Popper 1979), although this stringent approach has been critiqued by many as providing clear answers to less interesting questions (e.g., Lakatos et al. 1980; reviewed by Hilborn & Mangel 1997). In writing this review, I allowed my imagination to run freely in identifying the most important upcoming opportunities for future research in plant-pollinator phenological mismatches and climate change, then attempted to ground these ideas in carefully thought-out experimental designs. Admittedly, the experiments I proposed were not described in explicit detail, which would require significantly more space than available in a single manuscript. Those who may attempt to do these experiments will need to refine the hypotheses and predictions, stemming from the questions posed, and will almost certainly suffer unforeseen logistical difficulties that come with exciting, unexpected findings.

This brings me to the second part of my thesis: the original research. One should be wary of those who make grand recommendations but have done little original work themselves. As the author of the aforementioned review, I do not purport to be any exception. My original research could be seen as an attempt to fill some of the gaps that I had identified in my review. Specifically, I tested the assumption that reproduction of plants (seed set, a common proxy for demographic impacts of mismatch) was pollen-limited at the scale of an alpine plant community, and considered a series of additional factors contributing to reproductive limitation in alpine plants. In the process of carrying out this experiment, I became keenly aware of the challenges inherent in doing original field work with limited time, funding, and personnel (Figure 4.1).

Figure 4.1- Field assistant Andrew Sherriff, becoming keenly aware of the challenges inherent in doing original field work in mid July, 2011, at Marriott Basin, Coast Range, British Columbia.

My field season was short because of record high snowpack, and only seven of the nine plants I experimented on yielded good-quality data because of losses to what are often considered background or “noise” effects: thunder storms, hail, and marmots (all of which are indeed noisy when sleeping in a tent). My results on insect abundance came from pan traps, which interacted strongly with trapping date, suggesting high variability over the course of the season. I did not have time to do systematic surveys of background abundance of flowers to create flowering curves and establish dates of “peak” flowering, or visitation rates of pollinators at the three elevations. Nor was I able to systematically estimate emergence schedules of bumble bees in a quantitative fashion. I also did not have time to capture pollinators at flowers, identify them to species, estimate their pollen loads (i.e., to measure effectiveness, or importance). Thus, many challenges remain for future research. But I have described in detail several alternative hypotheses that still require testing, including the possible influence of 1) pollinator neophobia and 2) phenology of *Bombus* as drivers of reproductive limitation in alpine plant communities.

Although it is too early to make definitive statements about the consequences of climate change for plant-pollinator interactions in alpine British Columbia, this thesis offers a new perspective on the problem. First, I have highlighted some underappreciated ways in which plant-pollinator interactions might be resilient to the effects of climate change, which still need to be investigated (Chapter 2). Seed set of plants is not pollen-limited in all cases (see Chapter 3), and more work is necessary to determine whether demography of pollinators is limited by floral resources. Flowers stay open longer when they aren't pollinated, and climate-driven shifts in phenology might lead to longer flowering periods or growing seasons with greater number and duration of flight periods for pollinators. Heritability of flowering time and selective pressures favouring synchrony (or sometimes asynchrony) suggest that rapid evolution or phenotypic plasticity of phenology might allow species to maintain important pollination interactions through time. Flexibility of interactions in the long term, and potential for 're-wiring' of pollination networks suggests that novel interactions might preclude severe reproductive limitation driven by mismatched historical interactions. Populations might also be maintained in mountainous environments where pollinators are mobile and phenology is "patchy."

The new data I have presented (Chapter 3) suggest that responses of alpine species to climate change are likely to vary based on characteristics of life-history and phylogeny. Taxa that are self-fertilizing and fly-pollinated (e.g., *Anemone occidentalis*) might be guaranteed high seed set regardless of flowering time. Taxa that are pollinated by *Bombus* (e.g., *Claytonia lanceolata, Erythronium grandiflorum, Lupinus arcticus,* and *Vaccinium membranaceum*) might be prone to mismatches with their pollinators when they flower before the emergence of queens, or between the nesting time of queens and emergence of workers. Some taxa (e.g., *Arnica latifolia*) are limited by seed-predation rather than pollination and warmer conditions with fewer frost hours might be associated with higher risks of seed-predation, which would have negative consequences for reproduction. Extreme events such as high snow accumulation, late-season frosts, and hail storms can also have negative impacts on reproduction of alpine plants.

Over the course of this work, several important underlying themes emerged that have not yet been highlighted, but are useful lessons for future work:

1) Studies must be explicit about what they want to measure, and how they are measuring it. This is necessary to show that what they think they are measuring is what they are actually measuring.

Pollination ecologists studying the demographic consequences of mismatches must be explicit about the definition of mismatches. In some cases, it has been concluded based on simulations done by Memmott et al. (2007) that phenological mismatches between plants and pollinators are expected to occur, which could have severe consequences for pollination services (e.g., Lawler et al. 2008; Tylianakis et al. 2008; Berg et al. 2010; Gilman et al. 2010). There are several problems with this conclusion, which would not have been asserted by Memmott et al. (2007). First, the term “phenological mismatch” is controversial for reasons outlined in my review: mismatched phenologies might actually be adaptive, and dates of first flowering or emergence may not be the best ways of representing phenology or mismatch. Second, there is a distinction to be made between mismatches and the consequences of mismatches. In a grander sense, this is a failure to relate a pattern to its underlying process. It is necessary to establish a link between services provided by plants or pollinators to their mutualistic partners (pollen transfer or floral resources) and demographic changes (Hegland et al. 2009; Miller-Rushing et al. 2010). This can only be done compellingly when considering the potential for ‘rewiring’ of interactions, which might include phenotypic plasticity and/or microevolution, and emergence of novel (or restoration of historical) interactions. Finally, a great deal of work has yet to be done in understanding how the effects of climate change on plant-pollinator phenological mismatch will be mediated by other patterns, such as the range of interactions between competition and facilitation and how it will vary over range of elevation and latitude.

2) The emphasis on the "plant" side of plant-pollinator interactions

As an undergraduate, I was warned by my entomology professor that, to a lot of people (and the largest sources of funding), doing entomology essentially meant constantly being asked "What is the best way to kill insects?" Studying pollination is a fantastic exception to this rule, since the important role of pollination in providing goods and services for humans is widely acknowledged (MEA 2003). However, the overwhelming emphasis still tends to be on plants, because they are much easier to study, and insects are seen as pollinators rather than plants being seen as pollen-providers. Charles Darwin (1841) struggled with exactly this problem, writing that he would "lament to see these industrious, happy-looking creatures punished with the severity proposed by [some gardeners]" who were upset at observing bees destroying flowers to access pollen or nectar (Pearn 2012). Darwin was also an exceptional thinker in that he had a rare ability to consider mutualistic interactions from the perspective of both partners. Indeed, in an 1872 letter to Herman Müller, he describes a particularly entertaining method for monitoring the movement of pollinators in the field:

> I repeatedly stationed five or six of my children, each close to a buzzing place, and told the one farthest away to shout as soon a bee buzzed there: "here is a bee", and so on with the other children one after another, the words "here is a bee" were passed on from child to child without interruption, until the bees reached the buzzing place where I myself was standing.

And clearly, many people agree that watching bees and flowers can be fascinating. Long-term data sets are now becoming available online, and recent years have seen an explosion of "crowd-sourced" citizen science initiatives such as eButterfly (eButterfly 2012), and the USA National Phenology Network (Thomas et al. 2010). As there is a rapid increase in available data, the key will be to use these data to ask important questions that are based on sound ecological theory. By carefully considering what we think we know about plant-pollinator interactions and climate change, what we *want* to know, and what we *can* know given the available data, there is a great deal we will be able to learn.

References Cited

Ackerman, J.D. (1989). Limitations to sexual reproduction in *Encyclia krugii* (Orchidaceae). *Systematic Botany*, 101–109.

Aguilar, R., Ashworth, L., Galetto, L. & Aizen, M.A. (2006). Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. *Ecology Letters*, 9, 968–980.

Akaike, H. (1973). Information theory and an extension of the maximum likelihood principle. In: *Second international symposium on information theory*. pp. 267–281.

Aldridge, G., Inouye, D.W., Forrest, J.R.K., Barr, W.A. & Miller-Rushing, A.J. (2011). Emergence of a mid-season period of low floral resources in a montane meadow ecosystem associated with climate change. *Journal of Ecology*, 99, 905–913.

Altermatt, F. (2010a). Tell me what you eat and I'll tell you when you fly: diet can predict phenological changes in response to climate change. *Ecology Letters*, 13, 1475–1484.

Altermatt, F. (2010b). Climatic warming increases voltinism in European butterflies and moths. *Proceedings of the Royal Society B: Biological Sciences*, 277, 1281 –1287.

Anderson, M.G. & Ferree, C.E. (2010). Conserving the stage: climate change and the geophysical underpinnings of species diversity. *PLoS ONE*, 5, e11554.

Arroyo, M.T.K., Primack, R. & Armesto, J. (1982). Community studies in pollination ecology in the high temperate Andes of central Chile. I. Pollination mechanisms and altitudinal variation. *American Journal of Botany*, 69, 82–97.

van Asch, M. & Visser, M.E. (2007). Phenology of forest caterpillars and their host trees: the importance of synchrony. *Annual Review of Entomology*, 52, 37–55.

Ashman, T.-L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., et al. (2004). Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. *Ecology*, 85, 2408–2421.

Augspurger, C.K. (1981). Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators in *Hybanthus prunifolius* (Violaceae). *Ecology*, 62, 775–788.

Baker, H.G. (1963). Evolutionary mechanisms in pollination biology. *Science*, 139, 1275.

Bartomeus, I., Ascher, J.S., Wagner, D., Danforth, B.N., Colla, S., Kornbluth, S., et al. (2011). Climate-associated phenological advances in bee pollinators and bee-pollinated plants. *Proceedings of the National Academy of Sciences*, 108, 20645 – 20649.

Bascompte, J., Jordano, P., Melián, C.J. & Olesen, J.M. (2003). The nested assembly of plant–animal mutualistic networks. *Proceedings of the National Academy of Sciences of the United States of America*, 100, 9383 –9387.

Bazzaz, F.A. (1990). The response of natural ecosystems to the rising global CO_2 levels. *Annual Review of Ecology and Systematics*, 21, 167–196.

Bell, J.M., Karron, J.D. & Mitchell, R.J. (2005). Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. *Ecology*, 86, 762–771.

Bender, E.A., Case, T.J. & Gilpin, M.E. (1984). Perturbation experiments in community ecology: theory and practice. *Ecology*, 65, 1–13.

Berg, M.P., Kiers, E., Driessen, G., van der Heijden, M., Kooi, B.W., Kuenen, F., et al. (2010). Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, 16, 587–598.

Bokhorst, S., Bjerke, J.W., Bowles, F.W., Melillo, J., Callaghan, T.V. & Phoenix, G.K. (2008). Impacts of extreme winter warming in the sub-Arctic: growing season responses of dwarf shrub heathland. *Global Change Biology*, 14, 2603–2612.

Bond, W.J. (1994). Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 344, 83–90.

Both, C., Bouwhuis, S., Lessells, C.M. & Visser, M.E. (2006). Climate change and population declines in a long-distance migratory bird. *Nature*, 441, 81–83.

Bowers, M.A. (1985). Bumble bee colonization, extinction, and reproduction in subalpine meadows in northeastern Utah. *Ecology*, 66, 914–927.

Bowers, M.A. (1986). Resource availability and timing of reproduction in bumble bee colonies (Hymenoptera: Apidae). *Environmental Entomology*, 15, 750–755.

Bray, J.R. & Curtis, J.T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27, 325–349.

Brittain, C. & Potts, S.G. (2011). The potential impacts of insecticides on the life-history traits of bees and the consequences for pollination. *Basic and Applied Ecology*, 12, 321–331.

Brody, A.K. (1997). Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology*, 78, 1624–1631.

Burd, M. (1994). Bateman's principle and plant reproduction: The role of pollen limitation in fruit and seed set. *The Botanical Review*, 60, 83–139.

Callaway, R.M., Brooker, R., Choler, P., Kikvidze, Z., Lortie, C.J., Michalet, R., et al. (2002). Positive interactions among alpine plants increase with stress. *Nature*, 417, 844–848.

Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., et al. (2011). Patterns of widespread decline in North American bumble bees. *Proceedings of the National Academy of Sciences*, 108, 662 –667.

Campbell, A., Frazer, B.D., Gilbert, N., Gutierrez, A.P. & Mackauer, M. (1974). Temperature requirements of some aphids and their parasites. *Journal of Applied Ecology*, 11, 431–438.

Cartar, R.V. & Dill, L.M. (1990). Colony energy requirements affect the foraging currency of bumble bees. *Behavioral Ecology and Sociobiology*, 27, 377–383.

Chalcoff, V.R., Aizen, M.A. & Ezcurra, C. (2012). Erosion of a pollination mutualism along an environmental gradient in a south Andean treelet, *Embothrium coccineum* (Proteaceae). *Oikos,* 121, 471–480.

Clarke, K.R. (1993). Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology,* 18, 117–143.

Cleland, E.E., Allen, J.M., Crimmins, T.M., Dunne, J.A., Pau, S., Travers, S.E., et al. (2012). Phenological tracking enables positive species responses to climate change. *Ecology*, 93, 1765–1771.

Colles, A., Liow, L.H. & Prinzing, A. (2009). Are specialists at risk under environmental change? Neoecological, paleoecological and phylogenetic approaches. *Ecology Letters*, 12, 849–863.

Colwell, R.K., Brehm, G., Cardelús, C.L., Gilman, A.C. & Longino, J.T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. *Science,* 322, 258 –261.

Cook, B.I., Wolkovich, E.M. & Parmesan, C. (2012). Divergent responses to spring and winter warming drive community level flowering trends. *Proceedings of the National Academy of Sciences*, 109, 9000–9005.

Cooper, E.J., Dullinger, S. & Semenchuk, P. (2011). Late snowmelt delays plant development and results in lower reproductive success in the High Arctic. *Plant Science*, 180, 157–167.

Cushing, D.H. (1990). Plankton production and year-class strength in fish populations: an update of the match/mismatch hypothesis. *Advances in Marine Biology*, 26, 249–293.

Darwin, C. (1841). Gardeners' Chronicle: Letter 607.

Darwin, C. (1859). *The origin of species by means of natural selection: or, the preservation of favored races in the struggle for life*. J. Murray.

Darwin, C. (1862). *On the various contrivances by which British and foreign orchids are fertilised by insects: and on the good effects of intercrossing*. J. Murray.

Darwin, C. (1872). Correspondence.

Devictor, V., van Swaay, C., Brereton, T., Brotons, L., Chamberlain, D., Heliölä, J., et al. (2012). Differences in the climatic debts of birds and butterflies at a continental scale. *Nature Climate Change*, 2, 121–124.

Devoto, M., Medan, D. & Montaldo, N.H. (2005). Patterns of interaction between plants and pollinators along an environmental gradient. *Oikos*, 109, 461–472.

Didham, R.K., Tylianakis, J.M., Gemmell, N.J., Rand, T.A. & Ewers, R.M. (2007). Interactive effects of habitat modification and species invasion on native species decline. *Trends in Ecology & Evolution*, 22, 489–496.

Diez, J.M., Ibáñez, I., Miller-Rushing, A.J., Mazer, S.J., Crimmins, T.M., Crimmins, M.A., et al. (2012). Forecasting phenology: from species variability to community patterns. *Ecology Letters*, 15, 545–553.

Donnelly, A., Caffarra, A. & O'Neill, B.F. (2011). A review of climate-driven mismatches between interdependent phenophases in terrestrial and aquatic ecosystems. *International Journal of Biometeorology*, 55, 805–817.

Doorn, W.G. van. (1997). Effects of pollination on floral attraction and longevity. *Journal of Experimental Botany*, 48, 1615–1622.

Dorken, M.E. & Eckert, C.G. (2001). Severely reduced sexual reproduction in northern populations of a clonal plant, *Decodon verticillatus* (Lythraceae). *Journal of Ecology*, 89, 339–350.

Droege, S., Tepedino, V.J., Lebuhn, G., Link, W., Minckley, R.L., Chen, Q., et al. (2010). Spatial patterns of bee captures in North American bowl trapping surveys. *Insect Conservation and Diversity*, 3, 15–23.

Dunne, J.A., Harte, J. & Taylor, K.J. (2003). Subalpine meadow flowering phenology responses to climate change: integrating experimental and gradient methods. *Ecological Monographs*, 73, 69–86.

Dunnell, K.L. & Travers, S.E. (2011). Shifts in the flowering phenology of the northern Great Plains: Patterns over 100 years. *American Journal of Botany*.

eButterfly. (2012). eButterfly [WWW Document]. *eButterfly.ca*. URL http://ebutterfly.ca/

Edwards, M. & Richardson, A.J. (2004). Impact of climate change on marine pelagic phenology and trophic mismatch. *Nature*, 430, 881–884.

Ehrlén, J. (1992). Proximate limits to seed production in a herbaceous perennial legume, *Lathyrus vernus*. *Ecology*, 73, 1820–1831.

Eickwort, G.C., Eickwort, J.M., Gordon, J., Eickwort, M.A. & Wcislo, W.T. (1996). Solitary behavior in a high-altitude population of the social sweat bee *Halictus rubicundus* (Hymenoptera: Halictidae). *Behavioral Ecology and Sociobiology*, 38, 227–233.

Eickwort, G.C. & Ginsberg, H.S. (1980). Foraging and mating behavior in Apoidea. *Annual Review of Entomology*, 25, 421–446.

Elberling, H. & Olesen, J.M. (1999). The structure of a high latitude plant-flower visitor system: the dominance of flies. *Ecography*, 22, 314–323.

Ellwood, E., Diez, J., Ibáñez, I., Primack, R., Kobori, H., Higuchi, H., et al. (2012). Disentangling the paradox of insect phenology: are temporal trends reflecting the response to warming? *Oecologia*, 168, 1161–1171.

Fabbro, T. & Körner, C. (2004). Altitudinal differences in flower traits and reproductive allocation. *Flora - Morphology, Distribution, Functional Ecology of Plants*, 199, 70–81.

Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R. & Thomson, J.D. (2004). Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*, 35, 375–403.

Fitter, A.H. & Fitter, R.S.R. (2002). Rapid changes in flowering time in British plants. *Science*, 296, 1689–1691.

Forrest, J. (2011). Plant-pollinator interactions in a changing climate [PhD Dissertation]. URL https://tspace-library-utoronto-ca.ezproxy.library.uvic.ca/handle/1807/29723

Forrest, J. & Miller-Rushing, A.J. (2010). Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3101 –3112.

Forrest, J. & Thomson, J.D. (2009). Pollinator experience, neophobia and the evolution of flowering time. *Proc. R. Soc. B*, 276, 935–943.

Forrest, J. & Thomson, J.D. (2010). Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower. *American Journal of Botany*, 97, 38–48.

Forrest, J.R.. & Thomson, J.D. (2011). An examination of synchrony between insect emergence and flowering in Rocky Mountain meadows. *Ecological Monographs*, 81, 469–491.

Franks, S.J., Sim, S. & Weis, A.E. (2007). Rapid evolution of flowering time by an annual plant in response to a climate fluctuation. *Proceedings of the National Academy of Sciences*, 104, 1278–1282.

Fründ, J., Dormann, C.F., Holzschuh, A. & Tscharntke, T. (2012). Mechanisms of pollinator diversity effects on pollination in experimental plant communities. Presented at the Ecological Society of America 2012, Portland, Oregon.

Fründ, J., Dormann, C.F. & Tscharntke, T. (2011). Linné's floral clock is slow without pollinators – flower closure and plant-pollinator interaction webs. *Ecology Letters*, 14, 896–904.

Galen, C. (1985). Regulation of seed-set in *Polemonium viscosum*: Floral scents, pollination, and resources. *Ecology*, 66, 792–797.

Galen, C. & Stanton, M.L. (1991). Consequences of emergence phenology for reproductive success in *Ranunculus adoneus* (Ranunculaceae). *American Journal of Botany*, 978–988.

Galen, C. & Stanton, M.L. (1993). Short-term responses of alpine buttercups to experimental manipulations of growing season length. *Ecology*, 1052–1058.

García-Camacho, R. & Totland, Ø. (2009). Pollen limitation in the alpine: a meta-analysis. *Arctic, Antarctic, and Alpine Research*, 41, 103–111.

Gilman, S.E., Urban, M.C., Tewksbury, J., Gilchrist, G.W. & Holt, R.D. (2010). A framework for community interactions under climate change. *Trends in Ecology & Evolution*, 25, 325–331.

Gordo, O. & Sanz, J.J. (2005). Phenology and climate change: a long-term study in a Mediterranean locality. *Oecologia*, 146, 484–495.

Gordo, O. & Sanz, J.J. (2006). Temporal trends in phenology of the honey bee *Apis mellifera* (L.) and the small white *Pieris rapae* (L.) in the Iberian Peninsula (1952–2004). *Ecological Entomology*, 31, 261–268.

Goulson, D. (2003). Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution, and Systematics*, 34, 1–26.

Goulson, D. (2010). *Bumblebees: behaviour, ecology, and conservation*. Oxford University Press.

Greenleaf, S.S., Williams, N.M., Winfree, R. & Kremen, C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia*, 153, 589–596.

Gross, R.S. & Werner, P.A. (1983). Relationships among flowering phenology, insect visitors, and seed-set of individuals: experimental studies on four co-occurring species of goldenrod (*Solidago*: Compositae). *Ecological Monographs*, 95–117.

Gulías, J. & Traveset, A. (2012). Altitudinal variation in the reproductive performance of the Mediterranean shrub *Rhamnus lycioides* L. *Journal of Plant Ecology*.

Gutierrez, A., Ponti, L., d' Oultremont, T. & Ellis, C. (2008). Climate change effects on poikilotherm tritrophic interactions. *Climatic Change*, 87, 167–192.

Haig, D. & Westoby, M. (1988). On limits to seed production. *The American Naturalist*, 131, 757–759.

Harder, L.D. & Aizen, M.A. (2010). Floral adaptation and diversification under pollen limitation. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 529 –543.

Harder, L.D. & Thomson, J.D. (1989). Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *The American Naturalist*, 133, 323–344.

Harrison, R.D. (2000). Repercussions of El Niño: drought causes extinction and the breakdown of mutualism in Borneo. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 267, 911 –915.

Heard, M.J., Riskin, S.H. & Flight, P.A. (2011). Identifying potential evolutionary consequences of climate-driven phenological shifts. *Evolutionary Ecology*, 1–9.

Hedges, L.V., Gurevitch, J. & Curtis, P.S. (1999). The meta-analysis of response ratios in experimental ecology. *Ecology*, 80, 1150–1156.

Hegland, S.J., Nielsen, A., Lázaro, A., Bjerknes, A.L. & Totland, Ø. (2009). How does climate warming affect plant-pollinator interactions? *Ecology Letters*, 12, 184–195.

Hegland, S.J. & Totland, Ø. (2008). Is the magnitude of pollen limitation in a plant community affected by pollinator visitation and plant species specialisation levels? *Oikos*, 117, 883–891.

Heikkinen, R., Luoto, M., Leikola, N., Pöyry, J., Settele, J., Kudrna, O., et al. (2010). Assessing the vulnerability of European butterflies to climate change using multiple criteria. *Biodiversity and Conservation*, 19, 695–723.

Heinrich, B. & Raven, P.H. (1972). Energetics and pollination ecology. *Science*, 176, 597–602.

Hendrickx, F., Maelfait, J.-P., Van Wingerden, W., Schweiger, O., Speelmans, M., Aviron, S., et al. (2007). How landscape structure, land-use intensity and habitat diversity affect components of total arthropod diversity in agricultural landscapes. *Journal of Applied Ecology*, 44, 340–351.

Hilborn, R. & Mangel, M. (1997). *The ecological detective: confronting models with data*. Princeton University Press.

Hodkinson, I.D. (2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews*, 80, 489–513.

Hoover, S.E.R., Ladley, J.J., Shchepetkina, A.A., Tisch, M., Gieseg, S.P. & Tylianakis, J.M. (2012). Warming, CO_2, and nitrogen deposition interactively affect a plant-pollinator mutualism. *Ecology Letters*, 15, 227–234.

Hothorn, T., Zeileis, A., Millo, G. & Mitchell, D. (2012). lmtest: testing linear regression models. *R-Project.org*.

Hülber, K., Winkler, M. & Grabherr, G. (2010). Intraseasonal climate and habitat-specific variability controls the flowering phenology of high alpine plant species. *Functional Ecology*, 24, 245–252.

Hunter, F.F., Burgin, S.G. & Woodhouse, A. (2000). Shattering the folklore: black flies do not pollinate sweet lowbush blueberry. *Canadian Journal of Zoology*, 78, 2051–2054.

Inouye, D., Morales, M. & Dodge, G. (2002). Variation in timing and abundance of flowering by *Delphinium barbeyi*; Huth (Ranunculaceae): the roles of snowpack, frost, and La Niña, in the context of climate change. *Oecologia*, 130, 543–550.

Inouye, D. w. (2000). The ecological and evolutionary significance of frost in the context of climate change. *Ecology Letters*, 3, 457–463.

Inouye, D.W. (1978). Resource partitioning in bumblebee guilds: experimental studies of foraging behavior. *Ecology*, 59, 672–678.

Inouye, D.W. (2008). Effects of climate change on phenology, frost damage, and floral abundance of montane wildflowers. *Ecology*, 89, 353–362.

Inouye, D.W., Barr, B., Armitage, K.B. & Inouye, B.D. (2000). Climate change is affecting altitudinal migrants and hibernating species. *Proceedings of the National Academy of Sciences*, 97, 1630–1633.

Intergovernmental Panel on Climate Change (IPCC). (2007). *Climate Change 2007: The Physical Science Basis : Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press.

Irwin, R., Brody, A. & Waser, N. (2001). The impact of floral larceny on individuals, populations, and communities. *Oecologia*, 129, 161–168.

Jamieson, M.A., Trowbridge, A.M., Raffa, K.F. & Lindroth, R.L. (2012). Consequences of climate warming and altered precipitation patterns for plant-insect and multitrophic interactions. *Plant Physiology.*

Janzen, D.H. (1971). Euglossine bees as long-distance pollinators of tropical plants. *Science*, 171, 203–205.

Johansen, C.A. (1977). Pesticides and pollinators. *Annual Review of Entomology* 22, 177–192.

Johnson, D.H. (1980). The comparison of usage and availability measurements for evaluating resource preference. *Ecology*, 61, 65.

Johnson, J.B. & Omland, K.S. (2004). Model selection in ecology and evolution. *Trends in Ecology & Evolution*, 19, 101–108.

Kaiser-Bunbury, C.N., Muff, S., Memmott, J., Müller, C.B. & Caflisch, A. (2010). The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. *Ecology Letters*, 13, 442–452.

Kameyama, Y. & Kudo, G. (2009). Flowering phenology influences seed production and outcrossing rate in populations of an alpine snowbed shrub, *Phyllodoce aleutica*: effects of pollinators and self-incompatibility. *Annals of Botany*, 103, 1385–1394.

Kawai, Y. & Kudo, G. (2008). Effectiveness of buzz pollination in *Pedicularis chamissonis*: significance of multiple visits by bumblebees. *Ecological Research*, 24, 215–223.

Kearns, C.A. & Inouye, D.W. (1993). *Techniques for pollination biologists*. University Press of Colorado.

Kearns, C.A. & Inouye, D.W. (1994). Fly pollination of *Linum lewisii* (Linaceae). *American Journal of Botany*, 81, 1091–1095.

Kearns, C.A. & Inouye, D.W. (1997). Pollinators, flowering plants, and conservation biology. *BioScience*, 47, 297–307.

Kearns, C.A., Inouye, D.W. & Waser, N.M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*, 29, 83–112.

Kevan, P.G. (1972). Insect pollination of high arctic flowers. *Journal of Ecology*, 60, 831–847.

Kim, J. & Thorp, R.W. (2001). Maternal investment and size-number trade-off in a bee, *Megachile apicalis*, in seasonal environments. *Oecologia*, 126, 451–456.

Kimball, S.L. & Salisbury, F.B. (1974). Plant development under snow. *Botanical Gazette*, 135, 147–149.

Klady, R.A., Henry, G.H.R. & Lemay, V. (2011). Changes in high arctic tundra plant reproduction in response to long-term experimental warming. *Global Change Biology*, 17, 1611–1624.

Knight, T.M., Steets, J.A., Vamosi, J.C., Mazer, S.J., Burd, M., Campbell, D.R., et al. (2005). Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics*, 36, 467–497.

Koh, L.P., Dunn, R.R., Sodhi, N.S., Colwell, R.K., Proctor, H.C. & Smith, V.S. (2004). Species coextinctions and the biodiversity crisis. *Science*, 305, 1632–1634.

Körner, C. (2007). The use of “altitude” in ecological research. *Trends in Ecology & Evolution*, 22, 569–574.

Kudo, G. & Hirao, A. (2006). Habitat-specific responses in the flowering phenology and seed set of alpine plants to climate variation: implications for global-change impacts. *Population Ecology*, 48, 49–58.

Kudo, G., Nishikawa, Y., Kasagi, T. & Kosuge, S. (2004). Does seed production of spring ephemerals decrease when spring comes early? *Ecological Research*, 19, 255–259.

Lakatos, I., Worrall, J. & Currie, G. (1980). *The methodology of scientific research programmes*. Cambridge University Press.

Lambert, A.M., Miller-Rushing, A.J. & Inouye, D.W. (2010). Changes in snowmelt date and summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily; Liliaceae). *American Journal of Botany*, 97, 1431–1437.

Larl, I. & Wagner, J. (2006). Timing of reproductive and vegetative development in *Saxifraga oppositifolia* in an Alpine and a subnival climate. *Plant Biology*, 8, 155–166.

Larson, B.M.H. & Barrett, S.C.H. (2000). A comparative analysis of pollen limitation in flowering plants. *Biological Journal of the Linnean Society*, 69, 503–520.

Lawler, J.J., Tear, T.H., Pyke, C., Shaw, M.R., Gonzalez, P., Kareiva, P., et al. (2008). Resource management in a changing and uncertain climate. *Frontiers in Ecology and the Environment*, 8, 35–43.

Lawton, J.H., Bignell, D., Bolton, B., Bloemers, G., Eggleton, P., Hammond, P., et al. (1998). Biodiversity inventories, indicator taxa and effects of habitat modification in tropical forest. *Nature*, 72–75.

Lee, T.D. & Bazzaz, F.A. (1982). Regulation of fruit and seed production in an annual legume, *Cassia fasciculata*. *Ecology*, 63, 1363–1373.

Lindsey, A.A. & Newman, J.E. (1956). Use of official weather data in spring time temperature analysis of an Indiana phenological record. *Ecology*, 37, 812–823.

Lovell, S., Hamer, M., Slotow, R. & Herbert, D. (2007). Assessment of congruency across invertebrate taxa and taxonomic levels to identify potential surrogates. *Biological Conservation*, 139, 113–125.

Manly, B.F.. (1993). Comments on design and analysis of multiple-choice feeding-preference experiments. *Oecologia*, 93, 149–152.

Martén-Rodríguez, S. & Fenster, C.B. (2010). Pollen limitation and reproductive assurance in Antillean Gesnerieae: a specialists vs. generalist comparison. *Ecology*, 91, 155–165.

Mayer, C., Adler, L., Armbruster, S., Dafni, A., Eardley, C., Huang, S., et al. (2011). Pollination ecology in the 21st century: key questions for future research. *Journal of Pollination Ecology*, 3, 8–23.

McCall, C. & Primack, R.B. (1992). Influence of flower characteristics, weather, time of day, and season on insect visitation rates in three plant communities. *American Journal of Botany*, 79, 434–442.

McNamara, J.M., Barta, Z., Klaassen, M. & Bauer, S. (2011). Cues and the optimal timing of activities under environmental changes. *Ecology Letters*, 14, 1183–1190.

McNickle, G.G., St. Clair, C.C. & Cahill Jr, J.F. (2009). Focusing the metaphor: plant root foraging behaviour. *Trends in Ecology & Evolution*, 24, 419–426.

Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007). Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*, 10, 710–717.

Memmott, J., Waser, N.M. & Price, M.V. (2004). Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271, 2605 –2611.

Millennium Ecosystem Assessment (MEA). (2003). *Ecosystems and human well-being: a framework for assessment*. Island Press.

Miller-Rushing, A.J., Høye, T.T., Inouye, D.W. & Post, E. (2010). The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3177.

Miller-Rushing, A.J. & Inouye, D.W. (2009). Variation in the impact of climate change on flowering phenology and abundance: An examination of two pairs of closely related wildflower species. *American Journal of Botany*, 96, 1821–1829.

Miller-Rushing, A.J., Inouye, D.W. & Primack, R.B. (2008). How well do first flowering dates measure plant responses to climate change? The effects of population size and sampling frequency. *Journal of Ecology*, 96, 1289–1296.

Miller-Rushing, A.J. & Primack, R.B. (2008). Global warming and flowering times in Thoreau’s Concord: a community perspective. *Ecology*, 89, 332–341.

Mitchell, R.J., Flanagan, R.J., Brown, B.J., Waser, N.M. & Karron, J.D. (2009). New frontiers in competition for pollination. *Annals of Botany*, 103, 1403 –1413.

Moeller, D.A. (2004). Facilitative interactions among plants via shared pollinators. *Ecology*, 85, 3289–3301.

Molnár, A., Tökölyi, J., Végvári, Z., Sramkó, G., Sulyok, J. & Barta, Z. (2012). Pollination mode predicts phenological response to climate change in terrestrial orchids: a case study from central Europe. *Journal of Ecology*, 100, 1141–1152.

Monterroso, V.A. & Wien, H.C. (1990). Flower and pod abscission due to heat stress in beans. *Journal of the American Society of Horticultural Science*, 115, 631–634.

Morales, C.L. & Traveset, A. (2009). A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. *Ecology Letters*, 12, 716–728.

Morandin, L.A., Winston, M.L., Franklin, M.T. & Abbott, V.A. (2005). Lethal and sub-lethal effects of spinosad on bumble bees (*Bombus impatiens* Cresson). *Pest Management Science*, 61, 619–626.

Motten, A.F. (1986). Pollination ecology of the spring wildflower community of a temperate deciduous forest. *Ecological Monographs*, 56, 21–42.

Motten, A.F., Campbell, D.R., Alexander, D.E. & Miller, H.L. (1981). Pollination effectiveness of specialist and generalist visitors to a North Carolina population of *Claytonia virginica*. *Ecology*, 62, 1278–1287.

Nealis, V.G., Jones, R.E. & Wellington, W.G. (1984). Temperature and development in host-parasite relationships. *Oecologia*, 61, 224–229.

Olesen, J.M., Bascompte, J., Dupont, Y.L., Elberling, H., Rasmussen, C. & Jordano, P. (2011). Missing and forbidden links in mutualistic networks. *Proceedings of the Royal Society B: Biological Sciences*, 278, 725 –732.

Olesen, J.M., Bascompte, J., Elberling, H. & Jordano, P. (2008). Temporal dynamics in a pollination network. *Ecology*, 89, 1573–1582.

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, 37, 637–669.

Parmesan, C., Ryrholm, N., Stefanescu, C., Hill, J.K., Thomas, C.D., Descimon, H., et al. (1999). Poleward shifts in geographical ranges of butterfly species associated with regional warming. *Nature*, 399, 579–583.

Parmesan, C., Yohe, G. & others. (2003). A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, 421, 37–42.

Pateman, R.M., Hill, J.K., Roy, D.B., Fox, R. & Thomas, C.D. (2012). Temperature-dependent alterations in host use drive rapid range expansion in a butterfly. *Science*, 336, 1028–1030.

Pearn, A. (2012). A tale of two bees [WWW Document]. *Darwin Correspondence Project*. URL http://www.darwinproject.ac.uk/a-tale-of-two-bees

Petanidou, T., Kallimanis, A.S., Tzanopoulos, J., Sgardelis, S.P. & Pantis, J.D. (2008). Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters*, 11, 564–575.

Pojar, J. (1974). Reproductive dynamics of four plant communities of southwestern British Columbia. *Canadian Journal of Botany*, 52, 1819–1834.

Pojar, J. & MacKinnon, A. (1994). *Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia & Alaska*. Lone Pine Publications.

Popper, K. (1979). *Objective knowledge*. Cambridge University Press.

Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O. & Kunin, W.E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, 25, 345–353.

Price, M.V. & Waser, N.M. (1998). Effects of experimental warming on plant reproductive phenology in a subalpine meadow. *Ecology*, 79, 1261–1271.

Rafferty, N.E. & Ives, A.R. (2011). Effects of experimental shifts in flowering phenology on plant–pollinator interactions. *Ecology Letters*, 14, 69–74.

Rafferty, N.E. & Ives, A.R. (2012). Pollinator effectiveness varies with experimental shifts in flowering time. *Ecology*, 93, 803–814.

Rathcke, B. (1983). Competition and facilitation among plants for pollination. *Pollination biology*, 305–329.

Regal, P.J. (1982). Pollination by wind and animals: ecology of geographic patterns. *Annual Review of Ecology and Systematics*, 13, 497–524.

Richards, A.J. (1997). *Plant breeding systems*. Garland Science.

Rigney, L.P., Thomson, J.D., Cruzan, M.B. & Brunet, J. (1993). Differential success of pollen donors in a self-compatible lily. *Evolution*, 915–924.

Roa, R. (1992). Design and analysis of multiple-choice feeding-preference experiments. *Oecologia*, 89, 509–515.

Salt Spring Island Publishing. (2012). 14th Annual Apple Festival cancelled due to weather-related poor apple crops [WWW Document]. *14th Annual Apple Festival cancelled due to weather-related poor apple crops*. URL http://www.saltspringmarket.com/apples/

Sánchez-Lafuente, A.M., Rodríguez-Gironés, M.A. & Parra, R. (2011). Interaction frequency and per-interaction effects as predictors of total effects in plant–pollinator mutualisms: a case study with the self-incompatible herb Linaria lilacina. *Oecologia*, 1–13.

Sargent, R.D., Kembel, S.W., Emery, N.C., Forrestel, E.J. & Ackerly, D.D. (2011). Effect of local community phylogenetic structure on pollen limitation in an obligately insect-pollinated plant. *American Journal of Botany*, 98, 283–289.

Scheidel, U., Röhl, S. & Bruelheide, H. (2003). Altitudinal gradients of generalist and specialist herbivory on three montane Asteraceae. *Acta Oecologica*, 24, 275–283.

Schemske, D.W. (1977). Flowering phenology and seed set in *Claytonia virginica* (Portulacaceae). *Bulletin of the Torrey Botanical Club*, 104, 254–263.

Schemske, D.W., Mittelbach, G.G., Cornell, H.V., Sobel, J.M. & Roy, K. (2009). Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics*, 40, 245–269.

Schemske, D.W., Willson, M.F., Melampy, M.N., Miller, L.J., Verner, L., Schemske, K.M., et al. (1978). Flowering ecology of some spring woodland herbs. *Ecology*, 59, 351–366.

Schweiger, O., Biesmeijer, J.C., Bommarco, R., Hickler, T., Hulme, P.E., Klotz, S., et al. (2010). Multiple stressors on biotic interactions: how climate change and alien species interact to affect pollination. *Biological Reviews*, 85, 777–795.

Schweiger, O., Heikkinen, R.K., Harpke, A., Hickler, T., Klotz, S., Kudrna, O., et al. (2012). Increasing range mismatching of interacting species under global change is related to their ecological characteristics. *Global Ecology and Biogeography*, 21, 88–99.

Schweiger, O., Settele, J., Kudrna, O., Klotz, S. & Kühn, I. (2008). Climate change can cause spatial mismatch of trophically interacting species. *Ecology*, 89, 3472–3479.

Sevruk, B. (1997). Regional dependency of precipitation-altitude relationship in the Swiss Alps. *Climatic Change*, 36, 355–369.

Sheffield, C.S., Westby, S.M., Smith, R.F. & Kevan, P.G. (2008). Potential of bigleaf lupine for building and sustaining *Osmia lignaria* populations for pollination of apple. *Can. Entomol.*, 140, 589–599.

Sherry, R.A., Zhou, X., Gu, S., Arnone, J.A., Schimel, D.S., Verburg, P.S., et al. (2007). Divergence of reproductive phenology under climate warming. *Proceedings of the National Academy of Sciences*, 104, 198–202.

Silvertown, J. (2008). The evolutionary maintenance of sexual reproduction: evidence from the ecological distribution of asexual reproduction in clonal plants. *International Journal of Plant Sciences*, 169, 157–168.

Singer, M.C. & Parmesan, C. (2010). Phenological asynchrony between herbivorous insects and their hosts: signal of climate change or pre-existing adaptive strategy? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3161 –3176.

Starzomski, B.M. (In press). Climate change and novel ecosystems. In: *Novel ecosystems: when and how do we intervene in the new ecological world order?* (eds. Hobbs, R.J. & Higgs, E.). Oxford: Wiley-Blackwell.

Stebbins, C.L. (1950). Variation and evolution in plants.

Steffan-Dewenter, I., Potts, S.G. & Packer, L. (2005). Pollinator diversity and crop pollination services are at risk. *Trends in Ecology & Evolution*, 20, 651.

Tauber, M.J., Tauber, C.A., Nyrop, J.P. & Villani, M.G. (1998). Moisture, a vital but neglected factor in the seasonal ecology of insects: hypotheses and tests of mechanisms. *Environmental Entomology*, 27, 523–530.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., et al. (2004). Extinction risk from climate change. *Nature*, 427, 145–148.

Thomas, J.. (2005). Monitoring change in the abundance and distribution of insects using butterflies and other indicator groups. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 339 –357.

Thomas, K.A., Denny, E.G., Miller-Rushing, A.J., Crimmins, T.M. & Weltzin, J. (2010). The National Phenology Monitoring System v0.1. *USA-NPN Technical Series*, 001.

Thomson, D.M. (2006). Detecting the effects of introduced species: a case study of competition between *Apis* and *Bombus*. *Oikos*, 114, 407–418.

Thomson, J.D. (1980). Skewed flowering distributions and pollinator attraction. *Ecology*, 61, 572–579.

Thomson, J.D. (1981). Spatial and temporal components of resource assessment by flower-feeding insects. *The Journal of Animal Ecology*, 49–59.

Thomson, J.D. (1982). Patterns of visitation by animal pollinators. *Oikos*, 39, 241–250.

Thomson, J.D. (2010). Flowering phenology, fruiting success and progressive deterioration of pollination in an early-flowering geophyte. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365, 3187 –3199.

Thomson, J.D., Maddison, W.P. & Plowright, R. (1982). Behavior of bumble bee pollinators of *Aralia hispida* Vent.(Araliaceae). *Oecologia*, 54, 326–336.

Thomson, J.D., Price, M.V., Waser, N.M. & Stratton, D.A. (1986). Comparative studies of pollen and fluorescent dye transport by bumble bees visiting *Erythronium grandiflorum*. *Oecologia*, 69, 561–566.

Thórhallsdóttir, T.E. (1998). Flowering phenology in the central highland of Iceland and implications for climatic warming in the Arctic. *Oecologia*, 114, 43–49.

Totland, ørjan & Schulte-Herbrüggen, B. (2003). Breeding system, insect flower visitation, and floral traits of two alpine *Cerastium* species in Norway. *Arctic, Antarctic, and Alpine Research*, 35, 242–247.

Totland, Ø. (1994a). Intraseasonal variation in pollination intensity and seed set in an alpine population of *Ranunculus acris* in southwestern Norway. *Ecography*, 17, 159–165.

Totland, Ø. (1994b). Influence of climate, time of day and season, and flower density on insect flower visitation in alpine Norway. *Arctic and Alpine Research*, 26, 66–71.

Totland, Ø. (1997). Limitations on reproduction in alpine *Ranunculus acris*. *Canadian Journal of Botany*, 75, 137–144.

Totland, Ø. (1999). Effects of temperature on performance and phenotypic selection on plant traits in alpine *Ranunculus acris*. *Oecologia*, 120, 242–251.

Totland, Ø. (2001). Environment-dependent pollen limitation and selection on floral traits in an alpine species. *Ecology*, 82, 2233–2244.

Totland, Ø. & Alatalo, J. (2002). Effects of temperature and date of snowmelt on growth, reproduction, and flowering phenology in the arctic/alpine herb, *Ranunculus glacialis*. *Oecologia*, 133, 168–175.

Tylianakis, J.M., Didham, R.K., Bascompte, J. & Wardle, D.A. (2008). Global change and species interactions in terrestrial ecosystems. *Ecology Letters*, 11, 1351–1363.

Vamosi, J.C., Knight, T.M., Steets, J.A., Mazer, S.J., Burd, M. & Ashman, T.-L. (2006). Pollination decays in biodiversity hotspots. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 956–961.

Vamosi, J.C., Sargent, R.D. & Elle, E. (2012). Pollination biology research in Canada: perspectives on a mutualism at different scales. *Botany*, 90, v–vi.

Vander Kloet, S.P. (1988). The genus *Vaccinium* in North America. *Agriculture Canada*, 201p.

Vázquez, D.P., Morris, W.F. & Jordano, P. (2005). Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters*, 8, 1088–1094.

Visser, M.E. (2008). Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proceedings of the Royal Society B: Biological Sciences*, 275, 649–659.

Visser, M.E. & Both, C. (2005). Shifts in phenology due to global climate change: the need for a yardstick. *Proceedings of the Royal Society B: Biological Sciences*, 272, 2561 –2569.

Visser, M.E. & Holleman, L.J.M. (2001). Warmer springs disrupt the synchrony of oak and winter moth phenology. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 268, 289 –294.

Visser, M.E., Noordwijk, A.J. van, Tinbergen, J.M. & Lessells, C.M. (1998). Warmer springs lead to mistimed reproduction in great tits (*Parus major*). *Proceedings of the Royal Society B: Biological Sciences*, 265, 1867–1870.

Vuong, Q.H. (1989). Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica: Journal of the Econometric Society*, 307–333.

Wall, M.A., Timmerman-Erskine, M. & Boyd, R.S. (2003). Conservation impact of climatic variability on pollination of the federally endangered plant, *Clematis socialis* (Ranunculaceae). *Southeastern Naturalist*, 2, 11–24.

Wang, T., Hamann, A., Spittlehouse, D.L. & Murdock, T.Q. (2012). ClimateWNA-High resolution spatial climate data for western North America. *Journal of Applied Meteorology and Climatology*, 51, 16–29.

Warren, S.D., Harper, K.T. & Booth, G.M. (1988). Elevational distribution of insect pollinators. *American Midland Naturalist*, 120, 325–330.

Waser, N.M. (1979). Pollinator availability as a determinant of flowering time in ocotillo (*Fouquieria splendens*). *Oecologia*, 39, 107–121.

Waser, N.M. & Real, L.A. (1979). Effective mutualism between sequentially flowering plant species, 281, 670–672.

White, I.M. (1987). The Linnaean species of the family Tephritidae (Insecta: Diptera). *Zoological Journal of the Linnean Society*, 90, 99–107.

Widén, B. (1991). Phenotypic selection on flowering phenology in *Senecio integrifolius*, a perennial herb. *Oikos*, 205–215.

Williams, P.H., Araújo, M.B. & Rasmont, P. (2007). Can vulnerability among British bumblebee (*Bombus*) species be explained by niche position and breadth? *Biological Conservation*, 138, 493–505.

Willis, C.G., Ruhfel, B., Primack, R.B., Miller-Rushing, A.J. & Davis, C.C. (2008). Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences*, 105, 17029 –17033.

Willmer, P. (2012). Ecology: pollinator–plant synchrony tested by climate change. *Current Biology*, 22, R131–R132.

Xerces Society. (2011). Farmers nationwide plant bee-friendly habitat to attract native pollinators, bolster honeybees [WWW Document]. *The Xerces Society*. URL http://www.xerces.org/2011/10/21/farmers-nationwide-plant-bee-friendly-habitat-to-attract-native-pollinators-bolster-honeybees/

Yang, L.H. & Rudolf, V.H.W. (2010). Phenology, ontogeny and the effects of climate change on the timing of species interactions. *Ecology letters*, 13, 1–10.

Zeileis, A., Kleiber, C. & Jackman, S. (2008). Regression models for count data in R. *Journal of Statistical Software*, 27, 1–25.

Zimmerman, M. & Pyke, G.H. (1988). Reproduction in *Polemonium*: assessing the factors limiting seed set. *The American Naturalist*, 131, 723–738.

Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S. & Dorn, S. (2010). Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation*, 143, 669–676.

Zuur, A.F., Ieno, E.N., Walker, N., Saveliev, A.A., Smith, G.M., Zuur, A.F., et al. (2009). Zero-truncated and zero-inflated models for count data. In: *Mixed effects models and extensions in ecology with R*, Statistics for Biology and Health. Springer New York, pp. 261–293.

Appendix A
Floral Visitation

Visitors incidentally observed at common alpine flowers at Marriott Basin, BC, in 2011. Species in parentheses ( ) are uncommon visitors, and species in double parentheses (( )) are rare visitors with no more than three observations. The most common *Bombus* species included both *B. insularis* and *B. melanopygus*.

Floral Species	Visitors
Erythronium grandiflorum	*Bombus* sp., Muscidae, Anthomyiidae, (Syrphidae), ((*Selasphorus rufus*))
Claytonia lanceolata	*Bombus* sp., Syrphidae, Muscidae, Anthomyiidae.
Anemone occidentalis	Muscidae, Anthomyiidae, Syrphidae, Phoridae, Tenthredinidae, ((*Bombus* sp.)).
Arnica latifolia	Muscidae, Anthomyiidae, *Bombus* sp., Tenthredinidae, *Euphydryas* sp., Syrphidae
Lupinus arcticus	*Bombus* sp., Syrphidae,
Caltha leptosepala	Muscidae, Anthomyiidae, Hesperiidae, (*Bombus* sp.)
Sorbus sitchensis	Muscidae, Anthomyiidae, *Formica* sp., *Bombus* sp., *Andrena* sp., *Osmia* sp., Syrphidae, Tenthredinidae
Valeriana sitchensis	Muscidae, Anthomyiidae, *Bombus* sp., Syrphidae
Vaccinium membranaceum	*Bombus* sp., *Formica* sp., Empididae, Vespidae, (Muscidae).

Appendix B
Effect sizes of pollen manipulation

Log response ratios of plants to experimental pollen-manipulation (calculated following Hedges et al. 1999), pooled across a 400 m elevation gradient at Marriott Basin, B.C., in 2011. PS = pollen-supplemented, B = bagged, O = open (control).

Species	Pollen supplementation (PS/B)	Pollen quality (PS/O)	Bagging (B/O)
Erythronium grandiflorum	0.383	-0.647	-1.031
Arnica latifolia	0.508	0.201	-0.307
Lupinus arcticus	1.093	-1.113	-2.206
Caltha leptosepala	1.414	0.059	-1.355
Claytonia lancelata	1.686	-0.256	-1.941
Vaccinium membranaceum	1.890	-1.045	-3.080
Anemone occidentalis	0.146	-0.371	-0.517

Appendix C
Morphospecies list and functional groups

Alphabetical list of morphospecies, by family group. I indentified all my morphospecies and assigned them into rough functional groups using the following keys and guides:

Marshall, S. (2007). Insects: Their Natural History and Diversity. Updated Reprint Edition. Firefly Books.

Marshall, S. (2012). Flies: The Natural History and Diversity of Diptera. Firefly Books.

Shepard, J. & Guppy, C. (2001). Butterflies of British Columbia: Including Western Alberta, Southern Yukon, the Alaska Panhandle, Washington, Northern Oregon, Northern Idaho, and Northwestern Montana. UBC Press.

Photographic key to the Syrphid genera of North America:
Jeff Skevington, Christian Thomson and Stephen Marshall, unpublished

Agaristidae
(1 mSp.)
Andrenidae
(2 mSp.)
Anthomyiidae
(2 mSp.)
Aphididae
(2 mSp.)
Braconidae
(2 mSp.)
Cantharidae
(3 mSp.)
Cecidomyiidae
(3 mSp.)
Ceratopogonidae
(1 mSp.)
Chironomidae
(6 mSp.)
Chloropidae
(6 mSp.)
Chrysididae
(1 mSp.)
Cicadellidae
(3 mSp.)
Coccinellidae
(1 mSp.)
Coccoidea
(1 mSp.)
Crabronidae
(1 mSp.)
Culicidae
(1 mSp.)
Dolichopodidae
(2 mSp.)
Empididae
(6 mSp.)
Fanniidae
(2 mSp.)
Formicidae
(3 mSp.)
Halictidae
1 mSp. (*Lasioglossum*)
Hesperiidae
Hesperia comma
Ichneumonidae
(7 mSp.)
Misc. Larvae
(2 mSp.)

Lycaenidae
Glaucopsyche lydamus columbia
Lycosidae
(3 mSp.)
Microhymenopteran
(29 mSp.)
Mymaridae
(3 mSp.)
Nymphalidae
(*Euphydryas* sp.)
Pentatomidae
(1 mSp.)
Phoridae
(5 mSp.)
Pieridae
Pieris marginalis
Pipundiculidae
(2 mSp.)
Psylloidea
(1 mSp.)
Sarcophagidae
(2 mSp.)
Scathophagidae
(1 mSp.)
Sciaridae
(2 mSp.)
Mlepidoptera
(1 mSp.)
Muscidae
(2 mSp.)
Mycetophilidae
(6 mSp.)
Tachinidae
(2 mSp.)
Tenthredinidae
(3 mSp.)
Tephritidae
(1 mSp.)
Thomisidae
(2 mSp.)
Tipulidae
(2 mSp.)
Sciaridae
(5 mSp.)
Simuliidae
(2 mSp.)
Sminthuridae
(2 mSp.)
Staphylinidae
(1 mSp.)

Syrphidae
Dasysyrphus
(1 sp.)
Platycheirus
(2 sp.)
Other genera
(12 mSp.)
Mites (Acari)
(3 mSp.)
Thysanoptera
(5 mSp.)
Calyptratae ('Black fly')
(1 mSp.)
Small Calyptratae ('Sm. black fly')
(1 mSp.)
Unk. (Clusiidae, or Agromyzidae?)
(1 mSp.)
UnkOrthoptera
(3 mSp.)
Collembola
(3 mSp.)

I distinguished 'morphospecies' within known families or genera based on easily-recognized morphological characteristics such as size, colour, and distinctive markings. In some cases, species richness might have been overestimated by splitting males and females of highly dimorphic and little-known species, but underestimated because of failure to distinguish cryptic species (e.g., within the Phoridae). For the purpose of analysis, many morphospecies ended up being lumped within the same functional groups (see below).

Taxa included in each functional group.

Group	Taxa
Predator & Pollinator	Symphyta, Cantharidae, Crabronidae
Pollinator	Tenthredinidae, Syrphidae, Apoidea, Lepidoptera (adult)
Parasitoid	Ichneumonidae, microhymenoptera, mymaridae,
Phloem-feeder	Aphididae, Coccoidea, hemiptera, Thysanoptera
Detritivore	Scathophagidae, Sarcophagidae
Herbivore	Lepidoptera (larvae), Orthoptera
Predator	Araneae, Dolichopodidae
Mycetovore	Mycetophilidae, Sciaridae
Omnivore	Formicidae
BlackFly	Calyptratae, Small calyptratae
Unknown	Muscidae
FlowerVisitor	Coccinellidae
Nonfeeding	Tipulidae, some lepidoptera

Code used to designate functional groups in Microsoft Excel:

```
=IF(I1388="Syrphidae","Pollinator",IF(I1388="Tenthredinidae","Pollinator",IF(I1388="Halictidae","Pollinator",IF(I1388="Andrenidae","Pollinator",IF(H1388="Lepidoptera","Pollinator",IF(I1388="Microhymenopteran","Parasitoid",IF(H1388="Hemiptera","Phloem-feeder",IF(I1388="Mymaridae","Parasitoid",IF(I1388="Cantharidae","Predator&Pollinator",IF(H1388="Araneae","Predator",IF(H1388="Thysanoptera","Phloem-feeder",IF(H1388="Orthoptera","Herbivore",IF(I1388="Dolichopodidae","Predator",IF(I1388="Sarcophagidae","Detritivore",IF(I1388="Empididae","Predator",IF(I1388="Formicidae","Omnivore",IF(I1388="Sciaridae","Mycetovore",IF(I1388="Mycetophilidae","Mycetovore",IF(I1388="Ichneumonidae","Parasitoid",IF(I1388="Braconidae","Parasitoid",IF(I1388="Crabronidae","Predator&Pollinator")))))))))))))))))))))
```

Appendix D
Power analysis

Post hoc statistical power analysis for Mann-Whitney U, Kruskal-Wallis, and Linear Multiple Regressions (broken down by species, and tests for particular effects). All values given are for a critical value of α = 0.05. O = open (control), B = bagged (pollinators excluded), F = pollen-supplemented (hand-pollinated).

Test (effects of?)	Effect size	Power (1 – β)	N	df (num/den)
Mann-Whitney U: (*Arnica*: seed pred.)	0.7106335	0.9999031	86	84
K-W (*Arnica*: seed pred. x elev.)	0.4681032	1.0000000	286	2
Erythronium grandiflorum (Treatment)	0.1729436	0.8314584	351	2/348
(Elevation): O only	0.1820541	0.3955468	39	2/114
(Elevation): B only	0.1723477	0.3573403	39	2/114
(Elevation): F only	0.2355587	0.6070464	39	2/114
Anemone occidentalis (Treatment)	0.3809594	0.9986877	198	2/195
(Elevation): O only	0.0098634	0.0559416	33	61
(Elevation): B only	0.0534460	0.0756372	33	61
(Elevation): F only	0.2819429	0.2952528	33	61
Caltha leptosepala (Treatment)	0.5377916	0.9757266	66	2/63
(Elevation): O only	1.180356	0.8319729	11	19
(Elevation): B only	0.3298717	0.1799423	11	19
(Elevation): F only	0.793364	0.5430488	11	19
Claytonia lanceolata (Treatment)	0.4015918	0.9999974	297	2/294
(Elevation): O only	0.1821254	0.3389811	99	2/96
(Elevation): B only	0.2115023	0.4419333	99	2/96
(Elevation): F only	0.1882689	0.3596863	99	2/96
Vaccinium membranaceum (Treatment)	0.281545	0.9840911	252	2/249
(Elevation): O only	0.2036683	0.3555845	84	2/81
(Elevation): B only	0.1845066	0.2981088	84	2/81
(Elevation): F only	0.4956779	0.9847118	84	2/81
Arnica latifolia (Treatment)	0.3750304	0.9998471	253	2/250
(Elevation): O only	0.3459183	0.8021464	84	2/81
(Elevation): B only	0.1717783	0.2628098	84	2/81
(Elevation): F only	0.5064469	0.9880126	84	2/81
Lupinus arcticus (Treatment)	0.401573	0.9999833	263	2/260
(Elevation): O only	0.2220231	0.4280199	87	2/84
(Elevation): B only	0.0352392	0.0579089	87	2/84
(Elevation): F only	0.0947564	0.1111086	87	2/84
Eryt (regression) (open only)	0.15	0.9674558	117	2/114
Anem (regression)(open only)	0.15	0.7912009	66	2/63
Vacc (regression) (open only)	0.15	0.6473411	84	2/82
Lupi (regression) (open only)	0.15	0.8984305	87	2/84
Clay (regression) (open only)	0.15	0.9346964	99	2/96
Arni (regression) (open only)	0.15	0.8869306	84	3/81
Calt (regression) (open only)	0.15	0.3042371	22	2/19