MECHANISMS OF POLLINATION: QUANTIFYING INSECT AND PLANT CONTRIBUTIONS

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Biology

at the University of Canterbury

by

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Acknowledgements

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Abstract

Global agricultural production is reliant on insect-mediated pollination, which is largely provided by the European honey bee (*Apis mellifera* L.). Recent concern about the health of honey bees has raised significant concern about the future of food production and, as a result, alternate pollinators have been explored to provide these services. However, identifying which insect species are efficient pollinators of a particular plant species is challenging and labor-intensive. Additionally, even if an alternate pollinator is identified, its services may be insufficient to prevent pollination failure, which may be due to other factors.

This thesis explores different measures that can be used to assess a species' effectiveness as a pollinator and the causes of pollination failure. Particularly, it addresses four main questions: 1) whether insect behavior or pollen transport can be used to predict single-visit pollen deposition (and thus pollinator efficiency) in four vegetable seed crops 2) whether examining pollen transport at scales finer than species (i.e. sexes, individuals, and body parts) using network approaches improves the predictive ability of pollen transport for single-visit deposition, and whether this differential pollen deposition across the insect body facilitates coexistence 3) whether there are phylogenetic trends in pollen longevity and 4) to assess the underlying cause of pollination failure in hybrid carrot seed production, a system traditionally considered to be pollinator-limited, and how the system is likely to be affected in a warming climate.

The key findings of this thesis are that there are differences in insect behavior and pollen transport at the body-part scale which, when accounted for, yield better predictions of single-visit pollen deposition than existing methodologies and result in higher estimates of species coexistence. It also appears that pollen longevity and its response to environmental conditions may be generalizable at the genus-level, potentially enabling estimations of longevity for unsampled taxa. Last, I found that a constellation of plant-related factors were implicated in pollination failure of hybrid carrot seed, and that increasing temperatures may decrease floral volatile emissions,

potentially affecting plant attractiveness. Each of these findings could fruitfully be incorporated into future models of pollination, potentially yielding better predictions of pollination systems.

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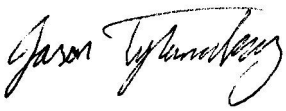
Name: *Jason Tylianakis* Signature:  Date: *8 Jan 2018*

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Please detail the nature and extent (%) of contribution by the candidate:

Melissa Broussard's contribution is ~85%. Melissa Broussard led the development of the study design, conducted the study, analyzed the data, and wrote the paper. Flore Mas provided study design for the volatile collection and assisted with the chemical analyses and the writing of the methods sections for those analyses. Mass spectroscopy was done by a third party. All authors assisted with the editing of the manuscript.

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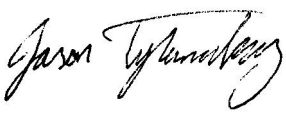
Name: *Jason Tylianakis* Signature:  Date: *8 Jan 2018*

Chapter I: Introduction

Over 60% of the most common agricultural crops require insect-vectored pollination for optimal production (Klein et al. 2007), comprising approximately one-third of the global food supply (Aizen et al. 2009). Honey bees (*Apis mellifera* L.) have been heavily relied upon to provide these pollination services, but numerous diseases and parasites have reduced their numbers globally (Neumann & Carreck 2010). Their loss has been partially compensated for by managed and wild pollinators (Garibaldi et al. 2013), but there is evidence that a number of wild pollinator species are also declining (Grixti et al. 2009; Potts et al. 2010; Cameron et al. 2011), likely due to loss or degradation of habitat (Potts et al. 2010). This is concerning, as pollinator diversity is linked to higher-quality pollination services through both additive and synergistic effects (Winfree et al. 2007; Rader et al. 2009; Carvalheiro et al. 2010; Isaacs & Kirk 2010; Brittain et al. 2013; Garibaldi et al. 2013) and cannot be easily replaced once lost.

Globally, the land area devoted to many pollinator-dependent crops is increasing in order to maintain yield, often resulting in the conversion of marginal areas to intensive agricultural land uses (Aizen et al. 2009). As non-managed pollinators tend to decrease as remnant habitat decreases, this trend has the potential to further reduce their diversity and abundance (Carvalheiro et al. 2010; Garibaldi et al. 2011a; Concepcion et al. 2012; Kennedy et al. 2013). The potential future pollination shortage has been approached from several different angles: examining honey bee disease and disease management (Evans & Schwarz 2011), determining whether non-managed pollinators can provide sufficient pollination in the face of declining honey bees (Winfree et al. 2007; Garibaldi et al. 2013), implementing hand-pollination by humans (Partap & Ya 2012), and selective plant breeding, including the development of self-fertile cultivars (Bekefi 2006; Hegedus 2006; Ledbetter 2010). As honey bee disease has been adequately explored elsewhere, and hand-pollination by humans does not appear to be a viable long-term strategy (Partap & Ya 2012), a focus

on alternate pollinators is necessary. A growing body of literature examines the efficiency of alternative pollinators, focusing on native and introduced bee species (McGregor 1976; Kennedy et al. 2013), with flies (Jauker & Wolters 2008; Rader et al. 2009; Blaauw & Isaacs 2014) and other invertebrates (Howlett et al. 2009a; Carvalheiro et al. 2010) gaining more consideration in recent years. Some groups of bees and flies outperform honey bees in single-visit comparisons (Jauker & Wolters 2008; Rader et al. 2009), making them ideal candidates for alternative pollinators.

Much of the work done to date regarding agricultural pollination has been locally specific, looking at which pollinators in that particular area or region might be most suitable for the crop of interest (e.g. Rader et al. 2009). As such, most findings from studies done to date are not generalizable, with reports on the same crop in different regions yielding different results (Garibaldi et al. 2013). Nevertheless, we know that pollinator-limited crops exhibit declining yield growth despite increased inputs (Garibaldi et al. 2011b), increasing pollinator diversity has benefits regardless of honey bee abundance (Garibaldi et al. 2013), and that integrating visitation rates and single-visit pollen deposition yields a reliable estimate of pollinator species' efficacy (King et al. 2013). However, we come up short when it comes to understanding what traits make an insect a good pollinator for a particular crop. In order to prevent future pollination failure, we must understand what mechanisms cause insect species-level differences in pollination efficiency, and whether plant physiological characteristics constrain fruit or seed set independent of pollination.

When seed or fruit set is low, pollinators are often blamed (Delaplane & Mayer 2000). Each crop may only have a few suitable pollinators in the landscape because many non-*Apis* pollinator species forage for a short period of time each year and at specific times of day (Hoehn et al. 2008), which may not coincide with peak bloom. While a number of insects may visit the crop of interest, the identification of effective pollinators has typically been done in terms of single-visit pollen deposition, as insect visitation rates alone are not well correlated to efficacy (King et al. 2013). The process of collecting single-visit pollen deposition data is laborious and time-consuming, but easier-

to-measure traits such as insect behavior have thus far only yielded equivocal results (Vaissière et al. 1996; Thomson & Goodell 2001; Adler & Irwin 2005; King et al. 2013). Indeed, so poorly understood are the behavioral and physiological traits that make an insect a good pollinator that the state-of-the-art is to measure single-visit pollen deposition rates and visitation rates for all common flower-visiting insect species on each plant species of interest in each region of interest to determine the relative importance of the available pollinators (King et al. 2013). Understanding the mechanisms underlying the observed interspecific differences in pollination efficiency would greatly aid the identification of these key pollinators.

Additionally, data linking efficient pollinators to plant species are collected on a variety of different scales, from local (e.g. Tanács et al. 2008) to regional (e.g. Rader et al. 2011), and nearly always aggregated by insect species. However, it is a standing question whether individuals of generalist pollinator species actually have a much narrower range of host plants than the pollinator species as a whole (Brosi 2016). If this is the case, insects which appear to have high fidelity at a local scale may appear to have poor fidelity at larger scales. It is also possible that there are differences not only between individuals, but within an individual, with pollen being collected and deposited on discrete body parts by plant species (e.g. Singer and Cocucci 1999). Therefore, not only is it unknown what determines the efficacy of different pollinators on different plants, but there may even be differences among individuals of a pollinator species and efficacy may depend on the pollen on a specific region of the insect body.

While pollen must be deposited on a stigma for successful pollination, numbers alone are not sufficient. As soon as pollen is exposed to the environment, it begins the decline to becoming inviable, a process which can take minutes to months, depending on the plant species (Dafni & Firmage 2000). If an insect deposits thousands of conspecific grains onto a stigma, but only a handful are viable, it does the plant little more good than an "inefficient" pollinator (as measured by single-visit pollen deposition) depositing a handful of pollen grains, all of which are viable. If these

pollen grains are distributed heterogeneously across the insect body, there may be additional implications for pollination efficacy.

Pollen longevity has been considered in a variety of contexts, but surprisingly rarely with respect to pollinators. An early paper examined the longevity of pollen on honey bees compared with stored pollen from flowers, and found that pollen on insects decayed faster than pollen left in ambient conditions (Mesquida & Renard 1989). These results were echoed in a more recent study of pollen stored on moths (Richards et al. 2005). Rader et al. (2011) found that pollen viability was different on bees and flies, likely due to the differing amounts of time each taxon spends between floral visits. Given that pollen viability on insects has been examined for so few plant species, but thousands of papers have been published on pollen viability and longevity under other conditions, there is the potential to use the latter to create estimations of the former. Prior to that being possible, however, it is necessary to synthesize the data on pollen viability present in the literature to assess how longevity is affected by different environmental conditions. The most recent review of pollen viability and the mechanisms of poor pollen quality (Dafni & Firmage 2000) gathers some of this information together, but represents only a tiny fraction of the taxa that have been studied. A systematic review and meta-analysis of these data is likely to reveal what the previous reviews only touch on: trends in pollen viability across taxa and under different conditions. This would finally allow estimation of pollen quality, a prerequisite for male fitness, in the field.

Pollen quality is only one part of the more complex issue of pollination, however. Numerous factors, from high levels of incompatible pollen (Wilcock & Neiland 2002) to poor genetic condition (Charlesworth & Willis 2009), to temperature (both too high and too low) can also result in low yield (Hedhly 2011). There is also some evidence that floral receptivity changes significantly over the course of the day (B. Howlett, personal communication). It is possible that the previously discussed changes in pollen due to environmental factors could interact with the daily changes in female receptivity. If environmental conditions affect these two factors differently, changing

conditions could lead to a mismatch or shortened window for pollination. Together, the above could identify whether the plant of interest is indeed pollinator-limited, and, if so, provide a framework to assess which insects will be active at the critical window for efficient pollination.

Taken all together, these factors (insect behavior, scale of measurement, pollen viability, and plant receptivity) could help form a more holistic, mechanistic model for how pollination works.

Outline and objectives

The themes outlined above are explored in depth in the chapters that follow, each of which is presented as a standalone manuscript. In **Chapter II**, I examine the relationship between insect species' behavior, the pollen they carry, and pollen deposition in order to elucidate a more mechanistic understanding of what makes particular insect species good pollinators. Both behavior and pollen transport are broken down to the body-part level in order to explore how these fine-scale differences might explain the bigger picture of pollen deposition. **Chapter III** explores how the scale at which interactions are studied affects the apparent characteristics of plant-pollinator networks. By examining the same data aggregated at the insect species-scale, the insect individual-scale, and the insect body-part-scale, the extent to which individuals of generalist pollinator species are also generalists will become apparent, as well as whether or not plant species utilize different regions of the insect body, potentially reducing the risk of pollen cross-contamination between plants. All of this information is used in the construction of a model which assesses how this partitioning affects competition and coexistence compared to the traditional species-aggregated data. **Chapter IV** is a systematic meta-analysis of pollen longevity across the English language literature. The relationship between pollen longevity and environmental factors, such as temperature, is examined. Comparisons are made between closely-related and distantly-related plant species, as well as between wild and cultivated plant species. Longevity estimates are presented for the plant families examined. **Chapter V** explores a system traditionally considered to be pollinator-

limited (hybrid carrot). Data on hand-pollination, pollen viability, floral volatiles, and nectar quality are examined to assess the cause of the low seed set observed in some cultivars in the field, and different temperature regimes are examined to determine if existing variation will be worsened in a warming climate. Finally, **Chapter VI** is the overall discussion, which synthesizes the findings from the preceding chapters and recommends areas for future study.

Chapter II: Pollinator behavior influences pollen viability and single-visit pollen deposition across multiple pollinator dependent crops

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2.1 Summary

1. The risks of relying on a single pollinator, the European honey bee (*Apis mellifera*) have led to worldwide interest in alternate pollinator taxa. The relative effectiveness of these taxa has been measured via visitation rate, pollen transport, and single-visit pollen deposition but, to date, there has been no analysis of the relationships between these metrics that accounts for the strong interspecific variation across metrics observed in the field.

2. We evaluated insect visitation behavior, pollen transport, and pollen deposition across four crop plants (carrot, onion, pak choi, and radish) in New Zealand. Interspecific differences in each metric were examined singly and in relation to the other metrics. Both insect behavior and pollen transport were examined at the whole insect level, and by six body regions (the top and bottom of the head, thorax, and abdomen) to test whether behavior (in terms of which body parts contact floral reproductive structures) predicted pollinator species' differences in pollen viability and transport, and whether this affected single-visit pollen deposition.

3. We found that observations of both insect behavior and pollen counts on the insect body were significant predictors of deposition potential for insect species, with the top of the insect's head

being the most relevant region across pollinator taxa. The head also had the highest proportion of viable pollen.

4. The amount of time each body part spent contacting floral reproductive structures, was predictive of both the pollen transported on that body part and pollen deposition. In contrast, the total proportion of time the insect spent contacting floral structures was only predictive of pollen transport. Our model explained up to 57% of the interspecific variation in pollen collected on each body part, and 54% of the interspecific variation in pollen deposition. Of this, ~40% of variation in pollen collected or deposited was explained by body part, and ~16% was explained by the crop plant being foraged on.

5. These findings indicate that examining insect behavior and pollen transport at the body part level has potential as a predictive tool for estimating pollen deposition rates in the field for novel taxa. This could substantially decrease the cost in both time and monetary resources in evaluating pollinators for target plant species at both local and regional scales, giving land managers greater ability to make locally-appropriate management choices.

2.2 Introduction

Wild and managed insects serve an important role as pollinators and are vital to continued agricultural and natural ecosystem functioning (Aizen & Feinsinger 2003; Klein et al. 2007; Buchmann & Nabhan 2012). Recent concerns about the health (Neumann & Carreck 2010; Potts et al. 2010) and efficacy (Brittain et al. 2013; Garibaldi et al. 2013; Rader et al. 2016) of the European honey bee (*Apis mellifera*), which is used to pollinate a large fraction of the world's agricultural crops (Dafni & Firmage 2000), have led to extensive investigations of alternate pollinators and their relative contributions to pollination success. These studies indicate that alternate pollinator taxa, primarily non-*Apis* bees (Adler & Irwin 2005; Sahli & Conner 2006; Brittain et al. 2013), but also flies (Rader et al. 2009; Munawar et al. 2011; Howlett 2012), may be as efficient as honey bees. These works have used visitation rate (or interaction frequency; Sahli & Conner 2006; Winfree et

al. 2008; Rader et al. 2009; Alarcón 2010; King et al. 2013), pollen transport by insects (Mesquida & Renard 1989; Vaissière et al. 1996; Howlett et al. 2011), and pollen deposition (Maloof & Inouye 2000; Rader et al. 2009; Davidson et al. 2010; Brittain et al. 2013; King et al. 2013) as metrics with which to compare the pollination efficacy of different insect species.

However, relatively few studies have compared more than one of these metrics, and those that do often highlight the limitations of using any one of them alone. For example, visitation rate (Alarcón 2010; King et al. 2013) and the total amount of pollen transported by a pollinator species (Adler & Irwin 2005) can be poor predictors of the deposition of conspecific pollen. Howlett et al. (2011) found that, while the number of pollen grains on the insect body was correlated with the amount of pollen deposited on plants, there was high variance among insect species, and the difference in magnitude between pollen transport and pollen deposited may make generalizing the predictive power of pollen transport challenging. As a result, single-visit pollen deposition remains the standard for assessing pollinator efficacy (Ne'eman et al. 2010; King et al. 2013).

Collecting single-visit pollen deposition has significant drawbacks, however, as virgin flowers must be watched until a target insect visits, or using the “interview stick” method (Thomson 1988) to present a flower to a target insect in hopes of it visiting (Howlett et al. 2017). In addition, because of the poor generalizability of the findings across plant species, measures must be taken for each plant species of interest. Therefore, compared with visitation rate or collecting insects from the field, pollen deposition data are time-consuming and costly to collect. Additionally, as previous works have found significant, but unexplained, differences between insect species' pollination efficiencies (Alarcón 2010; Howlett et al. 2011; King et al. 2013), it seems likely that contemporary study designs are failing to account for the variables that are responsible for these differences.

A possibility not widely explored is that species-level differences in flower handling may lead to pollen being deposited on different portions of the insect body. Some plant species are known to deposit pollen on specific insect body parts (Singer & Cocucci 1999), and insect species may forage in similarly idiosyncratic ways; for example, nectar-robbing insects often result in lower pollen deposition and seed set than active foragers (Maloof & Inouye 2000), presumably because their behaviors result in less pollen being carried by the insect. The way an insect approaches a flower may also have an effect; honey bees approaching apple flowers laterally deposit less pollen (Thomson & Goodell 2001), though the same study saw no differences between pollen- and nectar-foragers' rates of pollen deposition. Pollen viability is also potentially affected by behavior (such as foraging for pollen v. nectar) though results across studies are inconsistent (Vaissière et al. 1996; King et al. 2013). There is also some preliminary evidence that pollen viability differs across insect body parts (Mesquida & Renard 1989). Given these findings, it appears probable that variation in quality and quantity of pollen across the insect body may be explained by behavior on the one hand, and account for interspecific differences in pollen deposition on the other. If, on average, the body parts of different insect species spend different amounts of time touching the anthers, and thus end up carrying different quantities of pollen, which are then differentially deposited back onto conspecific flowers, there may be a convincing mechanism linking observations of insect behavior to predictions about pollen deposition. This linkage may explain a portion of the strong insect species-level differences in pollen deposition observed in the field, why those differences are inconsistent amongst plant species, and potentially produce a more robust correlation between visitation behavior, pollen transport, and pollen deposition by including body-part scale data. To our knowledge, no study has previously linked interspecific differences in flower-visiting behavior to pollen deposition.

Pollen deposition alone is insufficient to ensure fertilization, however. As soon as the pollen grains

are exposed to the environment, their viability begins to decline, a process which can take minutes or months, depending on the plant species (Dafni & Firmage 2000). An insect carrying inviable pollen grains will be unable to perform pollination services regardless of the number of grains it deposits. If the proportion of viable pollen varies between insect species and between insect body parts, this may further explain insect species-level differences in single-visit pollination success.

In the event that pollinator efficiency varies more within an insect species than between species (a hypothesis laid out in Brosi 2016), a large sample size would be necessary to obtain species-level trends and avoid spurious inferences based on idiosyncrasies of individuals. Comparing the three metrics of pollination efficiency (visitation behavior, pollen carried, and pollen deposition) at the insect species level across datasets (i.e. datasets with no overlap in individuals) is a way to further ensure that any trends found are measuring species rather than individual differences; such tests would be more conservative than if all three metrics were measured with the same insect but, by the same token, any findings will be robust at the species level.

To determine to what extent the different metrics of pollination efficiency are interrelated and if their relationships are able to explain some of the interspecific variation observed in previous literature, we analyzed data on insect behavior, the amount, viability, and composition of pollen carried, and single-visit pollen deposition, while also examining variation between body parts.

We had two primary objectives:

1) to test if insect species differed consistently in the amount of time each body part spent contacting floral reproductive structures, the quantity or viability of pollen carried on those body parts and

2) to test if an insect's behavior (the amount of time spent contacting floral structures) predicted the

amount and location of transported pollen, and whether this in turn explained interspecific differences in pollen deposition.

2.3 Methods

To assess the relationship between insect behavior, pollen transport, and pollen deposition, and the extent to which interspecific pollinator efficiency differs across plant species, we examined insect pollinators of four focal crop species. Carrot, radish, pak choi, and onion were chosen because yields of these crops are greatly improved by insect pollination, they have a relatively diverse community of local pollinators, and vary in their floral morphology. As there is little overlap between the flowering of each crop, each was sampled in succession. All samples were taken during full bloom in the Canterbury Plains of New Zealand.

2.3.1 Insect behavior

Videos of insect behavior on crop plants were recorded using a hand-held video recorder (Sony Handy Cam DCR-HC85E). Recordings were made in 2010 for carrot, 2006 – 2007 for pak choi and radish and 2007 and 2010 for onion. All recordings were made at peak bloom, during daylight hours (0900h – 1700h) in fine weather conditions, with air temperature 15.2 – 25.6ºC, wind gusts under 10 km/h, and light intensity 750 – 1250 W/m^2. Each recording session followed an individual insect visiting crop flowers, ending when the insect left the immediate area; as a result, video clips were inconsistent in length, ranging from a few seconds to several minutes. Within each crop, observations were collected throughout the day, alternating between insect species to maximize the diversity of recorded observations. To further this end, up to 15 minutes were spent after each recording in search of an insect species that had not recently been recorded. Recordings were taken over two days in each of the four crops.

Following recordings, videos were analyzed, and key events recorded using the software BORIS (Friard & Gamba 2016). For each individual insect, we assessed the time spent per inflorescence, the number of flowers (or umbellets) visited per inflorescence, the time spent per flower (or umbellet), the proportion of time on a flower spent touching floral reproductive structures (anthers and stigma), and the proportion of time each body part spent touching floral reproductive structures.

2.3.2 Pollen transport

Each of the four crops was sampled on three different days in the 2014/2015 austral field season. Each sample day, insects were collected by hand-netting in the morning, afternoon and early evening (i.e. nine samples in total for each crop). Up to six individuals of each insect species were collected per sampling time. Within 15 minutes of collection, insects were placed in cryotubes and stored in liquid nitrogen. Once back in the laboratory, specimens were transferred to a -80ºC freezer and kept there until processing.

We then assessed the quantity, composition, and viability of pollen on different body parts. Field-collected insects were removed from the freezer, dissected into head, abdomen, and thorax (head, mesosoma, and metasoma for bees), and then thawed for 2 – 5 minutes. Six slides were prepared per insect, one for each body part: the top and bottom of the head, abdomen, and thorax (Fig. 1). Each slide was given a drop of fluorescein diacetate (FDA) solution (0.25% w/v FDA, 20% w/v sucrose) and one of the six body parts was dipped 20 times in the dye solution to release pollen from the body part onto the slide. The forceps were washed between each body part. Each slide was then sealed with a coverslip and examined with a UV light microscope, where up to 200 total pollen

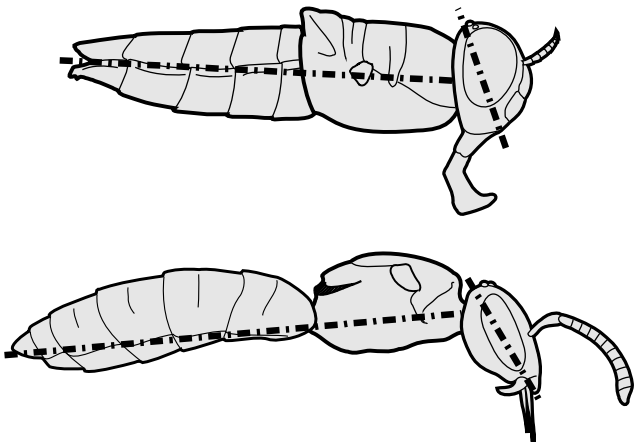

Figure 1: Regions of the body for pollen analysis for flies (top) and bees (bottom)

grains were counted across longitudinal transects of the slide. Both crop and non-crop pollen were assessed for viability. Pollen was considered viable if it fluoresced bright green (Heslop-Harrison & Heslop-Harrison 1970).

2.3.3 Pollen deposition

We measured the pollen deposited by different insect species on field-growing plants of each of the four crop species. Virgin, unopened inflorescences were caged in fine mesh (50 μm) to exclude pollinators (details in Walker et al. 2009). These inflorescences were either from male-sterile varieties (carrot, onion, radish), or the anthers were excised prior to use (pak choi). When the caged inflorescence reached peak bloom (>80% of flowers had fully developed stigmas), the cage was removed and the flower (onion, radish, pak choi) or umbel (carrot) was excised and presented to nearby floral visitors. To ensure adequate replication and increase the odds of pollen being deposited, flowers were presented only to common floral visitors of the crop species (Howlett et al. 2009b; Howlett, Lankin-Vega & Pattemore 2015) foraging on male-fertile flowers that had dehisced anthers, and were held 3 – 5 cm away to avoid startling the insects (Howlett et al. 2017).

Once an insect had landed on the presented flower, it was allowed to forage until it left the inflorescence or, in the case of carrot, until it had visited 3 – 5 umbellets. The first flower (onion, radish, pak choi) or umbellet (carrot) the insect visited was carefully excised and placed into an open Eppendorf tube suspended in a specimen container. An unvisited flower or umbellet was also excised as a negative control. Each inflorescence was allowed to be visited by only one insect. Flowers were stored in the dark at 4ºC until analysis.

In the laboratory, the style (or styles) were excised from each flower by cutting above the top of the ovary and mounted on a slide by pressing the stigmas into a small cube (~3 mm^3) of gelatine fuchsin, which was then melted and sealed with a cover slip (Kearns & Inouye 1993). Once cooled,

the styles were examined under a compound microscope at 200x magnification, and all conspecific pollen grains in contact with the stigma and style were counted. Carrot data were collected in January 2010, onion data were collected in January 2007 and December 2013 (Howlett et al. 2017), pak choi data were collected from December 2006 to February 2007 (Rader et al. 2009), and radish data were collected in January 2014.

2.3.4 Statistical analysis of individual datasets (Objective 1)

Preliminary analyses were conducted on the three datasets (insect behavior, pollen transport, and pollen deposition) individually to identify the extent of interspecific differences, and how those changed when looking at the whole insect versus body parts of the insect. The nature of crop bloom times required data to be collected over different dates, potentially creating the risk of non-independence within crop types. Therefore, we used generalized linear mixed-effect models (GLMMs) using the function *glmer* from the R package *lme4* (Bates et al. 2014). Final p-values for GLMM parameter estimates were obtained with the Satterthwaite method of denominator synthesis, which can produce non-integer degrees of freedom, implemented within the *lmerTest* package (Kuznetsova et al. 2015).

The insect behavior data were analyzed using three GLMMs to assess whether there were observed differences between insect species, broad differences between bees and flies, and whether there was a relationship between the proportion of time each body part spent touching floral reproductive structures and the proportion of time the whole insect spent touching floral reproductive structures. The first model had proportion of time spent touching floral reproductive structures as a response variable, and insect species and crop as crossed random effects; the significance of insect species was examined by removing it from the model and comparing the two models with a likelihood ratio test. The second model was the same as the first, but had insect order (Hymenoptera or Diptera) as a

fixed effect. To assess which body parts spent the highest proportion of time contacting floral structures, we used the proportion of time each body part spent as the response variable, and body part (a factor with six levels) as the fixed effect, with crossed random effects of insect species and the flower ID nested within crop type (to account for multiple body parts belonging to the same insect in the same floral visit). The overall test of whether body parts differed in the amount of time spent touching floral reproductive structures was conducted with a likelihood ratio test of models with and without body parts (but keeping random effects the same).

We also analyzed the pollen transport dataset with GLMMs, except for analyses involving the quantity of pollen, as this was censored at 200 (as per the methods above). For pollen quantity, we used a Cox mixed-effects model (CMM), which is designed to handle censored data, from the R package *coxme* (Therneau 2015). The results from the CMM were broadly similar to those we would have obtained with a GLMM (though the latter failed to converge), so we present results from the CMM only. The response variable was the quantity of pollen grains, with values of 200 marked as being censored, and the predictor variables were insect order (Hymenoptera and Diptera) and insect body part (a factor with six levels), as well as their interactive effect. Crop species and individual insect ID (to group body parts of a given insect) were included as crossed random effects to account for the temporal differences in pollinator communities between crop species, and to reflect the nested nature of the insect body part data. We ran GLMMs for the two pollen variables, both of which were modeled as a proportion with a binomial error distribution and a logit link function. The first was a model of the proportion of crop to non-crop pollen, with body part as a fixed effect, and crop, insect species, and individual insect ID as random effects. The second was a model of the proportion of viable to non-viable crop pollen, with the same random and fixed effects. Both of these GLMMs were run only for body parts with greater than 10 pollen grains so that proportions could be quantified with less error.

The pollen deposition data included many instances where insects failed to deposit pollen grains, introducing numerous zeros. While not over-dispersed, the dataset did not conform well to the Poisson distribution and so was analyzed with a zero-inflated negative binomial GLMM in the *glmmADMB* package (Bolker et al. 2014). The number of pollen grains per stigma was the response variable, and insect order was the fixed effect, while crop and insect species were random effects.

2.3.5 Statistical analyses of paired datasets (Objective 2)

After preliminary analyses of each of the three datasets, we compared them with each other to explore whether different sources of data provided congruent estimates of pollinator efficiency (Fig. 2). Each pairwise comparison we conducted involved creating a merged dataset, which contained only the insect species present in the two datasets being compared. Where relevant, models were checked for over-dispersion (where error distributions were not Gaussian) or normality of residuals and homoscedasticity (for Gaussian models). To check the fit of the GLMMs, we calculated both the marginal R-squared value (R^2m) and conditional R-squared value (R^2c) using the *r.squaredGLMM* function in the R package *MuMIn* (Barton 2014).

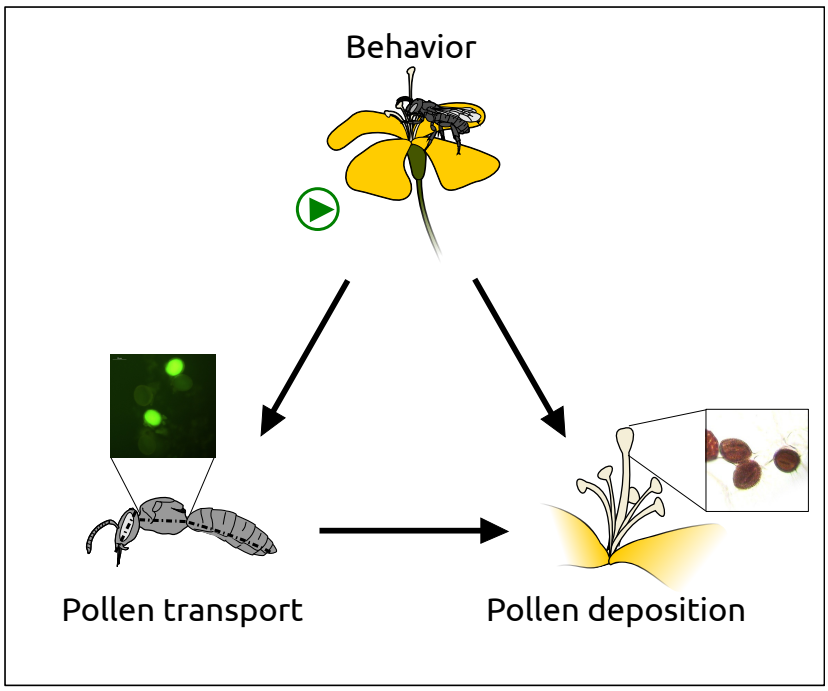

Figure 2: Dataset comparisons examined in our analysis. Each arrow represents a set of models we ran, with arrows pointing away from predictor variables toward response variables.

The comparison between behavior and pollen transport was analyzed with three models, similarly to the pollen dataset itself. We used a CMM to compare the proportion of time each body part spent touching floral reproductive structures (fixed effect) to the amount of pollen on that body part (response variable), with crop, insect species and individual insect ID as random effects. We used two GLMMs with binomial error distributions and logit link functions for the proportion models. The first was a model of crop to non-crop pollen per body part, with the proportion of time each body part spent touching floral reproductive structures a fixed effect, along with the body part identifier (a fixed effect with six levels), and crop, insect species, and individual insect ID as random effects (the last two of which were nested). The second was a model that had the same random and fixed effects, but the response variable was the ratio of viable to non-viable crop pollen.

The comparison between pollen transport and pollen deposition was made via model averaging GLMMs because of the collinearity between the body part measurements, which prevented meaningful inference from the complete model. The initial model that was put through a stepwise

model selection procedure had the number of pollen grains deposited per stigma by individual insects as a response variable and a Poisson error distribution because the count of pollen grains was bounded by zero. The fixed effects were the scaled average number of pollen grains found on each of the six body parts per insect species per crop, and the random effects were the crop and insect species.

The comparison between behavior and pollen deposition was made similarly to the model in the previous comparison, except that the initial model had the average amounts of time each of the six body parts spent touching floral reproductive structures per insect species per crop as the fixed effects predicting single-visit pollen deposition of individual insects.

2.4 Results

2.4.1 Insect behavior

We recorded over 11 hours of video footage, containing 8.5 hours of insect behavior on flowers. Across the four crops of interest, there were 389 individual insect observations (across 1,227 inflorescences) of 30 insect species with a 10:7 ratio of bees to flies. Insect species varied in the total proportion of time they spent touching floral reproductive structures ($P < 0.001$; $X^2 = 123.060$; Wald test; Fig. 3) and the proportion of the time touching those structures with each of the six body parts ($P < 0.001$; $X^2 = 36.205$; Wald test). Across all crops and insect species, a greater proportion of time was spent touching the bottom of the head to the stamen and style than any other body part ($P < 0.001$; Tukey HSD; Fig. 4). Overall, flies spent slightly greater proportion of time touching floral reproductive structures than did bees ($P = 0.017$; Tukey HSD), but the difference was only 6%, with flies spending more time in radish and carrot, and less time in pak choi and onion. Floral visit length was not correlated with the average proportion of time an insect species spent touching floral reproductive structures ($P = 0.380$, $t = -0.878$; GLMM; e.g. Fig. 5).

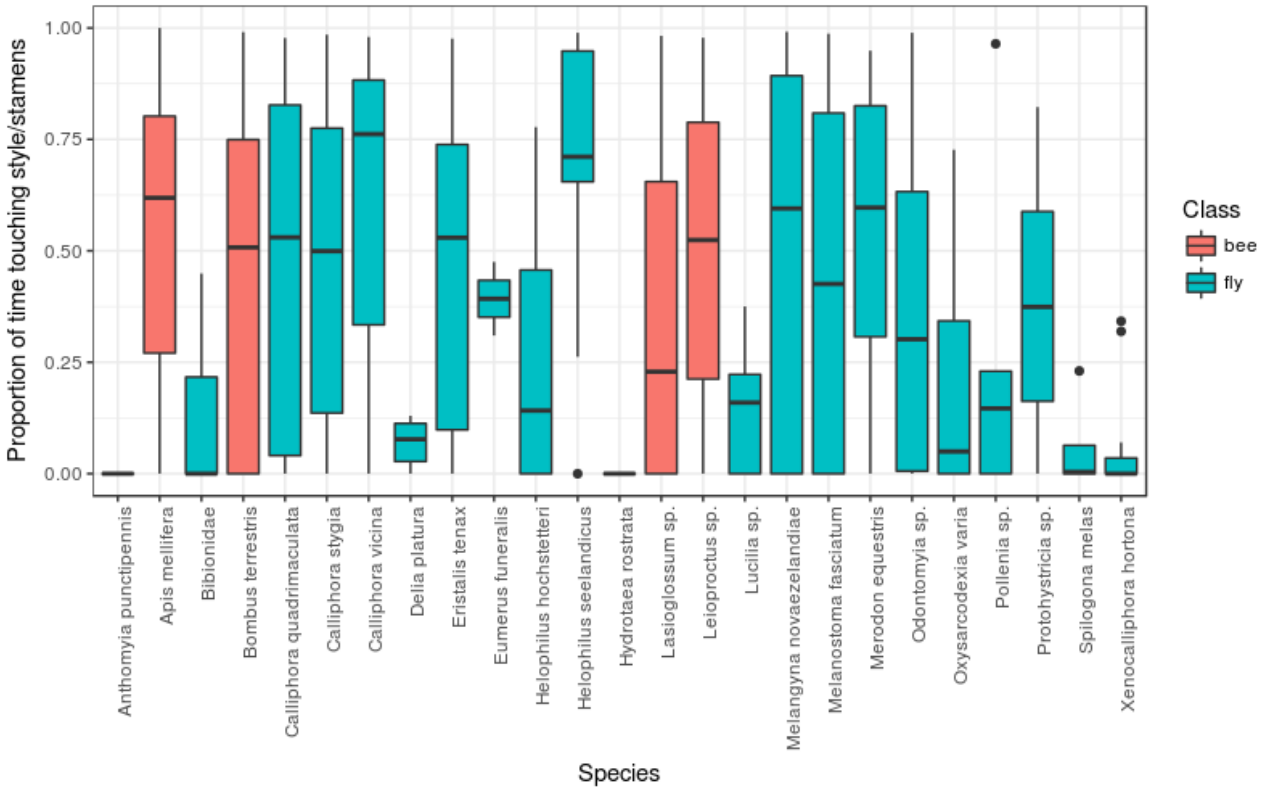

Figure 3: Proportion of floral visit spent touching reproductive structures for all insect species across all crops.

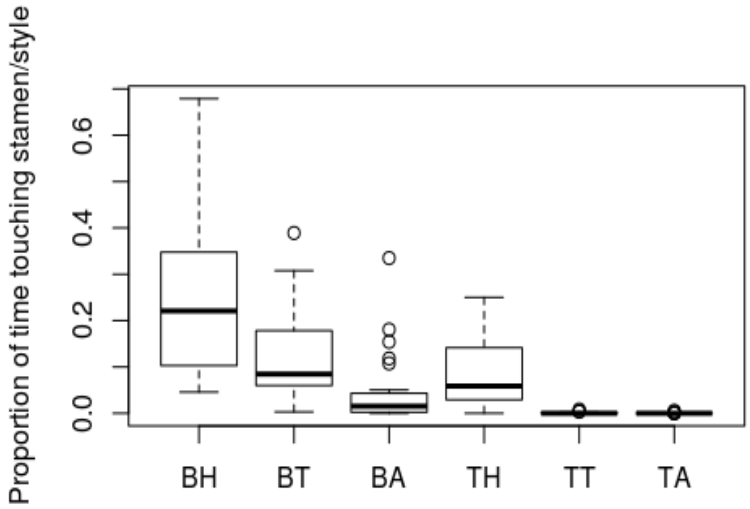

Figure 4: Proportion of time spent touching floral reproductive structures across all insect species and all four crops. BH = bottom head, BT = bottom thorax, BA = bottom abdomen, TH = top head, TT = top thorax, TA = top abdomen.

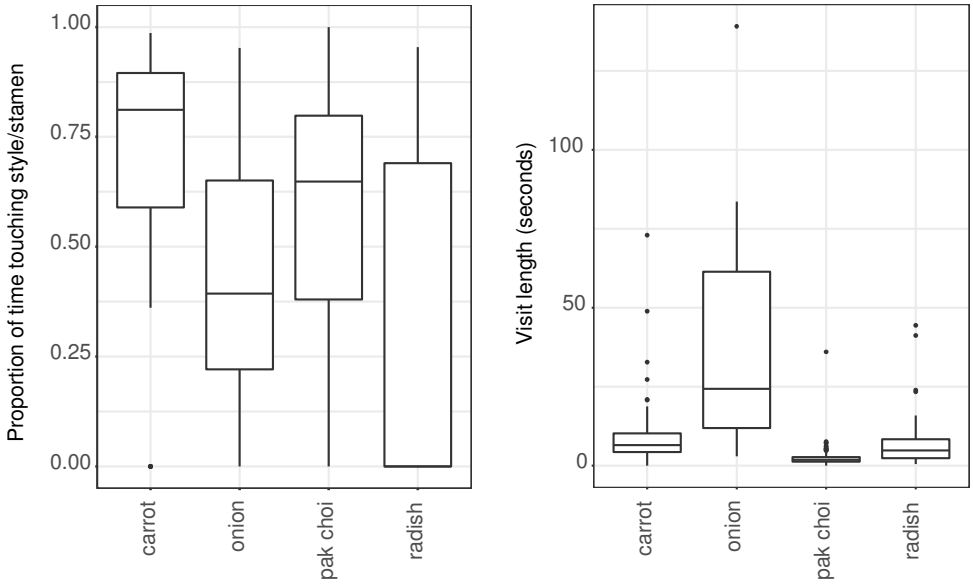

Figure 5: Total flower visit length and proportion of time touching floral reproductive structures for Apis mellifera.

2.4.2 Pollen transport

Across all four crops, we collected 53 insects, with an approximately 50:50 ratio of bees and flies. From these individuals, 278,141 pollen grains were counted, identified, and assessed for viability. In addition to the four crop species, 45 non-crop pollen morphospecies were identified. Across the four crops, bees were found to carry more pollen than flies ($P < 0.001$, $z = 4.22$; CMM), and significantly more pollen overall was carried on the bottom of the head ($P = 0.001$, -3.10; CMM) than on other body parts, while the top of the abdomen ($P < 0.001$, $z = 5.40$; CMM) and top of the thorax ($P < 0.001$, $z = 6.60$; CMM) carried significantly less pollen than other body parts. In addition, there were interactive effects between insect order and body parts, with flies carrying less pollen on the bottom ($P < 0.001$, $z = 4.27$; CMM) and top ($P < 0.001$, $z = 4.26$; CMM) of their heads than bees. The proportion of non-crop pollen to crop pollen was similar across all body parts, with the top of the head having a slightly higher proportion of crop pollen than other body parts ($P = 0.03$, $z = 2.980$; GLMM; Fig. 6), but the proportion of viable pollen varied substantially across body parts and insect order, with an interactive effect between the two (Fig. 7; Table 1). While flies had less pollen per body part than bees, they appeared to have a higher proportion of viable pollen overall, with the top of flies' heads generally having more viable pollen than the rest of the fly.

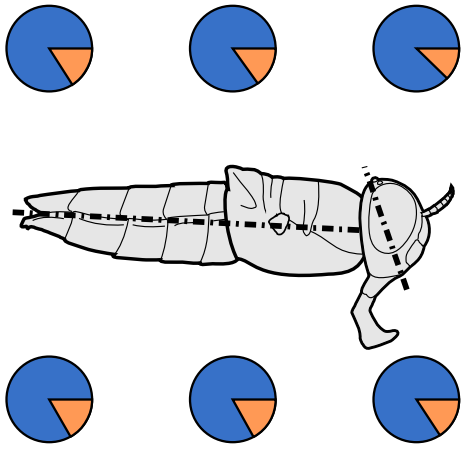

Figure 6: Proportion of non-crop pollen (orange) versus crop pollen (blue) on the top and bottom of the abdomen (left), thorax (center), and head (right) of all insects examined across all four crops. Pie charts include both viable and non-viable pollen.

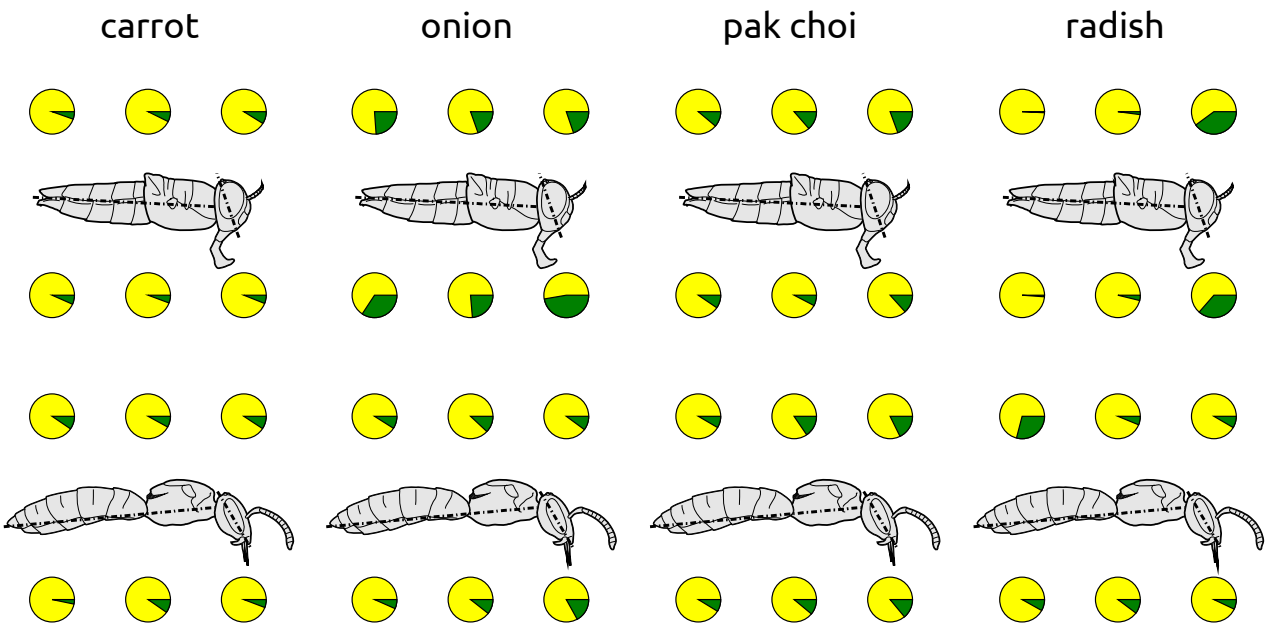

Figure 7: Proportion of viable crop pollen (green) to non-viable crop pollen (yellow) across crops and body parts, broken down by bees (bottom row) and flies (top row). Only crop pollen was included in this figure.

Table 1: Coefficients table for GLMM for pollen viability by body part. The intercept condition is the bottom of the abdomen of a bee. Crop, the individual insect ID, and the individual observations were random effects. TH = top head, TT = top thorax, TA = top abdomen, BH = bottom head, BT = bottom thorax

	Estimate	SE	z	P
(Intercept)	-4.223	0.294	-14.391	< 0.001 ***
BH	0.990	0.137	7.243	< 0.001 ***
BT	0.821	0.137	6.016	< 0.001 ***
TA	0.551	0.141	3.920	< 0.001 ***
TH	1.001	0.137	7.303	< 0.001 ***
TT	0.989	0.140	7.050	< 0.001 ***
Diptera	0.627	0.241	2.603	0.009 **
Diptera:BH	-0.828	0.201	-4.123	< 0.001 ***
Diptera:BT	-0.999	0.201	-4.978	< 0.001 ***
Diptera:TA	-0.595	0.208	-2.864	0.004 **
Diptera:TH	-0.258	0.203	-1.271	0.204
Diptera:TT	-0.582	0.206	-2.833	0.005 **

Significance codes: * < 0.05, ** <0.01 *** <0.001

2.4.3 Pollen deposition

We observed 504 pollination events across the four crops, involving 19 insect species. Individual

observations were at a ratio of 7:10 bees to flies. Pollen deposition per insect averaged 7.2 grains in carrot, 10.2 in onion, 76.4 in pak choi, and 33.1 in radish. Across the crops, pollen deposition was strongly predicted by insect species ($P < 0.001$). Overall, flies deposited less pollen than bees ($P < 0.001$, $z = -3.39$; GLMM).

2.4.4 Insect behavior versus pollen transport

The average proportion of time an insect species spent touching floral reproductive structures with different body parts in the behavioral dataset was a significant predictor of the amount of pollen on that body part ($P < 0.001$; $z = -8.61$; CMM) and ratio of crop to non-crop pollen ($P = 0.033$; $z = 2.129$; GLMM), but not the viability of crop pollen ($P = 0.124$; $z = -1.537$; GLMM) found on body parts of that species in the pollen transport dataset.

2.4.5 Pollen transport versus pollen deposition

The average amount of pollen carried by an insect species was predictive of individuals' pollen deposition across the four crops ($P < 0.001$, $z = 3.653$, $R^2m = 0.29$, $R^2c = 0.51$; GLMM). When examining pollen on body parts within individuals, each body part alone was a significant predictor of the pollen deposition, but the bottom and top of the head explained the most variance (Fig. 8; Table 2) – more variance than the total amount of pollen on the insect alone. When all six body parts were examined together, only the top of the head was significant, but both the top and bottom of the head were retained in the final model and both were significant (TH $P < 0.001$, $z = 4.043$; BH $P = 0.035$, $z = -2.103$). In the 6 models (out of 64) within AIC of 2 of the best model, all included the top of the head, and all but one included the bottom of the head.

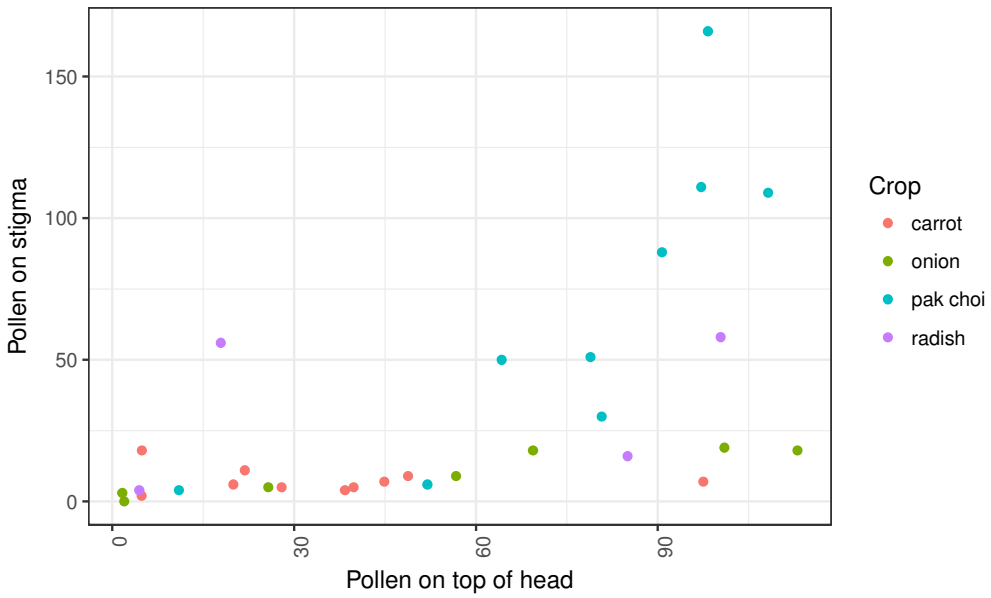

Figure 8: Relationship between pollen carried on the top of the head and single-visit pollen deposition in the four crops examined.

Table 2: Coefficients table for GLMM between species average pollen carried per body part and pollen deposition. Each pair of rows is a model with a single body part as a fixed effect, and the crop and individual observation as a random effect. TH = top head, TT = top thorax, TA = top abdomen, BH = bottom head, BT = bottom thorax, BA = bottom abdomen

	Estimate	SE	z	P	R^2m	R^2c
(Intercept)	2.632	0.296	8.892	< 0.001 ***	0.41	0.57
TH	0.788	0.179	4.414	< 0.001 ***		
(Intercept)	2.638	0.336	7.841	< 0.001 ***	0.21	0.42
TT	0.565	0.197	2.874	0.004 **		
(Intercept)	2.641	0.369	7.162	< 0.001	0.18	0.45
TA	0.532	0.184	2.889	0.004 **		
(Intercept)	2.646	0.303	8.747	< 0.001 ***	0.39	0.57
BH	0.763	0.172	4.435	< 0.001 ***		
(Intercept)	2.668	0.377	7.073	< 0.001 ***	0.17	0.44
BT	0.530	0.204	2.592	0.010 *		
(Intercept)	2.650	0.405	6.541	< 0.001 ***	0.13	0.45
BA	0.465	0.183	2.539	0.011 *		

Significance codes: * < 0.05, ** <0.01 *** <0.001

2.4.6 Insect behavior versus pollen deposition

The average proportion of time an insect species spent touching floral reproductive structures of a flower (ignoring the differences among body parts) was not predictive of individuals' pollen

deposition (P = 0.365, z = 3.911; GLMM). However, when all six body parts were examined, both the top of the head and the bottom of the abdomen were significant predictors of pollen deposition. In the best selected model, only these two body parts were retained, and were both significant (TH P < 0.001, z = 4.450; BA P = 0.003, z = -0.373). In the 6 models (out of 64) within 2 AIC of the best model, all contained the top of the head and the bottom of the abdomen. These two body parts predicted a considerable amount of variance within pollen deposition (R^2m = 0.12, R^2c = 0.44). Of note is the fact that insects spent a higher proportion of time touching the bottom of the head to the stamen and style, and about the same amount of time touching with the bottom of the thorax (Fig. 4), but neither of these was a significant predictor of pollen deposition, presumably because they were consistently high, and thus there was little variability across insect species with which to explain species differences.

2.5 Discussion

We found that insect behavior was a significant predictor of pollen transport and pollen deposition. In particular, we found that there were differences between insect body parts in the amount of time spent contacting floral reproductive structures and the amount of pollen carried on those body parts, and that these differences were significant predictors of pollen deposition. Body parts that touched floral structures frequently (the bottom and top of the head, and the bottom of the thorax) correspond well with the body parts that had high proportions of viable pollen (the top and bottom of the head, and the top of the thorax). This congruence may be due to body parts with frequent, short contacts with floral structures having higher pollen turnover, and thus proportionally more viable pollen, corroborating previous theoretical work on how accessible pollen collected at different times is on the insect body (Harder & Barrett 2012).

King et al. (2013) found that visit duration was not correlated to pollen deposition, which our results

support. The same paper put forward an argument that measuring behavior during floral visits, such as touching floral reproductive structures (cf. Gibson et al. (2011) is unlikely to be effective. However, our results indicated that this strategy may be a key to understanding the interspecific variation that vexed King et al. (2013) and others. While the total time a given insect species spent touching floral reproductive structures on average did not correlate with its pollen deposition, when those data were examined at the body-part level, trends became apparent, with the top of the head and bottom of the abdomen explaining 44% of the variance in pollen deposition.

Likewise, pollen transport was predictive of pollen deposition, in agreement with previous literature (Howlett et al. 2011). Similarly, pollen removal has been found to be predicted by behavior; for example, Adler and Irwin (2005) found that the largest forager, *Xylocopa virginica,* carried the most pollen grains on its body but transferred very few to flowers because of its nectar-robbing behavior. Indeed, we found that the way insects foraged on a flower was predictive of the pollen on the insect body; the more time an insect spent touching floral reproductive structures with a body part, the more pollen it tended to accumulate on that body part, and the more viable the pollen. The pollen found on different parts of the insect, and the head in particular, was then able to explain 41% of the variance in pollen deposition. Given that the head of most insect species spent the highest proportional amount of time touching floral reproductive structures, it follows that these regions could be responsible for pollen deposition, though the strength of the correlation may depend on floral morphology (as in Fig. 8).

Notably, pollen viability was particularly high on the heads of flies, despite their overall lower pollen counts. This contrast suggests that even though we found that flies deposited less pollen than bees, it is possible that the pollen deposited was of higher quality, making them more effective pollinators than they appear to be on the basis of single-visit pollen deposition alone. This finding

adds to the growing body of literature suggesting that flies are potentially important, but often overlooked, pollinators (Rader et al. 2016). Future work examining the viability of pollen deposited on plant stigmas may prove to be a more accurate measure of pollinator quality, as it has long been known that inviable pollen not only is of no use for pollination, but may actually prevent viable pollen from adhering to the stigma (Smith-Huerta & Vasek 1984; Wilcock & Neiland 2002).

Our findings suggest that, despite concern that insect visitation behavior is an unreliable signal of pollinator quality, and despite the risk of pollen being lost from the insect body (Harder & Routley 2006), both observations of insect behavior and pollen counts may be used successfully to estimate pollen deposition potential for insect species, with the insect's head being the most relevant region across pollinator taxa. The ability to rely on either of these measures, rather than single-visit pollen deposition, could result in a significant reduction in labor to collect data on the most effective pollinators of crop and unmanaged plant species, which could in turn benefit agriculturalists and ecologists by providing local data.

However, our data also underscore the findings of previous studies that differences in pollinator performance depend on the context within which they occur (e.g. Garibaldi et al. 2013). Even though we found strong predictive power of insect behavior and pollen transport at the body part level, ~16% of the variance in pollen deposition was explained by the crop species being foraged upon and just over 40% was from unexplained sources (Table 2; Fig. 7). The most common pollinating insect in our dataset, *Apis mellifera*, varied widely in its behavior (Fig. 4), the pollen it collected (Supplement Table 1), and the amount of pollen it deposited per visit across the four crop species. These findings could provide a mechanism for the context dependency between plant species, wherein crops better served by non-*Apis* pollinators may be those with morphology that is less compatible with *A. mellifera* behavior. Perhaps unsurprisingly, Garibaldi et al. (2013) found

different efficacies for honey bees in the same crop in different countries. This means that, while the use of these body-part-based metrics can give an impression of how an insect will perform (e.g. more pollen on the top of the head is correlated with higher pollen deposition), it will not eliminate the need to take measurements for each plant species and locale of interest. It may, however, make taking these measures less laborious. With respect to insect behavior, it may even become possible to measure passively with video traps on flowering plants, or estimate efficiency in the field through visual observation, which would help land managers with limited resources to better make management decisions.

Chapter III: Specialization of pollinator individuals and their body parts promotes species coexistence

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3.1 Abstract

Plant-pollinator mutualisms are critically important to ecosystem functioning, but are not well understood. Current network theory estimates that mutualistic networks become unstable at numbers of species that are significantly lower than those observed in nature. In this study, we explore a mechanism that could allow this coexistence: the partitioning of insect pollinators at scales finer than species, including by sex, individual, and body part. We examined four plant-pollinator networks collected from four cropping systems and found that, across all of them, plant pollen was distributed non-randomly amongst sexes, individuals, and body parts of insect species, allowing plants to reduce the effect of indirect competition. Uniquely, the distribution of pollen across body parts in particular appears to allow plants to experience the benefits of shared pollinators while mitigating the risks (e.g. heterospecific pollen deposition), increasing both the stability and feasibility of networks compared to those examined in the traditional, species-

aggregated fashion.

3.2 Introduction

Mutualisms, including plant-pollinator interactions, are fundamental to the functioning of ecosystems. Examining these interactions with a network approach has revealed that communities of mutualists have a unique structure which allows a large number of species to coexist and buffers the system against extinctions (Memmott et al. 2004; Bastolla et al. 2009; Thébault & Fontaine 2010). However, although the benefits of direct interactions among species of different trophic levels (e.g. plants and pollinators) define the mutualism, the indirect interactions among species at the same level (e.g. plants that share pollinators) are less clear. In the long term, plants could benefit from large populations of pollinators that have been supported by other plant species. These positive indirect effects of mutualists on each other appears to increase coexistence in dynamic models (e.g. Bastolla et al. 2009). In contrast, species may compete for interaction partners, particularly over short timescales, such that sharing of mutualist partners with other species could be a disadvantage. For example, an abundant plant species may attract and support large pollinator populations in an area (a positive indirect effect on other plants), but the benefit of sharing pollinators with that plant species could be negated if individuals of those pollinators carry only its pollen (a negative competitive effect; Lopezaraiza-Mikel et al. 2007). There has been difficulty accounting for this trade-off: networks generated based on the present understanding of mutualisms become unstable with fewer species than observed in nature (Bastolla et al. 2005a,b).

One possible explanation for the observed coexistence is that plants compete with each other less than would be assumed looking at species-level metrics. If a generalist pollinator species is divided into groups (e.g. by sex; Cane et al. 2010) or individuals (Tur et al. 2014) which show high fidelity

to a single plant species, then that plant will benefit from more effective pollen transport despite the generalism (Brosi 2016). Pollinator generalism may also benefit the community of plant species, because other plant species can support the pollinator populations at different times of year (Encinas-Viso et al. 2012). Thus, the balance of specialization at the species level vs. individual level will determine the extent to which plant species benefit or compete with one another.

Moreover, it is possible that even an individual organism may not be the appropriate scale at which to measure the nature of interactions, because at finer scales individuals may express partner fidelity within a certain spatial or temporal range. For example, certain plant species are known to attach their pollen to a specific part of an insect body (e.g., Singer and Cocucci 1999). If, on average, different plant species' pollen tends to occupy different parts of the insect body or is asymmetrically collected by individuals of a single species, this partitioning of the pollinator resource could increase the net benefit of sharing pollinators with other plant species. However, it remains unknown whether plants reduce competition by partitioning insect bodies or individuals in this way. Additionally, the consequences of this partitioning or individual- vs. species-level specialization for community stability remains unclear, because network models of stability typically treat species, rather than individuals or parts of individuals, as nodes.

Here we identify the pollen transported on different sexes, individuals, and body parts of insects to understand the nature of indirect effects among plants that share pollinators. Specifically, we test if 1) plant species partition pollinators at scales finer than insect species, including insect sexes, individuals, and body parts, 2) whether this partitioning is scale dependent, such that measures of pollinator specialization change when data are aggregated by insect body part vs. individual vs. species. 3) Finally, we explore how the observed partitioning at different scales influences the stability and feasibility (the range of parameter-space in which all species in the network have

nonzero abundance) of plant-pollinator networks.

3.3 Methods

To test how measures of insect species specialization change at different scales, we collected field data for four pollen-transport networks and then used those networks to explore our three objectives (above).

3.3.1 Data Collection

Insects were collected from crop species growing in the Canterbury plains, New Zealand. Carrot, onion, pak choi, and radish fields were each sampled on three different days in the 2014/2015 growing season. A different site was selected each time, with the exception of radish, where only one field was available, which was sampled successively. As there is little overlap between the peak flowering of each crop, the four crops were sampled in succession. Each sample day, insects were collected individually by hand-netting in the morning, afternoon and early evening. Up to six insects of each species per sampling time were used for analysis, or six per line (male fertile vs. male sterile) for hybrid crops (carrot, pak choi, onion). Insects were frozen individually until pollen analysis.

To assess the pollen communities on different parts of the insect body, the captured insects were dissected into head, abdomen, and thorax (head, mesosoma, and metasoma for bees), and then thawed for 2 – 5 minutes. Six slides were prepared, each with a drop of 20% sucrose (w/v) solution. In succession, the top and bottom of the head, abdomen, and thorax (Fig. 1) were each dipped 20 times in the solution to release pollen onto their own slide. The forceps were washed between handling each body part. Each slide, containing the pollen from the top or bottom of a single body part, was sealed with a coverslip and examined with a UV light microscope. Up to 200 pollen grains

were identified across longitudinal transects of the slide. If there were fewer than 200 grains present, all were identified. As a key to local weedy pollen was not available, non-crop pollen was cataloged and assigned to one of 45 morphospecies groups (S1). Morphospecies were assigned according to differences in key characteristics used in pollen identification, including number of apertures, type of apertures, surface sculpturing, shape, and size.

Because our analysis made comparisons within individual insects (rather than e.g. comparing sites), we pooled all sites of a given crop together. Crops were analyzed separately as there was low overlap between plant-insect communities between the four data sets. This approach allowed us to use each crop as a separate replicate to determine the robustness of our results.

3.3.2 Network Creation

Four bipartite insect-pollen species networks of different scales were created from the data collected in each of the four crops. All networks generated were quantitative, with links representing the number of pollen grains carried by insects at the different scales of aggregation, thereby ensuring that the same measure could be used for each. We make no inference about the importance of the number of pollen grains for pollination success, though it provides a measure of reproductive investment by each plant species. The first scale we examined (hereafter called “species network”) was the standard species-aggregated network, with each insect species recorded as interacting with all the plants of which at least one pollen grain was carried by at least one insect. The second network (hereafter called “body-part-by-species network”) was aggregated by species and body part, breaking up each insect species into six nodes with the sum total of all plants' pollen that had ever been recorded from the top or bottom of the head, thorax and abdomen of that species. The third network (hereafter called “individual-insect network”) was created by aggregating the data by individual insect, with all the pollen species each insect collected as connections. The final network

(hereafter called “body-part-within-individual network”) was the finest possible scale of aggregation, a large matrix whereby each insect node represented one of the six body parts from an individual insect, connected to the plant pollen found on them.

3.3.3 Statistical Analysis

Question 1: Do plant species partition pollinators at scales finer than insect species?

First, we tested whether pollen species composition varied systematically across insect body parts. To do this, we used a permutational multivariate ANOVA (PERMANOVA) procedure (function '*adonis*') with Bray-Curtis dissimilarity from the R package *vegan* (Oksanen et al. 2013). Because the pollen composition changed considerably across the pollinator communities sampled on each of the four crops (and was dominated by crop pollen), we analyzed each crop separately. This also provided an opportunity to test whether any effects were generalizable across plant communities. For each of the four analyses, the pollen transport matrix (presence and abundance of pollen of each plant species) on each insect body part was the response variable, and insect species, body part, and their interaction were predictor variables. We also looked at a similar model with insect order instead of species to see if there were broad differences between bees and flies, but these models provided less explanatory power (S2 Tables 5 – 12). To compare the pollen composition across body parts within each insect, the individual insect's ID number was included as a blocking factor (via the strata argument in *adonis*). Any body part with enough pollen to make an estimate of community composition, here defined as having 10 or more pollen grains, was included in these analyses (2034 out of 2988 individual insect body parts). This analysis compared the species composition of pollen across body parts, but did not test whether the total amount or presence of pollen differed across body parts. In order to test the latter, we used Cox mixed models (CMMs) with Poisson errors, generated with the R library *coxme* (Therneau 2015). The total number of pollen grains found on each body part for each insect was used as the response variable. The

predictor variables were the insect species (with honey bees set as the intercept for comparison) and insect body part, with each insect's unique ID included as the random effect to group body parts of a given insect.

We examined the flies from our dataset to test whether body part differences were influenced by insect sex. Bee species were excluded as there were too few males for detailed analysis. We then re-ran the models as described above, but with sex added to each as a predictor variable and interaction term with both body part and insect species.

Question 2: Is plant partitioning of insects scale-dependent?

Second, we tested whether specialization at the species level was correlated with specialization at finer scales. For each crop and each network scale, we calculated two metrics of specialization, one binary (presence/absence of pollen species) and one quantitative (number of grains per pollen species): degree, the number of plant species associated with an insect, and the paired differences index (PDI), a measure which takes into account the magnitude as well as number of insect-pollen interactions compared to the total number of possible interactions to produce a value which estimates the specialization of the insect species (Poisot et al. 2012):

$$PDI = \sum_{R}^{i=2} \frac{(P_1 - P_i)}{R-1} \qquad \text{Eqn 1}$$

where P_i represents the number of pollen grains for a given plant-insect species combination (i.e. network link), with P_1 being the strongest link and R the number of plant species in the dataset. Both measures were calculated using the R package *bipartite* (Dormann et al. 2008). To test whether insect species-level specialization is correlated with specialization at finer scales of aggregation, we used each of the metrics in turn as the response variable in GLMMs with the aggregated species-level specialization metric as a continuous predictor and insect species as a

random effect (to group individuals and body parts from a given species). To check the fit of the GLMMs, we calculated both the marginal R-squared value (R^2m: the variance explained by fixed effects alone) and conditional R-squared value (R^2c: the variance explained by fixed and random effects) using the *r.squaredGLMM* function in the R package *MuMIn* (Barton 2014).

To test whether the observed trends in degree and PDI were not artifacts of the differing number of data points between the networks, we compared the observed data to a null model. In our null model, all of the pollen collected by individuals of an insect species was pooled together and then each grain (which keeps the plant species identity) was randomly assigned to an individual and body part. This was accomplished with the function *permatfull* in the package *vegan* (Oksanen et al. 2013), which generated 100 community matrices for each of the four crops, preserving column sums (pollen grains per plant species) for each insect species. Using the methods above, we created a GLMM for how well the degree and PDI of each shuffled matrix was predicted by the original species matrix, resulting in distributions of R-squared values to which our empirical values could be compared.

Question 3: What are the effects of partitioning at different scales on species coexistence?

Finally, we evaluated whether partitioning of the pollination service among insect individuals and body parts affects the stable coexistence of plant and insect species in the community. For that, we employed the approach of Rohr, Saavedra and Bascompte (2014) which disentangles the conditions of both dynamical and structural stability (also called feasibility) of a steady state in the community. Dynamical stability refers to the ability of the community to return to an initial equilibrium point, whereas feasibility refers to the range of demographic parameters necessary for the stable coexistence of all species in the community.

We use the following form of a linear Lotka-Volterra model to approximate the system dynamics

(Saavedra et al. 2016a):

$$\begin{cases} \frac{dP_i}{dt} = P_i\left(r_i^{(P)} - \sum^{i} \alpha_{ij}^{(P)} P_j + \sum^{j} \gamma_{ij}^{(P)} A_j\right) \\ \frac{dA_i}{dt} = A_i\left(r_i^{(A)} - \sum^{i} \alpha_{ij}^{(A)} A_j + \sum^{j} \gamma_{ij}^{(A)} P_j\right) \end{cases} \quad \text{Eqn 2}$$

where P_i and A_i are the abundance of plants and pollinator species i, respectively. r_i is the species' intrinsic growth rate. In such a model, the interaction strength matrix

$$B = \begin{bmatrix} \alpha^{(P)} & -\gamma^{(P)} \\ -\gamma^{(A)} & \alpha^{(A)} \end{bmatrix} \quad \text{Eqn 3}$$

can be used to estimate the feasibility of a community. In B, the α sub-matrices encapsulate the competition among species within a guild given by α_{ij}, and γ encapsulates the benefits, γ_{ij}, conferred by the mutualistic interactions.

We use a mean field approximation for the intraguild competition of both plants and pollinators such that $\alpha_{ij}^{(A)} = \alpha_{ij}^{(P)} = \rho_{ij}$ for $i \neq j$ and $\alpha_{ij} = 1$ for $i = j$. As in Rohr et al. (2014), we define $\gamma^{(P)}$, the mutualistic benefit of plants on pollinators to be composed by $\gamma_{ij} = \gamma_0 y_{ij} / d_i^{\delta}$ where γ_0 is the mean level of mutualistic strength, y_{ij} is 1 if the species interact or 0 otherwise, d_i is the degree of i, and δ is the mutualistic trade-off (Saavedra et al. 2013). In this case d_i is simply the number of species i interacts with. Because we are interested in the effects of pollen partitioning we define the mutualistic effect of pollinators on plants as $\gamma^{(A)} = \Gamma Y^T$, where Γ is a matrix that contains information about the mutualistic benefit provided by each pollinator subunit (e.g. species, sex, individual, or body part) on plant species and Y is a mapping matrix that indicates the relative contribution of each partition to the species.

We define $\Gamma_{i j_k}$ as the mutualistic benefit that plant i receives from depositing pollen on partition k of pollinator j. When pollen is exclusively partitioned across pollinator species there is

effectively one partition per pollinator species, and so Γ_{ij_k} is identical to $\gamma_{ij}^{(P)}$ such that $\Gamma_{ij}=\gamma_0 y_{ij}/d_i^{\delta}$. In this case, Y is the identity matrix, and the effective degree d_i is the number of pollinator species plant i interacts with. When pollen is partitioned within a species we define $\Gamma_{ij_k}=c_{ij}\gamma_0 y_{ij_k}/d_i^{\delta}$ where y_{ij_k} is 1 if plant i deposits pollen on partition k of pollinator j or 0 otherwise, and c_{ij} is a compensation coefficient. This additional term is defined as $c_{ij}=\sum y_{ij_k}/K_j$, where K_j is the number of body parts considered for pollinator j. The compensation coefficient c_{ij} accounts for the fact that if a plant species deposits pollen in only one out of, for example, two pollinator body parts we would not expect the mutualistic benefit conferred by the pollinator to be halved. When pollen is partitioned, the effective degree d_i is the number of partitions in which plant i deposits pollen weighted by the relative contribution of the partition as defined by Y. For example, if plant i deposits pollen only in one out of two possible groups of the same insect species, then $d_i=1/2$. When pollen is partitioned across individuals, we assume that each individual represents a distinct group in the pollinator species population. In this case the mutualistic benefit is the same as in the body part partitioning, except that $c_{ij}=1$ as we can expect a reduction in the mutualistic benefit if a plant engages only with a portion of the pollinator population. The case in which pollen is partitioned across both individuals and body parts is a combination of the two last cases.

Once we have B, we estimate the community stability and feasibility following Saavedra et al. (2016a). They show that stability can be directly related to the upper limit of the mean mutualistic strength $[\lambda_{ij}]$ below which the real part of the eigenvalues of B reaches zero and therefore is globally stable. In turn, the feasibility is estimated as the solid angle Ω of the hypervolume cone formed by the growth rates under which positive abundances of all species is maintained. We calculate the feasibility domain for a level of mutualism that is half of the critical level necessary for dynamic stability in the community (Saavedra et al. 2016b; Saavedra et al. 2016a).

3.4 Results

Across the four crops, we found that plants appear to partition insects at the individual and body part level and that the intensity of plant-plant competition decreased at finer scales.

Question 1: Do plant species partition pollinators at scales finer than insect species?

The quantity of pollen carried varied across both body parts and insect species in all crops (S2 Tables 1 – 4). The species composition of carried pollen also differed significantly across insect species in all four crops we examined ($P = 0.002$ for radish and < 0.001 for the other three; PERMANOVA; Fig. 9), and across body parts in carrot, onion, and pak choi (S2 Tables 5 – 12). Because the results are broadly consistent across crops, we present figures and tables here only for radish, as it has fewer plant and insect species, and is therefore easier to visualize trends; additional information for the remaining crops is presented in the supplementary information.

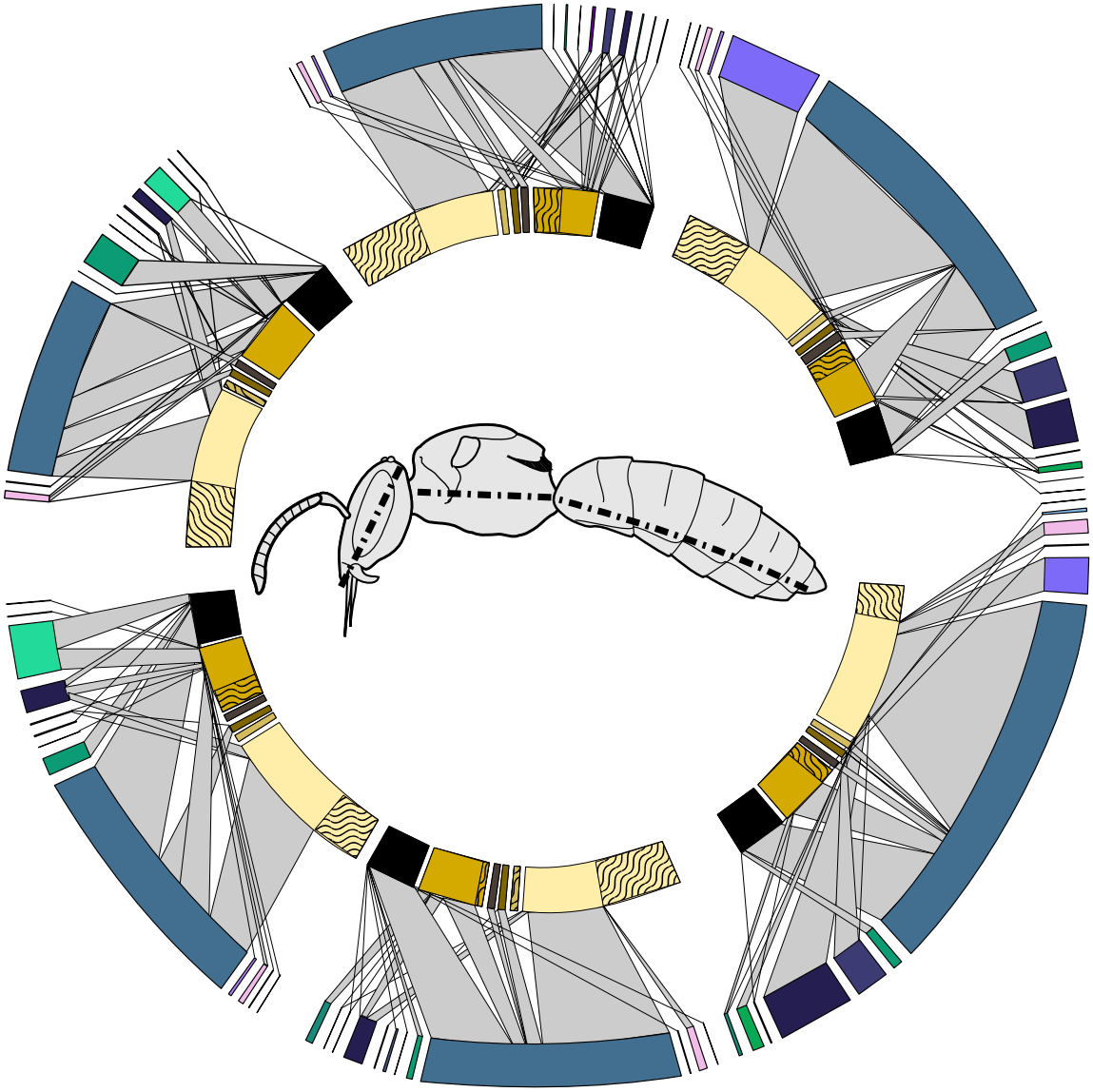

Figure 9: Pollen transport network by body part from insects foraging on radish. The outer ring represents pollen species and the inner ring represents insect species. Each network is next to the body part it represents—the top and bottom of the head, thorax or abdomen (head, mesosoma and metasoma for bees). Bars on the inner and outer rings are scaled by number of insects and number of pollen grains, respectively. The thickness of the connections represents the proportion of insects (inner ring) and number of pollen grains (outer ring) that the link represents. Wavy line shading indicates the proportion of observations (insect body parts) with zero pollen grains. In the outer ring, the large blue bars represent radish pollen.

While the abdomen had more pollen on average than the head or thorax (S2 Tables 1 – 4), and

despite differences in pollen composition across body parts, the number of pollen species was

similar across all body parts for most insect species.

In our analysis of insect sex (which could only be conducted for flies), the differences in pollen composition across insect species remained significant (onion $P = 0.006$, carrot, pak choi, and radish $P < 0.001$; PERMANOVA; S2 Tables 13 – 16) as did the differences across body parts in carrot ($P < 0.001$) and pak choi ($P < 0.001$), after accounting for insect sex. Pollen composition differed significantly across sexes in every crop except onion (all $P < 0.001$; onion $P = 0.998$; PERMANOVA), which had relatively few male insects, and proportionately fewer flies than the other crops. In carrot and pak choi, which contained many flies (respectively 456 and 405 fly body parts with greater than 10 pollen grains), there was also a significant interaction effect between species and sex (both $P < 0.001$; PERMANOVA). These models explained 34 – 77% of the variation in the pollen composition between body parts. Figure 10 illustrates the variation in the size and composition of pollen communities by body part and sex for a representative fly species.

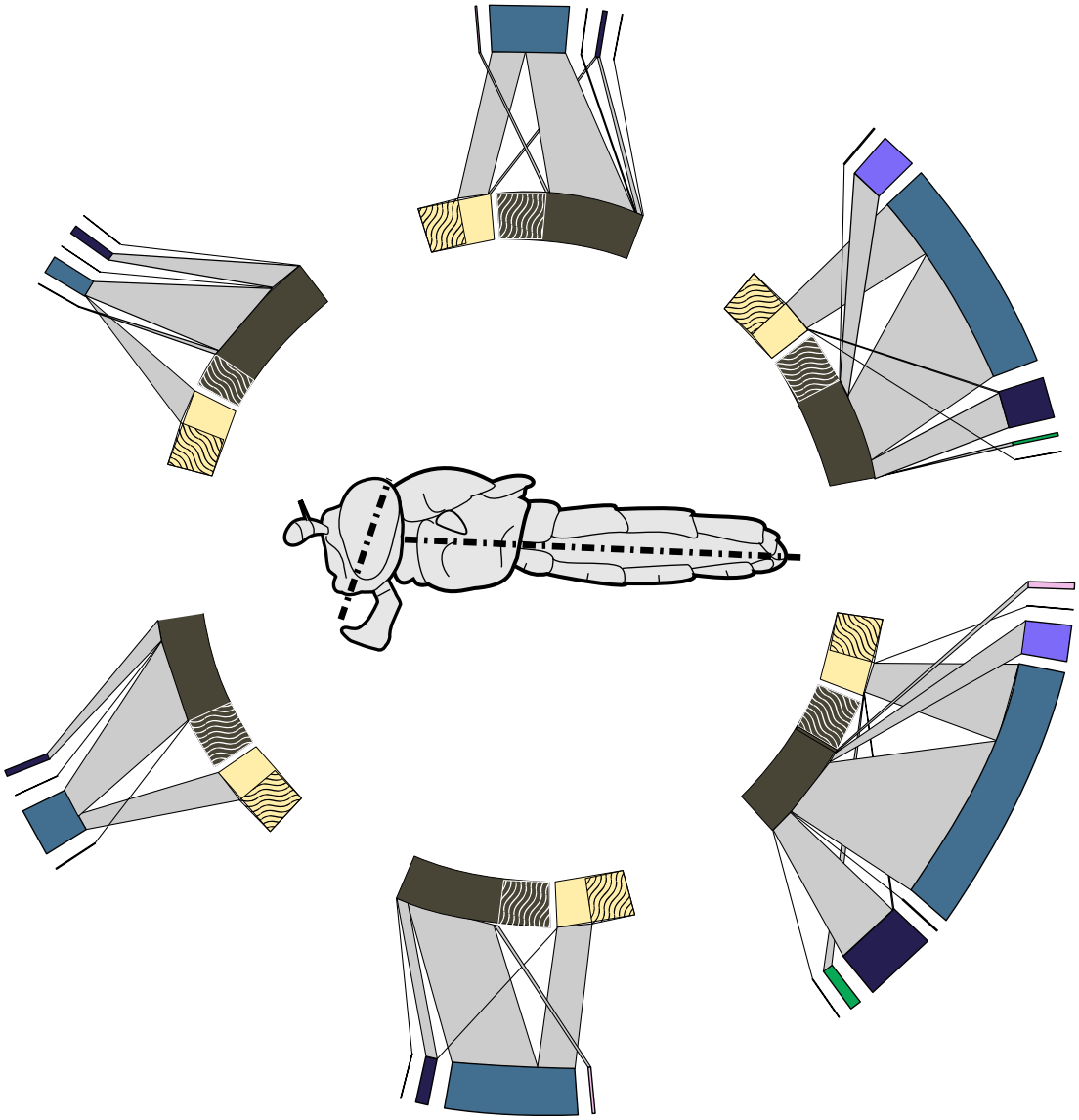

Figure 10: Pollen transport network by body part and sex for a representative fly species, Melanostoma fasciatum*, in samples from radish. The outer ring represents pollen species and the inner ring represents insect sex: black boxes females (n=18) and yellow boxes males (n=9). In the outer circle, the large blue boxes represent radish pollen. Each network is next to the body part it represents—the top and bottom of the head, thorax and abdomen (head, mesosoma and metasoma for bees). Inner and outer rings are scaled by number of insects and number of pollen grains, respectively. Wavy lines indicate observations (insect body parts) with zero pollen grains.*

Question 2: Is plant partitioning of insects scale-dependent?

We tested the extent to which the specialization of pollinators, measured as degree (the number of interaction partners), at the species level (as is standard in ecological networks) predicted specialization at smaller scales (i.e. the body-part-by-species, individual-insect, and body-part-by-

individual networks). We found that an insect species' degree was a strong predictor of the body-part-by-species degree ($P > 0.001$; $t = 11.433$; $R^2m = 0.77$; $R^2c = 0.85$; GLMM; Fig 11a), indicating that all body parts of a generalist insect species tended to carry diverse pollen at the population scale. However, the predictive ability of species-level degree was weaker, though still significant, at the scale of individual insects ($P = 0.046$; $t = 2.217$; $R^2m = 0.06$; $R^2c = 0.19$; GLMM; Fig 11b), and a poor predictor of degree at the finest scale, body parts within individuals ($P = 0.982$; $t = -0.022$; $R^2m = 0.00$; $R^2c = 0.27$; GLMM; Fig 11c). For all four crops, the trend was that, as the scale gets finer, fewer of the species' total plant partners are represented and the variability among sample units increases (see increased variance from left to right in Fig 11). At the finest scale, individual insects of generalist species had a similar number of pollen species per body parts as did more specialized species. The results for a quantitative measure of specialization (PDI) were similar to degree, with decreasing variance explained (measured as R^2m and R^2c) by species-level specialization at finer scales (Fig 11 d-f; S2 Tables 17 – 20). Graphs and figures are presented here for carrot, as the trend is clearer with more insect species, but the results for radish, onion and pak choi are similar (S2 Figs 5 – 8).

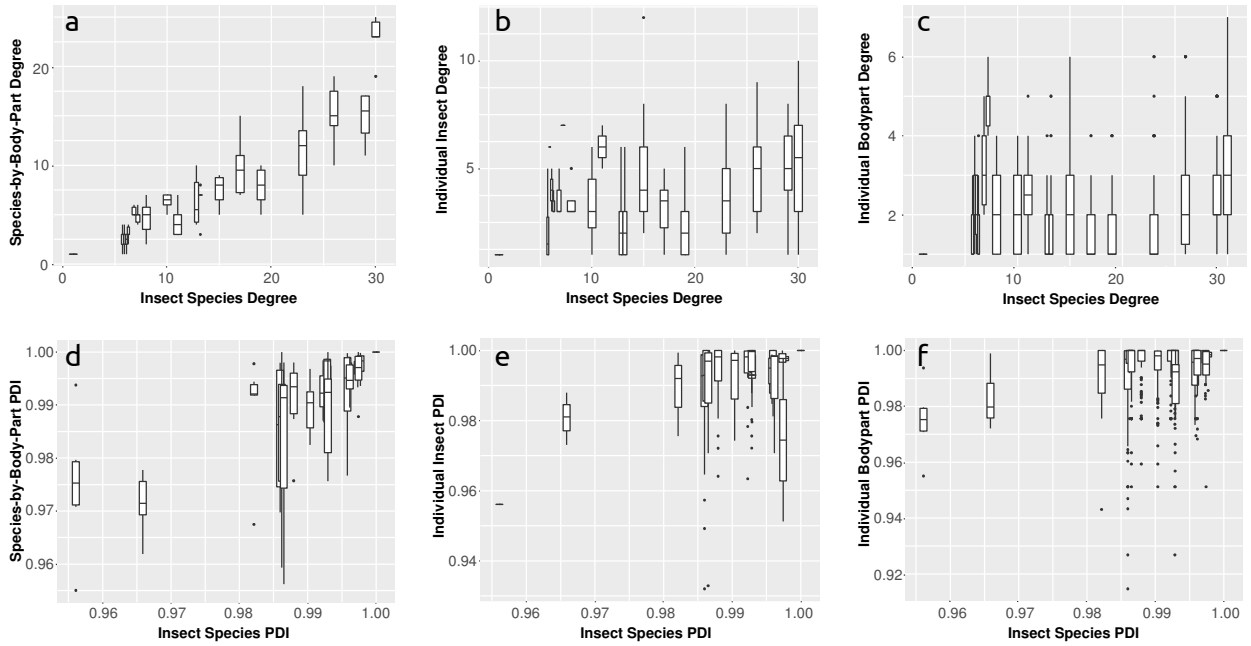

Figure 11: Bar graph/scatter plots for binary (degree, top), and quantitative (PDI, bottom) measures of insect specialization samples from in carrot. Left to right: species-level network versus aggregated by body part by species, species-level network versus aggregated by individual, species-level network versus individual body part. Each box represents the degree of an insect species.

While rarefaction curves indicated that our sampling effort was not sufficient to reach saturation of for a number insect species observed across the four crops (S2 Figs 1 – 4), our findings deviate substantially from the null model, where the pollen observed at the species-level is randomly allocated to individuals of that species and their body parts (Fig. 12), indicating that plant pollen is non-randomly deposited on insect individuals and their body parts. While the null model shows the same trend as the observed data (i.e. a decreasing predictive power of the species network for finer scales), it is more closely correlated with the species network at all scales than the observed data. This was true for all four crops examined (S2 Figs 8 – 10).

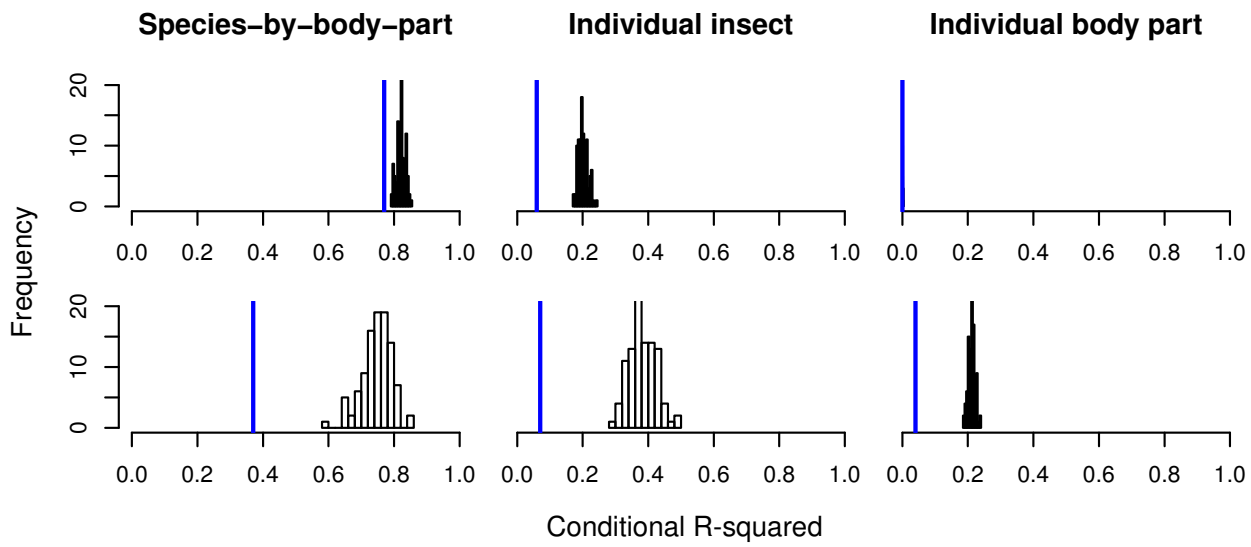

Figure 12: Histograms of the correlation (R2m) between the degree (top) and PDI (bottom) of the species network and the degree and PDI of 100 randomly-generated null models for each of the three finer network scales for carrot. Blue bars denote the correlation between the original species network and observed values at each scale. The histogram in the upper right has low variance and is close to zero, but is still separated from the bar.

Question 3: What are the effects of partitioning at different scales on species coexistence?

We found that pollen being differentially transported by individuals (or their body parts) of a given species impacted the stability and feasibility of the community, though this effect depended on the trade-off that plants experienced for interacting with an increasing number of pollinators (Fig 13). In addition, the effects of pollen partitioning were not homogeneous, and differed with the scale of the network. Individual insect networks tended to result in higher stability of the community versus traditional species networks for small levels of trade-off, but were also less feasible (Fig 13ad). In contrast, networks that accounted for body part differences, both within individuals and aggregated at the species level, tended to favor both the feasibility and the stability of the community and the impact increased with mutualistic trade-off (Fig 13bcef).

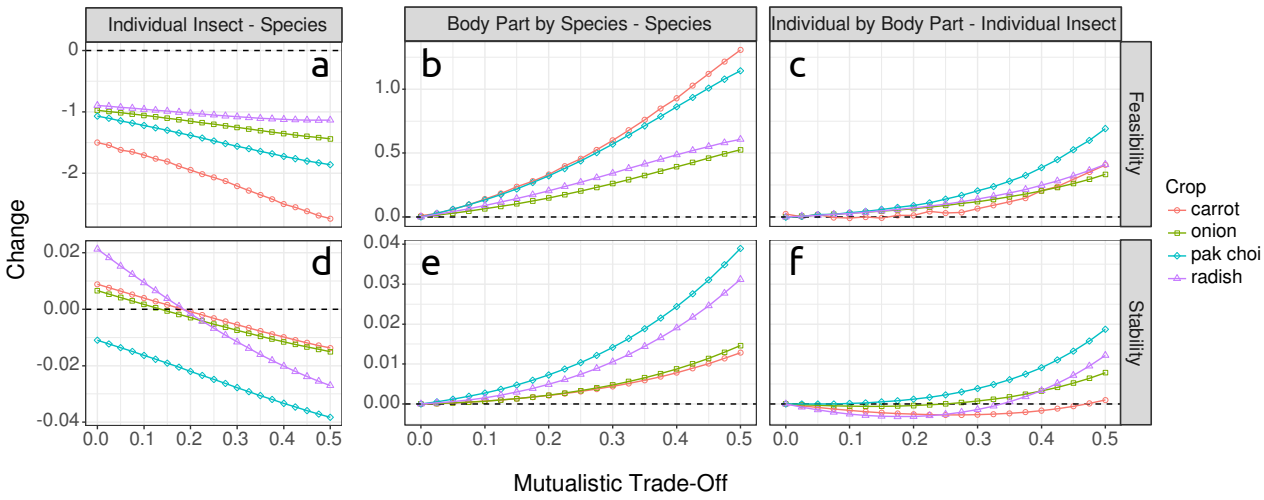

Figure 13: Change in feasibility and stability metrics when finer scales are considered: between species and individual insects (left), species aggregated by body part (center), and individual insects and body parts on individual insects (right) and insect species for different values of δ (mutualistic trade-off). Feasibility was generally increased by taking body parts into consideration.

3.5 Discussion

We have found that, while the pollen of numerous plant species may be found on an insect species, there was a systematic tendency for different plant species to deposit pollen across different regions of the insect body. Combined with the finding that insect individuals appear to be more specialized than species, this suggests that plants compete less for space on shared pollinators than would be expected if pollen were distributed randomly across an insect species' individuals and their body parts. This body-part partitioning increased the feasibility of networks when compared to traditional species-level networks.

A point to consider is that the pollination networks in this study were constructed from insects foraging on single plant species, which means that some species and links from the full network may be missing. However, those missing links represent additional specialization and compartmentalization of the network, likely adding to the robustness of our results.

A number of previous studies have attempted to address the paradox that networks appear to

become unstable with large numbers of species, despite the fact that real networks *have* large numbers of species (Bastolla et al. 2005a,b). These studies have found that network structure (Bastolla et al. 2009), phenology (Encinas-Viso et al. 2012), and individual specialization (Tur et al. 2014) reduce competition and increase the number of species which can coexist in these networks. While our findings on individual specialization are similar to Tur et al. (2014), our results indicate that pollen is partitioned *within* individuals as well, reducing estimates of plant-plant competition when examining data at either the body-part-by-species or body-part-by-individual scale, and that there was an additional effect of insect sex beyond body-part and individual differences. Because these differences were not well predicted using the species network, there is strong evidence that the standard method of representing plant-insect interactions with species as nodes gives a false appearance of high plant-plant competition for pollinators and lower stability and feasibility.

It has been hypothesized that if individuals within a generalist species are specialized, the species as a whole is insulated from the loss of interaction partners as only a relatively small portion of the population would be affected (Wolf & Weissing 2012). In contrast, Tur et al. (2014) argued that the population may then be susceptible to the loss of individuals, as links and individuals would be lost simultaneously. This trade-off, where losing a small proportion of the individuals of a generalist pollinator species has the potential to strongly impact a subset of plant interaction partners with little impact to the rest, could potentially explain why empirical networks are observed to have high feasibility at the expense of stability (Saavedra et al. 2016a). Interestingly, such a trade-off does not appear to occur when body parts are taken into consideration: by depositing pollen on different parts of the insect, plants are potentially able to reduce competition with each other, without suffering from the decrease in pollination services that utilizing only some individuals of a pollinator species would otherwise imply.

Our results suggest that plant-pollinator mutualisms are systems in which plant-plant competition is minimized via a number of processes, and that examining pollen transport networks at scales finer than species reveals greater potential for species coexistence than the typical species-scale approach. Importantly, plants depositing pollen on different regions of the insect body may reduce the chance for heterospecific pollen deposition, offering an explanation as to why, even in highly competitive environments, species receive more conspecific pollen than would be expected by chance (Emer et al. 2015). Taken together, our results suggest that individual and body-part specialization may be an answer to how plant-pollinator networks are able to maintain high levels of biodiversity and functioning despite their redundancies and potential for interspecific conflict. Accounting for between- and within-individual specialization may, then, result in more accurate models of mutualistic networks and potential disruptions to their functioning.

Chapter IV: Pollen longevity: a systematic meta-analysis

4.1 Abstract

Pollination is critical for the majority of the world's plant species, including the vast majority of plants grown for food. Pollen must be viable in order for pollination to occur, but this viability depends on a number of environmental conditions and varies between plant species and cultivars. This complicates models of pollen storage and transport. To address this shortcoming and summarize the large but disparate body of knowledge represented in the literature, we performed a systematic review and meta-analysis of the literature on pollen longevity including both cultivated and wild plants, gymnosperms and angiosperms. Using the data from 421 papers, we found that methodological choices, such as the type of method used to assess pollen viability, explained almost as much variation in the data as time since anthesis and temperature. We also found that plants within a genus tended to have similar longevity, and that there was some correlation within families, but there did not appear to be strong phylogenetic signal for pollen longevity in the greater phylogenetic tree. Our results indicate that it may be possible to impute pollen longevity for unsampled plant species from data for species in the same genus.

4.2 Introduction

Pollination is important for the majority of the world's plant species (Buchmann & Nabhan 2012) including most of the world's agricultural crops (Klein et al. 2007). In order for pollination to occur, pollen must travel from the male reproductive structures (catkins or anthers) to the female reproductive structures. This movement has been quantified using a variety of techniques, from capturing pollen from the air (Käpylä 1991) to removing pollen from flower-visiting insects (Rader et al. 2011), to examining pollen attached to the stigmas of target plant species (King et al. 2013). However, it has long been noted that pollen transfer is meaningless or even detrimental if the pollen

is not viable (Wilcock & Neiland 2002).

Many studies have tried to estimate pollen viability and longevity for various plant species for purposes including pollen storage for genebanking and plant breeding, assessment of plant tolerance to various environmental conditions, and investigations of pollen dispersal and how it affects gene (and transgene) flow (Abdul-Baki & Stommel 1995; Hanna & Towill 1995; Coast et al. 2016). Despite the research on how pollen viability responds to factors such as temperature and humidity in these papers, particularly those concerning pollen storage, relatively little has been done to assess the longevity of pollen under environmental conditions likely to be encountered in the field. The primary focus of the work on field conditions has been evaluating the pollen longevity of individual species at risk of spreading transgenes, with numerous studies in wind-pollinated crops such as wheat (Khan et al. 1971), rice (Coast et al. 2016), and maize (Fonseca 2004). In contrast, studies of insect-vectored pollination have tended to focus on pollen transport and single-visit pollen deposition (Mesa et al. 2013), with only a handful also examining pollen viability (e.g. Richards et al. 2005; Rader et al. 2011). Interpretation of pollen transport results could be greatly improved by the inclusion of pollen viability data, which exists in a piecemeal form in the literature. Thousands of studies on pollen viability have been published, which could potentially be used to fill in these gaps, but few assess more than a single plant species, and not all assess viability over different environmental conditions. Combining data from these disparate studies may enable the estimation of any general response to environmental factors affecting pollen viability.

A number of review articles have been written on pollen longevity, typically focusing on techniques for measuring viability (e.g. Stone, Thomson & Dent-Acosta 1995; Dafni & Firmage 2000) but, to our knowledge, there has not been a previous attempt to systematically summarize plant species' pollen longevity. Since pollen viability has a known mathematical relationship with time,

temperature, and humidity (Fonseca 2004), it should be possible to use viability values (and the conditions under which they occurred) reported in the literature to create a model of pollen viability across plant species. In addition to synthesizing information on individual plant species, this could reduce the likelihood of duplicated effort, highlight places where future work would be profitably directed, and potentially allow for the inference of pollen longevity values for unsampled species.

The objective of this systematic meta-analysis was to assess how pollen viability and longevity varied across plant taxa, how that variation was affected by environmental variables, particularly time since anthesis and temperature.

4.3 Methods

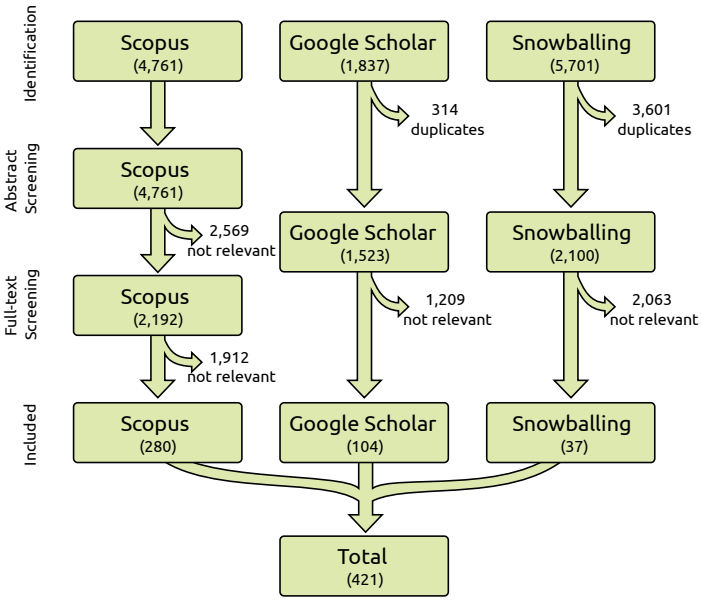

Figure 14: Flowchart of data acquisition process for this meta-analysis.

4.3.1 Article selection

In order to obtain articles on pollen longevity for our review, we searched both Scopus and Google Scholar in September, 2014. The Scopus query was:

```
TITLE-ABS-KEY((pollen W/2 (*viability OR *viable OR longevity OR aging OR age OR
quality OR germina*)) AND NOT("air quality") AND NOT(allerg*) AND NOT(rhinitis)
AND NOT(*conjunctivitis) AND NOT (Arabidopsis))
```

which yielded 4,761 articles, and the Google Scholar (GS) queries were:

```
“pollen AROUND(2) viability” -pollinosis -rhinitis -allergies -allergic
-allergen -conjunctivitis -Arabidopsis
```

and

```
“pollen AROUND(2) longevity” -pollinosis -rhinitis -allergies -allergic
-allergen -conjunctivitis -Arabidopsis
```

which yielded 1,939 articles, 1,837 of which were unique within the GS dataset, and 1,523 which were unique overall. We did not limit the timeframe from which we collected papers, but we did restrict results to papers written in English (papers written in other languages were rejected at various stages; Fig 14).

We collected a third set of articles from the references of papers selected through full-text screening (snowballing). References were only collected from Scopus papers as text could not reliably be scraped from selected GS papers. In total, there were 13,991 references, 5,701 of which were unique within the snowball dataset and contained the word “pollen”. Of these, 2,100 were unique overall, resulting in a total of 8,698 potentially relevant articles.

4.3.2 Abstract screening

Abstracts from Scopus were loaded into abstrackr (Wallace et al. 2012), a semi-supervised active machine-learning web application. Four people manually screened abstracts and marked them as relevant or not-relevant based on the following criteria:

1. The word “pollen” was near one of the following words: viability, viable, longevity, aging, age, quality, germination
2. The abstract appeared to indicate that pollen viability/germination was measured (rather than e.g. seed germination)
3. Keywords indicated that pollen viability/germination was measured, even if this information was not included in the abstract

In order to determine reliability of screening, paired assessments were done during which the project leader (also a screener) double-screened 20 papers for each other participant. Any conflicts were addressed. The remaining abstracts were single-screened.

Because abstrackr selects abstracts with high relevance probability to be reviewed next, we stopped screening when 50 abstracts in a row were judged by the human assessors as non-relevant and the machine-learning algorithm estimated that 0 of the remaining articles were relevant. We screened 3,750 out of the 4,761 Scopus articles.

Abstracts from GS and snowballed papers had to be retrieved manually, and thus could not be assessed via abstrackr. We manually screened all 3,623 of these abstracts with the same criteria as above.

4.3.3 Full-text screening

Papers deemed relevant at the abstract level were then acquired. To reduce the volume of papers selected to go through full-text screening, we used abstrackr again for the 2,192 Scopus papers, with

a similar procedure to above, stopping when 50 papers in a row were not relevant at the full-text level. In total, 2,891 full-texts were retrieved: 1,186 Scopus, 1,087 GS, and 618 snowballed. Interlibrary loans were requested if papers were not locally available. Some papers were unable to be sourced; 119 Scopus, 129 GS, and 331 snowballed (80% retrieval rate). A paper was selected as relevant for data extraction if it contained pollen viability data which varied in time, temperature or relative humidity (though we did not collect enough studies to analyze RH). If a study had data on multiple species or cultivars and measured at least two of time, temperature and RH, it was also selected for data extraction.

4.3.4 Data extraction

Data were extracted from the 421 papers that passed the full text screening (Fig 14) and entered into a spreadsheet. Each row recorded the mean from a treatment combination or critical temperature points from models. Pollen viability was converted from a percentage to a proportion where necessary so that it could be fit to binomial models. The variability measure (e.g. variance, standard error) was recorded and converted to a proportion as well (if appropriate). While a number of predictor variables were extracted, the key ones for our analyses are: temperature, humidity, time from anthesis, method used to determine pollen viability, and plant family, genus, and species. Where necessary, RH values were calculated for studies that reported using saturated salt solutions to control humidity in pollen storage treatments.

4.3.5 Statistical analysis

As the collected data formed an extremely sparse matrix, traditional meta-analysis metrics, such as hedge's d, could not be computed. In order to account for the variance and non-independence between studies, we used generalized linear mixed model (GLMM) in R using the package *lme4* (Bates et al. 2014). The response variable was pollen viability (a proportion from 0 to 1). Although

relative humidity has been shown to affect pollen viability, too few studies in our dataset examined it alongside time and temperature, so, we were only able to include time since anthesis and temperature as fixed effects. Because the literature has shown that there is a nonlinear response to temperature (Kakani et al. 2002), we included an additional quadratic and cubic temperature term as fixed effects, as well as the interaction term between all three temperature effects and time. The initial full model also included method used to assess pollen viability as a fixed effect (a factor with 5 levels—methods which were reported in at least 20 studies), whether or not the pollen was rehydrated prior to analysis (a factor with three levels: fresh, rehydrated, not rehydrated), and whether the anthers were collected pre- or post- dehiscence. Random effects in the full model were: the study ID and a nested random effect of plant family / plant genus / plant species. The model also included random slopes for time for the nested taxonomic random effect. The model had a logit-link function and binomial errors and each observation was weighted inversely to its variance. For observations that lacked a measure of variance, one was estimated using a binomial extension of Taylor's Law (Hughes & Madden 1992). The model was simplified by examining the AIC scores of all model subsets for the fixed effects. Then, the nested components of the family / genus / species random effect were dropped from the model and tested for significance. Random effects were excluded if their removal did not result in a significantly different model.

To test for phylogenetic signal in pollen longevity, the coefficients for the random slope for each species were compared against a phylogenetic tree generated for all taxa in our study. The tree was generated using the program Phylomatic (Webb & Donoghue 2005), and compared to the longevity slopes by the shuffling technique described in Blomberg, Garland Jr and Ives (2003) and accounting for variance, as in Ives, Midford and Garland Jr (2007), via the R function *phylosig* in the package *phytools* (Revell 2012).

4.4 Results

Our dataset of 421 papers contained information on the pollen viability of 549 plant species belonging to 222 genera and 84 plant families across 59 countries in every continent but Antarctica (Fig. 15). A variety of pollen viability assessment techniques were used, with *in vitro* germination being the most common (Table 3). Fluorescein diacetate was the second most represented method, with acetocarmine and tetrazolium stains tied for third. Although most studies examined a single species or cultivar, a considerable minority examined two or more (Fig. 16)

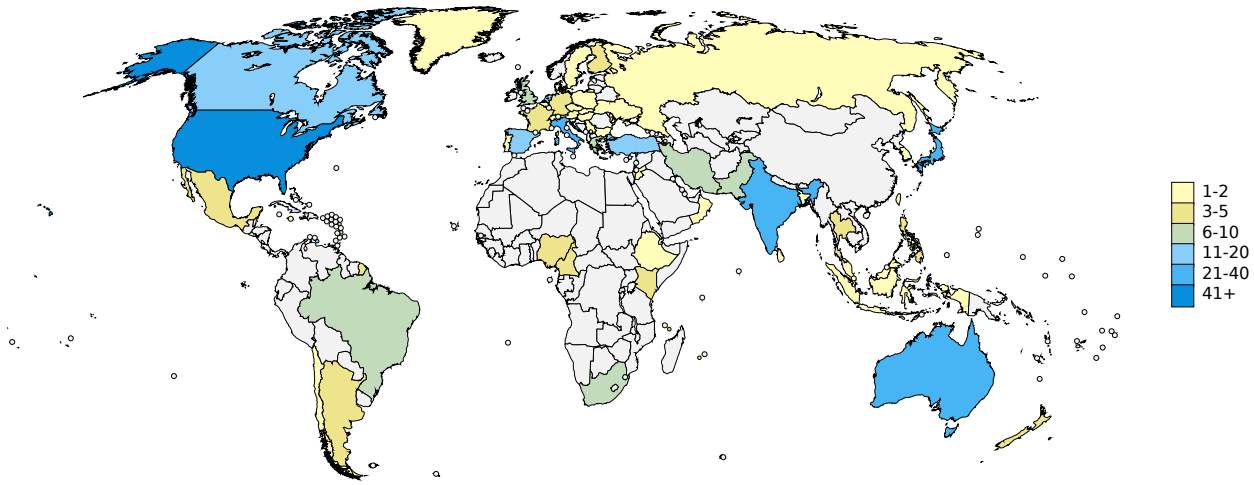

Figure 15: Map of studies included in the meta-analysis. Studies are placed in a country based on where they were conducted, not author location. In total, 21 studies came from Africa, 107 from Asia, 125 from Europe, 103 from North America, 32 from Oceania, and 15 from South America.

Table 3: Methods of assessing pollen viability, what they measure, and how many studies in our dataset used each

Pollen viability method	Type of test	Number of studies	Number of studies also using other method(s)
acetocarmine	Presence of nuclear material in cytoplasm	21	11
Alexander's stain	Aborted vs. non-aborted pollen	9	4
fluorescein diacetate (FDA / fluorochromatic reaction / FCR)	Intact plasma membrane	58	26
hydrogen peroxide	Peroxidase activity	5	2

benzidine test	Peroxidase activity	1	0
Baker's reagent	Alcohol dehydrogenase	1	1
in *vitro* germination	Germinability on artificial media	344	59
in *vivo* germination	Germinability on conspecific stigma	24	13
iodine staining (I_2-KI)	Presence of starch in cytoplasm	2	1
lactophenol cotton blue (LCB)	Presence of cytoplasm	3	1
nucleic acid precursors (^{3}H-thymidine, ^{3}H-uridine)	Rate of DNA replication	1	1
neutral red	Presence of cytoplasm	1	1
ppp test	Myeloperoxidase activity	1	0
tetrazolium	Dehydrogenase activity	21	15
X-gal	Galactosidase activity	1	1

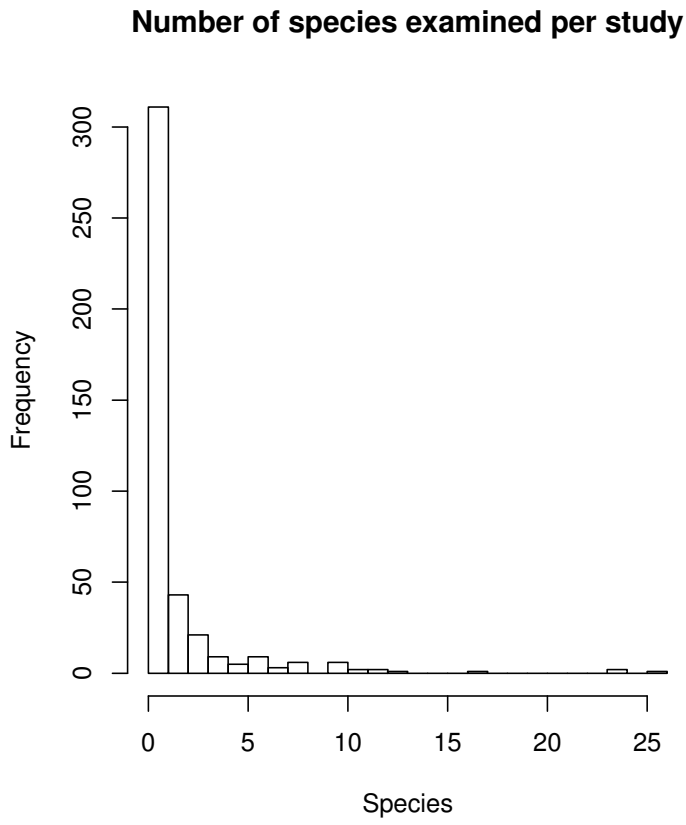

Figure 16: Histogram of the number of plant species examined in each of the extracted studies

The majority of studies had time and temperature data associated with pollen viability, but relatively fewer contained data on relative humidity or moisture content of pollen (Fig. 17).

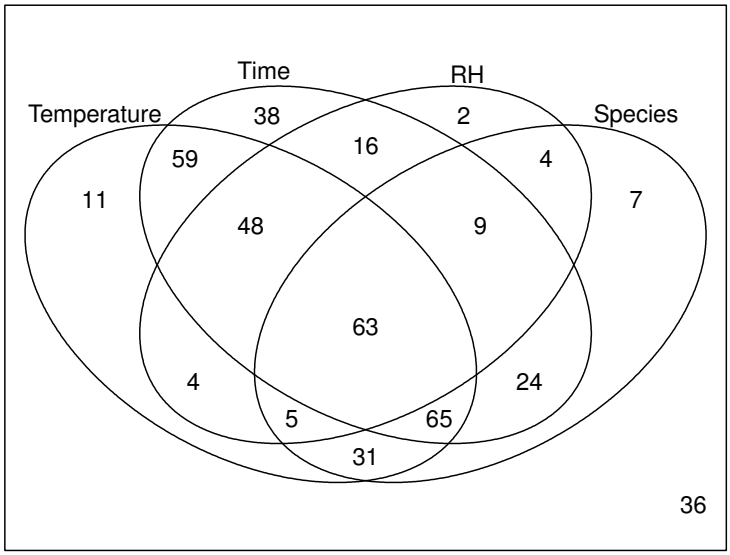

Figure 17: Venn diagram of number of studies comparing pollen viability across different variables. A study was placed into an ellipse if it compared multiple values of temperature, time, RH, or multiple cultivars/species. The number in the lower right-hand corner is the number of studies which passed full-text screening, but data could only be extracted for a single combination of treatments.

Our model showed a significant effect of temperature (linear term), time since anther dehiscence, and the interaction between temperature (linear, quadratic, and cubic terms) and time (Table 4; Fig. 18). These fixed effects explained 48% of the observed variance in pollen viability. The random effects, which included study ID, the method used to determine pollen viability, whether or not pollen was rehydrated prior to viability assessment, as well as a random slope for time since anther dehiscence for each of the nested family, genus, and species random effects, together with the fixed effects, explained 70% of the observed variation in pollen viability. The only random effect dropped from the model during model selection was whether the anthers were collected post or prior to

dehiscence.

Table 4: Coefficients table for GLMM of pollen viability. The intercept condition was the viability of fresh pollen measured with in vitro *germination.*

	Estimate	SE	Z	P
(Intercept)	-0.237	0.970	-0.282	0.778
Temperature	-4.200	1.114	-3.769	< 0.001 ***
Time	-2.679	1.434	-1.869	0.062 .
Temperature^2	-3.468	1.356	-2.558	0.011 *
Temperature^3	-3.810	1.241	-3.069	0.002 **
Acetocarmine	1.184	0.588	2.015	0.044 *
Fluorescein diacetate (FDA)	1.162	0.361	3.220	0.001 **
In vivo germination	2.297	0.430	5.341	< 0.001 ***
Tetrazolium	5.478	0.992	5.521	< 0.001 ***
Not rehydrated	-2.160	0.936	-2.308	0.021 *
Rehydrated	-0.633	0.932	-0.679	0.497
Time : Temperature	-17.319	4.536	-3.818	< 0.001 ***
Time : Temperature^2	-16.503	5.357	-3.081	0.002**
Time : Temperature^3	-15.899	4.801	-3.312	0.001 **

Significance codes: * < 0.05, ** <0.01 *** <0.001

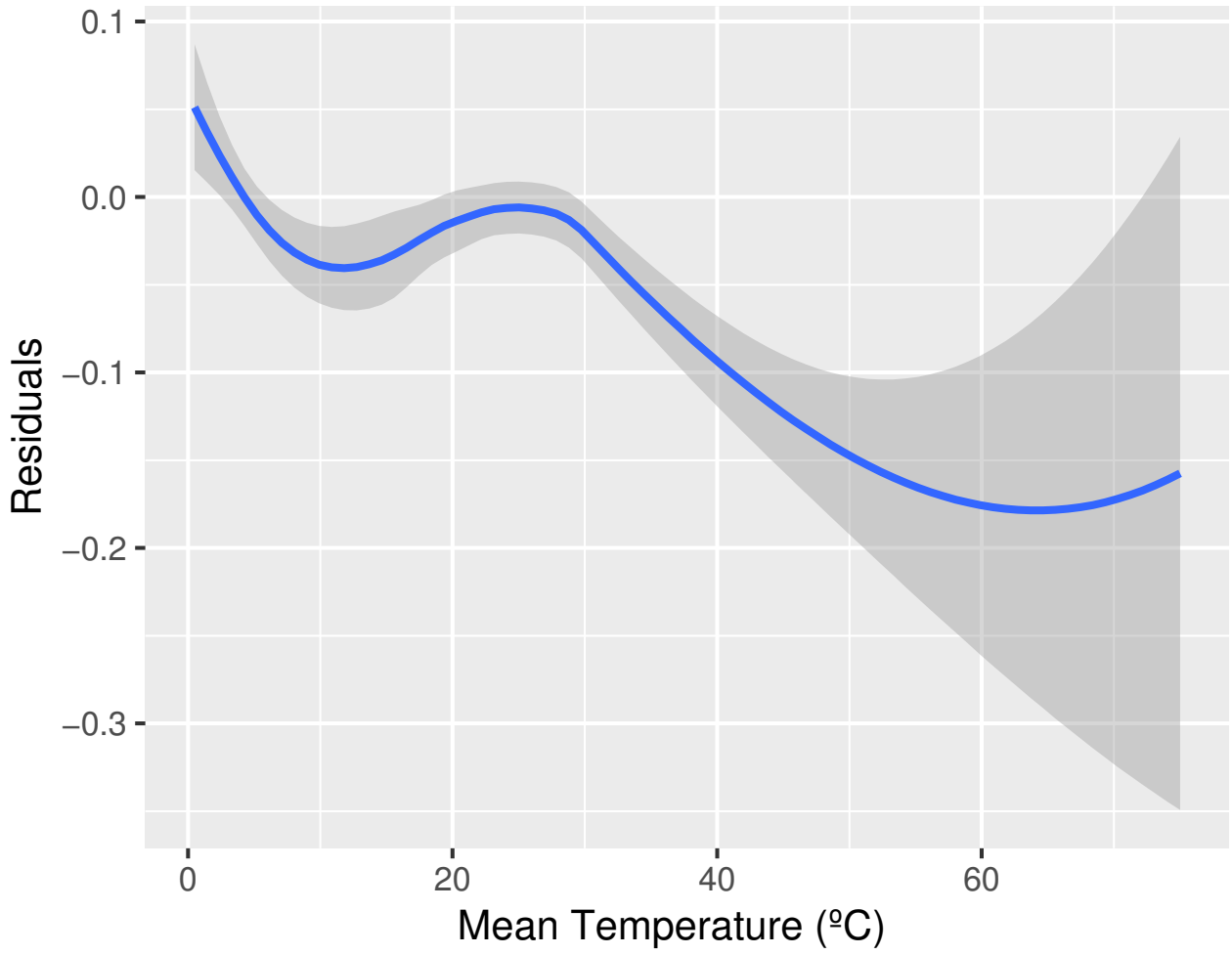

Figure 18: Residuals of model with no fixed effects plotted against mean temperature. The nonlinear relationship is clear, with an optimum temperature around 25ºC across all plant taxa examined. Temperatures below about 5ºC appear to have a preservative effect.

To generate a standardized measure of longevity for phylogenetic comparisons, we used the model coefficients to calculate the estimated hours to 50% viability at 20ºC for each of the 255 plant species included in the GLMM. While the nested family/genus/species effect explained a significant amount of variance in the GLMM ($P < 0.001$), there was no clear phylogenetic signal for pollen longevity ($P = 0.181$; Fig. 19).

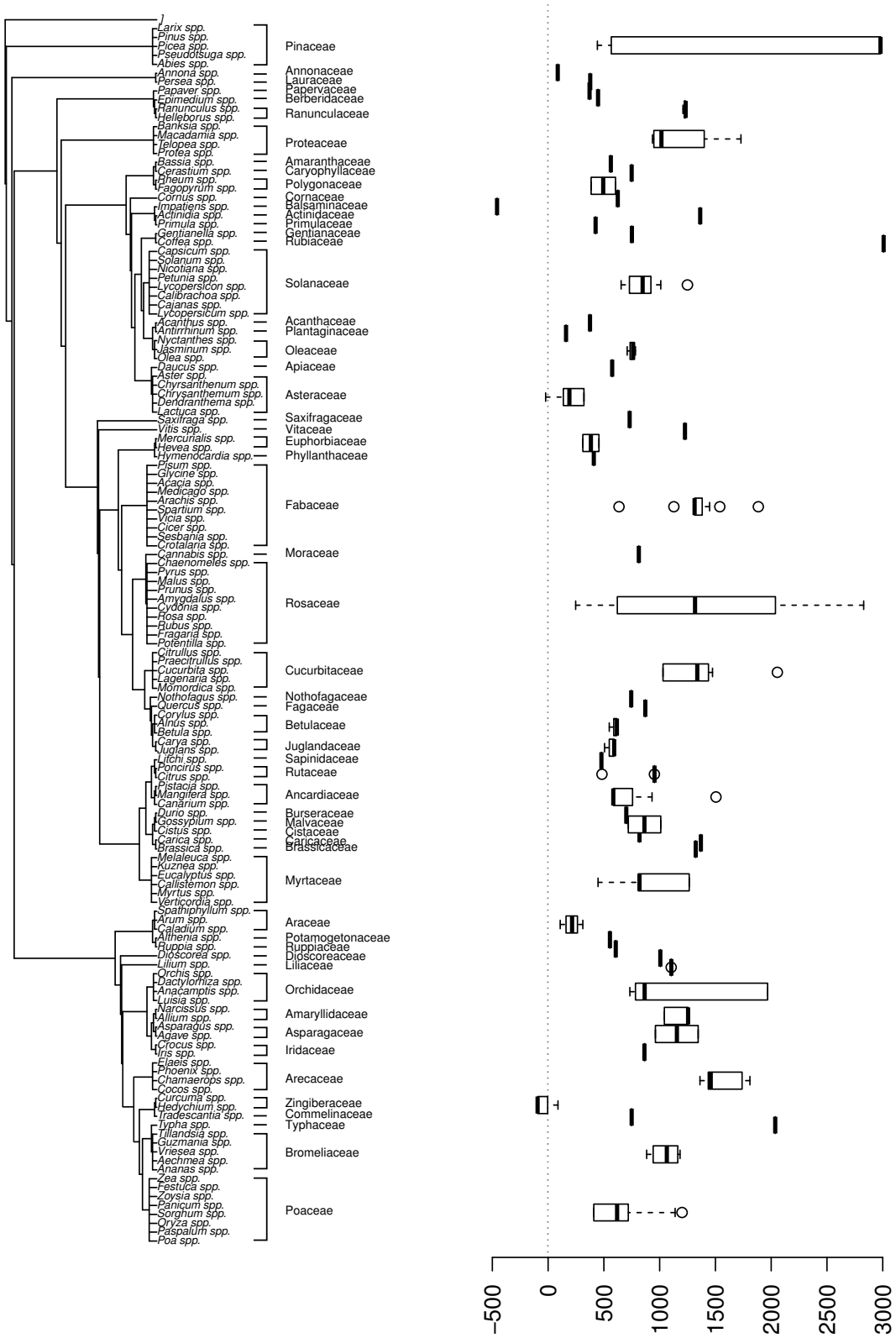

Figure 19: Predicted pollen longevity (in hours) at 20ºC for species across plant families with sufficient information to include in the model. Longevity was defined as time between anthesis and the pollen being reduced to 50% viability; negative values mean that the pollen was estimated to have less than 50% viability at anthesis at 20ºC. Values for each species were calculated from the back-transformed coefficients of a GLMM with a nested family/genus/species random effect. Variation between species within a genus was very low: many of the thin bars represent a number of species within the genus (being the only genus sampled in the family)—larger bars represent families with multiple sampled genera. In-vitro germination was used as the simulated methodology.

4.5 Discussion

We found that there was wide variation in pollen longevity amongst plant taxa, with family- and genus-level differences (random effects) explaining more variance than those between species, though all of these combined levels contributed significantly to the explanatory power of the model (i.e. they were retained during model selection). Given that we are aware of no previous study that examines both within-genus and between-family variation in pollen longevity, this is a novel observation. A number of previous works have noted that there is considerable variation in pollen viability and longevity between species (Shivanna et al. 1991; Pacini et al. 1997; Hedhly 2011), but, as these studies were generally sampling individual taxa from different families, they may have actually been observing between-genus and between-family variation. Studies that do sample numerous species within a genus (or cultivars within a species) often find differences in initial pollen viability (Bayazi et al. 2011), and sometimes responses to temperature (Husain et al. 2008), but the differences in longevity are typically of the same order of magnitude. Conversely, we did not find that the larger structure of the phylogenetic tree had an impact on pollen longevity (no phylogenetic signal); a result which is robust as our tree contains numerous polytomies, which tend to inflate estimates of phylogenetic conservatism (Davies et al. 2012). The combination of within-genus similarities and lack of phylogenetic signal means that it may be possible to estimate the pollen longevity for species in a genus which has one other measured species, but making predictions for novel genera and families may be difficult.

With respect to temperature, every study that examined viability versus temperature reported a nonlinear response (which was described in the study as quadratic, cubic, or bilinear). Our overall model also found that temperature had a significant (and nonlinear) effect on pollen viability and longevity as well, with an optimum temperature of about 25ºC, after which pollen viability declines across the species examined. Although individual studies report pollen being able to germinate after being exposed to temperatures as high as 80ºC (Nel, Van Staden & Bornman 2005) and *Petunia* has

been reported to maintain 19% germinability after 48 hours at 75ºC (Rao et al. 1995), exposure to high temperatures for more than a couple of hours tends to sharply reduce pollen viability (Rao et al. 1992; Abdul-Baki & Stommel 1995), and can have a constellation of other negative effects on plant vigor outside of pollen viability, even though pollen is somewhat sheltered from temperature effects within the anther (Young, Wilen & Bonham-Smith 2004; Hedhly 2011). These findings are potentially concerning as there are a number places on Earth which presently reach 50ºC, and there are likely to be more in the future—if 25ºC is a true optimum, then locations with growing-season temperatures of 25ºC and higher may experience decreases in pollen viability due to warming.

The variation in pollen viability explained by time since anthesis and temperature was, however, overshadowed by the variability explained by methodological considerations, such as whether the pollen was rehydrated prior to viability analysis, and which measure of pollen viability was used. Each of the most common methods produced significantly different baseline estimations of pollen viability, with *in vitro* germination providing the most conservative estimate of pollen viability, and tetrazolium the least conservative. Fluorescein diacetate (FDA), while producing higher viability scores than *in vitro* germination ($P = 0.002$; GLMM), was the method most closely reflecting *in vitro* germination, which is encouraging, as it was the second most common methodology. Likewise, rehydrating pollen prior to assessing viability had a significant positive effect on reported pollen viability and longevity. These trends are important particularly for studies assessing the risk of transgene flow, as *in vitro* and FDA do sometimes under-report “dead” pollen, which may be capable of germinating when introduced to a receptive stigma (Dafni & Firmage 2000). Likewise, failing to rehydrate pollen before viability assessment in such a situation is likely to lead to a lower apparent risk of escape.

While there have been reports of cultivated plants having lower viability than their wild relatives

(Abdul-Baki & Stommel 1995), we did not have enough studies making the comparison to run a model. However, of the ten studies which examine both wild and domestic cultivars of the same genus, four find that pollen viability is higher in wild plants (Abdul-Baki & Stommel 1995; Wheeler & McComb 2006; Daniel 2011; Sulusoglu 2014) two find that it is higher cultivated plants (Lyakh et al. 1991; Song et al. 2001), and four had equivocal results (Parzies et al. 2005; Husain et al. 2008; Kormuťák et al. 2010; Bayazit et al. 2011). Parzies et al. (2005) found that, while wild and cultivated barley were initially highly viable (80 – 96%), wild barley pollen lasted longer. Similarly, Song et al. (2001) found that wild rice pollen was initially less viable, but maintained viability for longer than cultivated rice.

In the most recent review of pollen longevity, Dafni and Firmage (2000) gathered a small pool of literature on the reported longevity of plant species, ranging from minutes for some grasses to several months for orchids. They put forth a theory that the fact that orchids produce pollenia, which bind numerous pollen grains together with viscin threads, may protect the pollen from dessication, one of the major factors reducing pollen viability. Interestingly, while orchids performed well in our model, our dataset yielded a different champion of pollen longevity; without refrigeration, *Pseudotsuga menziesii* (Pinaceae) maintained 15 – 47% viability after two years of storage at room temperature (Livingston & Ching 1967). Likewise, the Australian *Boronia molloyae* (Rutaceae) remained 32 – 45% viable after 15 months at room temperature (Astarini et al. 1999). Both of these plants have single pollen grains, which may have some other mechanism of maintaining viability for extended periods of time. The shortest-lived family in our study was in agreement with Dafni and Firmage (2000): Poaceae, the shortest-lived of which was that of *Panicum virgatum*, which was nearly completely inviable by the time one hour had elapsed (Ecker et al. 2013).

This study is the first we are aware of to gather together the values published in the literature and estimate longevity for a large number of plant species across numerous families. While we did not uncover trends in the innate longevity of pollen the phylogeny of all plants, our systematic meta-analysis did find that species within a genus tended to have similar longevity. The collated data include imputed longevity curves for a number of families, genera, and species, allowing the prediction of pollen viability at broader timescales and temperatures than presented in the literature. Additionally, this model has the potential to allow the imputation of longevity curves for species within a genus. Together, this information could be used in a variety of contexts, estimating pollen longevity across species in field conditions. Future directions include incorporating additional variables, such as pollen size, and whether the pollen is binucleate or trinucleate (Hoekstra & Bruinsma 1975), in order to improve predictive power.

Chapter V: Possible mechanisms of pollination failure in hybrid carrot seed and implications for industry in a changing climate

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5.1 Abstract

Approximately one-third of our food globally comes from insect-pollinated crops. The dependence on pollinators has been linked to yield instability, which could potentially become worse in a changing climate. Insect-pollinated crops produced via hybrid breeding (20% of fruit and vegetable production globally) are especially at risk as they are even more reliant on pollinators than open-pollinated plants. We already observe a wide range of fruit and seed yields between different cultivars of the same crop species, and it is unknown how existing variation will be affected in a changing climate. In this study, we examined how three hybrid carrot varieties with differential performance in the field responded to three temperature regimes (cooler than the historical average, average, and warmer that the historical average). We tested how temperature affected the plants' ability to set seed (seed set, pollen viability) as well as attract pollinators (nectar composition, floral volatiles). We found that there were significant intrinsic differences in nectar phenolics, pollen viability, and seed set between the carrot varieties, and that higher temperatures did not exaggerate those differences. However, elevated temperature did negatively

affect several characteristics relating to the attraction and reward of pollinators (lower volatile production and higher nectar sugar concentration) across all varieties, which may decrease the attractiveness of this already pollinator-limited crop. Given existing predictions of lower pollinator populations in a warmer climate, reduced attractiveness would add yet another challenge to future food production.

5.2 Introduction

Insect-pollinated crops comprise approximately one-third of the global food supply (Klein et al. 2007). Many of these plants owe their present uniformity (Dowker & Fennell 1981), disease resistance (Dowker & Fennell 1981), and high yields (Gonzalez et al. 1994; Niemelä et al. 2006; Mahli et al. 2007; Rogers & Wszelaki 2012) to hybrid production systems, including carrot, tomato, onion, melons, squash, brassicas, and eggplant (Tay 2002)—together totaling nearly 20% of global crop production (FAOSTAT 2009). Because these production systems rely on crossing two parent lines, one of which is rendered male-sterile by hand-emasculation or genetic techniques, they are even more reliant on insect pollinators than their open-pollinated counterparts, do not require insects to cross from one parent line to the other (Parker 1982; Delaplane & Mayer 2000; Steffan-Dewenter 2003; Greenleaf & Kremen 2006). Global reports of declines in many pollinator communities (Vanbergen 2013; Potts et al. 2016) , changing climate shifting pollinating insects' active time away from peak bloom (Memmott et al. 2007), and that pollinator reliance has been linked with reduced yield stability (Garibaldi et al. 2011b), indicate that hybrid systems may be at greater risk from additional disturbances than open-pollinated systems.

Yields of any given hybrid crop can vary significantly between varieties and also from year to year. In itself, this generates economic uncertainty, but it also makes determining which cultivars are best suited to the changing environment a difficult task. When hybrid crops that are grown for seed fail to produce adequate yields, pollinators are often blamed (Delaplane & Mayer 2000), particularly as

male-sterile plants, which have no pollen reward, are notoriously unattractive to honey bees (Erickson et al. 1979; Parker 1982; Greenleaf & Kremen 2006). However, it is unclear if the observed poor yields are due to the lack of pollen, other characteristics that have inadvertently been selected for during the process of crop breeding and selection, or a combination of plant traits and environmental variables.

Traits that affect the yield of insect-pollinated plants can broadly be placed into two categories: plant fertility and plant attractiveness. Characters surrounding plant fertility are intrinsic to the plant, such that increased pollinator activity will not improve yield. For example, if flowering of the male-fertile and male-sterile lines is poorly synchronized, yields can suffer as the cross-pollination window may not overlap sufficiently. In such cases, cultural measures to synchronize the lines even by a few days can substantially increase yield (Gracie 2011). Pollen viability has been a recurrent problem in hybrid crops as well, with inbreeding depression often causing pollen viability to drop to 50% or less before it ever leaves the flower (Ockendon & Gates 1976; Abdul-Baki & Stommel 1995; Geard et al. 2006). This initial difficulty can be further exacerbated by heat and water stress during critical periods of plant development, which can further degrade pollen fertility (Abdul-Baki & Stommel 1995; Young, Wilen & Bonham-Smith 2004). Female receptivity is also important, as flowers that are too young, too old, or damaged by suboptimal temperatures will set little seed even if pollen viability is high (Hedhly 2011).

Characters surrounding plant attractiveness, in contrast, are those which influence pollinator visitation. The quality of the nectar reward is foremost for ensuring return visits (Wolf et al. 1999; Nicolson & Thornburg 2007; Cakmak et al. 2010). Honey bees prefer nectar rewards between 30 and 50% sugar w/w (Nicolson & Thornburg 2007), but lower concentration sources at higher volumes per flower may be chosen over many low-volume, high-concentration sources (Nicolson

& Thornburg 2007). Nectar production is altered in high-temperature conditions, typically resulting in lower volumes and higher concentrations (Nicolson & Thornburg 2007), and can also be affected by rainfall—both too high and too low (Gillespie et al. 2015), but hot conditions can also alter the production of secondary compounds, such as phenolics (Petanidou & Smets 1996), which can result in a very different flavor palette to potential insect visitors. Volatile compounds emitted by the plant are also important, as they play a key role in attracting pollinators to the flower initially (Nicolson & Thornburg 2007). Although the volatiles comprising the floral bouquet of numerous plant species have been cataloged, little is known about how these compounds are perceived by pollinators, and less still is known about how they respond to changes in temperature, individually or in aggregate.

In order to achieve successful pollination, a plant must be both fertile (able to receive pollen and set seed), and attractive to pollinators. To understand how climate change may affect pollination, we must therefore look at factors relating to both overarching categories. To address these broad questions, we chose to focus on hybrid carrot production because, despite being a generalist flower pollinated by hundreds of insect species (Willis & Burkill 1895; Willis & Burkill 1903; Proctor et al. 1996; Gaffney 2011), hybrid carrot is known for its low seed set (Hart & Butler 2004) and lack of pollinator attractiveness (Galuszka & Goral 1989; Delaplane & Mayer 2000). In addition, seed production for carrot occurs in areas not optimal for carrot growth in order to avoid genetic contamination from wild carrot (Small 1984; Hauser & Bjørn 2001), which can readily cross into cultivated varieties and reduce agronomic quality of progeny (Small 1984; Hauser & Bjørn 2001). As a number of major carrot seed producing regions are located in temperate, semi-arid areas (OSA 2010; Howlett et al. 2015; IPPC 2016), additional temperature variability may negatively affect seed production. The combination of environmental stress and poor pollination in present-day hybrid carrot make it a promising model for future conditions experienced by hybrid crops, and

examining the mechanisms of current pollination failure may highlight future vulnerabilities both carrot and hybrid crops in general.

The objectives of this study were to 1) test the effect of temperature on temporal patterns of plant traits that might predict performance in the field, including bloom phenology, seed set, pollen viability, nectar quality (both sugars and phenolic compounds), and floral volatiles and 2) examine if these effects differ across varieties with a range of historical yields to test whether varietal differences are reduced or exaggerated by warming, and 3) use this information to determine which factors are important in present-day pollination failure, and which may be important given a changing climate.

5.3 **Methods**

This study was conducted in New Zealand as it is one of the world's largest producers of carrot seed (Hampton et al. 2012). We exposed carrot (*Daucus carota* L) plants to experimental temperature treatments, and measured characteristics relating to their innate ability to produce seed ('Plant Fertility Metrics', below) as well as several metrics that may affect their attractiveness to pollinators in the field ('Plant Attractiveness Metrics', below). To assess the extent to which these characters contribute to differences in yield and how they may respond to climate warming, we examined the correlations between each one and plant variety, temperature, and time-of-day in generalized linear mixed-effects models (GLMMs), generalized additive mixed-effects models (GAMMs) or ordination-based tests.

5.3.1 **Plant Material**

In order to determine how floral receptivity and pollen viability vary with time-of-day (a known source of variation in floral traits; Dudareva et al. 2003; Nicolson & Thornburg 2007) and temperature, we grew the male-fertile and cytoplasmically male-sterile (brown anther type) parents

of three lines of Nantes-type hybrid carrot for hand-pollination trials and measures of pollen viability. These three lines had previously been observed to perform poorly (172 ± 43 kg/ha), average (377 ± 17 kg/ha), and well (607 ± 87 kg/ha) in the field (hereafter referred to as 'poor', 'average' and 'excellent' lines); we chose this gradient to attempt to tease out the cause(s) of the differential performance in the field, and to have a range of yields to assess the effects of temperature. Seeds for each line were sown in trays in February 2015, during the southern hemisphere summer. When plants had germinated, we transplanted them individually into 3L pots filled with potting mix and slow-release fertilizer (Canterbury Landscape Supplies). Each line had 100 male-sterile plants and 75 male-fertile plants potted out, and these were kept outdoors in ambient conditions until flowering began.

In order to minimize the effect of temperature on plant physiological processes other than flowering, we moved plants to temperature treatments after the umbels had formed, and just prior to flower opening. The three temperature treatments simulated cool, average, and warm seasons via shade houses, unheated glasshouses and heated glasshouses, respectively. Two separate buildings were used for each temperature treatment, and the plants were equally divided between them. To accurately record conditions experienced by the carrot flowers, we placed temperature and relative humidity probes (onset HOBO Prov2 temp/RH meters) at chest height in each of the six locations. Temperatures were extracted from the data loggers and recorded as temperature at the sample time (S4 Fig. 2), average temperature during the 24-hour period prior to the sample time, and average temperature during each plant's time in the glasshouse prior to sampling. Models were run with each measure of temperature sequentially, but in all cases, the 24-hour period had the highest predictive value, so only this measure was used.

Every day, we checked plants for floral stage, and, when the petals had turned white and the outer

whorl of flowers had just begun to open, they were randomly assigned to a temperature treatment. Male-sterile plants were also assigned to one of seven time treatments (4am, 8am, 11am, 2pm, 5pm, 8pm, and 11pm) for hand-pollination. Selection of treatments was done without replacement, so that male-sterile plants were always equally distributed between the three temperature treatments and each time-temperature combination received the first replicate before proceeding to the second, third, and fourth (252 male-sterile plants total). Male-fertile plants were preferentially assigned to locations where male-sterile plants required pollination.

Once selected, we bagged the primary umbel of each male-sterile plant with 1mm mesh to prevent insect pollination and, as an extra precaution, placed plants into a $1.5m^3$ fine mesh cage in each of the six locations. Male-fertile plants were left unbagged inside the cage. As a further precaution, we placed yellow sticky cards in each cage to trap any flying insects that entered.

5.3.2 Plant Fertility Metrics

Phenology

In order to properly hybridize in the field, both the male-sterile and male-fertile lines must bloom simultaneously, and the male-fertile lines should, ideally, produce pollen for the duration of the male-sterile line's bloom time. To quantify this bloom synchrony, we checked the primary umbel of each potted carrot plant daily. Just prior to the opening of the outer whorl of umbellets, we recorded the date and assigned the plant to its treatment. We then calculated the number of days between seed sowing and flowering. Any plants that had not flowered after 365 days were recorded as having failed to bloom.

Seed Set

To quantify changes in stigma receptivity across lines, temperature treatments, and at different times of day, we conducted a hand pollination experiment. Once pollen from male-fertile plants started to dehisce, hand-pollinations began. Every morning at 8am, we surveyed caged plants and any plant where the stigmas appeared receptive throughout the umbel was pollinated that day at its pre-

selected time slot. To assist with visually identifying receptive stigmas, a photo guide was prepared the season prior by staining stigmas with alpha-napthyl acetate (Kearns & Inouye 1993). The primary umbel of each plant to be pollinated was unbagged and three umbellets were selected and tagged, one from each of the three whorls, as previous studies indicate that there may be differences in female fertility between the inner and outer umbellets (Koul et al. 1989). As the 'medium' male-fertile line was the only one blooming throughout the sample period, we used pollen from this line for hand-pollinations of all male-sterile lines. For each time slot where a flower needed to be pollinated, we bulked together pollen from all the 'medium' male-fertile plants in the building. We took a subsample of this pollen, put it in a cryotube, immediately placed it in liquid nitrogen for later pollen viability assessment, and applied the remainder with a paintbrush to each stigma of each floret of the tagged umbellets of all flowers in the building needing pollination at that time. Plants were then rebagged and left in the glasshouse for a further 72 hours, to allow pollen tubes to reach the ovaries (typically 24 – 48 hours; Delaplane & Mayer 2000), before being brought back outside to complete seed set.

Once the seed heads dried, we brought them back into the lab, and the seeds of the three tagged umbellets of each flower were counted. In addition, three untagged umbellets, one from each whorl, were examined as an unpollinated control for each plant.

Pollen Viability

Subsamples of bulked pollen were stored in cryotubes in liquid nitrogen until the final pollination for each day (typically 11pm or 4am), when we transferred it to a -80^{o}C freezer. When all pollen samples were collected, we transferred them to a second facility on dry ice and then immediately placed them in a second -80^{o}C freezer until processing.

Pollen viability was assessed with fluorescein diacetate (FDA), which has previously been shown to have a strong correlation with *in-vivo* germination in carrot (Spurr 2003). We thawed the cryotubes for 2 – 5 minutes and then washed the tube with 50µl FDA-sucrose solution (0.25% w/v FDA, 20% w/v sucrose) via a pipettor, with as much liquid as possible collected and slide mounted. We examined samples with a UV light microscope, counting 200 pollen grains across longitudinal transects of each slide. We categorized pollen as viable if it fluoresced bright green (Heslop-Harrison & Heslop-Harrison 1970).

5.3.3 Plant Attractiveness Metrics

Nectar Quality

After volatile collection (if applicable, see below), but prior to pollination, we sampled each male-sterile plant for nectar. We followed the protocol described by Gaffney (2011), dipping half of each umbel (~30 umbellets, with the umbel diameter being controlled for in analyses) into 40mL of distilled water twenty times, ensuring that the umbel was shaken off afterward to recover as much water as possible. We then immediately placed the dilute nectar in a freezer until further processing. To prepare the samples for high-performance liquid chromatography (HPLC), they were thawed, filtered to remove any large contaminants, and freeze-dried in 50mL falcon tubes. We then resuspended the samples in 1mL of methanol:water at a ratio of 1:1 and divided it in two parts: a 600 µL aliquot for nectar sugar analysis and a 400 µL aliquot for nectar phenolic analysis.

Sugar identifications were made via HPLC using a modified combination of the methods of Ruperez (Rupérez 1998), Knudsen (Knudsen 1986), and Knudsen and Li (Knudsen & Li 1991). The 600µL aliquot was centrifuged at 14,000 rpm for 10 minutes. A 250 µl aliquot of the supernatant was placed directly into an HPLC vial. We then carried out the HPLC analysis using a refractive index (RI) detector (Waters™ Alliance 2690 HPLC with Waters™ 2414 RI detector). HPLC-RI analysis was carried out by injecting 10 µl of sample into an isocratic mobile phase of 70%

acetonitrile in water with chromatographic separation (Econosphere™, Amino, 5µm, 4.6 x 250mm, Grace™) at 30 °C and RI detection at 40°C. Unknown sugars were identified using retention times and response factors of known sugars (Sigma).

Phenolic composition of the nectar was also analyzed via combined liquid chromatography-mass spectrometry (LC-MS). The previously prepared 400µl aliquots were filtered with a Single Step® vial 0.22 µm PVDF (Thompson™ Part No. 65531-200) filter. The LC-MS system consisted of a Thermo Electron Corporation (San Jose, CA, USA) Accela UHPLC pump, Thermo Accela Open Auto sampler (PAL HTC-xt with DLW), Finnigan Surveyor PDA plus detector and a ThermaSphere TS-130 column heater (Phenomenex, Torrance, CA, USA). Each of the 48 extracts was analyzed by two ion formation modes creating 96 data files, as follows. A 2µL aliquot of each prepared extract was separated with a mobile phase consisting of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) by reverse phase chromatography (Kinetex guard cartridge and Kinetex C18, 2.6 µ, 100 Å, 100 x 2.1 mm, Phenomenex, Torrance, CA, USA) maintained at 30 °C with a flow rate of 200 µl/min. A gradient was applied : as 0-10 min/95%A, 13 min/60%A, 15-20min/5%A, 23-28min/95%A. The eluent was scanned by API-MS (LTQ, 2D linear ion-trap, Thermo-Finnigan, San Jose, CA, USA) with electrospray ionization (ESI) in the negative mode. Data were acquired for precursor masses from m/z 120–1000 with up to MS3 product spectral tree formation. All data were processed with the aid of Xcalibur®2.20 (Thermo Electron Corporation) and an in-house Plant and Food Research database of chemical signatures.

Floral Volatiles

We collected volatile organic compounds (VOCs) from the headspace of plants after the outer whorl of florets opened, but prior to pollination. Due to resource limitations, we could not collect volatiles from every plant, so a subsample was taken across the treatments. To achieve a good cross-section

of the experimental treatments, we collected three separate datasets; one across varieties, one across temperatures, and one across times of day. For the variety dataset, we took 24-hour headspace collections for twelve plants (6 male-sterile, 6 male-fertile) from each of the three varieties in the average temperature treatment, totaling 36 samples. For the temperature dataset, we took 24-hour headspace collections from 6 'medium' male-sterile plants each in the cool and hot treatments, which were analyzed together with the 'medium' male-sterile samples in the previous dataset, totaling 18 samples (12 unique to this dataset). For the final dataset, in order to capture variation throughout the course of the day, we sampled six further 'medium' plants beginning at each of the seven time periods (3 – 5 hour headspace collection), for 42 total time-of-day samples.

Each headspace sample was collected *in situ* using the active sampling apparatus in Fig. 20. The primary umbel of each flower was fitted with a nylon oven bag and, insofar as it was possible, leaf material was excluded from the bag. In order to ensure floral volatiles rather than green leaf or ambient compounds in the air were being collected, each set of collections included a control where the bag was secured around a leaf. Each bag was fitted with a charcoal filter at the base to remove ambient VOCs. We used a pump with an airflow rate of 500mL/min, split four ways so that 125mL/minof air was pulled through each headspace collection apparatus and into a Tenax® filter, which adsorbed the floral VOCs. The Tenax® filter was constructed from a 15mm long, 10mm diameter glass tube containing 60mg of Tenax® 35/60 (Grace Davidson Discovery Sciences, VIC, Australia) held in place with silane-treated fiberglass (Grace Davidson Discovery Sciences, VIC, Australia). Tenax tubes were conditioned prior to use by heating for 3 hours at 250°C under a stream of nitrogen gas, and the charcoal filters were baked overnight at 150°C in a filtered-air oven. After VOC collection, each tenax was desorbed by solvent extraction with 1mL of n-hexane (Sigma-Aldrich, 99% purity).

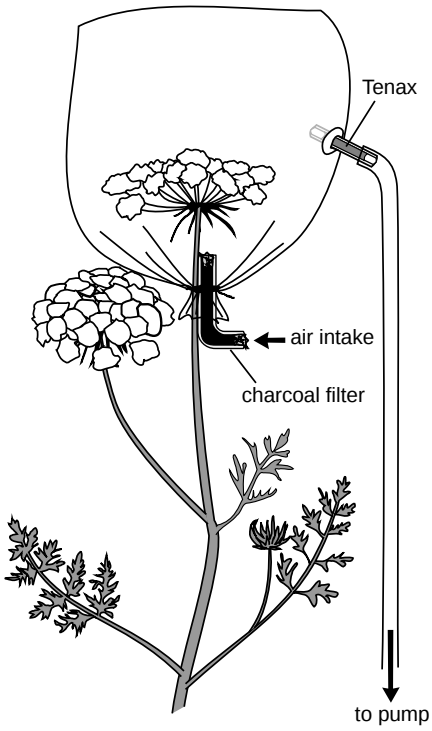

Figure 20: Apparatus for collecting carrot headspace volatiles

To obtain quantitative values of each VOC identified in the headspace samples, we added an internal standard of nonadecane (10 µg) to the 1 mL of n-hexane (Sigma-Aldrich, 99% purity) used to elute the tenax tubes. A sample containing 10 µg/mL of each of the identified compounds was run as an external standard.

We used one microliter of each headspace extract for gas chromatography coupled with mass-spectrometry (GC/MS). The subsample was injected into a Varian 3800 gas chromatograph (Varian Walnut Creek, CA, USA) with the injector port set at 250°C, and then run through a DB5-MS non-polar column (J&W Scientific Folsom, CA, USA) with dimensions 30m x 0.25mm id x 0.25µm film thickness. The column was raised from 40°C up to 280°C at a rate of 4°C/min, and then held at 280°C for 5 minutes. We used a constant flow of helium as a carrier gas (1 mL/min). Injections were splitless for 36 seconds. A Saturn 2200 mass spectrometer (MS, Varian Walnut Creek, CA, USA) ionized the molecules from a mass range of 29 m/z to 399 m/z, after the GC separated each compound present in the extract according to their volatility. The abundance of each compound was

then recorded as the area under each peak. After each sample was run, we identified compounds by comparing MS results to a database (NIST MS Search 2.2). Synthetic compounds were injected with the same GC-MS protocol to confirm identification.

To compare the compounds' abundance in each extract, we used the internal standard method to calculate quantities, multiplying the known amount of the internal standard nonadecane (10 ng) by the area of the compound of interest and the response factor (measured from an external standard run containing a known quantity, 10 ng, of both nonadecane and the compound of interest), then dividing by the area of the internal standard. Volatile collections were done over a different time period for each treatment (variety: 24 hours, time: 3 – 5 hours), so for comparison of each compound across all the experiments, we calculated the emission rate by dividing the quantity of each peak by the amount of time of collection in order to obtain value in µg/h.

5.3.4 **Statistical Analysis**

Phenology data were recorded as a count variable—the number of days between seed sowing and flowering. Because all plants were kept under the same conditions prior to blooming, we could compare them in a generalized linear model (GLM). We used a gamma error distribution to account for the observed variance-mean relationship. The predictor variables were plant variety and plant line.

Nectar, pollen viability, and seed set data were collected over multiple days, creating the risk of temporal autocorrelation. As the temperature treatments were conducted in two locations each, there was also the potential for spatial autocorrelation within each glasshouse. In order to account for this variance and non-independence, we used generalized linear mixed-effect models (GLMMs) with date sampled and location as crossed random effects. We used the *lme4* library (Bates et al. 2014) in

the R statistical programming language (Team 2014) to perform most of our analyses. We used the following model selection process to determine our best-fitting model. All permutations of the predictor variables (plant variety, temperature, and time-of-day, and their interactions) were used to create candidate models, and final models were selected if their AIC scores were within two points of the best-fit model; if multiple models met this criteria, we took a model average (Burnham & Anderson 1998) using the *MuMIn* package 1.15-6 (Barton 2014). In order to obtain p-values for the final models, we used the Satterthwaite method of denominator synthesis, implemented within the *lmerTest* package (Kuznetsova et al. 2015). It should be noted that this method calculates non-integer degrees of freedom. Where relevant, models were checked for over-dispersion (where error distributions were not Gaussian) or normality of residuals and homoscedasticity (for Gaussian models). In addition to differences in the mean response across treatments, initial examination of the data suggested that there may be differences in the variance of the response variables. To test for these differences, we used Levene's test.

Seed set data were recorded as a count variable, as it was not possible to count the number of initial florets once the seed heads dried to establish a proportion. Plants that failed to set seeds introduced numerous zeros to the data set. While not over-dispersed, the dataset did not conform well to the Poisson distribution and so was analyzed with a zero-inflated negative binomial GLMM in R, with the *glmmADMB* package (Bolker et al. 2014). Predictor variables in the initial model were the pollen viability (as a covariate to control for the quality of pollen used in each hand-pollination), temperature at the time of pollination, plant variety, and time of day.

Pollen viability data were recorded as a binomial variable, where individual pollen grains were either viable or not. The proportion of viable and inviable pollen grains were tested in a GLMM with binomial errors and a logit link function. Because pollen stored at -80^{o}C slowly loses viability

over time (Towill 1985; Hanna & Towill 1995), we included the number of days between collection and processing as a fixed covariate in final model to account for the between-sample variation in storage time. Other predictor variables in the initial model were the plant variety and temperature and time of day at the time of pollen harvest. As this model was significantly over-dispersed, we also included individual sample as a random effect (Browne et al. 2005).

Nectar sugars were measured as the concentration of fructose and glucose; no sucrose was found. As the concentrations of the two sugars were tightly correlated ($R^2 = 0.983$), only glucose was examined in a GLMM, with a gamma error distribution to account for the observed variance-mean relationship. Flowers varied in umbel diameter, which could influence the amount of nectar collected with the dipping methodology. Therefore, we controlled for flower size by adding the diameter of the umbel as a fixed covariate. Other predictor variables in the initial model were temperature at the time of nectar collection, plant variety, and time of day nectar samples were taken.

Nectar phenolics were expressed as concentrations. Three phenolic compounds were found in carrot nectar: caffeic acid, coumaric acid, and ferulic acid. As initial plots revealed that each compound reacted differently to different conditions, it was necessary to examine all three. To avoid multiple single-response models and to capture shifts in the combined phenolic bouquet, we conducted a nonmetric multidimensional scaling (NMDS) ordination within the R package *vegan* (Oksanen et al. 2013) with Bray-Curtis dissimilarity to account for the large differences in mean concentration. To test whether the three compounds varied across variety, temperature, and time of day, we used a permutation multivariate ANOVA (PERMANOVA) procedure (function '*adonis*') from the same package.

Floral volatiles were expressed as the concentrations of methyl salicylate, nonanal, and phenylacetaldehyde—the three compounds found in carrot floral volatiles which bees are able to sense (measured in previous work as a consistent electroantennograph response; S4 Fig. 1). To account for the three compounds simultaneously, we ran a NMDS ordination for each of the volatile datasets with variety, temperature, and time of day as predictor variables. As with the phenolic data, we used a PERMANOVA procedure to test for significance. To aid with interpretation of these multivariate results, we then conducted univariate analyses on each volatile. Data exploration revealed that there was a non-linear relationship between time-of-day and volatile concentration, so we conducted a generalized additive mixed-effects model (GAMM), which allows for non-linear relationships between predictor and response variables (Zuur et al. 2009). In the GAMM, time sampled was a smooth term, while plant ID was a random effect. The amount of smoothing was determined in the model using maximum-likelihood within the *gamm4* package (Wood & Scheipl 2009).

5.4 **Results**

We found numerous effects of plant variety, temperature, and time-of-day on measures of both plant fertility and attractiveness to pollinators (see Fig. 21 for a summary).

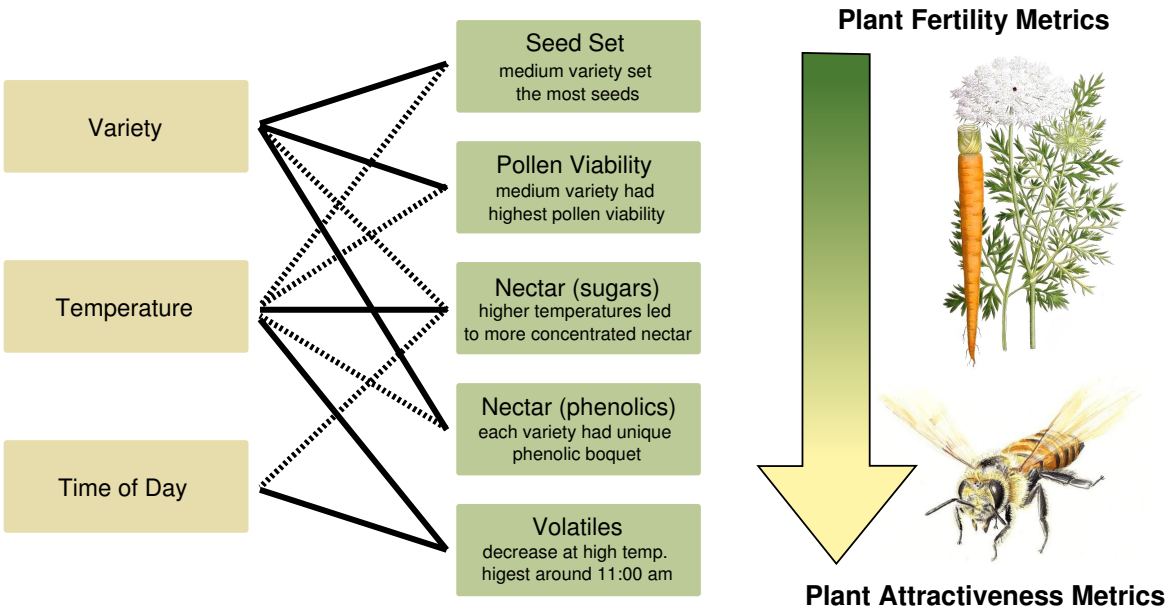

Figure 21: Relationships between different factors examined in this study. Solid lines indicate a statistically significant relationship. Dotted lines indicate factors that were conserved in the final selected models, but were not statistically significant. We did not find any significant interaction effects.

5.4.1 Plant Fertility Metrics

Phenology

A number of plants failed to flower at all: 5% of male-sterile and 22% of male-fertile carrots did not send up a flowering stalk after one calendar year. The rate for male-fertile plants was heavily influenced by the poor line, which accounted for 76% of failures (51% of these plants did not flower). Of the plants that did bloom, there was considerable spread in flowering time, with the first plant blooming on day 285 and the last on day 341. Male-fertile lines bloomed significantly later than male-sterile lines, and there was an interactive effect between variety and line, with the poor variety having the widest gap between the bloom time of the male fertile and male-sterile lines (Fig. 22, Table 5). The male-sterile excellent variety had the most tightly grouped flowering time ($P < 0.001$; $F = 8.560$; Levene's test for differences in variance)

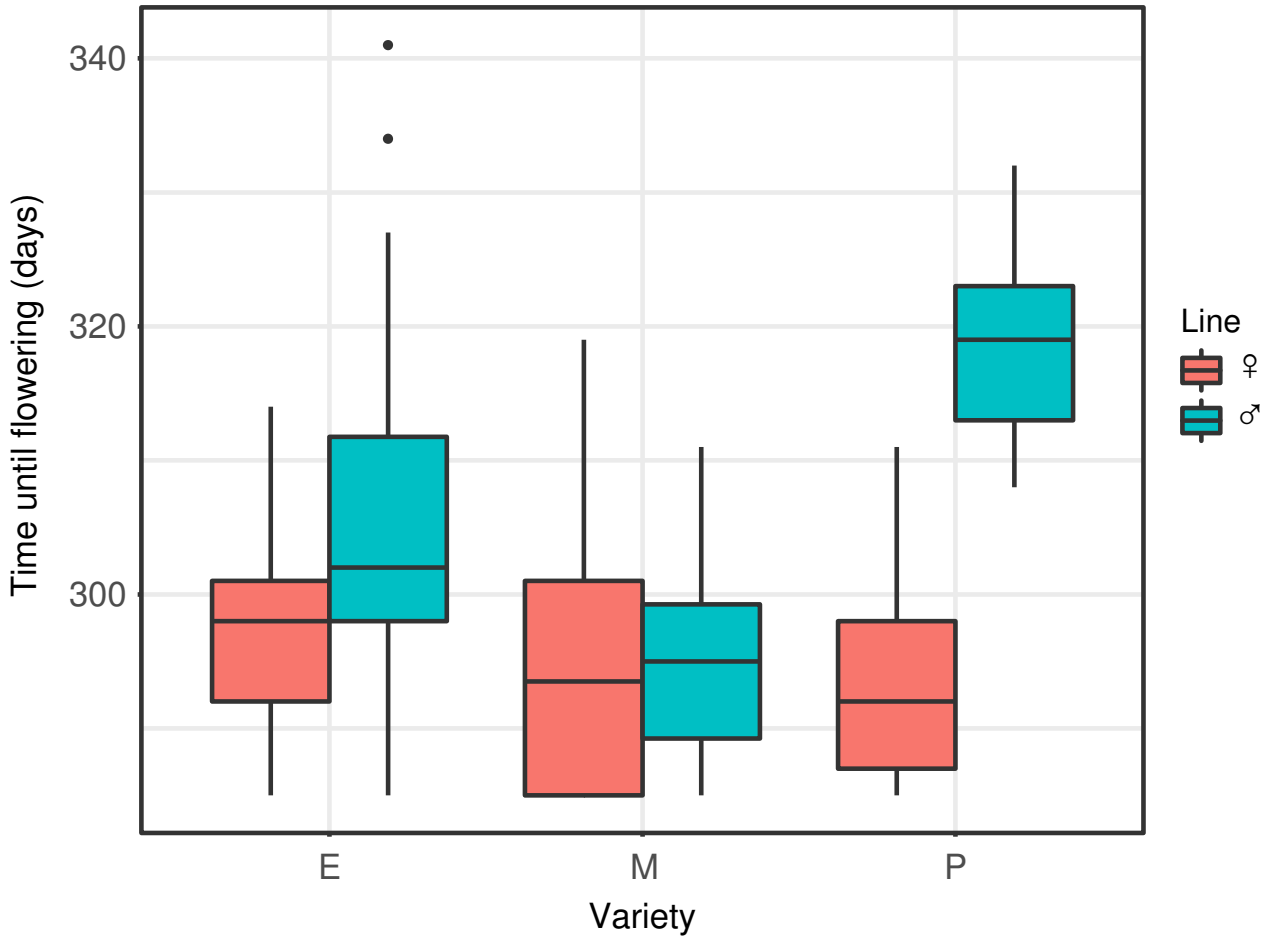

Figure 22: Number of days between seed sowing and flowering between the three carrot varieties. Variety: E = excellent, M = medium, P = poor. Line: male sterile (♀) and male fertile (♂) lines broken out for each variety. Data ceased being collected at 365 days. Boxes represent the middle 50% of the data, lines within boxes are the median.

Table 5: Coefficients table of GLM for carrot phenology. Variety: E = excellent, M = medium, P = poor. Line: male sterile (♀) and male fertile (♂). The intercept condition is the male-sterile, excellent line.

	Estimate	SE	t statistic	P value
intercept	3.373×10^{-3}	8.855×10^{-5}	380.908	< 0.001 ***
Variety (M)	2.675×10^{-5}	1.274×10^{-5}	2.099	0.036 *
Variety (P)	4.577×10^{-5}	1.267×10^{-5}	3.611	< 0.001 ***
Line (♂)	-9.521×10^{-5}	1.345×10^{-5}	-7.076	< 0.001 ***
Variety (M) : Line (♂)	8.971×10^{-5}	1.945×10^{-5}	4.612	< 0.001 ***
Variety (P) :	-1.783×10^{-4}	2.102×10^{-5}	-8.483	< 0.001 ***

Line (♂)				

Significance codes: * < 0.05, ** <0.01 *** <0.001

Seed Set

The rate of seed set was low, with fewer than half of the hand-pollinated umbels setting seed. This may have been due to poor weather, poor pollen viability, and, potentially, the presence of the brown shield bug (*Dictyotus caenosus* (Westwood), Hemiptera:Heteroptera), which was able to enter the seed heads through the exclusion mesh. Nearly every mesh pollinator exclusion bag contained at least one *D. caenosus*, and though, to our knowledge, there is no record of *D. caenosus* feeding on carrot seed, we cannot exclude the possibility that this generalist plant-feeder used the seed heads as a food source. Despite the overall low seed set, we still found differences between the carrot varieties, such that the medium-performing variety set significantly more seed than the other two varieties (Fig. 23), with an average of 1 additional seed per three umbellets (P = 0.005; z = 2.788; GLMM). Temperature (P = 0.995; z = 0.010; GLMM) and proportion of viable pollen (P = 0.383; z = 0.872; GLMM) were both retained in the best-fitting model, though neither was a significant predictor of seed set. The medium variety was also more variable in seed set than the other two varieties (P = 0.001; Levene's test), as would be expected for count data, where the variance often increases with increasing means.

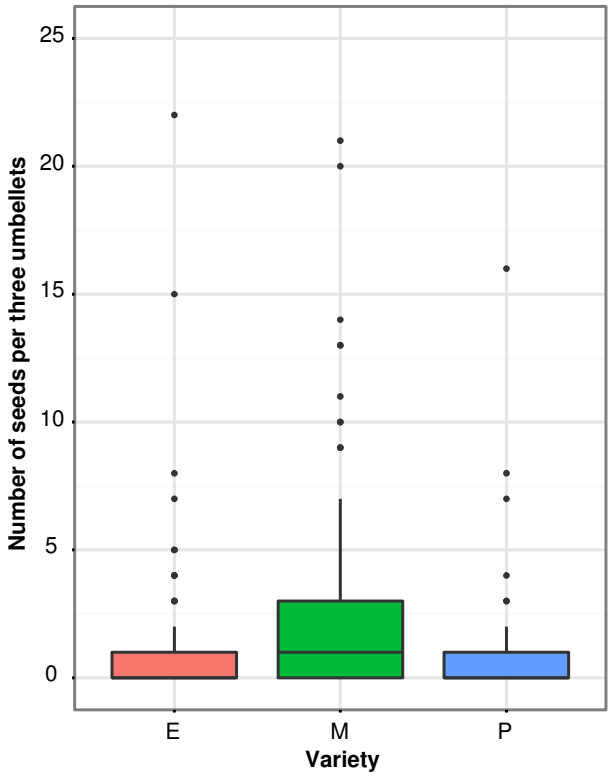

Figure 23: Seed set amongst the three carrot varieties. E = excellent, M = medium, P = poor. Boxes represent middle 50% of data, lines within boxes are the median.

Pollen Viability

Overall pollen viability was low, with a median of 14.5%. This is comparable to previous estimations of carrot pollen viability in New Zealand (S4 Text 1), but is low compared to the viability of cultivated carrot pollen elsewhere in the world (Spurr 2003; Geard et al. 2006; Song et al. 2010), and much lower than wild carrot pollen (~80%) (Hauser & Bjørn 2001). Temperature was retained in the final model, but was not a significant predictor of viability (P = 0.825; z = 0.22; GLMM). There was a significant difference in pollen viability between varieties (Fig. 24; P = 0.004; z = 2.866; GLMM), with the medium variety being the highest, about 45% higher than either of the other two (observed mean of 21.2% versus 14.3% for excellent and 15.9% for poor). The medium variety was also more variable than either the poor- or excellent-performing varieties (P = 0.023; Levene's test).

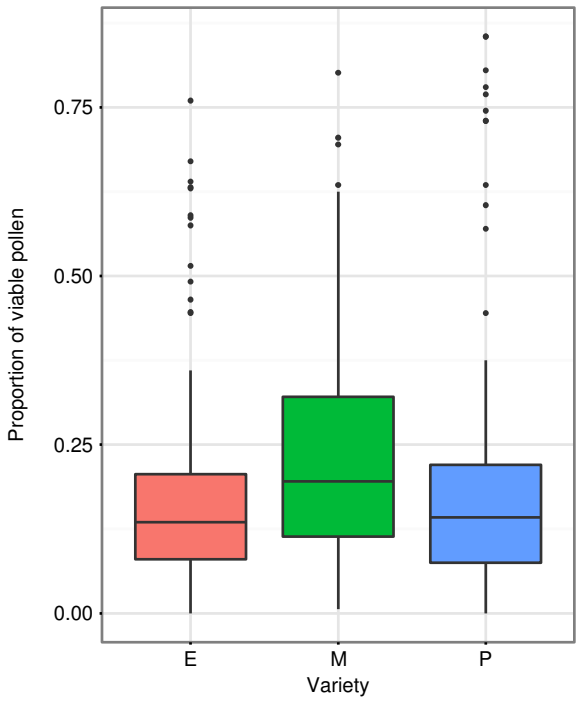

Figure 24: Each sample point represents all male-fertile flowers with pollen at the time of sampling (evenly spread throughout the 7 time and 3 temperature treatment combinations). Viability was calculated as a proportion viable out of 200 grains. E = excellent, M = medium, P = poor. Boxes represent middle 50% of data, lines within boxes are the median.

5.4.2 Plant Attractiveness Metrics

Nectar Quality

Glucose and fructose were found in a close to 1:1 ratio (1.019:1; R^2 = 0.983; LM). No sucrose was detected. There was a significant effect of temperature (P = 0.002; t = 3.091; GLMM) on glucose concentrations, with increasing temperature being correlated with higher concentrations of sugars (Fig. 25). Time-of-day, variety, and an interactive effect between time-of-day and variety were all retained in the final model, though none of them were significant predictors of sugar concentration. For phenolic compounds in the nectar, each of the three varieties had a different composition (P = 0.012; F = 3.393; PERMANOVA), with the excellent variety having high concentrations of caffeic acid, moderate concentrations of coumaric acid, and low concentrations of ferulic acid; the medium variety had low concentrations of caffeic acid, moderate concentrations of coumaric acid and moderate concentrations of ferulic acid; the poor variety had low concentrations of caffeic acid, high concentrations of coumaric acid and moderate concentrations of ferulic acid (Fig. 26). The

excellent variety was more variable in its concentration of caffeic acid than the other two varieties (P = 0.017; Levene's test). Temperature was not a significant predictor of nectar phenolic composition (P = 0.080; F = 2.566), but was retained in the final model.

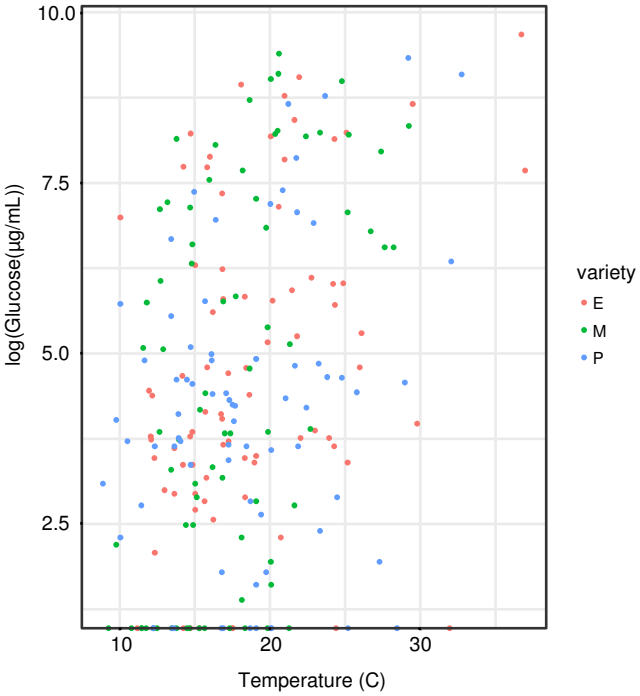

Figure 25: Log glucose concentration in nectar at different temperatures for the female lines of all three carrot varieties. E = excellent, M = medium, P = poor.

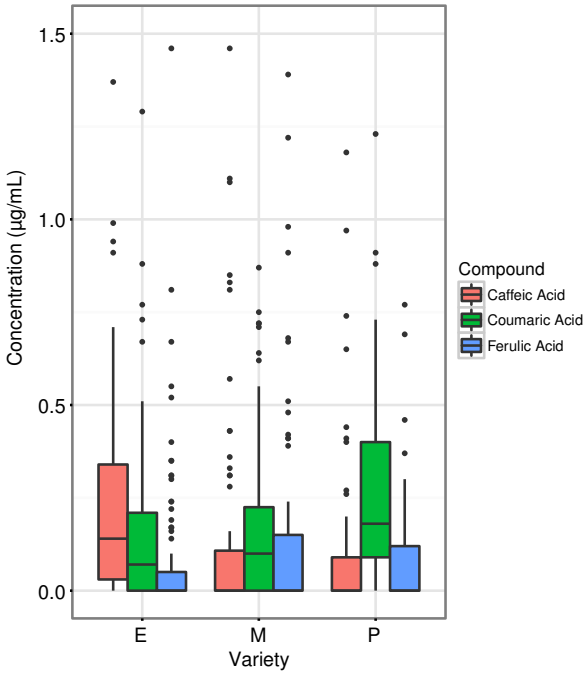

Figure 26: Concentrations of nectar phenolic compounds by carrot variety. E = excellent, M = medium, P = poor. Boxes represent middle 50% of data, lines within boxes are the median.

Floral Volatiles

Of the three compounds contained in the male-sterile carrot flowers' floral bouquets that honey bees are capable of sensing, only nonanal was present in all samples. There was no effect of variety on floral bouquet (P = 0.671; F = 0.467; PERMANOVA), but there was a significant effect of temperature (P = 0.028; F = 4.743; PERMANOVA), with higher temperatures corresponding to lower volatile emissions. As plants were tracked through the course of a 24-hour day, there was a significant spike in nonanal concentration at around 11:00am (Fig. 27; P < 0.001; t = 17.460; GAMM), just prior to the afternoon heat. Although there was no significant effect of variety, the medium variety was more variable than the other two (P = 0.009; Levene's test), meaning that the significant time-of-day result despite this background variability, which was tested using the medium variety, is likely robust.

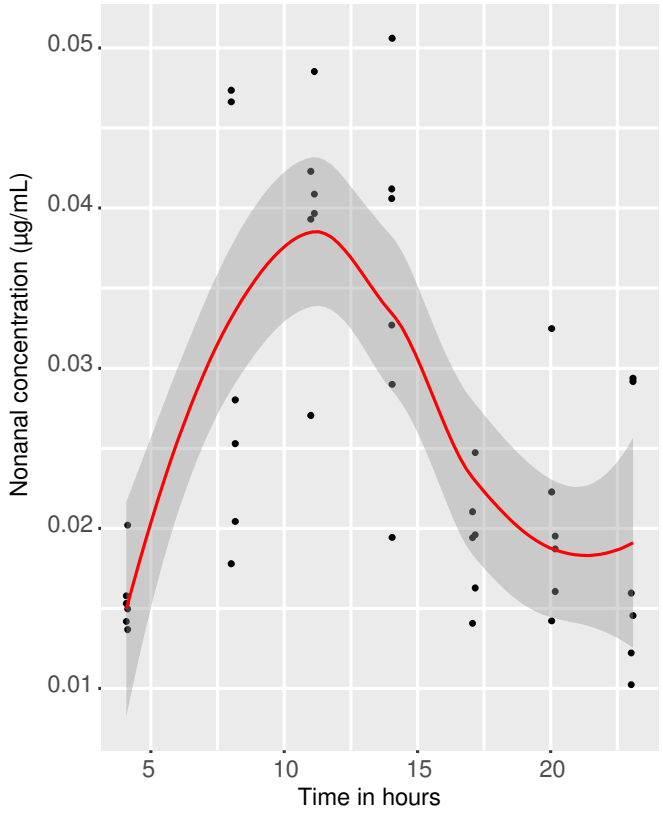

Figure 27: Concentration of nonanal versus time-of-day. Values are from six 'medium' plants sampled repeatedly over the course of 24 hours. The red line is the trend line created by the GAMM, the dark grey area is the 95% confidence interval.

5.5 **Discussion**

While environmental conditions at flowering time affected some plant fertility and attractiveness measures, we did not find any interaction effects between plant variety and temperature. This implies that the differences observed between the poor-, medium-, and excellent-performing carrots were due largely to innate plant characteristics (Fig. 21), which did not respond to temperature. Our data suggest numerous mechanisms of poor performance in the field, as the 'poor' variety consistently underperformed the other two: it bloomed late, 50% of the male-fertile plants failed to even initiate flowering, had the worst synchronization between male-sterile and male-fertile lines, relatively low seed set, and pollen viability typically below 20%. Additionally, the nectar phenolic profiles showed a wide gap between the varieties. The poor line's nectar was high in coumaric acid, which has been found to upregulate bee detoxification pathways (Mao et al. 2013), and ferulic acid, which, while commonly found in honey bee propolis (Cao et al. 2004), is thought to be an insect feeding deterrent (Arnason et al. 1992). Similarly, the poor line is low in caffeic acid, which is highly attractive to bees at modest concentrations (Hagler & Buchmann 1993).

Underperformance in multiple categories, from purely physiological characters to factors which influence plant attractiveness to pollinators, is a satisfying explanation for why a particular variety is observed to have low yield, but we have found that there is not a similarly easy explanation for the high yield of the best-performing variety. In fact, the excellent variety was observed to perform worse than the medium variety under controlled conditions: the excellent variety had somewhat worse synchronization between the male-sterile and male-fertile lines than the medium variety, and seed set and pollen viability on par with the poor variety. The excellent variety, however, distinguishes itself in its nectar phenolics, where it has high concentrations of the attractive caffeic acid, and is more consistent overall, with lower variances than the medium line in nearly every aspect, including bloom duration of the male-sterile line. Although there may be other differences

that we did not capture, these two may result in increased pollinator attractiveness and better conditions for those pollinators to cross-pollinate the hybrid lines under field conditions.

5.5.1 Implications for Industry

Our results are particularly important as previous studies have identified poor pollination as a major cause of low seed yields in hybrid carrot (Hawthorn et al. 1956; Spurr 2003; Gracie 2011). Varieties that are better able to attract pollinators may be better able to fare annual fluctuations in pollinator populations as they could draw in pollinators from the surrounding environment, a trend already observed in mass-flowering crops (Holzschuh et al. 2011). The importance of pollinator attractiveness for determining which varieties succeed or fail is magnified here by the very low pollen viability of these commercial hybrid carrot lines—typically less than 30%. If viability rates were closer to wild carrot (~80%; Hauser & Bjørn 2001), the required pollen deposition would be reduced by ½ to ¾, and thus pollination could be achieved with fewer insect visits and this would reduce the effect of differential attractiveness between the varieties.

We might join the numerous other authors that encourage the breeding of crops for increased insect attractiveness (Delaplane & Mayer 2000; Gracie 2011), or higher capacity for seed production (Stein & Nothnagel 1995), or suggest that pollen viability be selected for (Spurr 2003). However, it is important to keep in mind that vegetable seed crops are not bred for seed production, they are bred for the desirable characteristics of the plants raised from that seed. Indeed, these two goals are often at odds with each other. For example: an onion that produces two flower spikes will produce much more seed, but it will also produce low-grade onions with doubled hearts (Krontal et al. 2000). The result of this compromise between seed set and plant characters has been hybrid production systems, which produce vigorous, uniform progeny, but may set less seed than open pollinated systems, if for no other reason than some portion of the field must be occupied by the

pollinizer line, from which seed is not collected. As a result, hybrid carrots often yield less than 50% of the seed produced by open-pollinated carrots (Delaplane & Mayer 2000; Hart & Butler 2004).

Although the yield of hybrid carrot seed is likely to remain lower than open pollinated seed, there is obviously latitude for higher yield. In our experiment, synchrony of bloom was poor, as is common with hybrid crops (Verdial et al. 2001; Spurr 2003; Sugimoto et al. 2012). Male-sterile plants of all three varieties began blooming before the male-fertile line, meaning that some primary umbels, which typically have high seed yield (Koul et al. 1989), would have failed to set seed due to absence of available pollen. One cultural solution already underway in industry is to cut carrot plants early in the season to delay the flowering of one of the lines in order to synchronize bloom, which then increases yield (Spurr 2003; Gracie 2011). Cutting the plants at an early stage of flowering has been shown to delay bloom by 10 – 14 days (Gracie 2011)—however, this means that growers would need multiple cuttings to successfully synchronize poor male-fertile and male-sterile lines. Planting the male-fertile line even one month earlier than the male-sterile line isn't enough to fully align the two (South Pacific Seeds, Methven, New Zealand, pers. comm).

Another cultural solution is to increase the number of pollinators in the field. Carrot has a generalist flower type (Proctor et al. 1996; Delaplane & Mayer 2000) and is visited by hundreds of insect species (Willis & Burkill 1895; Willis & Burkill 1903; Bohart & Nye 1960; Proctor et al. 1996; Ahmad & Aslam 2002; Gaffney 2011), many of which can contribute significantly to successful pollination (Bohart & Nye 1960; Spurr 2003; Gaffney 2011; Howlett et al. 2011). Honey bees have traditionally been used to pollinate the crop with a stocking density of 5 – 8 hives/ha in New Zealand (Goodwin 2012; Howlett et al. 2015) and Australia (Spurr 2003). This is

considerably lower than the hive density used in the United States, which has stocking densities 2 – 4 times that (15 – 20 hives/ha; Rubatzky et al. 1999). Increasing the stocking density of honey bees may improve yield, but the discrepancy may represent the different pollinator communities in the two localities as honey bees do not appear to favor carrot as a forage source (Delaplane & Mayer 2000) and preferentially visit other attractive floral resources where possible (Galuszka & Goral 1989). Developing practices in which increase the numbers of other insect species shown to efficiently pollinate carrot, such as *Megachile rotundata (Davidson et al. 2010)* (Hymenoptera), *Calliphora vicina* (Howlett 2012), and *Eristalis tenax* (Pérez-Bañón, Petanidou & Marcos-Garcia 2007) (Diptera) may prove better options for New Zealand, though additional field trials would be necessary to verify a benefit.

In addition, there may be some room for improvement of the carrot varieties. Surprisingly, none of the cultivars we examined contained any detectable quantities of sucrose, which is much more attractive to bees than glucose or fructose alone (Waller 1972; Hagler et al. 1990), and has been detected in hybrid carrot varieties in other parts of the world (Erickson et al. 1979; Gaffney 2011). Additionally, all three cultivars examined in this study have very low pollen viability (<30%) compared to elsewhere in the world (~50%; Spurr 2003; Geard et al. 2006; Song et al. 2010). Inbreeding depression has been observed for numerous other agronomically important traits in carrot (Stein & Nothnagel 1995), and it may be the case that New Zealand hybrid carrots have poor pollen viability because of this. However, there is considerable genetic diversity within cultivated carrot globally (Bradeen et al. 2002)—including within groups sharing the same agronomic characters (Rubatzky et al. 1999). This being the case, introducing breeding stock from elsewhere in the world may alleviate some of the stress in the New Zealand hybrid carrot production system while still selecting for marketable qualities, and the added genetic variability could result in varieties more robust to changes in climate and weather patterns. Although hybrid seed crops are

not typically bred for the fitness of the parent lines, it may become necessary to do so if poor plant vigor, poor attractiveness, lower pollinator populations, and increased stress from a warming climate lead to seed sets much lower than they are today.

5.5.2 Implications for Pollination Under Climate Change

In the carrot seed producing region of New Zealand, climate change is forecast to decrease rainfall and increase surface temperature, leading to seasonal shifts and an increase in droughty conditions (IPPC 2016). Climate change has been linked to negative crop plant outcomes, including: increased susceptibility to insects and disease (Patterson 1995; Juroszek & Von Tiedemann 2011), decreased competitiveness versus weeds (Patterson 1995; Ziska et al. 2004), decreased effectiveness of herbicides on weed control (Ziska & Teasdale 2000; Ziska et al. 2004), reduced overlap between bloom and pollinators (Ziska & Teasdale 2000), reduced pollen viability (Young et al. 2004; Hedhly 2011), changes in volatile emissions (Sagae et al. 2008), and quantity and quality of nectar which may affect plant attractiveness (Petanidou & Smets 1996; Pacini et al. 2003; Gillespie et al. 2015). Previous work in New Zealand has identified that higher temperatures may result in increased foraging by honey bees, while reducing pollinator species richness (Howlett et al. 2013) —primarily native and introduced flies. As a recent meta-analysis has found that crop yields tend to increase with pollinator richness, independent of honey bee abundance (Garibaldi et al. 2013), this may result in an increased number of visits, but a decrease in average visit quality.

We found that there was an effect of temperature on nectar concentration and volatile emission, but no other plant characteristics in our study, although temperature was retained in the models for seed set and pollen viability (Fig. 21), meaning it added explanatory power. It is important to remember that we attempted to expose plants only at the pollen formation stage during flowering, rather than throughout development. Temperature has been shown to affect plant development at numerous

critical periods (Young et al. 2004), and exposure to high temperatures at an earlier point may have reduced plant vigor beyond the effects we observed here. However, the temperatures we exposed plants to in the hot treatment are slightly higher than the future temperature projections for the region (IPPC 2016), so there are unlikely to be further effects on pollen viability in a warmer climate, which is fortunate given the already low viability of the hybrid varieties. Hybrid crops such as carrot are highly susceptible to pollination disruption, due to their requirement for pollen transmission across pollinizer lines. Therefore, if a warming climate leads to fewer non-managed pollinators, this could potentially reduce yield. Given carrot's already modest attractiveness to pollinators compared with weedy species (Galuszka & Goral 1989; Delaplane & Mayer 2000), the potential increase in nectar concentration to above the attractive range of 30 – 50% (Nicolson & Thornburg 2007) and change floral scent could further limit its competitiveness. The situation may be exacerbated by the CO_2 and warming-induced increase in weed vigor predicted by other studies (Patterson 1995; Ziska et al. 2004; Juroszek & Von Tiedemann 2011), as it would increase competition for a more limited pool of pollinators, with a net negative effect on seed set.

5.5.3 Conclusions

The combination of lowered attractiveness with higher competition for pollinators and higher losses to weeds could prove to be a difficulty for future hybrid carrot seed production. If other carrot seed growing regions of the world experience a decline in unmanaged pollinators, as New Zealand is expected to, it could lead to a fragile production system through over-reliance on honey bees (Winfree et al. 2007; Garibaldi et al. 2013). As insurance against adverse pollination conditions, future hybrid production systems may have to balance agronomic traits with the plant's ability attract to pollinators and set seed or, potentially, domesticate currently unmanaged pollinator species.

Chapter VI: Discussion

The aim of this thesis was to examine the mechanisms underpinning pollination and provide a framework for assessing pollinator efficiency and what factors might be responsible for pollination failure—with an emphasis on determining the underlying factors that cause the considerable interspecific (and inter-varietal) variance observed in the field. Those factors fall into two categories: those relating to insects, and those to plants.

6.1 **Factors relating to insects**

Assessing pollinator efficiency has historically been a difficult and laborious process, where collecting single-visit pollen deposition for each locale of interest is required to compare pollinators (King et al. 2013). Easier-to-measure metrics, such as insect behavior, have often led to equivocal findings (Vaissière et al. 1996; King et al. 2013). In **Chapter II**, building on previous evidence that pollen quality differs across the insect body (Mesquida & Renard 1989), I was able to successfully link behavior (floral contact by body part regions) to pollen transport and deposition. The model was additionally able to explain some of the interspecific variation previous studies have noted in insect pollen transport (Howlett et al. 2011), and pollen deposition (Adler & Irwin 2005; Rader et al. 2009; King et al. 2013; Howlett et al. 2017). I found that insect species that tend to spend a greater proportion of time touching floral reproductive structures with a particular part of their body also collect large amounts of pollen on that body part. Likewise, insect species that had high single-visit pollen deposition tended to have more pollen on their bodies in general, and spend a greater proportion of their floral visit touching reproductive structures with their heads. Using measures taken at the body-part scale, it may be possible to use assessments of insect behavior or pollen transport to estimate the efficacy of a pollinator on a target plant species.

The use of the same body-part metrics also provided insight into the coexistence of plant-pollinator communities, with plants depositing pollen on different parts of the insect body. This trend makes sense broadly, as it has the potential to increase plant fitness by avoiding heterospecific pollen. Current methodologies used to model interactions within pollinator communities find that communities with large numbers of species become unstable (Bastolla et al. 2005b,a)—even though empirical data reveals that many ecosystems have even larger numbers of species than this theoretical limit. In **Chapter III** I found that plant pollen was distributed non-randomly amongst sexes, individuals, and body parts of insect species, potentially allowing plants to reduce the effect of competition. This may explain some reported phenomena, such as why, even in competitive environments, plant species receive more conspecific pollen than would be expected by chance (Emer et al. 2015). An insect carrying multiple types of pollen is not necessarily providing reduced pollination services merely by virtue of carrying multiple pollen types; the results suggest that its behavior and body-part-specific storage of the pollen may allow it to deposit conspecific pollen on multiple flowers in a single foraging bout.

6.2 **Factors relating to plants**

Transporting pollen is only part of the pollination equation, however. As soon as pollen is released from the anthers, viability begins to decline (Dafni & Firmage 2000). In addition to time, we know that pollen viability is influenced by a number of factors, including temperature (Chang and Struckmeyer, 1976b; Subedi et al., 1998) and humidity (Fonseca 2004). Literally thousands of studies have been published on pollen viability for numerous plant species under various conditions, but it had not been collated until the writing of **Chapter IV**. I conducted a systematic meta-analysis that identified that the coverage of the literature was relatively good, with 549 plant species belonging to 222 genera and 84 plant families represented in the dataset. Previous reviews (e.g. Dafni & Firmage 2000) indicated that orchid pollen is the longest-lived under field conditions, but I

found that pollen from the Australian genus *Boronia* lasted far longer (15 months v. 3 – 6 for orchids; Astarini et al. 1999). Although only ~25% of the studies we examined measured time since anthesis, temperature and RH together, which limited the inferences that could be made, we found relatively little variability between plant species within a genus, meaning that it may be possible to impute the pollen longevity of unmeasured plant species from known congeners. We also identified a number of gaps in the literature, including a definitive answer as to whether the viability of pollen transported on insects is generally lower than that stored in ambient conditions; the few studies that have been conducted provide suggestive evidence that this is the case (Mesquida & Renard 1989; Richards et al. 2005).

In addition to pollen aging differently on particular insects than in ambient conditions, there is also suggestive evidence that there are differences in pollen viability carried by different species (Rader et al. 2011). The mechanism underlying this trend may be similar to what I explored in **Chapter II,** where different insect species spend different amounts of time touching floral reproductive structures, potentially affecting the pollen turnover rate on the insect. Rader et al. (2011) found that flies carried less viable pollen than bees, which agrees with my findings, but I also found that flies carried a higher *proportion* of viable pollen on their heads. The fly species examined in Chapter II spent less time touching floral reproductive structures, often landing on a petal and resting or, when foraging, spending longer per flower than their bee counterparts, with the head rather than other parts of the body touching the reproductive structures. Thus, pollen turnover may have been much higher on the heads of flies than the rest of the body. If pollen ages faster on insects, this relatively small increase of transport time between body parts may have disproportionate effects on pollen viability, making the effective longevity of pollen on infrequently-contacted body parts shorter than would be estimated by many of the studies included in the meta-analysis.

Pollen longevity is only one way in which plants affect their pollination success, however. To

explore the mechanisms of pollination in **Chapter V**, we looked at a variety of plant traits in addition to pollen viability, including seed set, floral volatiles, and nectar constituents in hybrid carrot, a crop suspected to be pollinator-limited. We examined three varieties of carrot: one known to perform well, one known to perform at average levels, and one known to perform poorly. We found that plants with poor seed set observed in the field scored poorly across all of these metrics, while the variety that performed well was distinguished only by phenolic compounds in its nectar. Very little is known about how insects react to odor and taste compounds at this time, but there is some evidence that caffeic acid, which was abundant in the high-performing plants, is attractive to bees at modest concentrations (Hagler & Buchmann 1993). My study suggests that it is possible that coumaric acid may be a feeding deterrent. Coumaric acid is present in high concentrations in the poorly-performing variety, but not the average variety, which otherwise has an identical phenolic bouquet. Coumaric acid is known to up-regulate honey bees' detoxification pathways (Mao et al. 2013), so it is not unreasonable that such a compound may be avoided by foraging insects. Interestingly, we also found that pollen viability for all carrot varieties studied was quite low, with an overall mean of 14.5%. This is particularly concerning when compared to viability values reported elsewhere for cultivated carrot (~50%; Spurr 2003; Geard et al. 2006; Song et al. 2010) and wild carrot (~80%; Hauser & Bjørn 2001), and, although this is similar to differences between other wild and cultivated plants (13 – 98% loss; Abdul-Baki & Stommel 1995; Wheeler & McComb 2006; Daniel 2011; Sulusoglu 2014), the crops in these studies tend to have >20% viability. The low quality of cultivated carrot pollen may require the alteration of crop breeding programs to focus on pollen viability rather than exclusively agronomic traits.

In both **Chapter IV** and **Chapter V** temperature was underscored as an important factor in successful plant pollination. Temperatures that are too high have been noted to cause a number of problems in plant reproduction, including malformed flowers, altered nectar production, inviable

pollen, and failure to set viable seed (Abdul-Baki & Stommel 1995; Hedhly 2011; Annisa et al. 2013). My meta-analysis found that pollen viability in particular was sensitive to fluctuations in temperature, both for the initial formation of viable pollen and for the maintenance of that pollen viability through time. I also found that higher temperatures reduced the production of floral volatiles and increased viscosity of nectar, which could impede the ability of plants to attract pollinators. These factors are likely to interact with temperature-dependent effects on pollinator species, particularly as higher temperatures can also lead to reduced reproduction (Jump & Penuelas 2005; Cranston et al. 2015), reduced flowering (Saavedra et al. 2003), and changes in flowering phenology (Lambrecht et al. 2007; Munguia-Rosas et al. 2011; Wolkovich et al. 2012). Together, these may reduce overlap between plants and pollinators, increasing risk of both plant and pollinator extinctions (Memmott et al. 2007).

6.3 **Conclusions**

While it has been known for some time that insects perform pollination services (Darwin 1862), only recently, with concerns about pollinator extinctions and food shortages on the rise, and with significant computing power available, have complex models of pollinators and pollination services started appearing in the literature (Lonsdorf et al. 2009; Garibaldi et al. 2013; Kennedy et al. 2013; Benjamin et al. 2014). These models have a number of shortcomings, including predicting that larger body size corresponds to increased pollen deposition when this may not always be the case: Adler and Irwin (2005) found that the largest pollinator they examined, *Xylocopa virginica*, carried the fewest pollen grains due to its nectar-robbing behavior. Likewise, Sahli and Conner (2006) found that insect behavior was a much stronger predictor of pollen-removal ability than was body size. **Chapter II** underscored the importance of insect behavior as well, but also provided a way to link that behavior to pollinator efficiency through measuring body-part-level contact with plant reproductive structures. While this analysis was only done in a few crop species for a relatively

small number of insects, the fact that the findings are robust at the species level means that it is a promising tool for predicting pollinator efficiency. Although there is some debate about whether visitation or efficiency is more important for pollinator efficacy, several authors have made the relevant observation that if an insect deposits no pollen, it doesn't matter how often it visits, it will never be an effective pollinator (Thomson 2003; King et al. 2013). Likewise, many extant models assume that pollen deposited on the flower by a pollinator is viable, but, given that pollen may age faster on pollinators than in ambient conditions (Mesquida & Renard 1989; Richards et al. 2005), and particularly in light of the less-than-100% viability of pollen on insects found in **Chapter II** and the poor pollen viability found on plants in **Chapter V**, there is reason to doubt that this is the case. Taking a viability measure of pollen grains deposited from single-visit studies may improve models' ability to identify important pollinators and predict successful pollination. Pollen longevity results from **Chapter IV** could potentially also be used to improve models. Although the literature we were able to examine is only a fraction of global diversity, the results from the examined taxa are promising in that pollen longevity could be generalizable at the genus-level, enabling dynamic models of pollen transport that account for the decay in viability for novel plant species, which could be used for examining pollination and transgene flow, particularly for systems in which there is a long tail in pollen deposition from either wind or insect vectors. Finally, models that examine pollinators at the species level predict that fewer species can coexist than are observed in nature (Bastolla et al. 2005b,a). Examining pollen transport at the insect body-part level rather than the insect species level created much lower estimations of interspecific competition which, in turn, improved species coexistence in these models. Although each of the above would require more sampling effort and more complex models, I believe the trade-off would be worthwhile, particularly as a number of my findings have the potential to be generalized across large numbers of species, which would ultimately reduce the labor required while improving predictions of plant-pollinator systems. Pollination is a complex and multi-staged process, from the generation of viable pollen on

the stamen to transport and viability when deposited, and I believe that integrating these stages to yield more accurate predictions of pollination is a promising future direction.

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Appendix I: Supplementary material for Chapter II

Table 6: Coefficients table for CMM for pollen quantity by insect order and body part. The intercept condition is the bottom of the abdomen of a bee. Crop, the individual insect ID, and the individual observations were random effects. TH = top head, TT = top thorax, TA = top abdomen, BH = bottom head, BT = bottom thorax

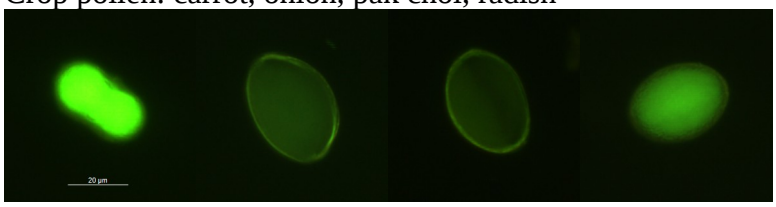
	Estimate	SE	z	P
Diptera	0.901	0.214	4.22	< 0.001 ***
BH	-0.474	0.153	-3.10	0.002 **
BT	-0.050	0.144	-0.35	0.730
TA	0.750	0.139	5.40	< 0.001 ***
TH	0.188	0.144	1.30	0.190
TT	0.899	0.136	6.60	< 0.001 ***
Diptera:BH	0.806	0.189	4.27	< 0.001 ***
Diptera:BT	0.137	0.181	0.75	0.450
Diptera:TA	-0.212	0.174	-1.22	0.220
Diptera:TH	0.765	0.179	4.26	< 0.001 ***
Diptera:TT	-0.267	0.173	-1.54	0.120

Significance codes: * < 0.05, ** <0.01 *** <0.001

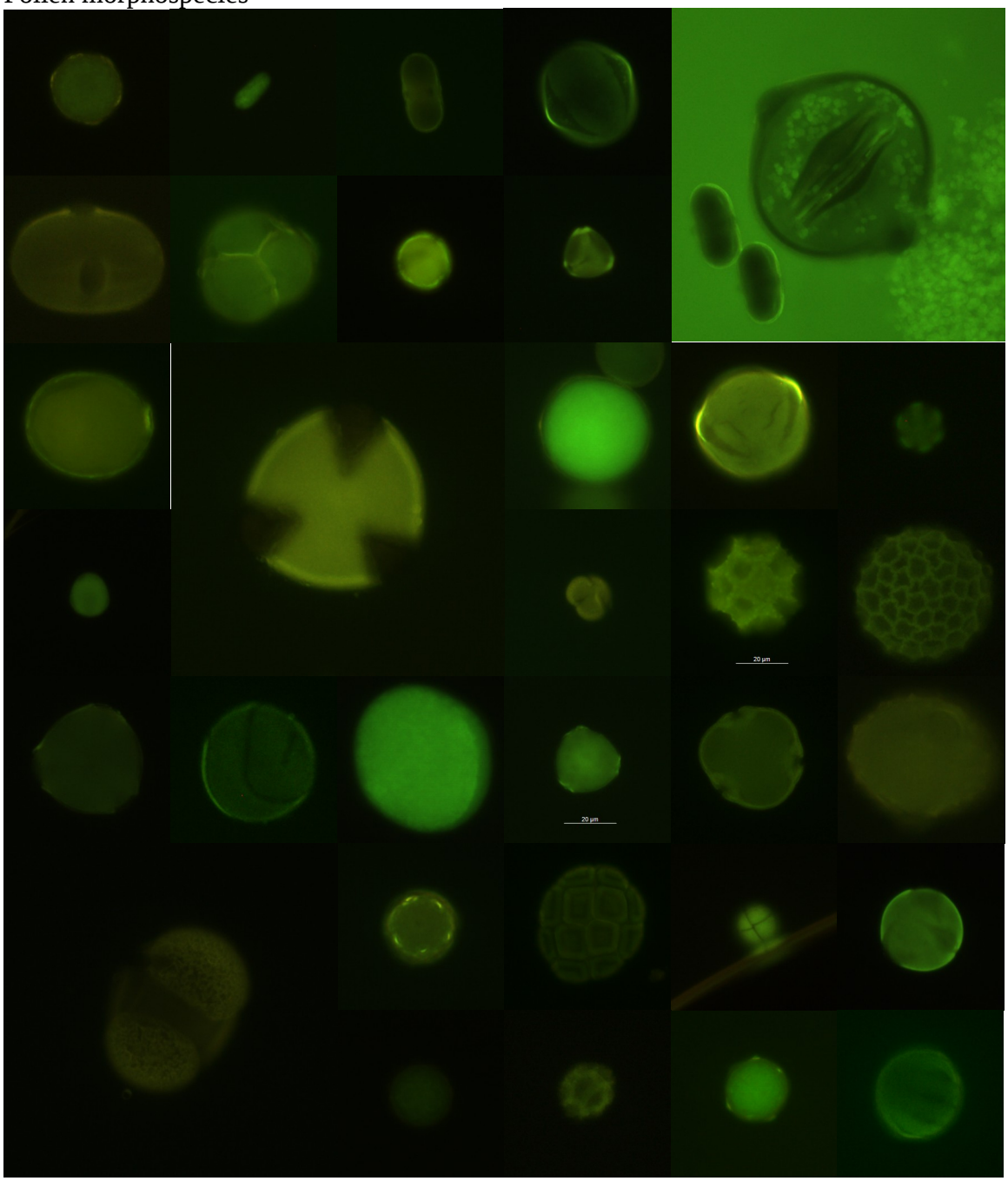
Appendix II: Supplementary material for Chapter III

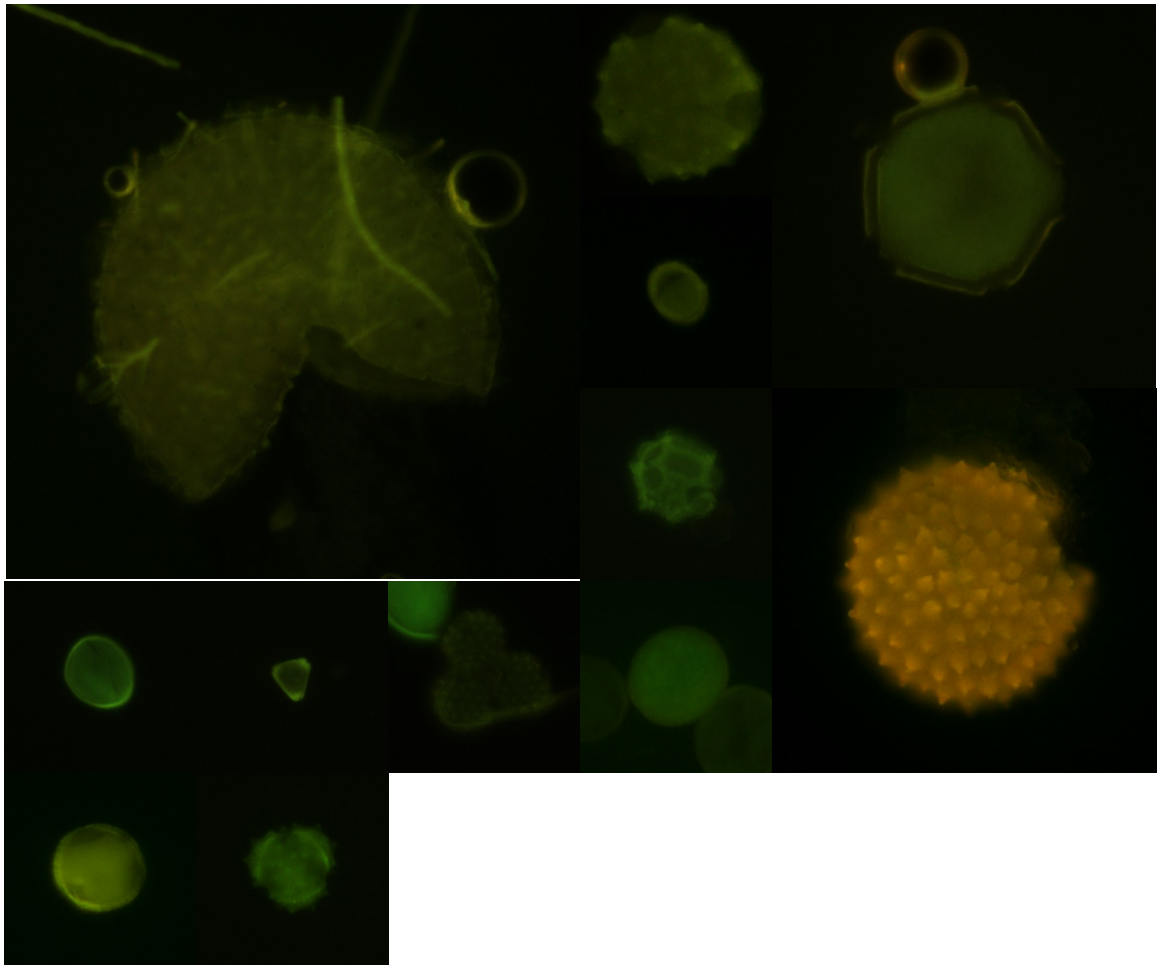
S1: Pollen species collected from insects in this study

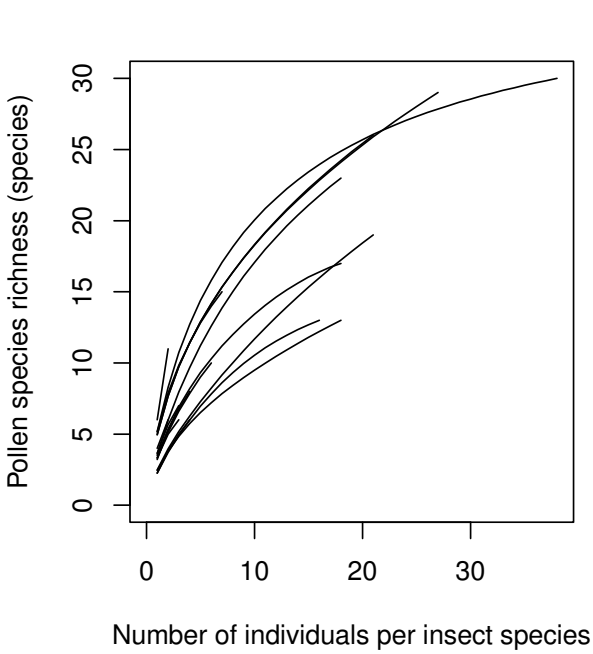
Crop pollen: carrot, onion, pak choi, radish

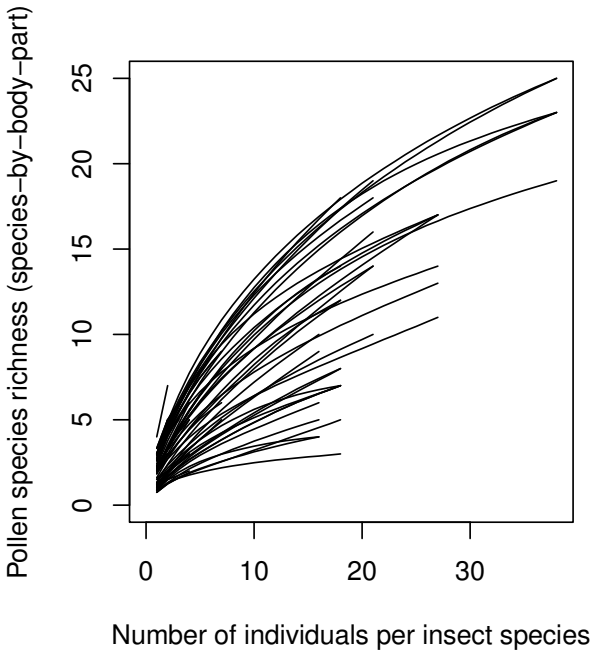


Pollen morphospecies

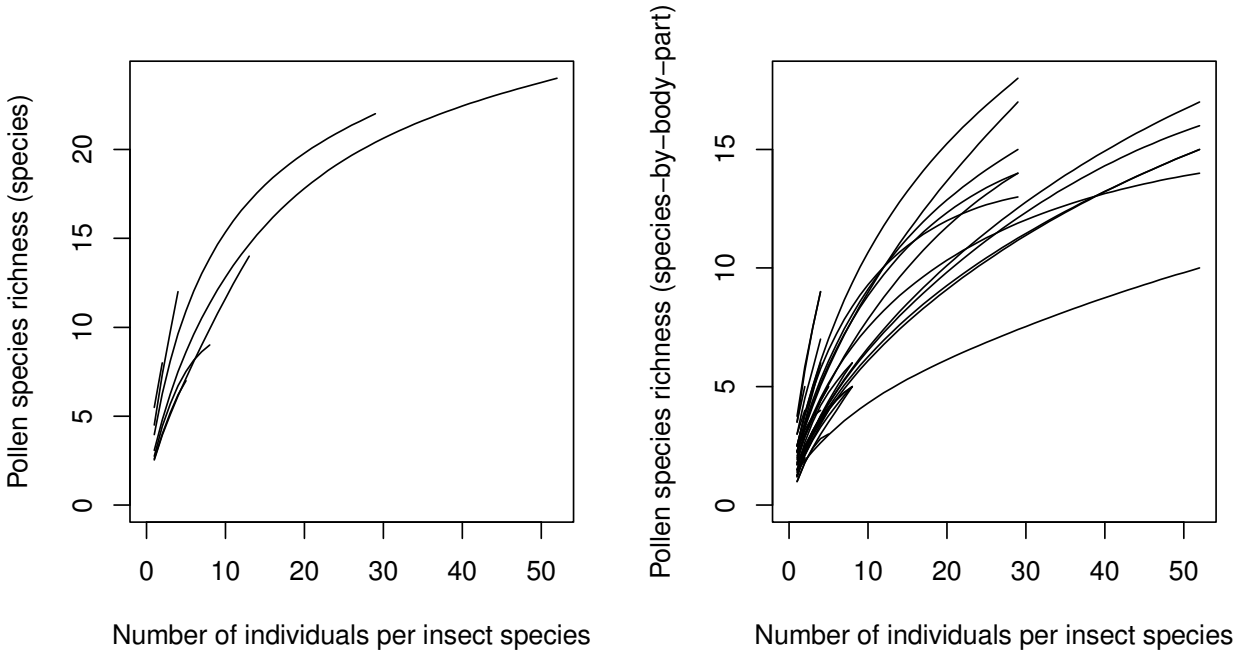




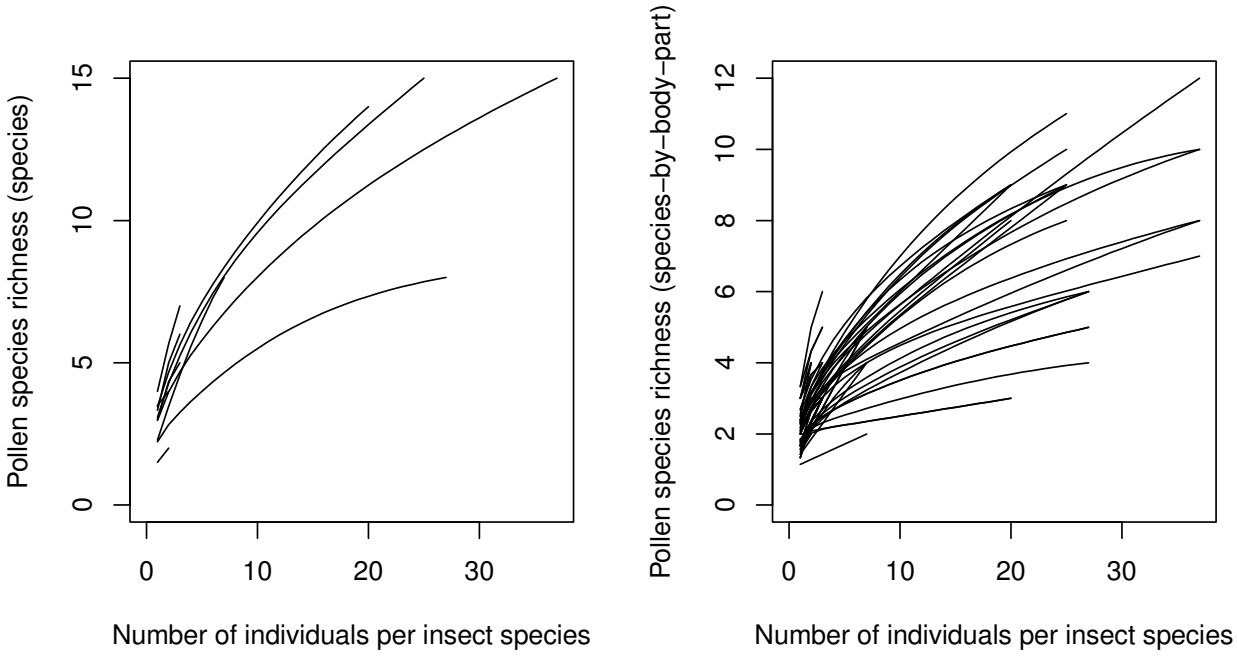

Pollen species richness (species)
0
5
10
15
20
25
30
0
10
20
30
Number of individuals per insect species


Pollen species richness (species–by–body–part)
0
5
10
15
20
25
0
10
20
30
Number of individuals per insect species

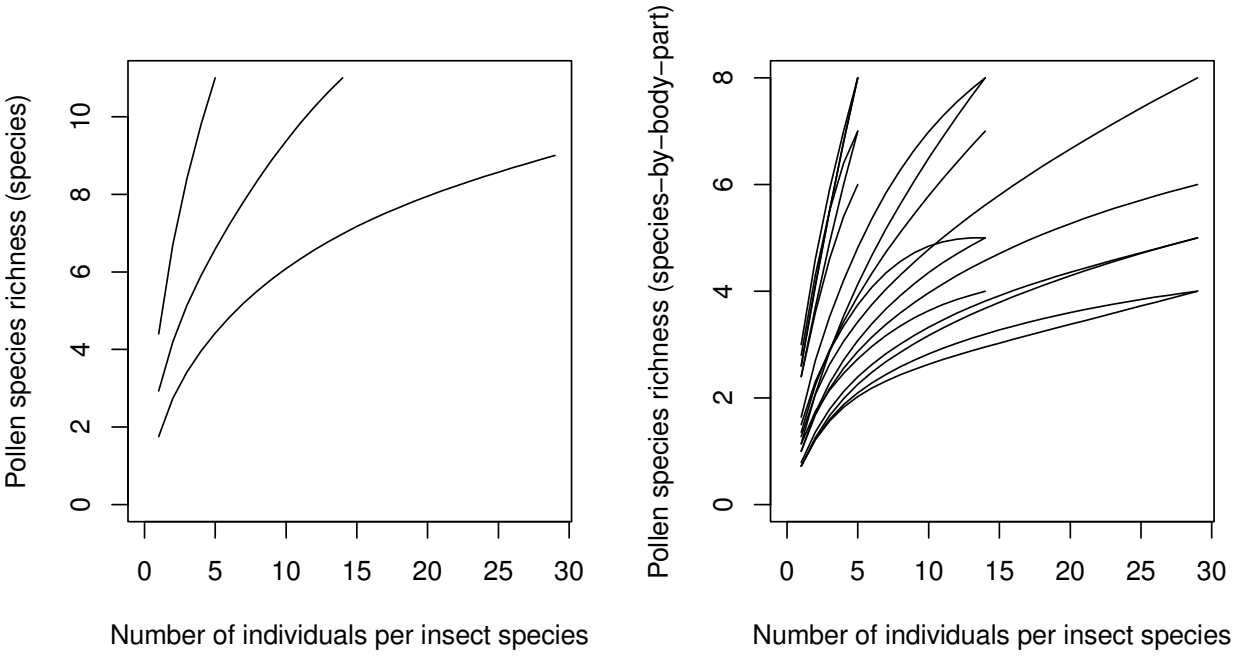
S2 Fig 1: Rarefaction curves for pollen carried by insect species in carrot at different network scales.



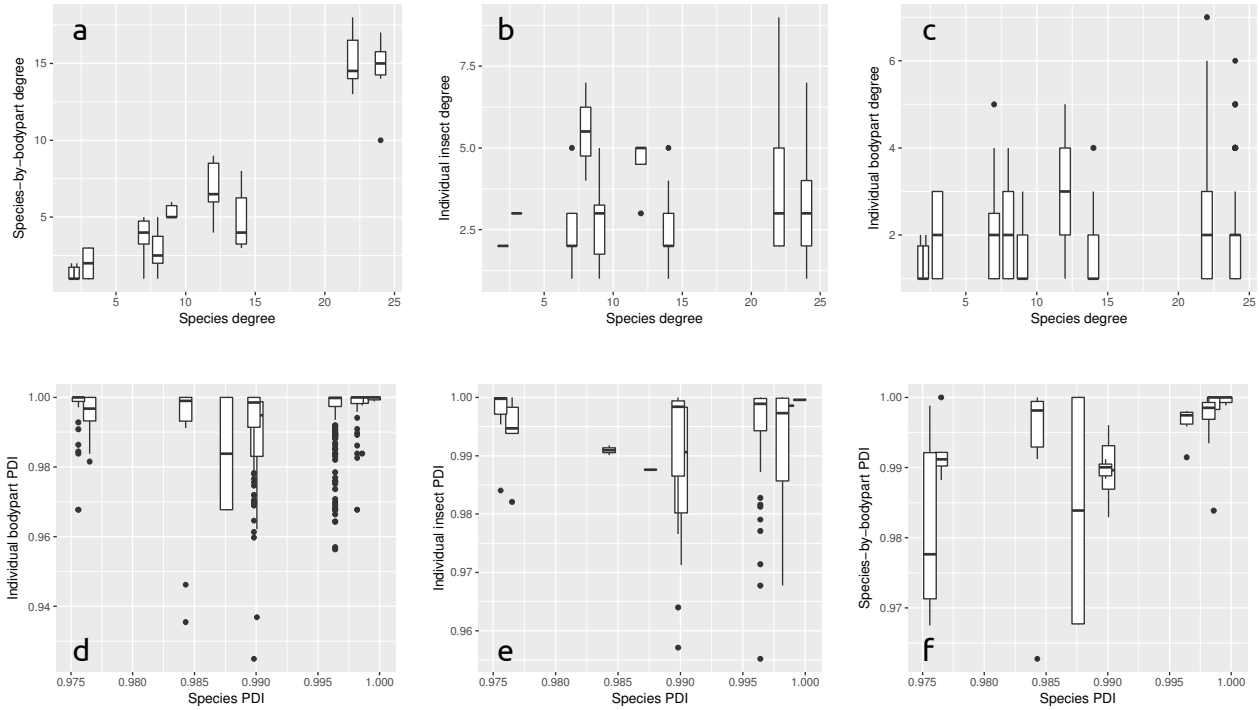
S2 Fig 2: Rarefaction curves for pollen carried by insect species in onion at different network scales.



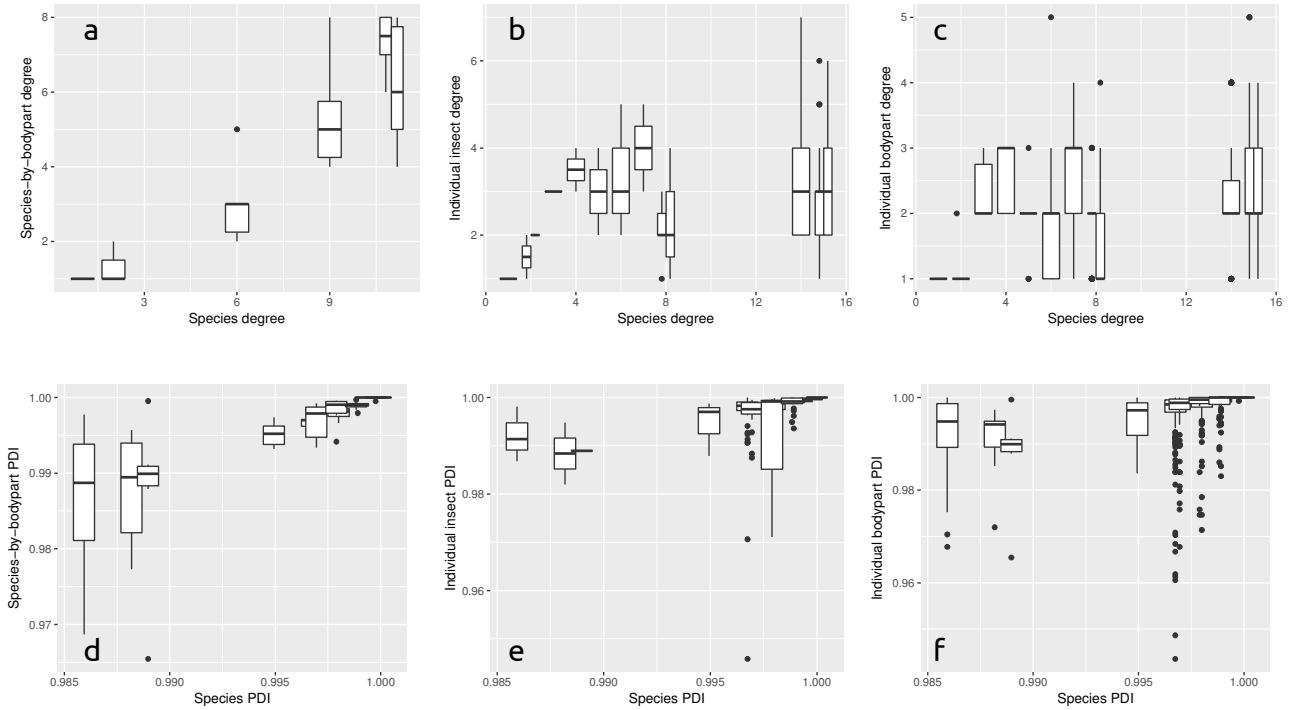
S2 Fig 3: Rarefaction curves for pollen carried by insect species in pak choi at different network scales.



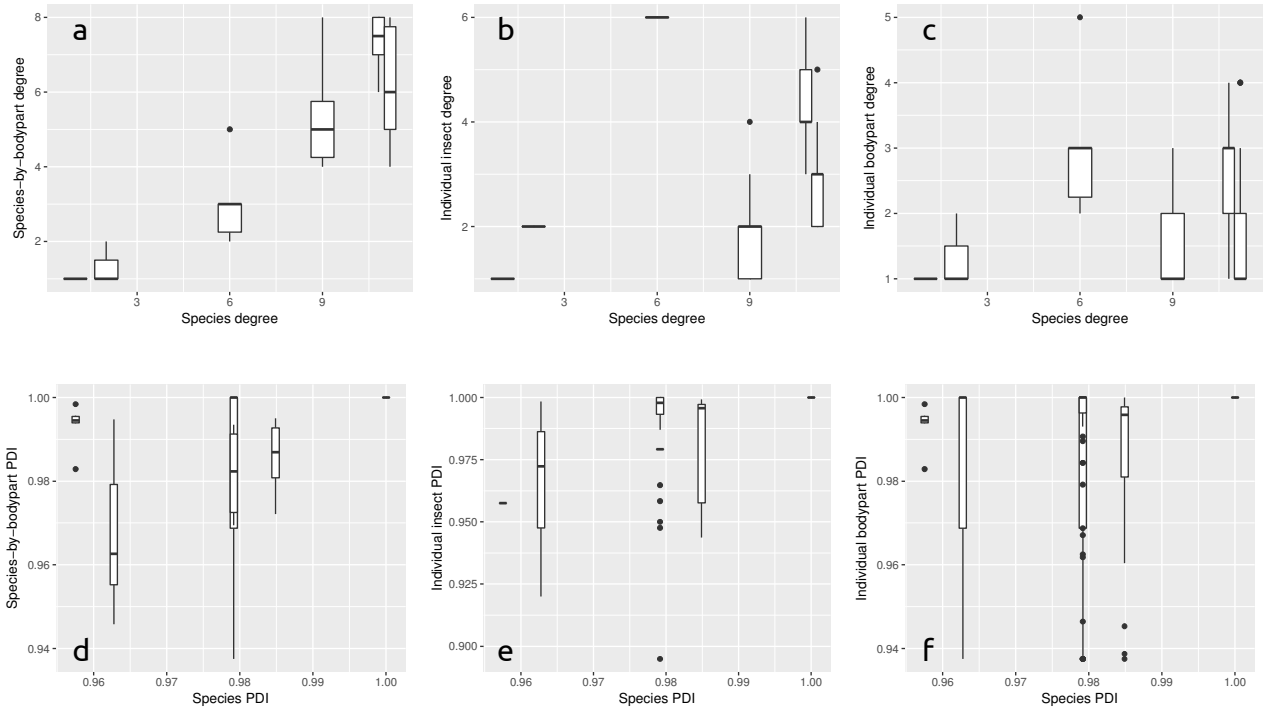
S2 Fig 4: Rarefaction curves for pollen carried by insect species in radish at different network scales.



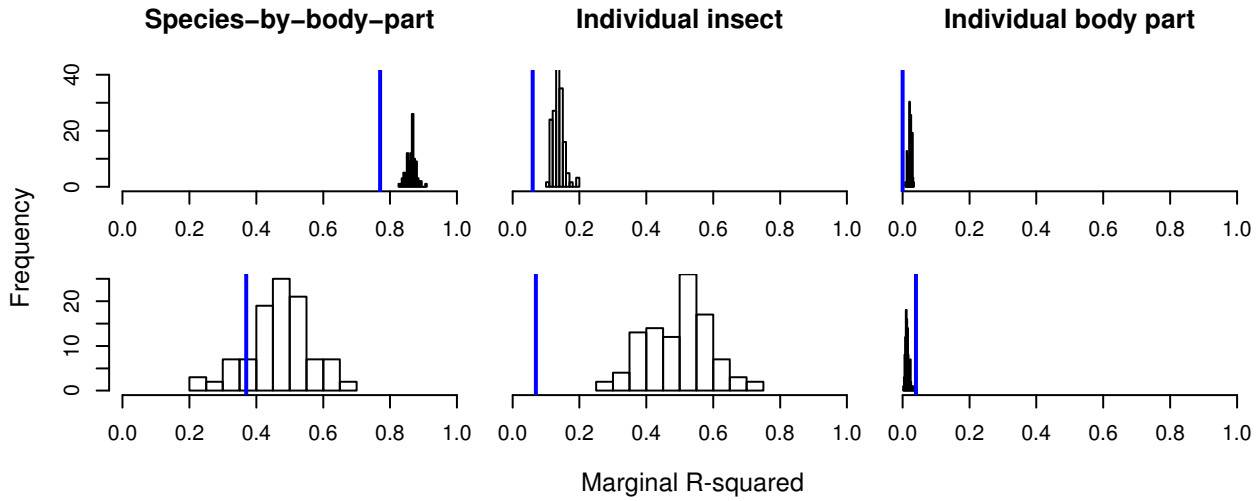
S2 Fig 5: Bar graph/scatter plots for binary (degree, top), and quantitative (PDI, bottom) measures of insect specialization samples from in onion. Left to right: species-level network versus aggregated by body part by species, species-level network versus aggregated by individual, species-level network versus individual body part. Each box represents the degree of an insect species.



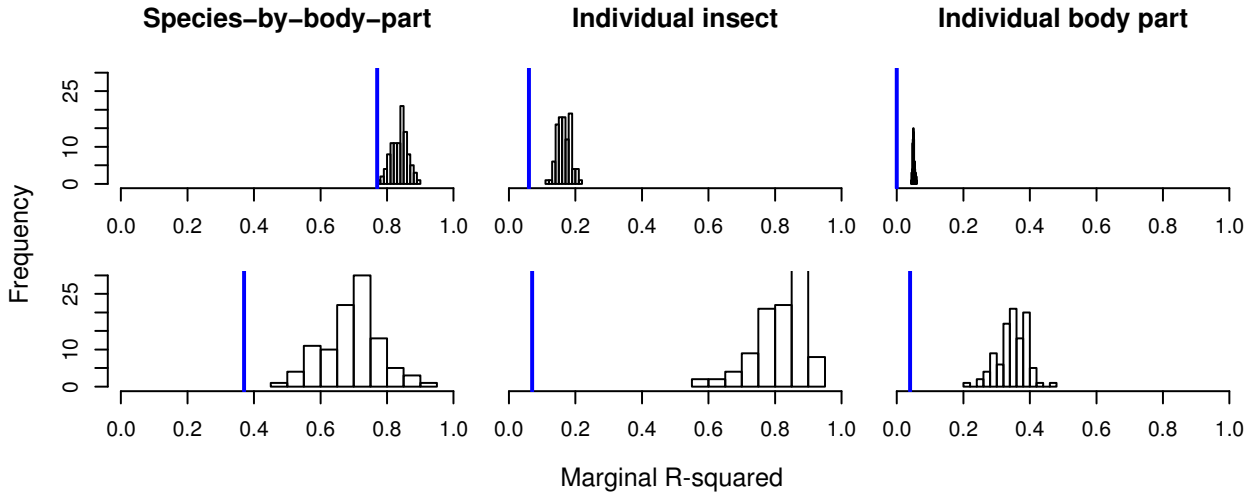
S2 Fig 6: Bar graph/scatter plots for binary (degree, top), and quantitative (PDI, bottom) measures of insect specialization samples from in pak choi. Left to right: species-level network versus aggregated by body part by species, species-level network versus aggregated by individual, species-level network versus individual body part. Each box represents the degree of an insect species.



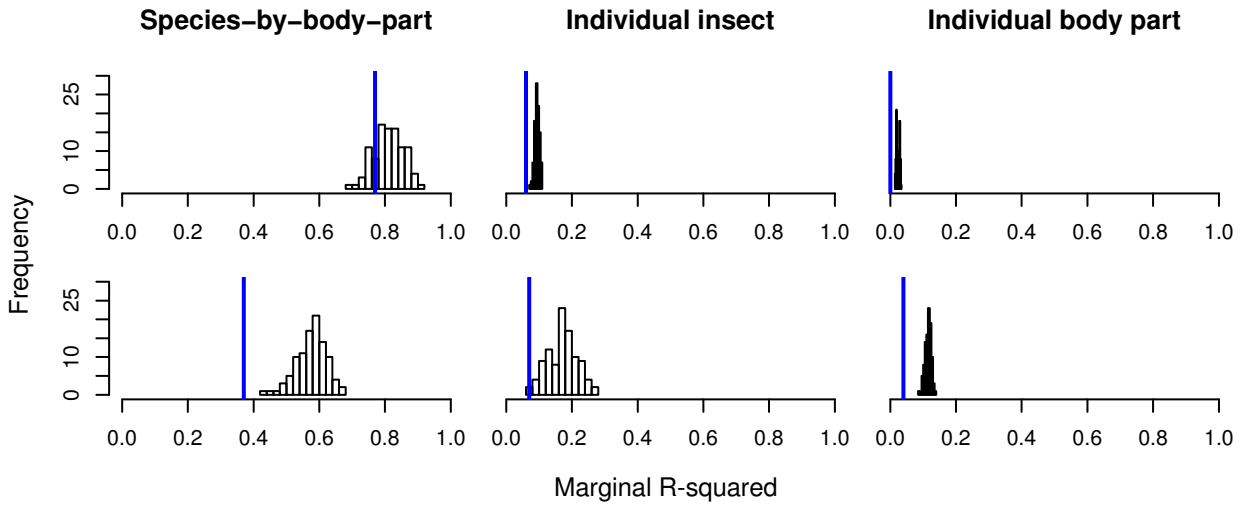
S2 Fig 7: Bar graph/scatter plots for binary (degree, top), and quantitative (PDI, bottom) measures of insect specialization samples from in radish. Left to right: species-level network versus aggregated by body part by species, species-level network versus aggregated by individual, species-level network versus individual body part. Each box represents the degree of an insect species.



S2 Fig 8: Histograms of the correlation (R^2m) between the degree (top) and PDI (bottom) of the species network and the degree and PDI of 100 randomly-generated null models for each of the three finer network scales for onion. Blue bars denote the correlation between the original species network and observed values at each scale.



S2 Fig 9: Histograms of the correlation (R^2m) between the degree (top) and PDI (bottom) of the species network and the degree and PDI of 100 randomly-generated null models for each of the three finer network scales for pak choi. Blue bars denote the correlation between the original species network and observed values at each scale.



S2 Fig 10: Histograms of the correlation (R^2m) between the degree (top) and PDI (bottom) of the species network and the degree and PDI of 100 randomly-generated null models for each of the three finer network scales for radish. Blue bars denote the correlation between the original species network and observed values at each scale.

S2 Table 1. Coefficients table from a Cox generalized linear mixed-effects model for pollen quantity (number of grains on a body part) predicted by insect body parts (a factor with six levels) and insect species (a factor with 17 levels) in carrot. The intercept condition is the bottom of the abdomen of a honey bee. The model had Poisson error. Insects with no variance in pollen grains observed per body part could not be assigned a p-value via the Wald method. A log-likelihood ratio test determined that the amount of pollen was predicted by body part (χ^2 = 152.9; P < 0.001), insect species (χ^2 = 86.9; P < 0.001), and their interaction (χ^2 = 260.0; P < 0.001).

	Estimate	SE	Z	P
bottom head	-1.054	0.307	-3.43	0.001 **
bottom thorax	-0.272	0.283	-0.96	0.336
top abdomen	1.159	0.272	4.27	< 0.001 ***
top head	0.455	0.278	1.64	0.101
top thorax	1.119	0.265	4.22	< 0.001 ***
Calliphora stygia	0.290	0.478	0.61	0.543
Melangyna novazealandiae	-0.176	0.520	-0.34	0.736
Bibionidae	0.196	1.046	0.19	0.852
Bombus terrestris	-28.772	1.425	-20.19	< 0.001 ***
Pollenia sp.	3.025	0.476	6.36	< 0.001 ***
Eristalis tenax	-1.720	0.503	-3.42	0.001 **
Calliphora vicina	1.166	0.924	1.26	0.207
Lucilia sp.	1.962	0.701	2.8	0.005 **
Lasioglossum sordidum	1.380	0.492	2.81	0.005 **
Muscidae	3.447	0.842	4.09	< 0.001 ***

Calliphora quadrimaculata	-1.594	1.659	-0.96	0.337
Melanostoma fasciatum	0.284	0.507	0.56	0.576
Odontomyia sp.	0.506	0.783	0.65	0.518
Oxysarcodexia varia	2.635	0.480	5.49	< 0.001 ***
Tachinidae	2.338	1.231	1.9	0.058 .
Scaptomyza flava	5.416	1.743	3.11	0.002 **
Xenocalliphora hortona	2.883	1.001	2.88	0.004 **
bottom head : *C. stygia*	0.296	0.484	0.61	0.541
bottom thorax : *C. stygia*	-1.340	0.461	-2.91	0.004 **
top abdomen : *C. stygia*	-0.706	0.428	-1.65	0.099 .
top head : *C. stygia*	-0.130	0.445	-0.29	0.770
top thorax : *C. stygia*	-1.632	0.434	-3.76	< 0.001 ***
bottom head : *M. novazealandiae*	2.046	0.509	4.02	< 0.001 ***
bottom thorax : *M. novazealandiae*	1.579	0.469	3.37	0.001 **
top abdomen : *M. novazealandiae*	-0.405	0.473	-0.86	0.391
top head : *M. novazealandiae*	0.877	0.489	1.79	0.073 .
top thorax : *M. novazealandiae*	0.582	0.461	1.26	0.206
bottom head : Bibionidae	-0.160	0.970	-0.17	0.869
bottom thorax : Bibionidae	-0.536	0.870	-0.62	0.538
top abdomen : Bibionidae	-1.838	0.863	-2.13	0.033 *
top head : Bibionidae	-0.892	0.871	-1.02	0.305
top thorax : Bibionidae	-2.424	0.956	-2.53	0.011 *
bottom head : *B. terrestris*	1.054	0.000	∞	
bottom thorax : *B. terrestris*	27.601	1.439	19.18	< 0.001 ***
top abdomen : *B. terrestris*	-1.159	0.000	-∞	
top head : *B. terrestris*	26.874	0.000	∞	
top thorax : *B. terrestris*	-1.119	0.000	-∞	
bottom head : *Pollenia* sp.	1.429	0.443	3.22	< 0.001 ***
bottom thorax : *Pollenia* sp.	0.398	0.428	0.93	0.352
top abdomen : *Pollenia* sp.	-0.739	0.424	-1.74	0.081 .
top head : *Pollenia* sp.	-0.082	0.426	-0.19	0.848
top thorax : *Pollenia* sp.	-1.051	0.424	-2.48	0.013 *
bottom head : *Eristalis tenax*	1.862	0.494	3.77	< 0.001 ***
bottom thorax : *Eristalis tenax*	1.396	0.473	2.95	0.003 **
top abdomen : *Eristalis tenax*	-0.346	0.482	-0.72	0.473

top head : *Eristalis tenax*	1.773	0.460	3.85	< 0.001 ***
top thorax : *Eristalis tenax*	1.143	0.448	2.55	0.011 *
bottom head : *Calliphora vicina*	0.082	0.837	0.1	0.922
bottom thorax : *Calliphora vicina*	0.150	0.791	0.19	0.849
top abdomen : *Calliphora vicina*	-0.674	0.764	-0.88	0.378
top head : *Calliphora vicina*	0.010	0.772	0.01	0.990
top thorax : *Calliphora vicina*	-0.673	0.766	-0.88	0.379
bottom head : *Lucilia* sp.	-0.176	0.649	-0.27	0.787
bottom thorax : *Lucilia* sp.	-0.154	0.612	-0.25	0.801
top abdomen : *Lucilia* sp.	-1.763	0.655	-2.69	0.007 **
top head : *Lucilia* sp.	-0.063	0.627	-0.1	0.920
top thorax : *Lucilia* sp.	-0.932	0.623	-1.5	0.135
bottom head : *L. sordidum*	1.886	0.480	3.93	< 0.001 ***
bottom thorax : *L. sordidum*	1.756	0.456	3.85	< 0.001 ***
top abdomen : *L. sordidum*	-0.747	0.463	-1.61	0.107
top head : *L. sordidum*	0.410	0.455	0.9	0.368
top thorax : *L. sordidum*	-0.135	0.449	-0.3	0.764
bottom head : Muscidae	2.180	0.722	3.02	0.003 **
bottom thorax : Muscidae	0.585	0.698	0.84	0.402
top abdomen : Muscidae	-0.833	0.700	-1.19	0.234
top head : Muscidae	0.017	0.729	0.02	0.981
top thorax : Muscidae	-0.823	0.697	-1.18	0.238
bottom head : *C. quadrimaculata*	2.401	1.450	1.66	0.098 .
bottom thorax : *C. quadrimaculata*	-26.806	0.000	-∞	
top abdomen : *C. quadrimaculata*	-28.237	0.000	-∞	
top head : *C. quadrimaculata*	1.973	1.443	1.37	0.172
top thorax : *C. quadrimaculata*	-0.707	1.439	-0.49	0.623
bottom head : *M. fasciatum*	3.423	0.495	6.92	< 0.001 ***
bottom thorax : *M. fasciatum*	1.304	0.480	2.72	0.007 **
top abdomen : *M. fasciatum*	-0.154	0.464	-0.33	0.739
top head : *M. fasciatum*	2.114	0.472	4.48	< 0.001 ***
top thorax : *M. fasciatum*	1.384	0.459	3.02	0.003 **
bottom head : *Odontomyia* sp.	0.642	0.745	0.86	0.389
bottom thorax : *Odontomyia* sp.	-0.459	0.739	-0.62	0.535
top abdomen : *Odontomyia* sp.	-0.461	0.696	-0.66	0.508

top head : *Odontomyia* sp.	-0.607	0.712	-0.85	0.394
top thorax : *Odontomyia* sp.	-0.811	0.688	-1.18	0.238
bottom head : *O. varia*	0.970	0.471	2.06	0.040 *
bottom thorax : *O. varia*	-0.253	0.453	-0.56	0.577
top abdomen : *O. varia*	-1.008	0.441	-2.29	0.022 *
top head : *O. varia*	0.205	0.441	0.46	0.643
top thorax : *O. varia*	-1.677	0.436	-3.84	< 0.001 ***
bottom head : Tachinidae	0.247	1.090	0.23	0.821
bottom thorax : Tachinidae	0.399	1.040	0.38	0.701
top abdomen : Tachinidae	-0.601	1.042	-0.58	0.564
top head : Tachinidae	-0.010	1.041	-0.01	0.992
top thorax : Tachinidae	-0.163	1.059	-0.15	0.877
bottom head : *S. flava*	1.054	1.447	0.73	0.466
bottom thorax : *S. flava*	0.272	1.442	0.19	0.850
top abdomen : *S. flava*	-2.346	1.441	-1.63	0.104
top head : *S. flava*	-0.455	1.441	-0.32	0.752
top thorax : *S. flava*	-1.119	1.439	-0.78	0.437
bottom head : *X. hortona*	1.736	0.883	1.97	0.049 *
bottom thorax : *X. hortona*	-0.451	0.914	-0.49	0.622
top abdomen : *X. hortona*	-0.862	0.861	-1.00	0.317
top head : *X. hortona*	-0.623	0.865	-0.72	0.471
top thorax : *X. hortona*	-2.239	0.887	-2.52	0.012 *

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 2. Coefficients table from a Cox generalized linear mixed-effects model for pollen quantity (number of grains on a body part) predicted by insect body parts (a factor with six levels) and insect species (a factor with 9 levels) in onion. The intercept condition is the bottom of the abdomen of a honey bee. The model had Poisson errors. A log-likelihood ratio test determined that the amount of pollen was predicted by body part (χ^2 = 71.3; P < 0.001), insect species (χ^2 = 1187.7; P < 0.001), and their interaction (χ^2 = 83.1; P < 0.001).

	Estimate	SE	Z	P
bottom head	-0.015	0.261	-0.06	0.955
bottom thorax	-0.346	0.269	-1.28	0.199
top abdomen	1.265	0.247	5.13	< 0.001 ***
top head	0.584	0.251	2.32	0.020 *
top thorax	0.780	0.260	3	0.003 **
Calliphora stygia	0.305	1.073	0.28	0.776

Bombus terrestris	-1.780	0.535	-3.33	0.001 **
Pollenia sp.	3.504	0.915	3.83	< 0.001 ***
Eristalis tenax	-2.573	1.592	-1.62	0.106
Eumerus funeralis	1.993	2.094	0.95	0.341
Lasioglossum sordidum	1.707	0.748	2.28	0.022 *
Delia platura	4.503	2.215	2.03	0.042 *
Odontomyia sp.	0.738	2.038	0.36	0.717
Oxysarcodexia varia	2.456	0.648	3.79	< 0.001 ***
bottom head : *C. stygia*	0.543	0.863	0.63	0.529
bottom thorax : *C. stygia*	-1.270	1.190	-1.07	0.286
top abdomen : *C. stygia*	-1.503	0.878	-1.71	0.087 .
top head : *C. stygia*	0.373	0.819	0.46	0.649
top thorax : *C. stygia*	-0.297	0.822	-0.36	0.718
bottom head : *B. terrestris*	-0.010	0.499	-0.02	0.984
bottom thorax : *B. terrestris*	0.767	0.477	1.61	0.108
top abdomen : *B. terrestris*	-0.653	0.470	-1.39	0.164
top head : *B. terrestris*	-0.684	0.514	-1.33	0.183
top thorax : *B. terrestris*	0.339	0.466	0.73	0.467
bottom head : *Pollenia* sp.	-0.732	0.714	-1.02	0.305
bottom thorax : *Pollenia* sp.	-0.710	0.667	-1.06	0.287
top abdomen : *Pollenia* sp.	-0.951	0.694	-1.37	0.170
top head : *Pollenia* sp.	0.667	0.678	0.98	0.325
top thorax : *Pollenia* sp.	-1.801	0.876	-2.06	0.040 *
bottom head : *E. tenax*	3.045	1.473	2.07	0.039 *
bottom thorax : *E. tenax*	3.376	1.475	2.29	0.022 *
top abdomen : *E. tenax*	2.502	1.564	1.6	0.110
top head : *E. tenax*	3.081	1.544	1.99	0.046 *
top thorax : *E. tenax*	4.020	1.277	3.15	0.002 **
bottom head : *E. funeralis*	2.148	1.445	1.49	0.137
bottom thorax : *E. funeralis*	0.299	1.440	0.21	0.835
top abdomen : *E. funeralis*	1.255	1.444	0.87	0.385
top head : *E. funeralis*	1.936	1.445	1.34	0.180
top thorax : *E. funeralis*	1.354	1.444	0.94	0.348
bottom head : *L. sordidum*	1.226	0.599	2.05	0.041 *
bottom thorax : *L. sordidum*	0.691	0.602	1.15	0.252
top abdomen : *L. sordidum*	-0.435	0.571	-0.76	0.446

top head : *L. sordidum*	0.825	0.589	1.4	0.161
top thorax : *L. sordidum*	-0.146	0.579	-0.25	0.801
bottom head : *D. platura*	0.853	1.441	0.59	0.554
bottom thorax : *D. platura*	3.162	1.446	2.19	0.029 *
top abdomen : *D. platura*	1.548	1.443	1.07	0.283
top head : *D. platura*	-0.130	1.438	-0.09	0.928
top thorax : *D. platura*	-0.326	1.439	-0.23	0.821
bottom head : *Odontomyia* sp.	0.709	1.441	0.49	0.623
bottom thorax : *Odontomyia* sp.	0.542	1.440	0.38	0.707
top abdomen : *Odontomyia* sp.	-0.014	1.437	-0.01	0.992
top head : *Odontomyia* sp.	0.667	1.439	0.46	0.643
top thorax : *Odontomyia* sp.	0.250	1.440	0.17	0.862
bottom head : *O. varia*	0.849	0.495	1.72	0.086 .
bottom thorax : *O. varia*	-0.117	0.520	-0.22	0.822
top abdomen : *O. varia*	-0.958	0.488	-1.96	0.050 *
top head : *O. varia*	0.508	0.480	1.06	0.290
top thorax : *O. varia*	-0.756	0.500	-1.51	0.130

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 3. Coefficients table from a generalized linear mixed-effects model for pollen quantity (number of grains on a body part) predicted by insect body parts (a factor with six levels) and insect species (a factor with 12 levels) in pak choi. The intercept condition is the bottom of the abdomen of a honey bee. The model had log-linked negative binomial errors.

	Estimate	SE	Z	P
(Intercept)	5.190	0.122	42.45	< 0.001 ***
bottom head	-0.043	0.127	-0.34	0.734
bottom thorax	-0.004	0.127	-0.03	0.974
top abdomen	-0.046	0.127	-0.36	0.720
top head	0.020	0.127	0.15	0.878
top thorax	-0.151	0.128	-1.18	0.237
Calliphora stygia	-0.559	0.473	-1.18	0.238
Melangyna novazealandiae	-3.219	0.531	-6.07	< 0.001 ***
Bibionidae	-0.410	0.162	-2.54	0.011 *
Hydrotaea rostrata	-1.567	0.663	-2.37	0.018 *
Bombus terrestris	0.093	0.176	0.53	0.598
Pollenia sp.	-2.384	0.419	-5.69	< 0.001 ***

Eristalis tenax	0.058	0.188	0.31	0.756
Hylaeus sp.	-4.514	0.961	-4.70	< 0.001 ***
Leioproctus sp.	0.401	0.390	-1.03	0.305
Lasioglossum sordidum	0.605	0.411	1.47	0.141
Melanostoma fasciatum	-3.396	0.763	-4.45	< 0.001 ***
Odontomyia sp.	-1.586	0.278	-5.71	< 0.001 ***
bottom head : *C. stygia*	0.743	0.495	1.50	0.133
bottom thorax : *C. stygia*	0.658	0.494	1.33	0.182
top abdomen : *C. stygia*	-0.151	0.499	-0.30	0.762
top head : *C. stygia*	0.125	0.486	0.26	0.798
top thorax : *C. stygia*	0.848	0.495	1.71	0.087 .
bottom head : *C. stygia*	1.047	0.564	1.85	0.064 .
bottom thorax : *C. stygia*	2.876	0.570	5.05	< 0.001 ***
top abdomen : *C. stygia*	-0.576	0.635	-0.91	0.364
top head : *C. stygia*	1.751	0.551	3.17	0.002 **
top thorax : *C. stygia*	0.717	0.575	1.25	0.212
bottom head : Bibionidae	0.242	0.168	1.44	0.151
bottom thorax : Bibionidae	0.460	0.168	2.74	0.006 **
top abdomen : Bibionidae	-0.161	0.169	-0.95	0.341
top head : Bibionidae	-0.016	0.169	-0.10	0.923
top thorax : Bibionidae	0.550	0.169	3.26	0.001 **
bottom head : *H. rostrata*	0.668	0.694	0.96	0.335
bottom thorax : *H. rostrata*	0.455	0.696	0.65	0.513
top abdomen : *H. rostrata*	-0.309	0.711	-0.43	0.664
top head : *H. rostrata*	-0.541	0.716	-0.76	0.450
top thorax : *H. rostrata*	1.841	0.687	2.68	0.007 **
bottom head : *B. terrestris*	0.082	0.183	0.45	0.655
bottom thorax : *B. terrestris*	-0.133	0.183	-0.73	0.468
top abdomen : *B. terrestris*	-0.073	0.183	-0.40	0.691
top head : *B. terrestris*	-0.063	0.183	-0.34	0.731
top thorax : *B. terrestris*	-0.163	0.184	-0.89	0.375
bottom head : *Pollenia* sp.	0.456	0.456	1.00	0.318
bottom thorax : *Pollenia* sp.	1.576	0.446	3.54	< 0.001 ***
top abdomen : *Pollenia* sp.	0.550	0.440	1.25	0.211
top head : *Pollenia* sp.	0.344	0.463	0.74	0.458
top thorax : *Pollenia* sp.	1.609	0.445	3.62	< 0.001 ***

bottom head : *E. tenax*	-0.065	0.197	-0.33	0.742
bottom thorax : *E. tenax*	-0.067	0.196	-0.34	0.732
top abdomen : *E. tenax*	-0.182	0.196	-0.93	0.352
top head : *E. tenax*	-0.193	0.197	-0.98	0.326
top thorax : *E. tenax*	-0.188	0.197	-0.96	0.339
bottom head : *Hylaeus* sp.	-0.660	1.402	-0.47	0.638
bottom thorax : *Hylaeus* sp.	-0.699	1.402	-0.50	0.618
top abdomen : *Hylaeus* sp.	0.046	1.210	0.04	0.970
top head : *Hylaeus* sp.	-0.723	1.402	-0.52	0.606
top thorax : *Hylaeus* sp.	0.151	1.210	0.12	0.901
bottom head : *Leioproctus* sp.	0.5704	0.406	1.41	0.160
bottom thorax : *Leioproctus* sp.	-0.4839	0.408	-1.19	0.236
top abdomen : *Leioproctus* sp.	0.4026	0.407	0.99	0.322
top head : *Leioproctus* sp.	0.4758	0.405	1.17	0.240
top thorax : *Leioproctus* sp.	-0.1731	0.419	-0.41	0.679
bottom head : *L. sordidum*	-1.550	0.449	-3.45	0.001 **
bottom thorax : *L. sordidum*	-1.513	0.454	-3.33	0.001 **
top abdomen : *L. sordidum*	-1.467	0.459	-3.20	0.001 **
top head : *L. sordidum*	-1.689	0.453	-3.73	< 0.001 ***
top thorax : *L. sordidum*	-1.541	0.450	-3.42	0.001 **
bottom head : *M. fasciatum*	-1.068	1.057	-1.01	0.312
bottom thorax : *M. fasciatum*	0.617	0.839	0.74	0.462
top abdomen : *M. fasciatum*	1.037	0.821	1.26	0.207
top head : *M. fasciatum*	0.594	0.839	0.71	0.479
top thorax : *M. fasciatum*	-1.660	1.272	-1.30	0.192
bottom head : *Odontomyia* sp.	1.572	0.304	5.16	< 0.001 ***
bottom thorax : *Odontomyia* sp.	1.039	0.293	3.55	< 0.001 ***
top abdomen : *Odontomyia* sp.	-0.597	0.297	-2.01	0.045 *
top head : *Odontomyia* sp.	0.812	0.290	2.80	0.005 **
top thorax : *Odontomyia* sp.	0.366	0.290	1.26	0.207

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 4. Coefficients table for radish from a Cox generalized linear mixed-effects model for pollen quantity (number of grains on a body part) predicted by insect body parts (a factor with six levels) and insect species (a factor with 5 levels) in radish. The intercept condition is the bottom of the abdomen of a honey bee. The model had Poisson errors. Insects with no variance in pollen

grains observed per body part could not be assigned a p-value via the Wald method. A log-likelihood ratio test determined that the amount of pollen was predicted by body part (χ^2 = 19.320; P = 0.002), insect species (χ^2 = 18.91; P = 0.002), and their interaction (χ^2 = 42.00; P = 0.018).

	Estimate	SE	Z	P
generalized linear mixbottom head	-0.542	0.419	-1.29	0.196
bottom thorax	-0.240	0.390	-0.61	0.539
top abdomen	0.090	0.392	0.23	0.818
top head	-0.332	0.389	-0.85	0.394
top thorax	0.325	0.384	0.85	0.398
Melangyna novazealandiae	-1.413	1.521	-0.93	0.353
Bombus terrestris	-1.894	0.790	-2.4	0.017 *
Pollenia sp.	0.758	1.533	0.49	0.621
Eristalis tenax	-28.821	411921.784	0.00	1.000
Melanostoma fasciatum	-1.449	0.530	-2.73	0.006 **
bottom head : *M. novazealandiae*	0.992	1.479	0.67	0.503
bottom thorax : *M. novazealandiae*	0.589	1.470	0.4	0.689
top abdomen *M. novazealandiae*	0.663	1.472	0.45	0.652
top head : *M. novazealandiae*	1.537	1.474	1.04	0.297
top thorax : *M. novazealandiae*	-0.347	1.466	-0.24	0.813
bottom head : *Bombus terrestris*	-1.621	1.208	-1.34	0.180
bottom thorax : *Bombus terrestris*	-0.105	0.864	-0.12	0.904
top abdomen : *Bombus terrestris*	-0.383	0.861	-0.44	0.656
top head : *Bombus terrestris*	-0.636	0.952	-0.67	0.504
top thorax : *Bombus terrestris*	-0.362	0.808	-0.45	0.654
bottom head : *Pollenia* sp.	1.723	1.478	1.17	0.244
bottom thorax : *Pollenia* sp.	1.421	1.470	0.97	0.334
top abdomen : *Pollenia* sp.	-0.090	1.467	-0.06	0.951
top head : *Pollenia* sp.	1.513	1.470	1.03	0.303
top thorax : *Pollenia* sp.	-0.837	1.467	-0.57	0.568
bottom head : *Eristalis tenax*	0.542	713517.536	0	
bottom thorax : *Eristalis tenax*	27.168	411921.784	0	
top abdomen : *Eristalis tenax*	-0.089	713518.052	0	
top head : *Eristalis tenax*	27.090	411921.784	0	
top thorax : *Eristalis tenax*	-0.324	0.000	-∞	
bottom head : *M. fasciatum*	1.870	0.528	3.54	< 0.001 ***
bottom thorax : *M. fasciatum*	1.496	0.504	2.97	0.003 **
top abdomen : *M. fasciatum*	0.263	0.503	0.52	0.602

top head : *M. fasciatum*	2.003	0.504	3.97	< 0.001 ***
top thorax : *M. fasciatum*	0.988	0.500	1.98	0.048 **

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 5. PERMANOVA results for pollen community on insect body parts in carrot.

	Df	**Sum of Squares**	**F**	**P**
Species	16	21.769	5.444	< 0.001 ***
Body	5	3.698	2.960	< 0.001 ***
Species:Body	77	14.580	0.758	1.000
Residual	605	151.191		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 6. PERMANOVA results for pollen community on insect body parts in onion.

	Df	**Sum of Squares**	**F**	**P**
Species	8	10.038	6.576	< 0.001 ***
Body	5	1.877	1.967	0.009 **
Species:Body	35	5.293	0.793	0.0952
Residual	478	91.209		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 7. PERMANOVA results for pollen community on insect body parts in pak choi.

	Df	**Sum of Squares**	**F**	**P**
Species	11	12.154	16.406	< 0.001 ***
Body	5	1.544	4.584	< 0.001 ***
Species:Body	51	6.284	1.830	< 0.001 ***
Residual	675	45.458		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 8. PERMANOVA results for pollen community on insect body parts in radish.

	Df	**Sum of Squares**	**F**	**P**
Species	4	3.509	3.380	< 0.001 ***
Body	5	0.595	0.458	0.996
Species:Body	18	2.838	0.608	0.995
Residual	74	19.204		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 9. PERMANOVA results for pollen on insect body parts in carrot.

	Df	Sum of Squares	F	P
Order	1	4.834	18.608	< 0.001 ***
Body	5	3.853	2.966	< 0.001 ***
Order:Body	5	2.764	2.128	0.002 **
Residual	692	179.787		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 10. PERMANOVA results for pollen on insect body parts in onion.

	Df	Sum of Squares	F	P
Order	1	2.329	9.697	< 0.001 ***
Body	5	1.886	1.908	0.009 **
Order:Body	5	0.699	0.490	0.837
Residual	515	103.503		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 11. PERMANOVA results for pollen on insect body parts in pak choi.

	Df	Sum of Squares	F	P
Order	1	3.811	47.332	< 0.001 ***
Body	5	1.437	3.570	< 0.001 ***
Order:Body	5	1.335	3.317	0.002 **
Residual	731	58.856		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 12. PERMANOVA results for pollen on insect body parts in radish.

	Df	Sum of Squares	F	P
Order	1	0.558	2.084	0.073
Body	5	0.593	0.443	0.996
Order:Body	5	0.918	0.687	0.883
Residual	90	24.078		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 13. PERMANOVA results for pollen on fly body parts in carrot.

	Df	Sum of Squares	F	P
Species	13	13.961	5.240	< 0.001 ***
Body	5	3.000	2.928	< 0.001 ***
Sex	2	4.174	10.185	< 0.001 ***
Species:Body	62	11.289	0.888	0.897
Species:Sex	10	9.998	4.879	< 0.001 ***
Body:Sex	10	1.592	0.777	0.886
Residual	386	79.106		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 14. PERMANOVA results for pollen on fly body parts in onion.

	Df	Sum of Squares	F	P
Species	5	3.964	3.914	< 0.001 ***
Body	5	0.762	0.752	0.779
Sex	2	0.672	1.658	0.112
Species:Body	20	3.181	0.785	0.872
Species:Sex	2	0.606	1.496	0.145
Body:Sex	10	1.059	0.523	0.990
Residual	30	6.078		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 15. PERMANOVA results for pollen on fly body parts in pak choi.

	Df	Sum of Squares	F	P
Species	7	7.497	11.916	< 0.001 ***
Body	5	2.146	4.774	< 0.001 ***
Sex	1	1.142	12.700	< 0.001 ***
Species:Body	31	4.034	1.448	0.022 *
Species:Sex	3	1.274	4.724	< 0.001 ***
Body:Sex	5	0.213	0.474	0.953
Residual	352	31.638		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 16. PERMANOVA results for pollen on fly body parts in radish.

	Df	Sum of Squares	F	P

Species	2	1.467	2.790	0.010 *
Body	5	0.785	0.597	0.928
Sex	2	2.729	5.190	< 0.001 ***
Species:Body	8	1.422	0.676	0.910
Species:Sex	0	0		
Body:Sex	9	1.523	0.644	0.948
Residual	30	7.887		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 17. Comparisons of whole network to finer scales with GLMMs in carrot

Comparison	**Metric**	**Fixed Effect**	**Estimate**	**Df**	**t**	**P**	**R^2m**	**R^2c**
Aggregate body part	degree	Intercept	-0.826 ± 0.853	18.588	-0.967	0.346	0.77	0.86
		whole	0.611 ± 0.054	17.938	11.433	< 0.001 ***		
	PDI	Intercept	0.392 ± 0.076	105.03	5.152	< 0.001 ***	0.37	0.37
		whole	0.604 ± 0.077	105.03	7.843	< 0.001 ***		
Individual insect	degree	Intercept	2.611 ± 0.587	20.550	4.446	< 0.001 ***	0.06	0.19
		whole	0.069 ± 0.031	12.291	2.217	0.046 **		
	PDI	Intercept	0.433 ± 0.143	209.04	3.028	0.003 **	0.07	0.07
		whole	0.564 ± 0.144	209.04	3.908	< 0.001 ***		
Individual body part	degree	Intercept	2.213 ± 0.345	15.540	6.386	< 0.001 ***	0.00	0.27
		whole	0.000 ± 0.021	13.031	-0.022	0.982		
	PDI	Intercept	0.590 ± 0.066	48.190	8.995	< 0.001 ***	0.04	0.06
		whole	0.407 ± 0.066	48.130	6.148	< 0.001 ***		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 18. Comparisons of whole network to finer scales with GLMMs in onion

Comparison	**Metric**	**Fixed Effect**	**Estimate**	**Df**	**t**	**P**	**R^2m**	**R^2c**
Aggregate body part	degree	Intercept	-0.907 ± 0.847	8.174	-1.070	0.315	0.85	0.92
		whole	0.651 ± 0.064	8.061	10.100	< 0.001 ***		
	PDI	Intercept	0.473 ± 0.148	6.996	3.198	0.015 *	0.21	0.24
		whole	0.524 ± 0.149	6.995	3.503	0.010 **		
Individual insect	degree	Intercept	1.704 ± 0.302	11.380	5.644	< 0.001 ***	0.01	0.16
		whole	0.016 ± 0.021	7.810	0.792	0.452		

	PDI	Intercept	1.030 ± 0.140	7.179	7.344	< 0.001 ***	0.00	0.01
		whole	-0.037 ± 0.141	7.083	-0.258	0.803		
Individual body part	degree	Intercept	2.818 ± 0.645	10.212	4.371	0.001 ***	0.01	0.12
		whole	0.031 ± 0.037	4.568	0.823	0.451		
	PDI	Intercept	0.875 ± 0.157	5.625	5.585	0.002 **	0.01	0.13
		whole	0.121 ± 0.158	5.628	0.764	0.476		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 19. Comparisons of whole network to finer scales with GLMMs in pak choi

Comparison	**Metric**	**Fixed Effect**	**Estimate**	**Df**	**t**	**P**	**R^2m**	**R^2c**
Aggregate body part	degree	Intercept	0.341 ± 0.298	11.000	1.143	0.277	0.82	0.83
		whole	0.554 ± 0.036	11.000	15.61	< 0.001 ***		
	PDI	Intercept	-0.049 ± 0.113	76.080	-0.435	0.665	0.53	0.53
		whole	1.049 ± 0.113	76.080	9.270	< 0.001 ***		
Individual insect	degree	Intercept	2.257 ± 0.390	14.206	5.780	< 0.001 ***	0.05	0.17
		whole	0.061 ± 0.036	6.747	1.690	0.136		
	PDI	Intercept	0.268 ± 0.221	130.080	1.211	0.228	0.08	0.08
		whole	0.731 ± 0.222	130.080	3.293	0.001 **		
Individual body part	degree	Intercept	1.510 ± 0.254	11.111	5.953	< 0.001 ***	0.06	0.35
		whole	0.055 ± 0.029	9.218	1.898	0.090		
	PDI	Intercept	0.249 ± 0.095	61.070	3.086	0.003 **	0.08	0.08
		whole	0.706 ± 0.095	60.690	7.396	< 0.001 ***		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S2 Table 20. Comparisons of whole network to finer scales with GLMMs in radish

Comparison	**Metric**	**Fixed Effect**	**Estimate**	**Df**	**t**	**P**	**R^2m**	**R^2c**
Aggregate body part	degree	Intercept	0.133 ± 0.525	4.884	0.253	0.801	0.79	0.81
		whole	0.573 ± 0.064	4.494	8.998	< 0.001 ***		
	PDI	Intercept	0.633 ± 0.322	3.889	1.968	0.123	0.09	0.38
		whole	0.361 ± 0.329	3.890	1.097	0.336		
Individual insect	degree	Intercept	1.987 ± 1.504	4.499	1.321	0.250	0.02	0.79
		whole	0.145 ± 0.181	3.824	0.801	0.470		
	PDI	Intercept	0.051 ± 0.380	47.00	0.133	0.895	0.11	0.11

		whole	0.952 ± 0.389	47.00	2.447	0.182 *		
Individual body part	degree	Intercept	1.346 ± 0.654	4.753	2.058	0.098 .	0.02	0.52
		whole	0.072 ± 0.079	4.166	0.905	0.415		
	PDI	Intercept	0.769 ± 0.188	4.799	4.086	0.010 **	0.01	0.05
		whole	0.226 ± 0.193	4.825	1.174	0.295		

Significance codes: * < 0.05, ** <0.01 *** <0.001

Appendix III: Supplementary material for Chapter IV

S3 Table 1: Plant species in the meta-analysis

Family	Species	Studies
Acanthaceae	Acanthus mollis	2
Actinidiaceae	Actinidia chinensis	3
Actinidiaceae	Actinidia deliciosa	4
Amaranthaceae	Bassia scoparia	1
Amaranthaceae	Beta vulgaris	2
Amaryllidaceae	Allium	1
Amaryllidaceae	Allium altyncolicum	1
Amaryllidaceae	Allium ampeloprasum	1
Amaryllidaceae	Allium cepa	2
Amaryllidaceae	Allium cernuum	1
Amaryllidaceae	Allium cyathophorum	1
Amaryllidaceae	Allium denudatum	1
Amaryllidaceae	Allium fistulosum	1
Amaryllidaceae	Allium flavescens	1
Amaryllidaceae	Allium insubricum	1
Amaryllidaceae	Allium kokanicum	1
Amaryllidaceae	Allium lusitanicum	1
Amaryllidaceae	Allium najafdaricum	1
Amaryllidaceae	Allium nutans	1
Amaryllidaceae	Allium obliquum	1
Amaryllidaceae	Allium oreoprasum	1
Amaryllidaceae	Allium paniculatum	1
Amaryllidaceae	Allium ramosum	1
Amaryllidaceae	Allium rupestre	1
Amaryllidaceae	Allium saxatile	1
Amaryllidaceae	Allium senescens	1
Amaryllidaceae	Allium sphaeroc	1
Amaryllidaceae	Allium stellarianum	1
Amaryllidaceae	Allium tuberosum	2
Amaryllidaceae	Allium turcomanicum	1
Amaryllidaceae	Amaryllis sp.	1
Amaryllidaceae	Ismene sp.	1
Amaryllidaceae	Narcissus	1
Amaryllidaceae	Narcissus poeticus	2
Amaryllidaceae	Narcissus pseudonarcissus	1
Amaryllidaceae	Tulbaghia violacea	1
Anacardiaceae	Anacardium occidentale	1
Anacardiaceae	Mangifera indica	8
Anacardiaceae	Pistacia atlantica	6
Anacardiaceae	Pistacia chinensis	1
Anacardiaceae	Pistacia integerrima	1
Anacardiaceae	Pistacia khinjuk	1
Anacardiaceae	Pistacia palaestina	1
Anacardiaceae	Pistacia sp.	1
Anacardiaceae	Pistacia terebinthus	6
Anacardiaceae	Pistacia vera	10
Annonaceae	Annona cherimola	5
Annonaceae	Annona cherimola x squamosa	1
Annonaceae	Annona squamosa	1
Apiaceae	Daucus carota subsp. sativus	1
Araceae	Anaphyllopsis americana	1
Araceae	Arum italicum	1
Araceae	Caladium x	1
Araceae	Monstera adansonii	1
Araceae	Montrichardia aborescens	1
Araceae	Montrichardia arborescens	1
Araceae	Philodendron melinonii	1
Araceae	Philodendron pedatum	1
Araceae	Philodendron solimoesense	1
Araceae	Philodrendron solimoesense	1
Araceae	Spathiphyllum floribundum	1

Family	Species	Studies
Araceae	Xanthosoma sagittifolium	1
Araliaceae	Panax ginseng	1
Araliaceae	Panax quinquefolium	1
Arecaceae	Chamaerops humilis	2
Arecaceae	Cocos nucifera	3
Arecaceae	Cocoz nucifera	1
Arecaceae	Elaeis guineensis	3
Arecaceae	Phoenix dacrylifera	1
Arecaceae	Phoenix dactylifera	4
Arecaceae	Phoenix reclinata	1
Arecaceae	Phoenix sylvestris	1
Arecaceae	Syagrus coronata	1
Arecaceae	Syagrus romanzoffiana	1
Arecaceae	Trachycarpus fortunei	1
Asparagaceae	Agave sp.	1
Asparagaceae	Asparagus officinalis	2
Asphodelaceae	Bulbine bulbosa	1
Asteraceae	Aster tripolium	1
Asteraceae	Chrysanthemum cinerariaefolium	1
Asteraceae	Chrysanthemum morifolium	1
Asteraceae	Chyrsanthenum pacificum	1
Asteraceae	Dendranthema grandiflorum	1
Asteraceae	Lactuca	2
Balsaminaceae	Impatiens glandulifera	1
Berberidaceae	Epimedium pubescens	1
Betulaceae	Alnus cordata	1
Betulaceae	Betula cordifolia	2
Betulaceae	Betula papyrifera	2
Betulaceae	Betula populifolia	1
Betulaceae	Betula sp.	1
Betulaceae	Betula verrucosa	1
Betulaceae	Corylus americana	1
Betulaceae	Corylus avellana	1
Bixaceae	Bixa orellana	1

Family	Species	Studies
Blandfordiaceae	Blandfordia grandiflora	1
Bombacaceae	Durio zibethinus	3
Boraginaceae	Borago officinalis	1
Brassicaceae	Arabidopsis thaliana	1
Brassicaceae	Brassica juncea	1
Brassicaceae	Brassica napus	4
Brassicaceae	Brassica oleracea	2
Brassicaceae	Brassica rapa	1
Brassicaceae	Brassica rapa x chinensis	1
Brassicaceae	Lepidium virginicum	1
Bromeliaceae	Aechmea chantinii	1
Bromeliaceae	Aechmea fasciata	2
Bromeliaceae	Ananas bracteatus	1
Bromeliaceae	Ananas comosus	1
Bromeliaceae	Guzmania	1
Bromeliaceae	Guzmania lingulata	1
Bromeliaceae	Guzmania x	1
Bromeliaceae	Pitcairnia herdee	1
Bromeliaceae	Tillandsia cyanea	1
Bromeliaceae	Vriesea	1
Bromeliaceae	Vriesea splendens	1
Bromeliaceae	Vriesea x	1
Burseraceae	Canarium schweinfurthii	1
Butomaceae	Butomus umbellatus	2
Cactaceae	Opuntia stricta	1
Capparaceae	Capparis spinosa	1
Caricaceae	Carica cauliflora	3
Caricaceae	Carica papaya	6
Caryophyllaceae	Cerastium uniflorum	1
Caryophyllaceae	Silene dioica	1
Casuarinaceae	Allocasuarina verticillata	1
Cistaceae	Cistus incanus	1
Combretaceae	Terminalia paniculata	1
Commelinaceae	Tradescantia virginiana	1
Convolvulaceae	Ipomoea batatas	1
Convolvulaceae	Ipomoea bonariensis	1

Family	Species	Studies
Convolvulaceae	Ipomoea pes-caprae ssp. brasilensis	1
Convolvulaceae	Merremia borneensis	1
Cornaceae	Cornus florida	1
Cucurbitaceae	Citrullus lanatus	4
Cucurbitaceae	Cucumis melo	1
Cucurbitaceae	Cucurbita maxima	1
Cucurbitaceae	Cucurbita moschata	2
Cucurbitaceae	Cucurbita pepo	4
Cucurbitaceae	Cucurbita sativus	1
Cucurbitaceae	Lagenaria siceraria	1
Cucurbitaceae	Momordica dioica	1
Cucurbitaceae	Praecitrullus fistulosus	1
Cupressaceae	Austrocedrus chilensis	1
Cupressaceae	Cupressus arizonica	1
Dioscoreaceae	Dioscorea alata	1
Dioscoreaceae	Dioscorea bulbifera	1
Dioscoreaceae	Dioscorea dumetorum	1
Dioscoreaceae	Dioscorea praehensilis	1
Dioscoreaceae	Dioscorea preussii	1
Dioscoreaceae	Dioscorea rotundata	2
Ebenaceae	Diospyros kaki	2
Ebenaceae	Diospyros lotus	1
Ebenaceae	Diospyros virginiana	1
Ericaceae	Vaccinium corymbosum	1
Euphorbiaceae	Hevea brasiliensis	1
Euphorbiaceae	Mercurialis annua	2
Euphorbiaceae	Ricinus communis	4
Fabaceae	Acacia auriculiformis	3
Fabaceae	Acacia baileyana	1
Fabaceae	Acacia brownii	1
Fabaceae	Acacia gracifolia	1
Fabaceae	Acacia iteaphylla	2
Fabaceae	Acacia karroo	1
Fabaceae	Acacia longifolia	1
Fabaceae	Acacia mangium	2

Family	Species	Studies
Fabaceae	Acacia mearnsiii	1
Fabaceae	Acacia retinodes	1
Fabaceae	Acacia rotundifolia	1
Fabaceae	Arachis batizocoi	1
Fabaceae	Arachis cardenasii	1
Fabaceae	Arachis chiquitana	1
Fabaceae	Arachis diogoi	1
Fabaceae	Arachis duranensis	1
Fabaceae	Arachis hoehnei	1
Fabaceae	Arachis hypogaea	4
Fabaceae	Arachis hypogea	1
Fabaceae	Arachis hypogeae	1
Fabaceae	Arachis ipaensis	1
Fabaceae	Arachis kempff-mercadoi	1
Fabaceae	Arachis major	1
Fabaceae	Arachis stenophylla	1
Fabaceae	Arachis stenosperma	1
Fabaceae	Cicer arietinum	2
Fabaceae	Clianthus formosus	1
Fabaceae	Crotalaria retusa	3
Fabaceae	Glycine canescens	1
Fabaceae	Glycine max	5
Fabaceae	Glycine tomentelia	1
Fabaceae	Lathyrus sativus	1
Fabaceae	Medicago sativa	2
Fabaceae	Pisum sativum	3
Fabaceae	Sesbania sesban	1
Fabaceae	Spartium junceum	2
Fabaceae	Trifolium pratense	1
Fabaceae	Trigonella foenum-graecum	1
Fabaceae	Vicia faba	1
Fagaceae	Castanea dentata	1
Fagaceae	Quercus coccinea	1
Fagaceae	Quercus ilex	1
Fagaceae	Quercus robur	1
Gentianaceae	Gentianella germanica	1
Geraniaceae	Geranium carolinianum	1

Family	Species	Studies
Haemodoraceae	Anigozanthos manglesii	1
Iridaceae	Crocus sativus	1
Iridaceae	Crocus variegatus	1
Iridaceae	Crocus vernus	1
Iridaceae	Crocus vernus subs. vernus	1
Iridaceae	Gladiolus hybridus	1
Iridaceae	Gladiolus sp.	1
Iridaceae	Gladiolus tristis	1
Iridaceae	Iris ensata	1
Iridaceae	Iris unguicularis	1
Juglandaceae	Carya illinoensis	5
Juglandaceae	Carya illinoinensis	1
Juglandaceae	Carya illinoisnensis	1
Juglandaceae	Juglans nigra	2
Juglandaceae	Juglans regia	3
Lamiaceae	Rosmarinus officinalis	1
Lamiaceae	Tectona grandis	1
Lauraceae	Persea americana	4
Liliaceae	Lilium auratum	1
Liliaceae	Lilium davidi	1
Liliaceae	Lilium formosanum	1
Liliaceae	Lilium japonicum	1
Liliaceae	Lilium longiflorum	5
Liliaceae	Lilium maculatum	1
Liliaceae	Lilium nobilissimum	1
Liliaceae	Lilium regale	1
Liliaceae	Lilium rubellum	1
Liliaceae	Lilium speciosum	1
Liliaceae	Lilium x	1
Liliaceae	Tulipa sp.	1
Lythraceae	Lagerstroemia fauriei	1
Lythraceae	Lagerstroemia indica	1
Lythraceae	Lagerstroemia limii	1
Lythraceae	Lagerstroemia speciosa	1
Lythraceae	Lagerstroemia subcostata	1
Lythraceae	Lagerstroemia x	1

Family	Species	Studies
Malvaceae	Alcea rosea	1
Malvaceae	Althaea officinalis	1
Malvaceae	Durio zibethinus	3
Malvaceae	Gossypium hirsutum	5
Malvaceae	Gossypium sp.	1
Malvaceae	Lavatera arborea	1
Moraceae	Cannabis sativa	2
Moraceae	Ficus carica	1
Myrtaceae	Acca sellowiana	1
Myrtaceae	Callistemon rigidus	1
Myrtaceae	Eucalyptus grandis	1
Myrtaceae	Eucalyptus marginata	1
Myrtaceae	Eucalyptus nitens	1
Myrtaceae	Eucalyptus regnans	1
Myrtaceae	Eucalyptus smithi	1
Myrtaceae	Kuznea pomifera	1
Myrtaceae	Melaleuca alternifolia	1
Myrtaceae	Myrtus communis	1
Myrtaceae	Syzygium aromaticum	1
Myrtaceae	Verticordia ethellana	1
Myrtaceae	Verticordia helichrysantha	1
Myrtaceae	Verticordia monadelpha	1
Myrtaceae	Verticordia picta	1
Myrtaceae	Verticordia sp.	1
Myrtaceae	Verticordia staminosa	1
Nothofagaceae	Nothofagus antarctica	1
Nothofagaceae	Nothofagus belutoides	1
Nothofagaceae	Nothofagus dombeyi	1
Nothofagaceae	Nothofagus obliqua	1
Nyctaginaceae	Mirabilis jalapa	1
Oleaceae	Fraxinus pennsylvanica	1
Oleaceae	Jasminum sumbac	1
Oleaceae	Nyctanthes arbor-tristis	1
Oleaceae	Olea europaea	7
Onagraceae	Oenothera organensis	1

Family	Species	Studies
Orchidaceae	Anacamptis morio	2
Orchidaceae	Anacamptis papilionacea	1
Orchidaceae	Anacamptis pyramidalis	1
Orchidaceae	Dactylorhiza fuchsii	1
Orchidaceae	Dactylorhiza maculata	1
Orchidaceae	Dactylorhiza sambucina	1
Orchidaceae	Luisia macrantha	1
Orchidaceae	Mystacidium venosum	1
Orchidaceae	Ophrys apifera	1
Orchidaceae	Ophrys bertolonii	1
Orchidaceae	Ophrys bombyliflora	1
Orchidaceae	Ophrys fusca	1
Orchidaceae	Ophrys incubacea	1
Orchidaceae	Ophrys lutea	1
Orchidaceae	Ophrys tenthredinifera	1
Orchidaceae	Orchis anthropophora	1
Orchidaceae	Orchis italica	1
Orchidaceae	Orchis mascula	2
Orchidaceae	Orchis provincialis	1
Orchidaceae	Serapias cordigera	1
Orchidaceae	Serapias vomeracea	1
Papaveraceae	Papaver rhoeas	3
Philesiaceae	Lapageria rosea	1
Phyllanthaceae	Hymenocardia acida	1
Phytolaccaceae	Phytolacca dodecandra	1
Pinaceae	Abies alba	2
Pinaceae	Abies cilicica	1
Pinaceae	Abies concolor	1
Pinaceae	Abies nordmanniana	1
Pinaceae	Abies numidica	1
Pinaceae	Abies pinsapo	2
Pinaceae	Abies procera	1
Pinaceae	Larix kaempferi	1
Pinaceae	Picea abies	4
Pinaceae	Picea glauca	1
Pinaceae	Picea omorika	1
Pinaceae	Picea pungens	1
Pinaceae	Picea sp.	1
Pinaceae	Pinus attenuata x radiata	1
Pinaceae	Pinus banksiana	1
Pinaceae	Pinus canariensis	1
Pinaceae	Pinus caribaea	1
Pinaceae	Pinus cembra	1
Pinaceae	Pinus contorta	1
Pinaceae	Pinus echinata	2
Pinaceae	Pinus elliottii	1
Pinaceae	Pinus greggii	1
Pinaceae	Pinus gregii var. australis	1
Pinaceae	Pinus griffithi	1
Pinaceae	Pinus koraiensis	2
Pinaceae	Pinus lambertiana	2
Pinaceae	Pinus mugo	1
Pinaceae	Pinus nigra	3
Pinaceae	Pinus patula	1
Pinaceae	Pinus peuce	1
Pinaceae	Pinus pinea	1
Pinaceae	Pinus ponderosa	3
Pinaceae	Pinus radiata	3
Pinaceae	Pinus resinosa	3
Pinaceae	Pinus silvestris	1
Pinaceae	Pinus sp.	1
Pinaceae	Pinus strobus	7
Pinaceae	Pinus sylvestris	4
Pinaceae	Pinus taeda	5
Pinaceae	Pinus tecunumanii	1
Pinaceae	Pinus uncinata	1
Pinaceae	Pseudotsuga menziesii	2
Plantaginaceae	Antirrhinum tortuosum	1
Plantaginaceae	Digitalis purpurea	1
Plumbaginaceae	Limonium perezii	1
Poaceae	Agrostis stolonifera	1
Poaceae	Avena sativa	1

Family	Species	Studies
Poaceae	Festuca arundinacea	3
Poaceae	Hordeum bulbosum	1
Poaceae	Hordeum vulgare	1
Poaceae	Hordeum vulgare ssp. spontaneum	1
Poaceae	Hordeum vulgare ssp. vulgare	1
Poaceae	Leymus chinensis	1
Poaceae	Oriza sativa	1
Poaceae	Oryza	1
Poaceae	Oryza rufipogon	1
Poaceae	Oryza rufipogon x sativa	1
Poaceae	Oryza sativa	4
Poaceae	Panicum virgatum	2
Poaceae	Paspalum vaginatum	1
Poaceae	Pennisetum glaucum	1
Poaceae	Poa pratensis	1
Poaceae	Saccharum officinarum	1
Poaceae	Secale cereale	2
Poaceae	Sorghum bicolor	4
Poaceae	Triticum aestivum	1
Poaceae	Triticum sp.	1
Poaceae	Zea mays	21
Poaceae	Zizania texana	1
Poaceae	Zoysia japonica	1
Polygonaceae	Fagopyrum esculentum	1
Polygonaceae	Rheum nobile	1
Potamogetonaceae	Althenia orientalis	1
Potamogetonaceae	Potamogeton crispus	1
Potamogetonaceae	Potamogeton distinctus	1
Potamogetonaceae	Potamogeton maackianus	1
Potamogetonaceae	Potamogeton malaianus	1
Potamogetonaceae	Potamogeton natans	1
Potamogetonaceae	Potamogeton pectinatus	1
Potamogetonaceae	Potamogeton perfoliatus	1

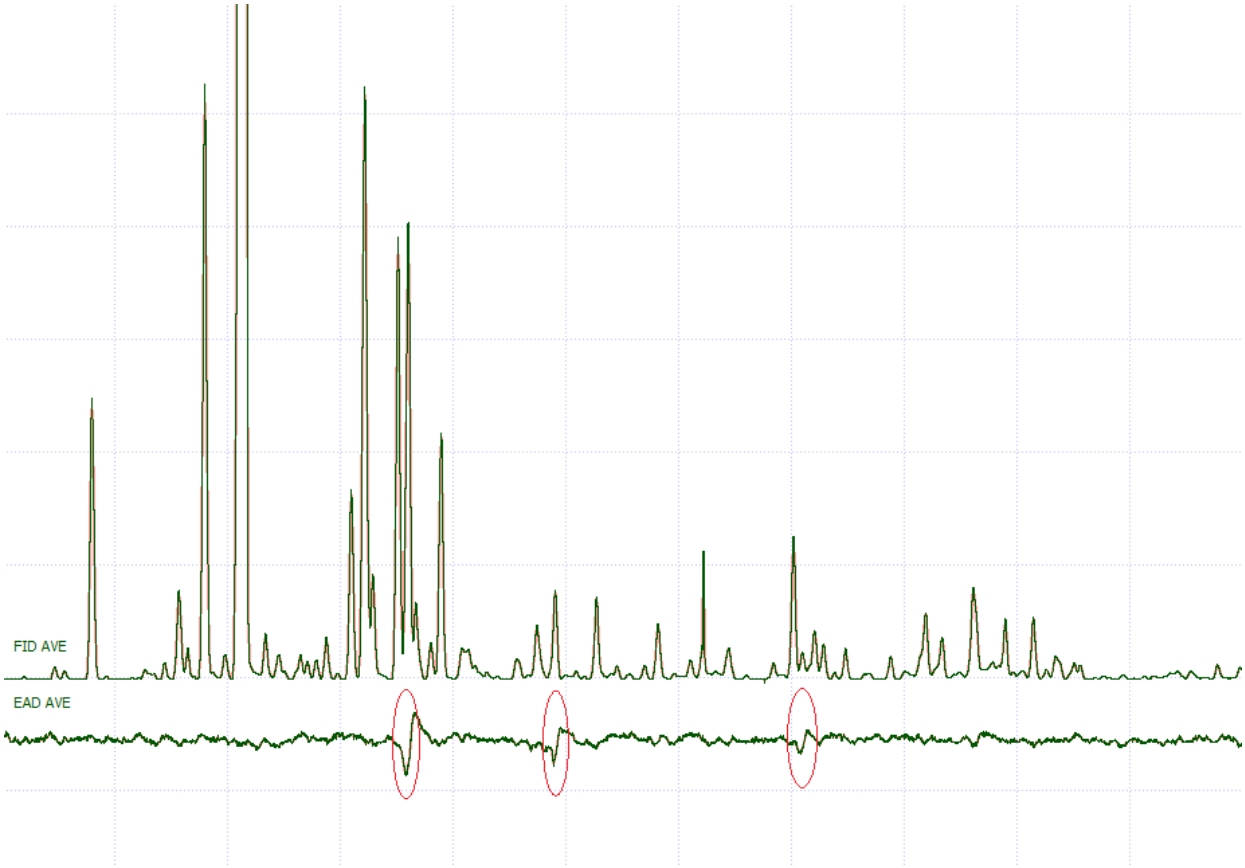
Family	Species	Studies
Primulaceae	Primula vulgaris	2
Proteaceae	Banksia menziesii	2
Proteaceae	Leucadendron conicum	1
Proteaceae	Leucadendron discolor	1
Proteaceae	Leucadendron salicifolium	1
Proteaceae	Macadamia integrifolia	1
Proteaceae	Protea amplexicaulis	1
Proteaceae	Protea humiflora	1
Proteaceae	Protea repens	1
Proteaceae	Telopea speciosissima	2
Ranunculaceae	Anemone rivularis	1
Ranunculaceae	Delphinium ajacis	1
Ranunculaceae	Delphinium belladonna	1
Ranunculaceae	Delphinium cardinale	1
Ranunculaceae	Delphinium cashmerianum	1
Ranunculaceae	Delphinium grandiflorum	1
Ranunculaceae	Delphinium hybridum	1
Ranunculaceae	Delphinium nudicaule	1
Ranunculaceae	Delphinium pentagynum	1
Ranunculaceae	Delphinium staphisagria	1
Ranunculaceae	Delphinium tatsienense	1
Ranunculaceae	Helleborus bocconei	1
Ranunculaceae	Helleborus foetidus	1
Ranunculaceae	Helleborus niger	1
Ranunculaceae	Ranunculus alpestris	1
Ranunculaceae	Ranunculus glacialis	1
Rosaceae	Amygdalus orientalis	1
Rosaceae	Amygdalus turcomanica	1
Rosaceae	Chaenomeles	1
Rosaceae	Cydonia oblonga	3

Family	Species	Studies
Rosaceae	Eriobotria japonica	1
Rosaceae	Fragaria √ó ananassa	2
Rosaceae	Fragaria ananassa	2
Rosaceae	Fragaria x ananassa	3
Rosaceae	Malus domestica	3
Rosaceae	Malus pumila	6
Rosaceae	Pistacia atlantica	6
Rosaceae	Potentilla fruticosa	1
Rosaceae	Potentilla rupestris	1
Rosaceae	Prunus amygdalus	2
Rosaceae	Prunus arabica	1
Rosaceae	Prunus armeniaca	3
Rosaceae	Prunus avium	8
Rosaceae	Prunus dulcis	8
Rosaceae	Prunus eleagnifolia	1
Rosaceae	Prunus glauca	1
Rosaceae	Prunus laurocerasus	1
Rosaceae	Prunus lycioides	1
Rosaceae	Prunus mume	1
Rosaceae	Prunus nigra	1
Rosaceae	Prunus orientalis	1
Rosaceae	Prunus persica	9
Rosaceae	Prunus reuteri	1
Rosaceae	Prunus salicina	4
Rosaceae	Prunus scoparia	1
Rosaceae	Prunus sp.	2
Rosaceae	Pyrus	1
Rosaceae	Pyrus commonis	1
Rosaceae	Pyrus communis	3
Rosaceae	Pyrus malus	1
Rosaceae	Pyrus phaeocarpa	1
Rosaceae	Pyrus pyrifolia	1
Rosaceae	Pyrus serotina	1
Rosaceae	Pyrus sp.	1
Rosaceae	Rosa	1
Rosaceae	Rosa canina	1
Rosaceae	Rosa damascena	1
Rosaceae	Rosa foetida	1
Rosaceae	Rosa hybrida	1

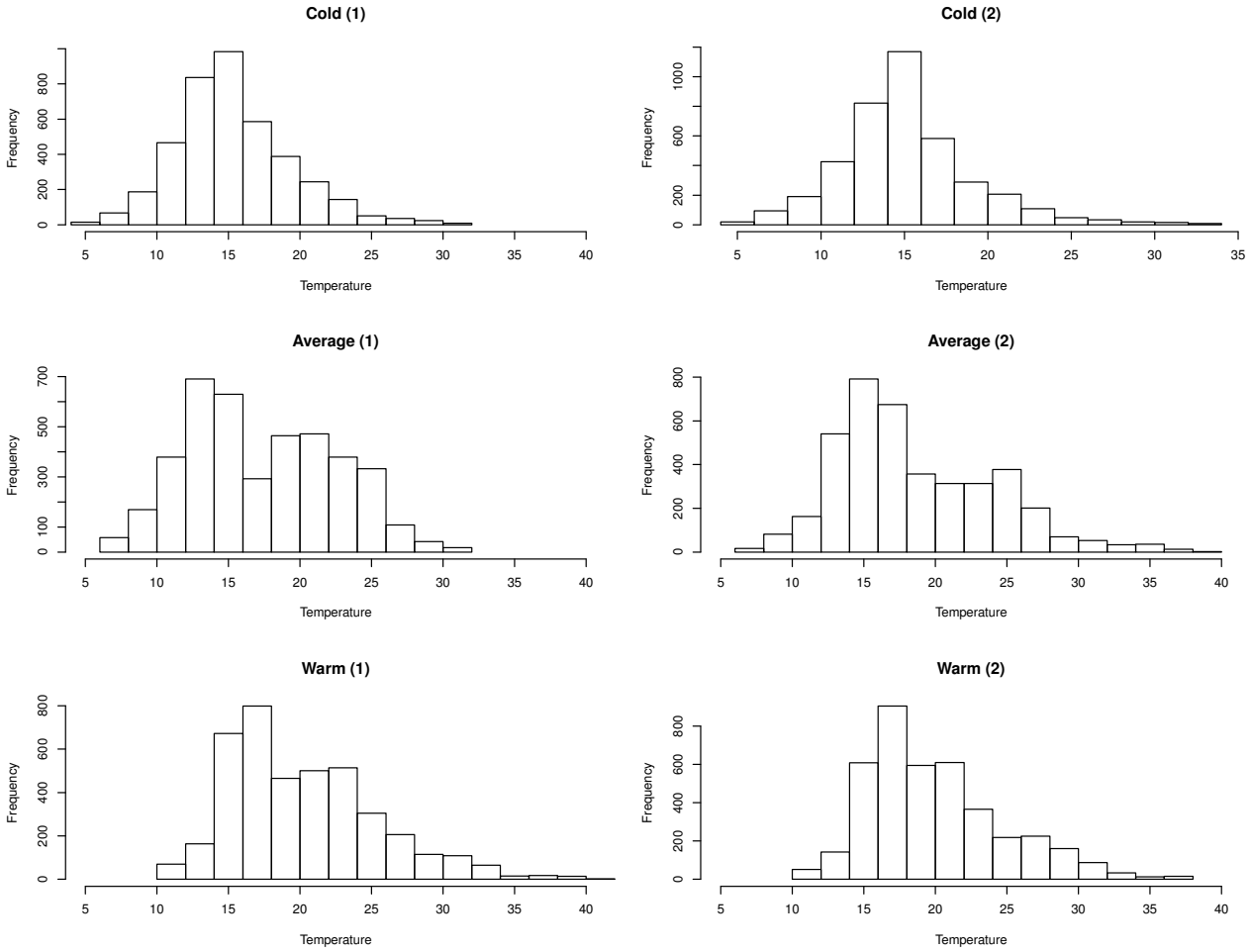
Family	Species	Studies
Rosaceae	Rosa moschata	1
Rosaceae	Rosa polyantha	1
Rosaceae	Rosa sp.	1
Rosaceae	Rosa x	1
Rosaceae	Rubus	1
Rosaceae	Rubus sp.	1
Rubiaceae	Coffea arabica	1
Ruppiaceae	Ruppia drepanensis	1
Ruppiaceae	Ruppia maritima	1
Rutaceae	Boronia	1
Rutaceae	Boronia crassipes	1
Rutaceae	Boronia crenulata	1
Rutaceae	Boronia deanei	1
Rutaceae	Boronia denticulata	1
Rutaceae	Boronia heterophylla	1
Rutaceae	Boronia megastigma	1
Rutaceae	Boronia molloyae	1
Rutaceae	Boronia purdieana	1
Rutaceae	Boronia ramosa	1
Rutaceae	Boronia stricta	1
Rutaceae	Boronia x	1
Rutaceae	Citrus √ó sinensis	1
Rutaceae	Citrus aurantifolia	1
Rutaceae	Citrus aurantium	2
Rutaceae	Citrus galgal	1
Rutaceae	Citrus grandis	2
Rutaceae	Citrus hassaku	2
Rutaceae	Citrus jambhiri	1
Rutaceae	Citrus karna	1
Rutaceae	Citrus limetta	1
Rutaceae	Citrus limettioides	1
Rutaceae	Citrus limon	5
Rutaceae	Citrus limonia	1
Rutaceae	Citrus natsudaidai	2
Rutaceae	Citrus paradisii	1
Rutaceae	Citrus pennivesiculata	1
Rutaceae	Citrus reticulata	2
Rutaceae	Citrus semperflorens	1
Rutaceae	Citrus sinensis	2

Family	Species	Studies
Rutaceae	Citrus x senensis	1
Rutaceae	Correa pulchella	1
Rutaceae	Poncirus trifoliata	1
Sapindaceae	Litchi chinensis	1
Saxifragaceae	Saxifraga bryoides	1
Saxifragaceae	Saxifraga caesia	1
Solanaceae	Cajanas cajan	1
Solanaceae	Calibrachoa caesia	1
Solanaceae	Calibrachoa humilis	1
Solanaceae	Calibrachoa ovalifolia	1
Solanaceae	Capsicum annuum	4
Solanaceae	Lycopersicon esculentum	5
Solanaceae	Lycopersicon pimpinellifolium	1
Solanaceae	Lycopersicum esculentum	2
Solanaceae	Nicotiana glutinosa	1
Solanaceae	Nicotiana tabacum	5
Solanaceae	Petunia hybrida	3
Solanaceae	Petunia x hybrida	1
Solanaceae	Pisum sativum	3
Solanaceae	Solanum alandiae	1
Solanaceae	Solanum ambosinum	1
Solanaceae	Solanum betaceum	1
Solanaceae	Solanum brachycarpum	1
Solanaceae	Solanum brevidens	2
Solanaceae	Solanum chacoense	1
Solanaceae	Solanum chancayense	1
Solanaceae	Solanum chomatophilum	1
Solanaceae	Solanum clarum	1
Solanaceae	Solanum colombianum	1
Solanaceae	Solanum gourlayi	1
Solanaceae	Solanum huancabambense	1
Solanaceae	Solanum kurtzianum	1
Solanaceae	Solanum lycopersicon	1
Solanaceae	Solanum lycopersicum	2
Solanaceae	Solanum marinasense	1
Solanaceae	Solanum melongena	2
Solanaceae	Solanum microdontum	1
Solanaceae	Solanum multidissectum	1
Solanaceae	Solanum oplocense	1
Solanaceae	Solanum pampasense	1
Solanaceae	Solanum pinnatisectum	1
Solanaceae	Solanum polyadenium	1
Solanaceae	Solanum raphanifolium	1
Solanaceae	Solanum stoloniferum	1
Solanaceae	Solanum tarijense	1
Solanaceae	Solanum trifidum	1
Solanaceae	Solanum tuberosum	6
Solanaceae	Solanum tuberosum x phureja	1
Solanaceae	Solanum venturri	1
Solanaceae	Solanum verrucosum	1
Solanaceae	Solanum x	1
Typhaceae	Typha latifolia	5
Ulmaceae	Ulmus carpinifolia	1
Ulmaceae	Ulmus glabra	1
Ulmaceae	Ulmus japonica	1
Ulmaceae	Ulmus laevis	1
Ulmaceae	Ulmus parvifolia	1
Ulmaceae	Ulmus villosa	1
Urticaceae	Parietaria judaica	2
Vitaceae	Vitis vinifera	5
Zingiberaceae	Curcuma alismatifolia	1
Zingiberaceae	Hedychium forrestii	1
Zingiberaceae	Hedychium x	1
Zingiberaceae	Zingiber officinale	1

Appendix IV: Supplementary material for Chapter V

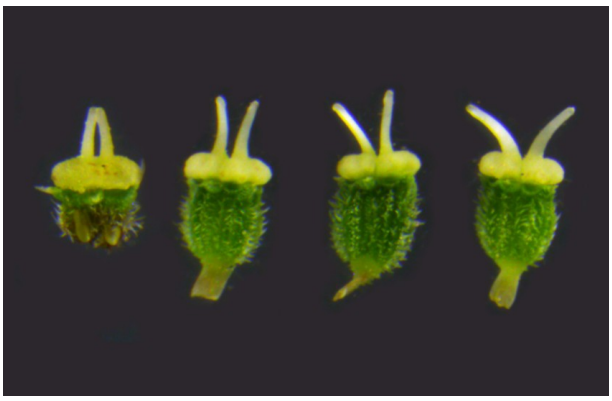


S4 Figure 1. Traces from the flame ionization detector (FID) of the gas chromatograph (top line) coupled with the electro-antennogram detector (EAD) responses (bottom line) from a honey bee antenna. Three electrophysiological responses were detectected from the EAD trace, circled in red: phenylacetaldehyde, nonanal, methyl salicylate, from left to right. The antenna was exposed to a carrot flower headspace sample that had been collected over a period of 24 hours.



S4 Figure 2. Temperatures experienced by each shadehouse and glasshouse treatment.

Histogram of temperatures collected at chest height from data loggers (onset HOBO Prov2 temp/RH meters) from the two enclosures for each of the three temperature treatments during the course of the experiment.



S4 Figure 3. Stages of development in carrot florets. Petals and anthers were removed from florets for photography.

S4 Table 1. Coefficients table of GLM for flower phenology. Relationship between time between sowing and blooming and the plant variety, and the plant line (male sterile vs. male fertile), with interactions and a gamma distribution. The intercept condition is the excellent variety, male sterile.

	Estimate	SE	t value	P value
intercept	3.373×10^{-3}	8.855×10^{-6}	380.908	< 0.001 ***
Variety (medium)	2.675×10^{-5}	1.274×10^{-5}	2.099	< 0.001 ***
Variety (poor)	4.577×10^{-5}	1.267×10^{-5}	3.611	0.036 *
Line (male fertile)	-9.521×10^{-5}	1.345×10^{-5}	-7.076	< 0.001 ***
Variety (medium) : Line (male fertile)	8.971×10^{-5}	1.945×10^{-5}	4.612	< 0.001 ***
Variety (poor) : Line (male fertile)	-1.783×10^{-4}	2.102×10^{-5}	-8.483	< 0.001 ***

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 2. Coefficients table of zero-inflated binomial GLMM for seed set. The final model retained temperature at the time of pollination, plant variety, and pollen viability as predictors of observed seed set per three umbellets. The intercept condition is the excellent variety.

	Estimate	SE	z value	P value
intercept	0.370	0.366	1.007	0.3138

Pollen Viability	-0.852	0.974	0.872	0.3833
Variety (medium)	0.973	0.347	2.788	0.0053 **
Variety (poor)	-0.319	0.362	0.877	0.3804
Temperature	1.118×10^{-4}	0.011	0.010	0.9918

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 3. Coefficients table of binomial GLMM for pollen viability. The final model retained temperature at the time of pollination, plant variety, and the number of days pollen was stored prior to processing as predictors of observed pollen viability in male fertile lines. The intercept condition is the excellent variety.

	Estimate	SE	z value	P value
intercept	-1.646	0.257	6.391	< 0.001 ***
Days Stored	-0.006	0.002	2.506	0.012 *
Variety (medium)	0.458	0.159	2.866	0.004 **
Variety (poor)	-0.012	0.204	0.057	0.954
Temperature	0.001	0.006	0.220	0.825

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 4. Coefficients table of LM for nectar glucose:fructose ratio. The intercept condition is nectar glucose (µg) per ½ umbel.

	Estimate	SE	t value	P value
intercept	58.365	21.387	2.729	0.007 **
Fructose (μg)	1.102	0.009	114.906	< 0.001 ***

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 5. Coefficients table of GLMM for nectar sugar composition. The final model retained time-of-day, temperature at the time of pollination, plant variety, and the interaction between time-

of-day and variety. The intercept condition is the excellent variety at the peak nectar emission time of 11:00am.

	Estimate	SE	t value	P value
intercept	1.237	1.469	0.842	0.400
Time (04:00)	1.371	1.107	1.238	0.216
Time (08:00)	0.246	1.085	0.226	0.821
Time (14:00)	-0.331	0.991	-0.334	0.739
Time (17:00)	0.491	0.994	0.494	0.622
Time (20:00)	1.312	1.039	1.263	0.206
Time (23:00)	0.826	1.095	0.755	0.450
Temperature	0.152	0.049	3.091	0.002 **
Variety (medium)	1.673	1.018	1.644	0.100
Variety (poor)	-0.224	1.037	-0.216	0.829
Time (04:00) : Variety (medium)	-2.782	1.427	-1.950	0.051 .
Time (08:00) : Variety (medium)	-2.426	1.395	-1.739	0.082 .
Time (14:00) : Variety (medium)	-2.178	1.409	-1.546	0.122
Time (17:00) : Variety (medium)	-1.623	1.423	-1.141	0.254
Time (20:00) : Variety (medium)	-1.843	1.423	-1.296	0.195
Time (23:00) : Variety (medium)	-1.383	1.379	-1.003	0.316
Time (04:00) : Variety (poor)	-0.478	1.438	-0.332	0.740
Time (08:00) : Variety (poor)	0.105	1.441	0.073	0.942
Time (14:00) : Variety (poor)	-1.113	1.404	-0.793	0.428
Time (17:00) : Variety (poor)	-0.610	1.415	-0.431	0.667
Time (20:00) : Variety (poor)	-2.301	1.412	-1.629	0.103
Time (23:00) : Variety (poor)	0.552	1.407	0.393	0.695

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 6. Coefficients table of ADONIS for nectar phenolic bouquet. The final model retained plant variety, temperature at the time of pollination, and time-of-day.

	Df	Sum of Sqs	F value	P value
Variety	2	6.695	3.393	0.011 *
Temperature	1	2.531	2.566	0.091 .
Time-of-day	6	6.814	1.151	0.294
Residual	226	222.960		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 7. Coefficients table of ADONIS for floral volatiles; variety trial.

	Df	Sum of Sqs	F value	P value
Variety	2	0.251	0.707	0.605
Residual	14	2.485		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 8. Coefficients table of ADONIS for floral volatiles; temperature trial.

	Df	Sum of Sqs	F value	P value
Temperature	1	0.441	3.128	0.013 *
Residual	16	2.256		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Table 9. Coefficients table of ADONIS for floral volatiles; time-of-day trial.

	Df	Sum of Sqs	F value	P value
Time	1	0.001	2.734	0.098 .
Residual	19	0.004		

Significance codes: * < 0.05, ** <0.01 *** <0.001

S4 Text 1. Preliminary carrot pollen viability sampling method and results

Carrot pollen was sampled for viability from a hybrid carrot field located near Waipara, Canterbury, New Zealand (43.055° S, 172.761° E) on 10 and 11 January 2014. Pollen samples were collected at 8 am, 11 am 2 pm, 5 pm 8 pm and 10 pm over two days. Six pollen dehiscing umbels were sampled at each time (except for 8 pm and 10 pm on 10 January where three umbels were sampled). From each umbel, three umbellets were sampled per umbel. They were selected from the outermost whorl, the innermost and a whorl equidistant from the outermost and innermost. On collection, florets were removed from each umbellet using forceps and placed directly into a cryotube that was sealed and placed immediately into into a dewar containing liquid N and transported to the laboratory for longer term storage in a -80°C freezer. Samples were assessed within three months for pollen viability. Methods for assessing the pollen viability are described in the methods section of this paper. We assessed the percentage pollen viability of 200 pollen grains per umbel. **The mean (±S. E.) pollen viability across all 66 samples was 9.1 ±1.4%.**