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The provision of pollination services to agroecosystems by a diverse assemblage of wild, unmanaged insect taxa

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For the degree of Doctor of Philosophy
in the School of Marine & Tropical Biology
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Table of contents

List of Tables

List of Figures

Abstract

The current global pollination crisis highlights the advantages of the provision of pollination services by a suite of core pollinators. Recent declines in honeybee populations have focused attention on the potential for unmanaged insects to replace them as pollinators of global food crops. The ability of unmanaged pollinators to replace services currently provided by honeybees requires understanding of their efficiency as pollinators as well as the spatial and temporal variability of their services, yet few quantitative assessments currently exist. In order to understand if unmanaged pollinator taxa are capable of providing equivalent pollination services to the honeybee I compared the pollination services provided by unmanaged taxa to the honeybee within a *Brassica rapa* mass flowering crop in a highly modified New Zealand agricultural landscape. First, I examined four functional traits of insect flower visitors in order to compare the pollination services provided: per visit pollen deposition, the probability of stigmatic contact, visitation rate, and capacity to transport pollen over distance. Second, I investigated how these traits vary between unmanaged pollinators and the honeybee both in space (between field locations) and time (within a day and between years) to examine whether unmanaged pollinators are capable of providing consistent, stable pollination services to a *B. rapa* mass flowering agricultural crop.

I observed a total of 41 managed and unmanaged taxa visiting flowers of *B. rapa*. In addition to *A. mellifera,* seven insect species visited flowers frequently. These were three other bees: *Lasioglossum sordidum*, *Bombus terrestris* and *Leioproctus* sp.; and four flies: *Dilophus nigrostigma*, *Melanostoma fasciatum*, *Melangyna novae-*

zelandiae and *Eristalis tenax*. Two bee species, *Bombus terrestris* and *Leioproctus* sp. and one fly, *Eristalis tenax* were as efficient as the honeybee and as effective (in terms of rate of flower visitation). A higher honeybee abundance however, resulted in it being the more effective pollinator overall. All frequently visiting pollinators were consistent in their visits over a four year period, even though individually visit frequencies varied between taxa. The pooled services of the unmanaged taxa were equal to or better than the honeybee in three of the four years.

Pollinators were active at different times of the day. Most unmanaged fly taxa were most active early in the morning and late in the afternoon/evening. Managed and unmanaged bee taxa and one fly (*D. nigrostigma*) were more active in the middle of the day. Overall visitation rate did not differ significantly between the hours within a day, indicating that changing taxonomic composition in assemblage structure was not accompanied by changes in potential pollination services. The contributions to visitation rate provided by fly taxa outside of standard survey hours resulted in a higher visitation rate at the end of the day.

Both managed and unmanaged pollinators transported viable pollen outside of a *B. rapa* crop. These consisted of three species from two bee families (Hymenoptera: Apidae and Halictidae), and seven species from four fly families (Diptera: Bibiondae, Stratiomyidae, Syrphidae and Tachinidae). Pollen viability varied between insect families and declined with distance from the crop but was nonetheless carried to at least 300m outside of the crop.

The results of this study suggest alternative pollinator taxa are capable of performing pollination services in a mass flowering crop equal to or better than the managed European honeybee. Alternative land management practices that increase the population sizes of unmanaged pollinator taxa to levels resulting in visitation frequencies as high as *A. mellifera,* have the potential to replace services provided by the honeybee. At the time of this study the *Varroa* sp. mite had not been recorded in the Canterbury region of New Zealand, where this study was conducted. It has since been recorded in this area, representing a significant possible threat to pollination services and hence crop production. My results have direct application suggesting that pollination services historically provided by managed honeybees might be replaced by those provided by other existing unmanaged pollinator taxa. Evidence from my study suggests that increases in the abundances of these alternative taxa should translate into greater pollination services.

To increase the population sizes of unmanaged pollinator taxa, I suggest “in-situ” management. This will require a change in land management practices in order to ensure year round refuge, feeding, nesting and other resource requirements of pollinator taxa are met.

Chapter 1: General Introduction

"If we didn't have pollinators, the plant world would deteriorate rapidly"- E.O. Wilson

Why study unmanaged insect pollinators?

Pollination is an ecosystem service that maintains function in natural systems as well as enhances the production of many crops (Free, 1993; Greenleaf and Kremen, 2006a). About 75% of the leading global food crops benefit from pollination, which represents at least 35% of global production volumes (Kremen *et al.*, 2007). The value of these pollination services are estimated to be worth over 100 billion dollars globally (De Marco and Coelho, 2004).

For decades, the managed European honeybee (*Apis mellifera* Linnaeus, 1758) has provided high quality pollination services to a range of global food crops (Klein *et al.*, 2007a). Many agricultural crops are currently wholly reliant on this single pollinator (Klein *et al.*, 2007a). In its absence, yields of some fruit, seed and nut crops decrease by up to 90% (Southwick and Southwick, 1992).

The combined impact of the *Varroa destructor* mite and Colony Collapse Disorder however, has led to a global decline in honeybee populations (Oldroyd, 2007; Stokstad, 2007). This decline has sparked renewed interest in the role of native insects as 'alternative' pollinators (Klein *et al.*, 2003; Greenleaf and Kremen, 2006a; Rader *et al.*, 2009) due to their provision of "pollination insurance" (Winfree *et al.*, 2007). Pollination insurance is provided by a suite of taxonomically diverse insect floral visitors attracted to agricultural fields that perform pollination roles (Walther-

Hellwig and Frankl, 2000; Westphal *et al.*, 2003; Morandin and Winston, 2005; Jauker and Wolters, 2008). These insects are "unmanaged" in that, unlike the honeybee, they are not actively transported in hives by people (Winfree, 2008) nor are their population sizes artificially maintained at high levels by way of intensive husbandry.

Habitat loss and fragmentation, however, has led to declines in unmanaged pollinator abundance and species richness in some pollinator communities (Kremen *et al.*, 2002; Thomas *et al.*, 2004; Ockinger and Smith, 2006). For example, the diversity and abundance of social bee pollinators is positively associated with the proportion of natural vegetation in a landscape (Kremen *et al.*, 2002; Klein *et al.*, 2003; Chacoff and Aizen, 2006; Greenleaf and Kremen, 2006a; Winfree *et al.*, 2007). As a result of this association, many social bee pollinators respond poorly to land use intensification (Ricketts *et al.*, 2008) as many of the resources are too transient (Corbet, 2000; Ricketts *et al.*, 2008; Winfree, 2008). Other taxa however, including solitary bees and flies, are capable of utilizing transient resources and can provide pollination services in agriculturally intensive locations isolated from natural habitats (Steffan-Dewenter, 2002; Westphal *et al.*, 2003; Brosi *et al.*, 2007; Winfree, 2007; Winfree *et al.*, 2008). For example, mass flowering crops can support diverse pollinator assemblages (Westphal *et al.*, 2003; Rader *et al.*, 2009) and bare earth associated with cultivated fields is a resource used by some bee taxa for nesting (Winfree, 2007).

Knowledge of the range of taxa that attend crops as well as their relative contribution to total pollination services is necessary in order to determine the potential for unmanaged taxa to act as potential honeybee replacements in the event of honeybee decline. In order to be economically viable, the contribution to pollination services by unmanaged taxa must be equivalent, or superior to the managed honeybee (Allsopp *et al.*, 2008). To accurately compare unmanaged to managed pollination services, four fundamental traits of pollinators need to be investigated. These are: (i) the efficiency in which pollen is transferred (i.e. pollen transfer efficiency), (ii) the frequency in which flowers are visited (pollinator visitation rate), (iii) the consistency in which flowers are visited over time (pollinator consistency) and (iv) the capacity to transfer viable pollen over distance (pollinator mobility).

Pollinator efficiency

Pollinator “efficiency” is defined as the capacity to transfer pollen (e.g. Kearns and Inouye, 1993; Madjidian *et al.*, 2008). It is often described as the quality of the pollination service (the type and amount of pollen transferred) provided by a single animal and it is generally assessed by examining stigmatic pollen loads (e.g. Kearns and Inouye, 1993; Madjidian *et al.*, 2008), although other measures exist. I use stigmantic pollen loads in this thesis, as well as the probability of stigmatic contact per flower visit as an additional component of efficiency. This is because not every visit results in a stigmatic contact; hence the proportion of successful stigmatic contacts will mediate the resulting efficiency of particular taxa.

Pollinator visitation rate

Pollinator visitation rate is defined as both the number of flowers visited per minute and the number of visits received per flower per time interval observed (Herrera, 1987; Herrera, 1989; Vazquez *et al.*, 2005; Madjidian *et al.*, 2008). It is an important quantitative component affecting pollination success (Herrera, 2000) and is therefore often used as a proxy for determining the contribution of individual taxa to total pollination services (Vazquez *et al.*, 2005).

Overall pollinator effectiveness is estimated by combining the efficiency of the pollinator (behavioural and morphological traits associated with individuals) with some measure of visitation rate to a population of flowers (a function of pollinator taxon population size (Herrera, 1987). It has been defined as the total contribution to plant fitness provided by particular taxa (Herrera, 1987) and is used in this study to ascertain total pollination services contributed by specific taxa.

Several studies have already demonstrated that unmanaged bee taxa can be superior pollinators to honeybees for some crop species (Klein *et al.*, 2003; Kremen *et al.*, 2004; Greenleaf and Kremen, 2006a; Greenleaf and Kremen, 2006b). For example native bees were responsible for a significantly greater number (62%) of flower visits than honeybees and were thus more effective pollinators of watermelon in North America (Winfree *et al.*, 2008). These studies however, concentrate on specific taxa and their association with native vegetation. Few studies exist which directly compare the efficiency and overall effectiveness of the honeybee to unmanaged taxa in intensive agricultural systems.

Pollinator consistency

While previous evidence has shown that unmanaged taxa provide services comparable to the honeybee with respect to pollen transfer (Rader *et al.*, 2009) and visitation rate (Winfree *et al.*, 2008) these studies have assessed alternative pollinators in crops over short periods (i.e. 1 year). Of equal importance in the widespread utilization of unmanaged taxa as crop pollinators in agricultural systems is their ability to provide reliable pollination services over time (Winfree and Kremen, 2009).

Unmanaged pollinator diversity potentially facilitates reliability in unmanaged assemblages (Kremen *et al.*, 2002; Ricketts, 2004). First, this is because assemblages consisting of a range of taxa increases the chance that an effective pollinator is present (Tilman *et al.*, 1997). Second, diverse assemblages ensure that fluctuations in the population size of any one individual species is buffered by the range of responses experienced across all species (Yachi and Loreau, 1999; McCann, 2000; Elmqvist *et al.*, 2003; Ricketts *et al.*, 2008). For example, Kremen *et al.* (2002) demonstrate that year-to-year variation in pollinator community composition had the capacity to buffer watermelon crops against population fluctuations of any one given pollinator species in any given year.

This capacity for diverse assemblages to provide reliable pollination services over time is a result of trait variation among taxa. Trait variation is the result of both intrinsic biological attributes as well as extrinsic pressures from the surrounding environment. Intrinsic traits include dietary preferences, foraging behaviours and thermoregulatory abilities all of which may differ between taxa and give rise to differences in population response to change (Roulston and Cane, 2000; Pereboom and Biesmeijer, 2003; Goulson and Darvill, 2004). Extrinsic factors include environmental pressures such as ambient temperature, wind velocity and direction (Stone, 1994; Comba, 1999; Stone *et al.*, 1999), pollen and nectar availability (Stone *et al.*, 1996; Stone *et al.*, 1998) as well as population pressures such as competition (Pinkus-Rendon *et al.*, 2005). The combination of both intrinsic traits and external pressures set species-specific tolerances (Herrera, 1990). The upper and lower limits of these tolerances present in the form of 'daily activity windows'. Activity windows determine the timing and duration of species-specific visits (Schlising, 1970; Gilbert, 1985; Hoehn *et al.*, 2008). Reliabilty in pollination services over time is therefore achieved via this variation in the timing and duration of species-specific visits, because it ensures the presence of pollinator taxa to perform the service irrespective of changing environmental conditions over time.

Pollinator mobility

Dispersal distances of insect pollinators are critical in defining their contribution to landscape-wide pollen movement and ultimately gene flow in natural and agricultural systems. The landscape context in which pollen mediated gene flow occurs however, is important in determining its potential impact. For example, In natural systems, pollen-mediated gene flow between native plant populations is beneficial as it facilitates gene flow between remnants, including isolated trees existing within agricultural or urban land uses (Ellstrand, 1992; Richards, 2000; Volis *et al.*, 2005; Ottewell *et al.*, 2009). In agricultural systems however, pollen-mediated gene flow between weeds or crop cultivars in agricultural systems can be undesirable due to facilitation of weed invasion and/or reduced purity in seed crops (Lavigne *et al.*, 2002; Wilkinson *et al.*, 2003; Fenart *et al.*, 2007), particularly in relation to the potential hybridization of genetically modified crops (Scheffler *et al.*, 1993; Rieger *et al.*, 2002; Devaux *et al.*, 2008).

While efficiency, effectiveness and temporal consistency are important, an insect must also be mobile for effective cross-pollination to occur (Kremen *et al.*, 2007). Further, of the many insect visitors that are mobile and carry pollen, not all are necessarily involved in pollen transfer or gene flow. Identifying the proportion of any given assemblage that transports viable pollen is more difficult than simple observations of flower attendance. Effects on pollen viability can differ according to the identity of insect pollen carriers (Kendall, 1973). To fully understand the potential for different species to act as pollinators and to assess the contribution of gene movement across distance, temporal retention of pollen viability must also be understood.

Defining the distances over which viable pollen is moved can be problematic in the majority of insect species. For example, insect size and life history traits restrict the use of mark-recapture techniques and tracking devices to large and/or social insects and short time periods. Thus, mark-recapture and tracking studies have been undertaken using large-bodied pollinators; such as honeybees (Beekman and Ratnieks, 2000; Steffan-Dewenter and Kuhn, 2003; Riley *et al.*, 2005) and bumble bees (Walther-Hellwig and Frankl, 2000; Osborne *et al.*, 2008). Where smaller-bodied pollinators have been investigated, attempts to produce estimates of movement distances for a range of different sized insect taxa have been based on the allometric relationship between body size and distance (i.e. Greenleaf *et al.*, 2007). This relationship is not always consistent however as some smaller bodied insects are capable of travelling farther distances than their larger bodied counterparts (Lewis, 1997; Pathak *et al.*, 1999; Schowalter, 2006). There is a need for new methods to be employed to assess the capacity for smaller bodied insects to transfer viable pollen from crops.

Pollen transfer is a primary source of gene flow and has direct influence on the level of genetic exchange within and between plant populations (Ellstrand, 1992; Burczyk *et al.*, 2004). It has become especially significant in landscapes that contain both transgenic and conventional crops as pollen transfer can allow genes to escape from genetically modified (GM) to non-GM crops or to related weeds (Scheffler *et al.*, 1993; Lavigne *et al.*, 2002; Wilkinson *et al.*, 2003; Kuparinen *et al.*, 2007). Most studies concerning insect-mediated pollen flow have shown that most pollen transfer occurs close to the crop edge within 1-50m (Levin and Kerster, 1974; Scheffler *et al.*, 1993; Lavigne *et al.*, 1996). Longer distance dispersal events (>100m) have been less studied, yet may prove to be highly relevant and have important consequences for risk assessment particularly when the organism is at a selective advantage compared to the residents (Lavigne *et al.*, 1996). Pollinator mobility is therefore a critical issue in understanding the contribution of unmanaged taxa to pollen flow. Ths is because pollinator mobility enables cross pollination to occur and can impact upon the gene flow outside of transgenic crops.

***Brassica rapa* as a model crop**

In this thesis, I examine the potential for unmanaged insect taxa to provide pollination services to mass flowering crops using *Brassica rapa* var. *chinensis* (Brassicaceae). *B. rapa* was chosen as a model system to investigate the pollination services by both managed and unmanaged taxa for several reasons. First, it is capable of forming hybrids with other Brassica oilseed/canola cultivars including *B.*

napus and *B. juncea* (Scheffler and Dale, 1994; Mikkelsen *et al.*, 1996; Hauser *et al.*, 1998; Ingram, 2000; Stewart, 2002), which are among the first crops to be genetically modified for herbicide resistance (Hauser *et al.*, 1998; Rieger *et al.*, 2002; Allainguillaume *et al.*, 2006). This addresses the potential for pollinator mobility to impact upon gene flow between transgenic and non-transgenic crops. Second, *B. rapa* exhibits increased seed set in the presence of insect pollinators (Free, 1993) and attracts a diverse assemblage of insects from many insect orders including Diptera, Lepidoptera, Coleoptera and Hymenoptera (Feldman, 2006; Goodell and Thomson, 2007). This enabled me to compare the efficiency and overall effectiveness of unmanaged insects to the honeybee and to examine if pollinator diversity facilitates improved production. Third, *B. rapa* is a global food crop within the Brassicaceae, a family of great economic value in a wide range of end products including raw vegetables, oils and animal fodder (Williams and Hill, 1986). This directly addresses the utility and capacity for unmanaged pollinators to provide pollination services to global food crops. Fourth, *B. rapa* is a crop that is grown within a modified agricultural system. Spatial heterogeneity in floral resources is therefore lower than that associated with more natural landscapes (Klein *et al.*, 2008; Tylianakis *et al.*, 2008). This is ideal in order to examine temporal differentiation in activity patterns in contrasting pollinator species. Finally, *B. rapa* is ubiquitous in most agricultural landscapes as a crop/environmental weed (Feldman, 2006; Sutherland *et al.*, 2006). This ensures the results of this study are directly applicable to a range of interest groups.

Justification for this research

This research compares unmanaged pollinator taxa to the managed honeybee using a range of functional traits (efficiency, effectiveness, consistency and mobility) exhibited by insect pollinator taxa. The results of this research will identify which taxa perform best, their reliability over time and their capacity to move pollen over distance. A greater understanding of the services provided by unmanaged pollinator taxa has the potential to increase seed/fruit quality and quantity in agricultural crops, reduce pollination service costs and reduce dependence on the introduced honeybee. A reduced dependence on the honeybee for crop pollination services is critical considering the financial losses associated with honeybee decline in New Zealand and North America.

Finally, understanding the movements of pollinators in the emerging world of GM crops is essential in order to assess any biosecurity risk they may pose in the transport of GM pollen between crops.

Components of this thesis

This thesis is divided into four data chapters and a synthesis chapter. Each data chapter is presented as a manuscript which has been submitted for publication or published as a result of the work conducted for this thesis, as indicated by the reference provided at the start of each chapter.

Chapter 2 identifies a range of potential unmanaged pollinators in a mass flowering crop and compares their services to the managed honeybee. This is achieved by evaluating overall pollinator effectiveness by separating the pollination service into two components: efficiency (i.e. per visit pollen deposition) and visit rate (i.e.

pollinator abundance per available flower and the number of flower visits per minute). By directly testing and comparing the efficiency and overall effectiveness of managed and unmanaged pollinators in a mass flowering crop, this chapter evaluates the utility of unmanaged flower visiting taxa in an intensive agricultural system by addressing unmanaged pollinator effectiveness. Chapter 3 compares unmanaged pollinator performance in space (between fields) and over time, both within a day (from 10am to 2pm) and across a four year period in an attempt to assess the consistency of unmanaged pollination services. This chapter also investigates the contribution of all unmanaged taxa as a group to evaluate how the combined services of all unmanaged taxa perform relative to the managed honeybee. The findings from this chapter demonstrate that unmanaged taxa are capable of providing reliable pollination services. These findings address the second requirement for evaluating the utility of unmanaged flower visitors in intensive agricultural systems by addressing unmanaged pollinator reliability. Chapter 4 investigates whether unmanaged pollinator diversity potentially facilitates reliability in unmanaged assemblages. This is achieved by examining the differences between bee and fly pollinator ‘activity windows’ to investigate functional complementarity between them across an entire day (6am to 8pm), as opposed to the centre of the day (10am to 2pm; Chapter 3), in order to examine the potential contribution of unmanaged taxa outside of standard survey hours. Chapter 5 investigates the capacity of unmanaged pollinators to transfer viable pollen over distance, an important trait which has implications for GM pollen flow. This chapter addresses the third requirement for evaluating the utility of unmanaged flower visitors in intensive agricultural systems by addressing unmanaged pollinator mobility. Finally, Chapter 6 provides a synthesis of the results from this study, the limitations of this study and possible research questions that could be addressed in the future.

Chapter 2: Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop.

This chapter has been published as follows:

Rader, R., B. G. Howlett, S. A. Cunningham, D. A. Westcott, L. Newstrom-Lloyd, M. Walker, D. Teulon, and W. Edwards. (2009). Alternative pollinator taxa are equally efficient but not as effective as the honeybee in a mass flowering crop. *Journal of Applied Ecology* **42**, 1080-1087.

Abstract

The honeybee *Apis mellifera* is currently in decline worldwide due to the combined impacts of Colony Collapse Disorder and the *Varroa destructor* mite. In order to gain a balanced perspective of the importance of both wild and managed pollination services, it is essential to compare these services directly, a priori, within a cropping landscape. This process will determine the capacity of other flower visitors to act as honeybee replacements.

In a highly modified New Zealand agricultural landscape, I compared the pollination services provided by managed honeybees to unmanaged pollinator taxa (including flies) within a *Brassica rapa* var. *chinensis* mass flowering crop.

I evaluate overall pollinator effectiveness by separating the pollination service into two components: efficiency (i.e. per visit pollen deposition) and visit rate (i.e. pollinator abundance per available flower and the number of flower visits per minute). I observed 31 species attending flowers of *B. rapa*. In addition to *A. mellifera,* seven insect species visited flowers frequently. These were three other bees: *Lasioglossum sordidum*, *Bombus terrestris* and *Leioproctus* sp.; and four

flies: *Dilophus nigrostigma*, *Melanostoma fasciatum*, *Melangyna novae-ze*landiae and *Eristalis tenax*. Two bee species, *Bombus terrestris* and *Leioproctus* sp. and one fly, *Eristalis tenax* were as efficient as the honeybee and as effective (in terms of rate of flower visitation). A higher honeybee abundance however, resulted in it being the more effective pollinator overall. Alternative land management practices that increase the population sizes of unmanaged pollinator taxa to levels resulting in visitation frequencies as high as *A. mellifera,* have the potential to replace services provided by the honeybee. To increase the population sizes of unmanaged pollinator taxa, I suggest “in-situ” management. This will require a change in land management practices in order to ensure year round refuge, feeding, nesting and other resource requirements of pollinator taxa are met.

Introduction

The value of managed versus wild pollinator services has recently been the focus of much attention (e.g. Allsopp *et al.*, 2008), particularly with reference to global food crops and their pollination requirements (Klein *et al.*, 2007a; Aizen *et al.*, 2008; Winfree, 2008). Honeybee *Apis mellifera* Linnaeus, 1758 colony viability is now a serious concern because many agricultural crops are reliant on this single pollinator species and consequently, the contribution of this species to food production is high (Morse and Calderone, 2000; Klein *et al.*, 2007a). This has sparked renewed interest in the function of unmanaged or wild pollinator taxa, particularly due to their provision of ‘pollination insurance’ (Winfree *et al.*, 2007).

Unmanaged pollinator taxa have proved to be superior pollinators when compared to honeybees in some crop species. For example, in a study of watermelon pollination in North America, native bees were responsible for a significantly greater number (62%) of flower visits than honeybees and pollen deposition at flowers was strongly correlated with native bee visitation, but not with honey bee visitation (Winfree *et al.*, 2007). Similarly, an increased abundance and diversity of native bees significantly improved fruit set in coffee plantations (Klein *et al.*, 2003) and seed set in canola (Morandin and Winston, 2005) .

These ‘free’ wild pollinator services, however, are ostensibly at risk from land use modification and pesticide use (Watanabe, 1994; Stokstad, 2006). The perceived value of these services to global food production, however, has been questioned (Watanabe, 1994; Allen-Wardell *et al.*, 1998; Ghazoul, 2005a; Steffan-Dewenter *et al.*, 2005; Stokstad, 2007; Aizen *et al.*, 2008; Allsopp *et al.*, 2008) leading to, in some respects, the understatement of their potential services to global crop production (Ghazoul, 2005a; Allsopp *et al.*, 2008).

This premise has developed from two critical concerns: the first is that a majority of global food production does not in fact depend on animal pollination and hence the attention directed toward declining wild pollinators is currently not warranted (Ghazoul, 2005a). The assumption that pollinator declines have yet to be translated into decreased food production is supported by a study by Aizen *et al*. (2008) which compared rates of yield increase between pollinator dependent and pollinator independent crops over the last 45 years. The second concern arises from the fact that studies that have assessed the value of wild pollinators, often fail to compare the managed and the unmanaged components to arrive at a balanced view of the net worth of their services (Allsopp *et al.*, 2008). There is a need for empirical studies directly comparing managed and unmanaged pollinator services (Ghazoul, 2005a; Ghazoul, 2005b; Steffan-Dewenter *et al.*, 2005) in order to demonstrate unequivocally the relative significance of their services in pollinating global food crops (Allsopp *et al.*, 2008).

Two components of pollination need to be assessed in order to directly compare the overall effectiveness of managed and unmanaged pollinator services, pollen transfer efficiency and visitation frequency. Pollen transfer efficiency describes the proficiency with which individual pollinators remove and transport pollen to conspecific stigmas (Primack and Silander, 1975; Herrera, 1987; Harder and Wilson, 1998). Visitation frequency is a function of both the abundance of the pollinator and the number of flowers it visits in a given time interval (Herrera, 1987; Herrera, 1989;

Vazquez *et al.*, 2005; Madjidian *et al.*, 2008). The most effective insect pollinator would therefore be one that is present in high numbers and moves rapidly from flower to flower (i.e. has a high visitation rate). It would also frequently contact the stigma, transferring many pollen grains (i.e. has high pollen transfer efficiency). Conversely, the least effective insect pollinator would have low abundance and move relatively slowly from flower to flower (i.e. have a low visitation rate). It would rarely contact the stigma while visiting a flower and transfer few pollen grains when it did (i.e. have low pollen transfer efficiency).

Surprisingly few studies have directly compared the effectiveness of managed honeybee services with unmanaged alternative pollinator services in intensively cultivated landscapes. Although numerous studies have compared the effectiveness of wild and managed pollinators in crop situations, most of these concern their relationship with native or semi natural vegetation surrounding crops (Steffan-Dewenter, 2002; Klein *et al.*, 2003; Blanche *et al.*, 2006), focus on species or genus-specific comparisons (e.g. Goodell and Thomson, 2007; but see Winfree *et al.*, 2008), have been conducted in closed systems (Sadeh *et al.*, 2007) or relate to introductions occurring away from the pollinator's area of geographic origin (Mayer and Lunden, 2001; Slaa *et al.*, 2006). These traits limit our ability to make meaningful comparisons.

Furthermore, it is not immediately clear how intensification of land use impacts upon wild pollinators. While some studies suggest that wild pollinators respond positively to intensive land use (Westphal *et al.*, 2003; Winfree, 2007), others suggest wild pollinators respond poorly to intensification because many of the resources are too transient to be utilized effectively (Corbet, 2000; Ricketts *et al.*, 2008; Winfree, 2008). High variation in pollination success in areas with intensive agriculture and isolated from natural habitats is presumably because many wild pollinators vary markedly in their response to landscape context (Steffan-Dewenter, 2002; Brosi *et al.*, 2007; Winfree *et al.*, 2008).

The lack of studies comparing managed and unmanaged services in situ, in intensive agricultural systems, is surprising as there is a clear link between diverse pollinator guilds and improved pollen loads, high fruit and seed set and increased offspring vigour (Schemske and Pautler, 1984; Herrera, 1987; Klein *et al.*, 2003; Gomez *et al.*, 2007). Intensive agricultural systems that support diverse unmanaged pollinator assemblages co-existing with managed honeybee hives prior to honeybee decline, are ideal systems to identify potential alternative taxa that might be used if honeybees decline.

I use a highly modified landscape in the Canterbury region of New Zealand, to ask the following questions: 1. Does pollen transfer efficiency (as measured by stigmatic pollen loads and the proportion of visits in which the stigma is contacted) differ between the honeybee and other flower-visiting taxa? 2. Does the rate of flower visitation (measured as both visitor abundance per number of available open flowers, and the number of flower visits per minute) differ between the honeybee and other taxa? 3. How do these differences translate into overall pollinator effectiveness? 4. Are any of the alternative pollinator taxa directly, or as a group, capable of replacing honeybee services in a mass flowering crop?

Materials and methods

Study species

Brassica rapa var. *chinensis* (Brassicaceae) or 'Pak Choi' is a mass flowering vegetable and forage crop grown commercially in New Zealand as a seed crop for export. It is an ideal study species for comparing alternative pollinator assemblages with the honeybee due to its mass flowering habit and its attractiveness to a generalist pollinator assemblage. At the time of this study, *Varroa destructor* was not present on the South Island of New Zealand and an average of two managed honeybee hives (range 1-4) were located within 0.75-3 km from the eleven fields sampled in this study. This study assumes that honeybee visits are from honeybees in managed hives (as opposed to feral honeybees) due to the large number of hives operating in close proximity to the study plots.

I measured two components of pollination that are important in determining which alternative species may have the potential to replace the honeybee in providing pollination services: pollen transfer efficiency and pollinator rate of visitation. A number of methods were used to assess these two components. To determine pollen transfer efficiency, I observed flower visitors and measured the quantity of pollen transferred by each taxon, and the likelihood that pollen was transferred to flower stigmas. To determine pollinator rate of visitation, I observed how often flowers were visited by different taxa within a specified time.

Observations of flower visitors

From 15 December 2006 to 20 February 2007 I observed flower visiting insects in eleven commercial *B. rapa* fields (range: 0.75 to 2.0 ha) in the Canterbury region of

New Zealand. I selected fields for observations at the time of peak flowering, defined as the period during which mature receptive female flower density exceeded 1000/m^2. Five observation quadrats (10x10 m) were established per field; one near each of the four corners in four directions and one in the field centre. In each of these observation quadrats, flower density was estimated within three smaller randomly located 1 m^2 quadrats by counting the number of individual plants within each quadrat, the number of inflorescences per plant on 10 randomly selected plants, and the number of flowers per inflorescence (on the same 10 randomly selected plants). Using these quadrat level estimates (mean number of flowers per 1 m^2), I extrapolated values to estimate the number of flowers observed in visitor survey transects (i.e. multiplied mean flowers per 1 m^2 by 10 to calculate density per 10 m^2). On average, flower density across all fields was estimated to be (mean ± SE) 1596.6 ± 149 flowers/m^2. Flower density differed significantly between fields ($F_{1,10} = 359.61$; $P < 0.001$).

Pollen transfer efficiency

I measured pollen transfer efficiency by recording pollen deposition on stigmatic surfaces and the proportion of flower visits that resulted in stigma contacts per insect visit.

Pollen deposition

Pollen deposition on stigmatic surfaces was estimated via manipulation experiments. I bagged virgin inflorescences in bud (fine mesh 50 x 50 µm) to exclude pollinators. At flower opening I removed the bag and observed flowers for the period required before an insect visited the flower and contacted the stigma in a single visit. After identifying each insect I removed the stigma by carefully severing it from the style using finely pointed forceps. The stigma was placed on a cube of gelatine-fuchsin (approx. 3x3x3 mm) and a coverslip was placed on top of the gelatine cube. Gentle pressure was applied, after which the gelatine was melted onto a microscope slide by applying heat (Dafni, 1992; Kearns and Inouye, 1993). Pollen loads were estimated by counting all *B. rapa* pollen grains surrounding the stigma under 20X magnification. In total I estimated pollen loads for each of the 456 stigmas collected from eleven fields. I did this for 18 -31 individual insect contacts for each of the eight frequent visitor species (in addition to other species).

For each stigma sampled in this way, I also collected a second control stigma from another flower (of the same bagged inflorescence), at a similar developmental stage, that had not been contacted by an insect. This enabled assessment of the possible influence of pollen drift between neighbouring flowers and hence on total pollen counted. The control stigma was processed using the same method.

To assess self-pollen movement due to insect foraging behaviour (i.e. pollen transfer within a flower) I removed anthers from filaments in a second treatment of control flowers (n=75) and compared the pollen loads of these stigmas with those flowers having intact anthers.

Stigmatic contact

The second component of pollinator efficiency is the reliability with which individuals transfer pollen during floral visitation. I determined the proportion of all flower visits that resulted in stigma contact per insect individual. I followed individual insects for each of eight species of visitor (the most frequent visitors in the entire assemblage) over a period required for them to visit 10 flowers and I used a hand-held video camera to record their behaviour while attending each flower visited. The recording enabled identification of the number of occasions (/10) in which an insect landed on a flower and successfully touched the stigmatic surface.

It was not possible to manipulate the experimental protocol in order to ensure that equal representation of taxa across each field for either measure of pollen deposition or stigmatic contact. I could not control which insects visited flowers and therefore sampling all taxa in all fields with equal frequency was not possible. Stigmatic pollen deposition and stigma contact data thus represent the product of natural variability across the fields. In total I collected data for 6-30 individuals for each of eight species of visitor (Table 2.2).

Visitation rate

I estimated visitation rate using two measures: visitor abundance per number of available open flowers (measured by visits to quadrat/ no. open flowers observed per 10 min) and flower visitation rate (number of flower visits by an individual pollinator per minute). To determine visitor abundance in the plots, visitors were surveyed for 2d at each field for three observation periods; 10.00-11.00, 12.00-13.00 and 14.00-15.00 h. The five observation quadrats (10x10 m) already established to determine flower density were used to conduct these visitor observations. Observations of floral visitors were made by walking along each of the four boundaries of each quadrat (i.e. 10 m x 1 m) and recording all insect species and abundances within the boundary during a 10 min time period. The time taken to complete five quadrats was therefore 50 min per time interval. All floral visitor observations were made on sunny or partly cloudy days when the temperature was >16ºC and wind speed < 5 ms^{-1}. I observed flower visitors for a total of 55 hours. The order that fields, and quadrats-within-fields were examined was randomised throughout the study period. Frequencies were then divided by the number of open flowers (estimated from the open flower estimates in each quadrat) to remove the confounding effect of differences in floral density between fields on visit frequency (see Ivey *et al.*, 2003). I then divided the unmanaged taxa into 2 groups; all flies and all bees (other than the honeybee) and compared visitation frequencies among these groups.

To determine the number of flower visits per minute I followed individual insects from flower to flower and recorded all the flower visits made by this individual within a 1 min period using a digital voice recorder. I recorded observations of 20-50 individuals of each taxon (*n*=479) to calculate the mean number of visits per flower per min for each field. All flower visitors were described to species level in the field where possible. Where species identity was not determined at the time of observation, specimens were collected between observation periods and taken back to the laboratory for identification.

To calculate overall pollinator effectiveness per day I multiplied pollen transfer efficiency for each taxon (median stigma load x proportion of successful stigma contacts) by the frequency of visits/hr (visitor abundance per number of open flowers x number of flowers visited per minute x $10min^{-1}$ x 6) (see Madjidian *et al.*, 2008).

Data Analysis

Stigmatic pollen loads were log transformed to improve normality and means were compared between taxa using univariate general linear models. Where significant differences were revealed, means were compared using Tukey's Honestly Significantly Different (HSD) tests, which control the experiment-wise error rate to α = 0.05. The variation between fields was included as a random factor in all analyses. The proportion of successful flower visits were compared between taxa using generalized mixed models with a binomial error distribution. I compared visitation frequency between taxa (abundance per flower per 10 min), between groups of taxa, and per flower visitation rate between taxa (number of flowers visited per minute per focal animal) using mixed models restricted estimates maximum likelihood variance analysis (REML) with taxa as a fixed effect. I also compared flower density estimates between fields using mixed models REML analysis. Post-hoc pair-wise comparisons were performed using Least Significant Difference (LSD) tests. All analyses were conducted using version 17, SPSS statistical package (SPSS, 2008).

Results

In total I observed 31 species attending flowers of *B. rapa* (Table 2.1). In addition to *A. mellifera,* seven insect species visited flowers often enough to be included in analyses. These were three other bees: *Lasioglossum sordidum* (Smith, 1853), *Bombus terrestris* (Linnaeus, 1758) and *Leioproctus* sp.; and four flies: *Dilophus nigrostigma* (Walker, 1848), *Melanostoma fasciatum* (Macquart, 1850), *Melangyna novae-ze*landiae (Macquart, 1855) and *Eristalis tenax* Linnaeus, 1758 (Table 2.1).

Pollen transfer efficiency

Pollen deposition

In 63% of control (i.e. unvisited) flowers, there were no pollen grains on stigmas. In the remaining control stigmas, there were <2 pollen grains (mean=1.96 ±0.01). I thus have no have reason to expect that pollen movement occurred without insect pollinators. There was no significant difference between emasculated flower stigmatic loads and intact flower pollen loads (t =-1.14, *P*=0.27). This suggests pollen transfer estimated in stigmatic pollen load calculations was not likely to be self-pollen.

In the absence of detailed data regarding pollinator behaviour however, (i.e. extent of grooming, amount of self pollen carried on insect body (Harder, 1990; Harder and Wilson, 1998; Aizen and Harder, 2007)) I cannot verify with certainty that self-pollen was excluded from pollen transfer estimates in stigmatic pollen load calculations.

Table 2.1: Taxa recorded visiting flowers in *Brassica rapa* fields in the Canterbury region, New Zealand

Order	Family	Species
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758
		Bombus terrestris (Linnaeus, 1758)
	Halictidae	*Lasioglossum sordidum* (Smith, 1853)
	Colletidae	*Leioproctus* sp.
	Ichneumonidae	Unidentified species
Diptera	Anthomyiidae	*Delia platura (*Meigen, 1826)
		*Anthomyia punctipennis (*Weideman, 1830)
	Bibionidae	*Dilophus nigrostigma* (Walker, 1848)
	Calliphoridae	*Calliphora hortona* (Walker, 1849)
		*Calliphora quadrimaculata (*Swedarius, 1787*)*
		Calliphora stygia (Fabricius, 1794)
		Calliphora vicina Robineau-Desvoidy, 1830
		Lucilia sericata (Meigen, 1826)
		Pollenia pseudorudis (Rognes, 1985)
	Drosophylidae	*Drosophila sp.*
	Ephydridae	Unidentified species 1
	Stratiomyidae	*Odontomyia spp.*
	Muscidae	*Spilogona melas* Schiner, 1868
		Hydrotaea rostrata Robineau-Desvoidy, 1830
	Syrphidae	*Eristalis tenax* Linnaeus, 1758
		Melanostoma fasciatum (Macquart, 1850)
		Melangyna novae-zelandiae (Macquart, 1855)
		Helophilus hochstetteri Nowicki, 1875
	Sarcophagidae	*Oxysarcodexia varia* (Walker, 1836)
	Tabanidae	*Scaptia sp.*
	Tachinidae	*Pales usitata* (Hutton, 1901)
		Pales marginata (Hutton, 1901)
		Protohystricia spp.
Coleoptera	Coccinelidae	*Coccinella undecimpunctata* (Linnaeus, 1758)
Hemiptera	Pentatomidae	*Glaucias amyoi (Dallas)*
Lepidoptera	Pieridae	*Pieris rapae (*Linneaus, 1758*)*

There were significant differences in the mean pollen load (log transformed) deposited onto stigmatic surfaces between species ($F_{9,132}$=7.646, *P*<0.0001, Figure 2.1, Table 2.2). In comparisons between taxa representing the unmanaged component of pollinating fauna, *A. mellifera* transferred significantly greater amounts of pollen per stigmatic contact than four of the native species; *Dilophus nigrostigma*, *Melanostoma fasciatum*, *Melangyna novaezelandiae* and *Lasioglossum sordidum* (Table 2.2, Figure 2.1). Three species from the unmanaged assemblage were not different in this respect to *A. mellifera; B. terrestris* and *Leioproctus* sp. and the fly *E. tenax,* (Figure 2.1, Table 2.2).

Stigmatic contact

The proportion of times that individuals contacted stigmatic surfaces when visiting flowers, differed between species (df = 7, Wald = 434.405, *P*<0.0001, Figure 2.2). The honeybee and three unmanaged taxa (*B. terrestris*, *Leioproctus* sp. and *E. tenax)* contacted stigmatic surfaces on significantly more occasions than non-contact occasions. Stigma contact was low in the remaining taxa (Table 2.2, Figure 2.2).

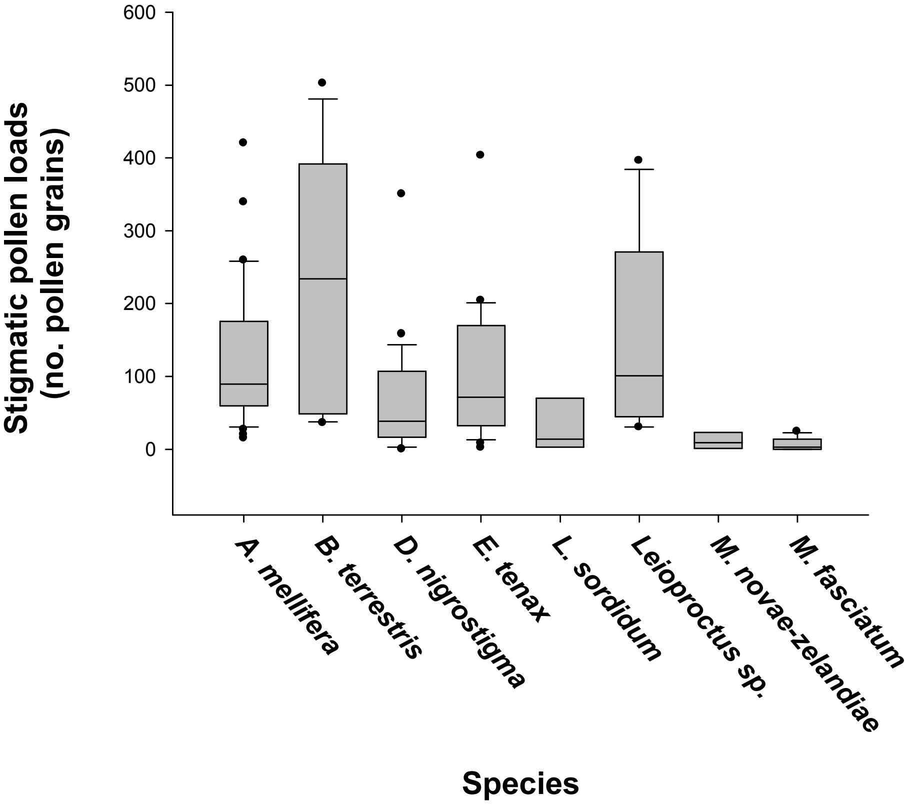


Figure 2.1: Boxplot of stigma pollen loads per flower visit for each species. Box indicates quartiles with median marked as a horizontal line; points are outliers.

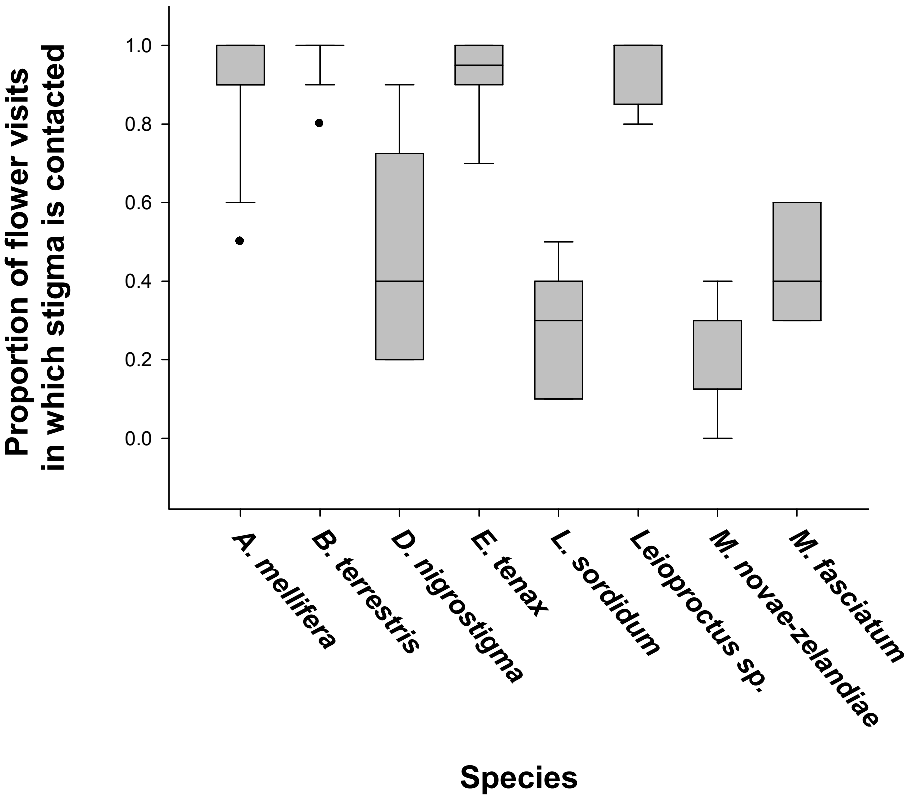


Figure 2.2: Boxplots of the proportion of stigma contact occasions per visit for 10 individual flower visits for each of n individuals. Box indicates quartiles with median marked as a horizontal line; points are outliers.

Table 2.2: Efficiency of the eight most frequent flower visitors to 11 B. rapa fields in the Canterbury region, New Zealand. No. in parenthesis represent n. Post-hoc pair-wise comparisons for pollen grains transferred are the P-values from Tukey's HSDs

Species	Stigmatic pollen grains transferred (mean ± SE)	Post-hoc comparisons with *A. mellifera* Tukey's HSD (*P*)	Proportion of successful stigma contacts (mean ± SE)	Post-hoc comparisons with *A. mellifera* LSD test (*P*)
Apis mellifera	123.03 ±16.42 (32)		0.88 ± 0.010 (230)	
Bombus terrestris	236.63 ± 43.69 (16)	0.052	0.97 ± 0.006 (110)	<0.001
Leioproctus sp.	153.20 ± 40.41 (10)	0.678	0.95 ± 0.01 (80)	0.059
Lasioglossum sordidum	30.14 ± 12.07 (7)	0.0001	0.28 ± 0.023 (50)	<0.0001
Eristalis tenax	106.64 ± 19.83 (22)	0.191	0.93 ± 0.011 (80)	0.292
Melangyna novae-zelandiae	16.13 ± 7.78 (8)	0.005	0.24 ± 0.014 (80)	<0.0001
Melanostoma fasciatum	6.36 ± 2.52 (11)	0.0001	0.43 ± 0.015 (70)	<0.0001
Dilophus nigrostigma	68.29 ± 15.43 (24)	0.007	0.45 ± 0.029 (80)	<0.0001

Table 2.3: Effectiveness of the eight most frequent flower visitors to 11 B. rapa fields in the Canterbury region, New Zealand. No. in parenthesis represent n. Post-hoc pair-wise comparisons for visit frequency and visit rate are the P-values from LSD tests.

Species	Visit frequency: visits flower^{-1} 10min^{-1} (mean ± SE)	Post-hoc comparisons with *A. mellifera* LSD test (*P*)	Visit rate: floral visits per minute (mean ± SE)	Post-hoc comparisons with *A. mellifera* LSD test (*P*)
Apis mellifera	2.35 x10^{-2} ± 0.0001		33.83 ± 3.07	
Bombus terrestris	1.28 x 10^{-3}± 0.00006	<0.001	69.12 ± 20.03	0.047
Leioproctus sp.	4.83 x 10^{-4}± 0.00007	<0.001	64.31 ± 20.16	0.148
Lasioglossum sordidum	3.74 x 10^{-4}± 0.00003	<0.001	10.03 ± 2.71	0.452
Eristalis tenax	3.39 x 10^{-3}± 0.0003	<0.001	19.42 ± 1.71	0.457
Melangyna novae-zelandiae	2.92 x 10^{-3}± 0.0003	<0.001	7.99 ± 1.07	0.286
Melanostoma fasciatum	1.87 x 10^{-3}± 0.0006	<0.001	6.38 ± 1.39	0.295
Dilophus nigrostigma	4.08 x 10^{-3}± 0.0003	<0.001	6.10 ± 1.67	0.394
All flies combined				
All bees combined (except	8.20 x 10^{-3}± 0.004	<0.001		
Apis mellifera)	9.25 x 10^{-4}± 0.004	<0.001		

Visitation rate

Taxon level visitation frequency (visitor abundance per number of available flowers) varied significantly ($F_{7,134}$ = 15.587, *P*<0.0001, Figure 2.3a). Honeybees visited flowers at a significantly higher rate than all other taxa (LSD tests: *P*<0.0001). When taxa were grouped, honeybee visitation frequencies were still significantly higher than both fly and bee groups ($F_{2,69}$ = 29.835, *P*<0.0001).

Visitation rate (no flower visits per min) also varied between taxa ($F_{1,416}$ = 2.013, *P* = 0.052, Figure 2.3b) but significance was marginal at *P*=0.052. Post hoc analysis suggests that this effect is due to *B. terrestris* visiting significantly more flowers per minute than the honeybee (LSD test: *P* = 0.047) while other taxa did not differ from the honeybee in this respect (LSD test: *P*>0.05; Table 2.3).

When both efficiency and visitation frequency were combined to produce an estimate of effectiveness (median stigma pollen load per visit x proportion of successful stigma contact x hourly rate of visitation), honeybees were the most effective single pollinator species. I estimated that honeybees accounted for the deposition of 7,879 pollen grains per hour which is more than three times greater than the next highest pollinator taxa, (*B. terrestris*: 2247 pollen grains transferred per hour). The overall effectiveness of the remaining taxa was as follows in pollen grains transferred per hour : *D. nigrostigma,* 22; *E. tenax,* 968; *L. sordidum, 2*; *Leioproctus* sp*., 300*; *M. fasciatum, 1*; *M. novae-zelandiae, 13*.

Discussion

This study revealed a diverse unmanaged component of the pollinator assemblage in *B. rapa* crops. I found that in terms of pollen transfer efficiency, the unmanaged component of the pollinator assemblage includes taxa that are capable of providing pollination services equal to those currently performed by honeybees. First, I found that mean pollen loads deposited on stigmas of virgin flowers by two bee species, *B. terrestris* and *Leioproctus* sp. and one fly species, *E. tenax*, were not significantly different to that deposited by the honeybee (Table 2.2, Figure 2.1). Second, these three species were as likely to touch stigmatic surfaces when attending flowers as *A. mellifera* (Table 2.2, Figure 2.2).

Rate of visitation is an important component affecting pollination success and determining the overall contribution of individual taxa to total pollination services (Vazquez *et al.*, 2005). In this study, I consider both visitation frequency (abundance per number of available flowers) and visitation rate (number of flower visits per min) separately for the purpose of demonstrating the potential of alternative pollinator taxa. Although honeybees visited flowers at significantly higher frequencies than any of the other visitors, they did not differ significantly in the number of flowers visited per minute when compared to all other taxa. I suggest that in the above measures of pollen transfer efficiency and floral visits per min, several alternative taxa are equal to the honeybee but are not common enough to make them more effective overall.

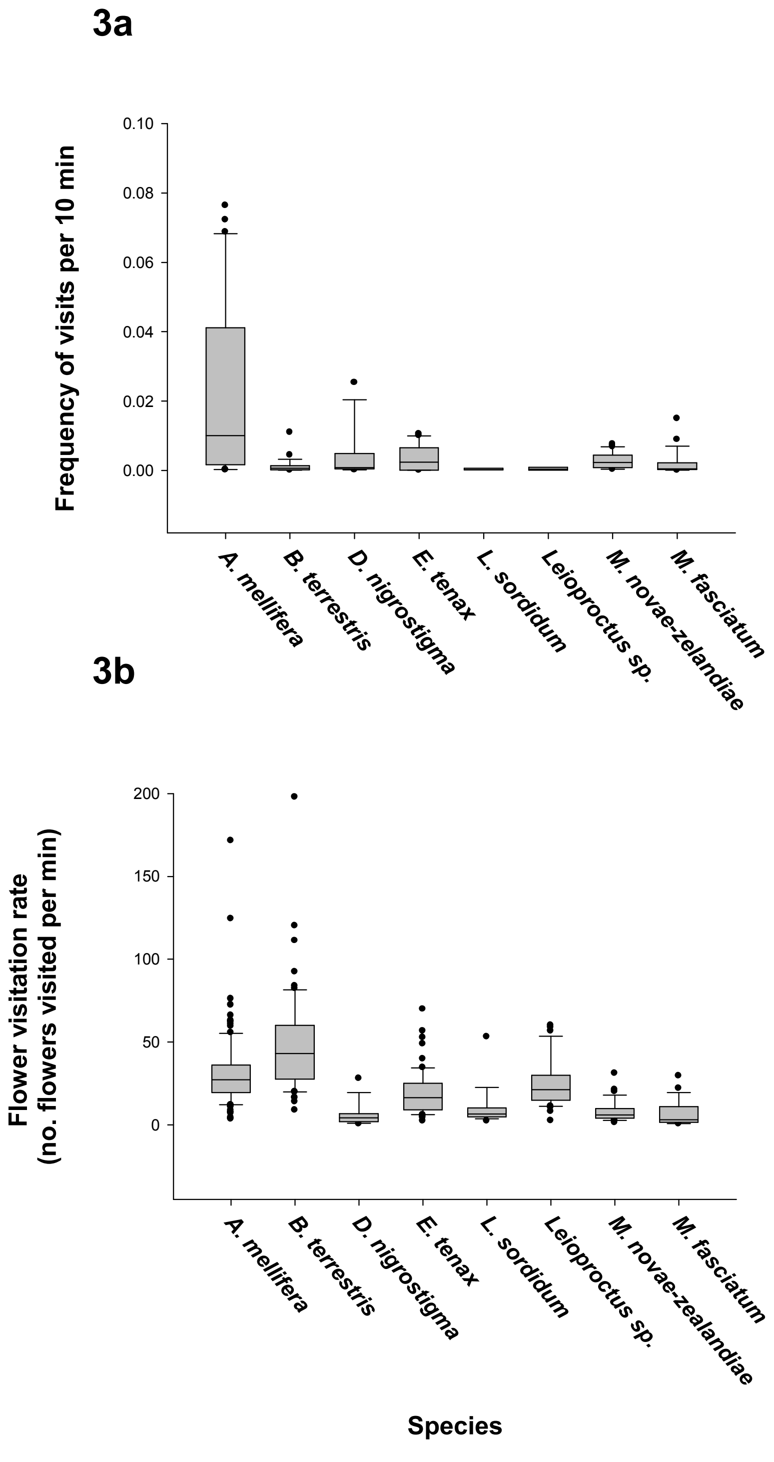


Figure 2.3: Boxplots of visitation rates: a refers to individual visitor frequencies (visits per flower) per 10 min period; b refers to number of flowers visited per minute. Box indicates quartiles with median marked as a horizontal line.

A higher abundance of honeybees ultimately resulted in overall greater effectiveness of honeybees as a single taxon. This result is similar to a study by Madjidian *et al*. (2005) in Argentina comparing a native and exotic bumblebee species. The higher visitation frequency of the exotic bumblebee *Bombus ruderatus* resulted in it being a more effective pollinator than the native bumblebee *B. dahlbomii*, even though the native bumblebee was more efficient.

The higher abundance of honeybees per number of available flowers relative to unmanaged taxa probably reflects managed and unmanaged status. By definition, honeybee populations are managed to maintain high population sizes whereas unmanaged taxa are not. This does not necessarily preclude currently unmanaged taxa from performing the same services. It is possible that even though the effectiveness of unmanaged taxa was lower, it may still result in maximum seed set. In the absence of seed set data, I cannot test this assumption.

If lower effectiveness results in lower seed set, unmanaged pollinators would need to be managed in order to increase population sizes in accordance with those of the honeybee, beyond that which exists naturally at this time in this system.
Managing a range of naturally existing pollinators in-situ is likely to be challenging for several reasons; First, we have become accustomed to ‘mobile’ as opposed to ‘in-situ’ pollination services. Honeybees are efficient, versatile and easily managed within transportable hives (Morse and Calderone, 2000; Klein *et al.*, 2007a; Winfree, 2008). In contrast, unmanaged pollinators are not as transportable and hence not as versatile (at present). Nonetheless, the effectiveness of flies (Syrphidae in particular) as crop pollinators is becoming increasingly evident (e.g. Feldman, 2006;

Pontin *et al.*, 2006). For example, pollination by *E. tenax* was shown to improve the shape and weight of sweet peppers in Canada (Jarlan *et al.*, 1997) while *Episyrphus balteatus* (also Syrphidae) significantly increased seed set and yield of an oilseed rape crop in cage experiments when compared to control cages (Jauker and Wolters, 2008).

Most of these 'recently managed' pollinator services however, are pollinator or crop-specific, as opposed to field-, system- or region-specific. Management in-situ will require a change in land management practices in order to ensure year round refuge, feeding, nesting and other resource requirements are met (Potts *et al.*, 2005; Klein *et al.*, 2007a; Williams and Kremen, 2007). Although other studies have demonstrated a positive relationship between pollinator diversity and existing vegetation in agro-ecosystems (Steffan-Dewenter, 2002; Klein *et al.*, 2003; Blanche *et al.*, 2006; Ricketts *et al.*, 2008), highly modified agricultural landscapes also have existing features which currently provide resources for insect pollinators. These include resource requirements for many solitary bees such as bare earth for nesting (Donovan, 2007; Winfree, 2007) and mass flowering crops (Westphal *et al.*, 2003) or other floral resources associated with agricultural field margins (Backman and Tiainen, 2002; Williams and Kremen, 2007), as sources of nectar and pollen.

Second, managing alternative pollinator taxa in situ to achieve high densities is challenging as it requires a thorough investigation of each taxon's intrinsic biology (Cane *et al.*, 2006). For instance, in this study system (and across most of New Zealand) the indigenous pollinating fauna lacks large social bees and is dominated by solitary bees and flies (Lloyd, 1985; Donovan, 2007). Solitary bees in particular have a short, fixed flying season, which is synchronized with the flowering time of certain host plants (Minckley *et al.*, 1994; Westerkamp and Gottsberger, 2000). This means that timing may not always be compatible with the flowering crop in need of pollination. In contrast, eusocial bees are capable of recruiting foragers quickly to high quality resources (Brosi *et al.*, 2007; Winfree, 2008)

Fundamental research into the intrinsic biology and life history traits of both solitary bees and flies is currently lacking in this, and most other systems (Klein *et al.*, 2007a). In order to understand which resources are needed for these taxa to maintain stable populations in agricultural landscapes we need to first understand their role and function in their current system.

In conclusion, the results of this study demonstrate that three species that currently exist as part of the unmanaged pollinator assemblage of *B. rapa* in the South Island of New Zealand are equally as efficient as the honeybee in providing pollination services. Effectiveness was higher in honeybees but this probably reflects the higher population sizes of a managed species giving rise to higher rates of visitation in honeybees. These results suggest that there is potential for other species to fulfil the pollination role of honeybees under management strategies that increase local population sizes and thus visitation rates.

Chapter 3: Spatial and temporal differences in pollinator effectiveness: Do unmanaged taxa have the capacity to provide long-term pollination services to mass-flowering crops?

This chapter has been submitted:

Romina Rader, Will Edwards, Saul A. Cunningham, David A. Westcott and Bradley G. Howlett. *Journal of Applied Ecology (submitted)*

Abstract

Recent declines in honeybee populations have focused attention on the potential for unmanaged insects to replace them as pollinators of global food crops. The ability of unmanaged pollinators to replace services currently provided by honeybees requires understanding of the spatial and temporal variability of these services, but few quantitative assessments currently exist. In this study, I first compared stigmatic pollen loads, stigmatic contact and visitation rates between managed and unmanaged taxa in four 'Pak Choy' (*Brassica rapa*) mass flowering crop fields in 2007 to investigate spatial variation in pollinator efficiency and overall pollinator importance. Second, I observed insect pollinators three times a day over four seasons from 2005-2008 in 43 *B. rapa* mass flowering fields to investigate temporal reliability in pollinator importance. The managed European honeybee and seven unmanaged taxa were identified as frequent and consistent floral visitors and were represented in all assemblages in all years. Stigmatic pollen loads and flower visits per minute were not significantly different between managed and unmanaged taxa

but stigmatic contact and rate of visitation were. All four pollinator traits differed spatially between fields. There was little temporal variation in visitation rate between times within a day (between 10am, 12pm and 2pm), between years (over a four year period) or between managed and unmanaged taxa. Pollinator importance did not change as a result of spatial and temporal variation. Individually, the honeybee was the most effective visitor, however, when unmanaged pollinator taxa were grouped there was little difference in overall effectiveness between honeybees and unmanaged taxa across fields or years. More importantly, there were no significant differences in unmanaged pollinator visitation rate within a day or between years. The results of this study suggest that a core assemblage of unmanaged flower visitors, are both spatially constant and temporally stable. These species may have the capacity to provide reliable pollination services and to potentially replace honeybees should population sizes decline.

Introduction

The current global pollination crisis highlights the advantages of the provision of pollination services by a suite of core pollinators (McCann, 2000; Klein *et al.*, 2007a). This is because the intrinsic differences in ecological tolerances that exist between taxa represent a range of thresholds to environmental disturbance. Because population sizes of individual species respond differently to perturbations, changes in abundance will not be temporally (or spatially) coordinated across species (Herrera, 1990; Tylianakis, 2005; Hoehn *et al.*, 2008).

Assemblages that contain a wide range of species with different ecological requirements could therefore maintain pollination services as environmental conditions change through time, if declines in abundance of some taxa are offset by increases in others (Yachi and Loreau, 1999; McCann, 2000; Elmqvist *et al.*, 2003). This is the basis of the biological insurance hypothesis with respect to pollination as an ecosystem service (Walker, 1992; Lawton and Brown, 1993; Naeem and Li, 1997; Naeem, 1998).

Ascertaining the reliability of a multi-species unmanaged pollinator assemblage requires an assessment of both the contribution to pollination by all species comprising the assemblage, as well as the upper and lower limits to the variability associated with each species (i.e. their reliability; Watanabe, 1994; Allen-Wardell *et al.*, 1998; Naeem, 1998; Elmqvist *et al.*, 2003; Memmott *et al.*, 2007; Stokstad, 2007). To date, much of the literature examining spatial and temporal variation in unmanaged pollinator assemblages has focused on native vegetation (e.g. Cane and Payne, 1993; Fishbein and Venable, 1996; Roubik, 2001; Price *et al.*, 2005; but see Tylianakis, 2005 and ; Hoehn *et al.*, 2008; Dupont *et al.*, 2009).

While the potential for unmanaged insects to act as crop pollinators has been addressed (Free, 1993; Klein *et al.*, 2003; Blanche *et al.*, 2006; Greenleaf and Kremen, 2006b; Klein *et al.*, 2007a; Winfree *et al.*, 2007; Hoehn *et al.*, 2008; Rader *et al.*, 2009; Winfree and Kremen, 2009), few studies have assessed the long term consistency of pollination services provided by unmanaged taxa (but see Winfree and Kremen, 2009). This is likely due to the inherent temporal variability in abundance estimates for unmanaged taxa (Cane and Payne, 1993; Roubik, 2001) that can obscure general patterns. For example, the variable nature of pollination services in space and over time (Gross and Werner, 1983; Ashman and Stanton, 1991; Wiggam and Ferguson, 2005) can lead to high species turnover (Tylianakis, 2005; Dupont *et al.*, 2009). Thus, the identity of the most effective pollinator(s) (Fishbein and Venable, 1996; Hoehn *et al.*, 2008; Olesen *et al.*, 2008) and/or the status of pollinator species as generalists or mutualists (Petanidou *et al.*, 2008; Dupont *et al.*, 2009) can be strongly influenced by the extent and duration of any investigation. These vagaries are especially prevalent in intensive agricultural systems since the ephemeral nature of floral resources (Corbet, 2000; Williams and Kremen, 2007; Ricketts *et al.*, 2008) can alter pollinator foraging behaviours (Diekotter *et al.*, 2010) and reduce the presence of habitat specialists (Tylianakis, 2005).

Nevertheless, an understanding of how visitation rates vary between taxa and the temporal stability of pollination services they provide is particularly relevant in agricultural systems where reliable productivity is directly linked to economic outcomes (Aizen *et al.*, 2008; Allsopp *et al.*, 2008; Gallai *et al.*, 2009).

For unmanaged pollinator services to be utilised in agricultural systems in the longer term, they need to be comparable in efficiency and reliability to existing managed services (Allsopp *et al.*, 2008). For decades, the European honeybee (*Apis mellifera* Linnaeus, 1758) has provided high quality pollination services to a range of global food crops (Klein *et al.*, 2007a). It's economic value to agricultural crops, versatility, efficiency and current use as a pollinator in many agricultural systems (Morse and Calderone, 2000) defines it as the benchmark against which to compare lesser known pollinators.

Comparisons of individual managed and unmanaged pollinator effectiveness in *B. rapa* crops have been studied elsewhere (Rader et al. 2009). The capacity for unmanaged taxa to provide consistent pollination services, however, has to my knowledge not been demonstrated previously. Specifically I ask the following questions:

1. Is there a group of unmanaged pollinator taxa that are spatially and temporally consistent in their visits to a mass flowering crop?
2. Do these taxa provide reliable pollination services (pollen transfer efficiency and/or overall effectiveness) where and whenever they occur?

3. Are these services likely to provide pollination rates equal to that of the managed honeybee across different locations and between years?

Materials and Methods

Brassica rapa var. *chinensis* (Brassicaceae) or 'Pak Choi' is a mass flowering crop. This and other species within the Brassicaceae are grown commercially in New Zealand for use as forage, seed, vegetable and oilseed production (Stewart, 2002).

Spatial variation in pollen transfer efficiency and rate of visitation

To examine spatial variation in both pollen transfer efficiency (i.e. stigmatic pollen deposition and stigmatic contact; Rader et al. 2009) and rate of visitation (i.e. visitor abundance per number of available open flowers and flower visitation rate, Rader et al. 2009), I observed managed and unmanaged taxa within four *Brassica rapa* commercial fields in the Canterbury region of New Zealand between November 2004 and February 2005. These four fields (two in Lincoln and two in Gore) represented the most abundant records of pollinators enabling samples of five or more individuals of most taxa (range 5-18).

I estimated pollen loads for each of 338 stigmas collected from 4 fields and observed 465 stigmatic contact occasions for 13–25 individuals of each of the seven frequent visitor species. To calculate rates of visitation in these four fields, five observation quadrats (10x10m) were established per field; one near each of the four corners in four directions (North West, North East, South

West, South East) and one in the field centre. Observations of floral visitors were made by walking along each of the four boundaries of the quadrat (i.e. 10m) and recording all insect species and abundances within 1m of the boundary observed during a 10min time period. Observations thus took approximately one hour to complete all five transect locations. Further details of specific methods to sample stigmatic pollen loads, stigmatic contacts and flower visits per minute are published elsewhere (Rader et al. 2009).

Temporal variation in pollinator visitation rate

Temporal variation in pollinator visitation was investigated by observing pollinators at three two-hourly intervals from 10am to 2pm across 8-12 commercial *B. rapa* fields per year for four years (43 fields in total) between November 2004 - January 2008. All fields were approximately rectangular (range: 0.75 and 2.0 ha). I selected fields for observations at the time of peak flowering, defined as the period during which mature receptive female flower density exceeded 1000/m^2. In three randomly located 1m^2 quadrats, flower density was estimated by counting the number of individual plants within each quadrat, the number of inflorescences per plant on 10 randomly selected plants, and the number of flowers per inflorescence (on the same 10 randomly selected plants). All floral visitor observations were made on sunny or partly cloudy days when the temperature was >16ºC and wind speed < 5 ms $^{-1}$.

All five quadrats in each field were observed for three observation periods (3h) throughout the day; 10am, 12pm and 2pm over a four year time period (2005-2008). The order that fields, and quadrats-within-fields were examined was randomised throughout the study period. Visitation rate was measured as the mean number of visits per flower per 10min for each field. All flower visitors were described to species level in the field where possible. Where species identity was not possible at the time of observation, specimens were collected and taken back to the laboratory for identification.

Overall pollinator effectiveness

I define overall pollinator effectiveness as the total contribution to pollination services provided by each taxa. This represents the product of taxa pollination efficiency at individual flowers (stigmatic pollen load x proportion of stigmatic contact) and the rate of visitation (visitor abundance per number of open flowers x flower visits per unit time).

Statistical Analyses

I investigated spatial differences in stigmatic pollen loads and visitation rate (i.e. flower visits per minute and frequency of visitation per 10min) between managed and unmanaged taxa and between fields using a mixed effects model using the nlme package (Pinheiro *et al.*, 2009). In all cases, the fixed factor “Taxa” represented the pooled visits for all unmanaged taxa present at each field. “Taxa” and “field” were considered fixed factors and “species” (the seven individual unmanaged taxa) as a random effect. Violation of homoscedasticity required the square root transformation of the dependent variables ‘flower visits per minute’ and ‘frequency of visitation per 10min’. This model was chosen as it enabled the incorporation of random effects

(Zuur *et al.*, 2009) and use of the REML method (Restricted Maximum Likelihood Method) which is robust to unbalanced designs (Payne, 2005).

Spatial differences in stigmatic contact between managed and unmanaged taxa were investigated using a generalized linear model (McCullagh and Nelder, 1989) with stigmatic contacts as the response variable and “taxa” and “field” as fixed factors. As the response variable consisted of successful and non successful occasions of stigmatic contact, I employed a model based on a binomial distribution and controlled for overdispersion by correcting the standard errors using a quasi-binomial model (Zuur *et al.*, 2009).

To examine temporal changes in visitation across 42 fields and over 4 years, I used a linear mixed effects model with visitation rate as the response variable and the following three fixed factors as explanatory variables: time of day (10am, 12pm and 2pm), year (y1-y4), and taxa. In this model I added “field” as random factor. I also ran this same model using only time and year as fixed factors with honeybee records excluded. I did this to determine if unmanaged taxa alone provide stable visitation rates within a day and across years. Statistical analyses were calculated with R Statistical Software (The R Foundation for Statistical Computing 2009).

Results

I observed 42,032 visits to *B. rapa* flowers over a four year period. These visits were made by a total of 43 insect species. Of these species, *A. mellifera* and seven other unmanaged insects, were frequent visitors in all years (observed in >10 time periods per year) and were responsible for 79.8% of all visitation records. The seven frequent visitors in addition to *A. mellifera* were one introduced bee, *Bombus terrestris, two native bees, Lasioglossum sordidum and Leioproctus sp. 1* and four flies, *Dilophus nigrostigma*, *Melangyna novae-zelandiae, Eristalis tenax* and *Melastoma fasciatum*.

Visitation rates of the remaining 36 species were highly variable. Fourteen species visited with high frequency (>10 time periods per year) in two or three of the four years, twelve were frequent in one year only while the remaining 10 species were always observed in low numbers (< 5 time periods) and occurred in one or more years (Table 3.1). These species were not considered further.

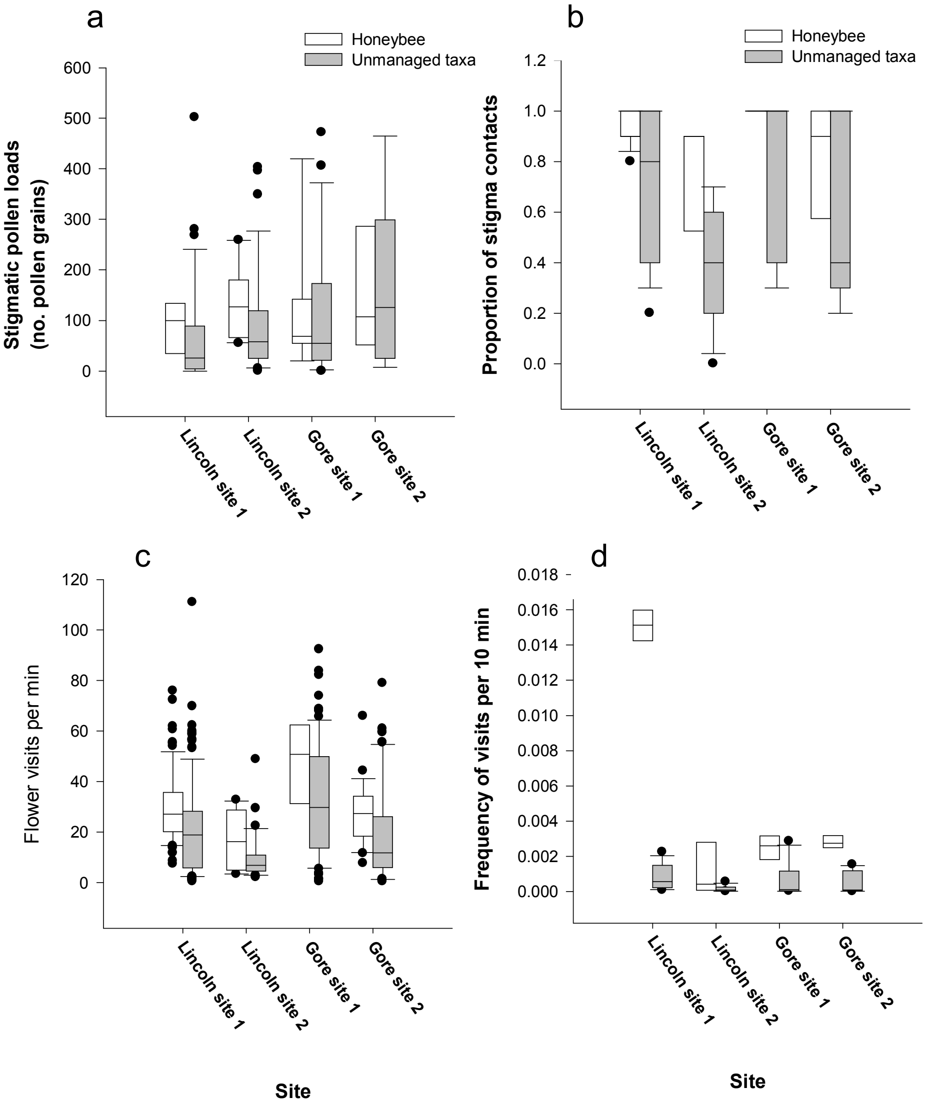


Figure 3.1 a-d: Boxplots of spatial differences in four pollinator traits used to determine overall effectiveness. Box indicates quartiles with median marked as a horizontal line; points are outliers. a: stigmatic pollen; b: stigmatic contact; c: flower visits per min; d: rate of flower visitation per available open flowers.

Spatial variation in pollen transfer efficiency

The honeybee did not differ from unmanaged taxa in the amount of stigmatic pollen transferred across the four field locations ($F_{1,6}$ = 0.1708; *P* = 0.6936) and stigmatic pollen loads did not differ between field locations ($F_{3,126}$ = 1.1813; *P* = 0.319). The proportion of stigmatic contacts by the honeybee however was significantly greater than that of unmanaged taxa across the four field locations (t = 6.550; *P* < 0.0001). Stigmatic contact also varied significantly between fields with Gore site 1 having a higher proportion of stigmatic contacts than Gore site 2 (t = -2.749; *P* = 0.0069) and Lincoln site 2 (t = -4.071; *P* < 0.0001), and Lincoln site 1 significantly greater than Lincoln site 2 (t = 3.503, *P* = 0.0006). No other site comparisons were significant.

Spatial variation in pollinator visitation rate

Flower visitation rate (the number of flower visits per minute) did not vary significantly between the honeybee and unmanaged taxa ($F_{1,6}$ = 0.8616, *P* = 0.3891). Flower visitation rate did, however, vary between field locations ($F_{3,408}$ = 5.63, *P* = 0.0009). The flower visitation rate at Lincoln site 1 was significantly higher than Lincoln site 2 (t = 3.573, *P* = 0.0004) and Gore site 1 was significantly higher than Lincoln site 2 (t = 3.148, *P* = 0.001). The honeybee also performed significantly better than unmanaged taxa in the second component of visitation (visitor abundance per number of available open flowers; $F_{1,6}$ = 15.117, *P* = 0.008). Visitor abundance per number of available open flowers differed significantly between sites ($F_{3,63}$ = 40.246, *P* < 0.0001). Both Lincoln site 1 (t = 10.922, *P* = 0.0001) and Lincoln site 2 (t = -

3.41, $P = 0.001$) had a significantly higher visitation rate than Gore site 1. Lincoln site 2 also had a significantly higher visitation rate than Lincoln site 1 ($t = 6.098$, $P = 0.0001$).

The honeybee was the most effective pollinator overall when considered against individual unmanaged taxa. Variations between taxa and across sites however, did little to alter the relative order of importance of unmanaged pollinators, particularly the four most effective taxa (Table 3.2).

Temporal variation in visitation rate

Visitation rate (i.e. visitor abundance per number of available open flowers) did not differ significantly between the honeybee and unmanaged taxa ($F_{1,880} = 3.375$; $P = 0.0665$), the hours within a day (“time-day” $F_{1,880} = 0.01785$, $P = 0.893$; Figure 3.1) or between years ($F_{1,31} = 1.216$, $P = 0.2977$). The significant interaction between taxa and year ($F_{1,880} = 65.272$; $P < 0.0001$) arose because the combined unmanaged taxa visited flowers at the same or higher rates than the honeybee in 2004 and 2005, while honeybees displayed highest visitation in 2006 and 2007 (Figure 3.2). Unmanaged taxa alone (i.e. with honeybee records excluded) also did not vary in visitation rate within a day ($F_{1,434} = 0.4048$, $P = 0.525$) or between years ($F_{1,31} = 4.069$, $P = 0.056$).

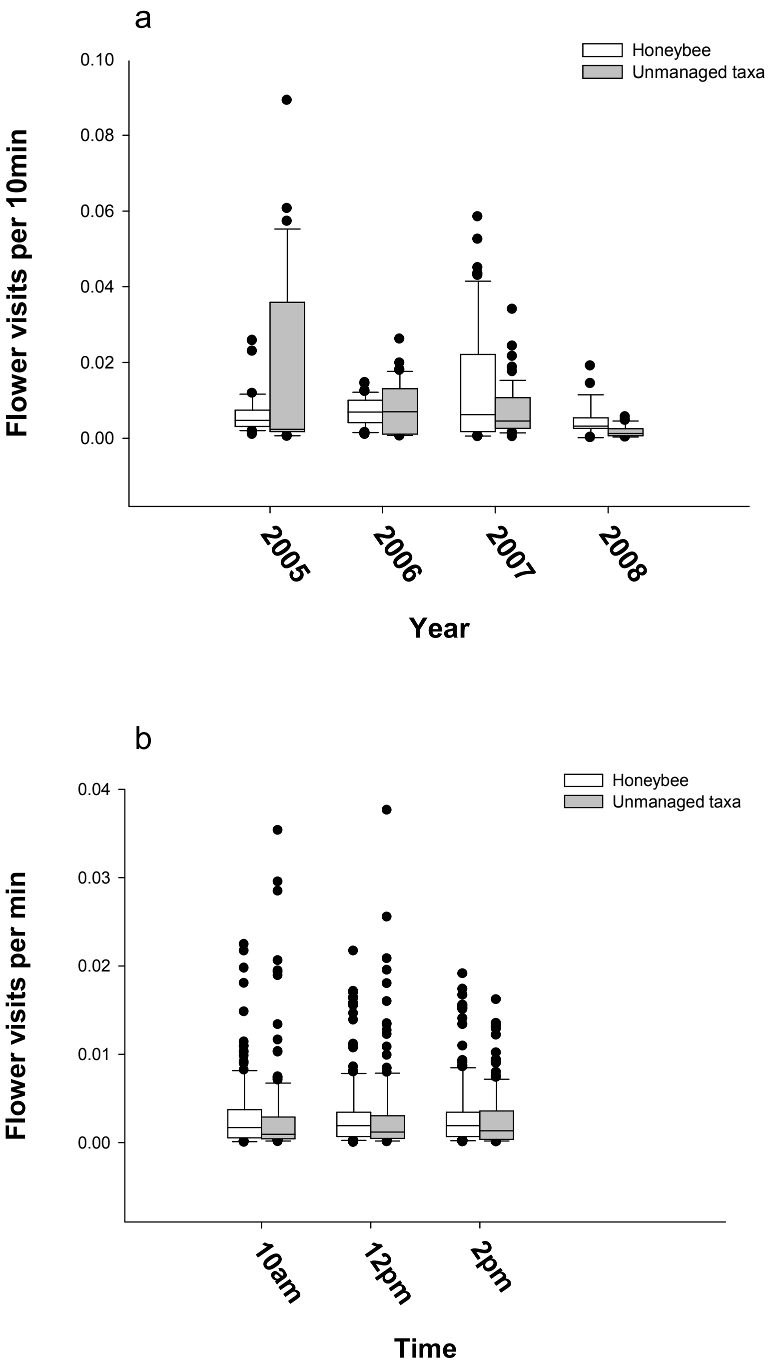


Figure 3.2 a-b: Boxplots of temporal differences between managed and unmanaged taxa in rate of flower visitation per available open flowers. Box indicates quartiles with median marked as a horizontal line; points are outliers. a: visitation across four year period; b: visitation within a day.

Table 3.1: Taxa recorded visiting flowers in Brassica rapa fields in the Canterbury region, New Zealand.

Order	Family	Species
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758
		Bombus terrestris
	Halictidae	*Lasioglossum sordidum* (Smith, 1853)
	Colletidae	*Leioproctus fulvescens*
		Leioproctus sp. 1
		Hylaeus sp.
	Ichneumonidae	Unidentified species
Diptera	Anthoymiidae	*Delia platura (*Meigen, 1826)
		*Anthomyia punctipennis (*Weideman, 1830)
	Bibionidae	*Dilophus nigrostigma* (Walker, 1848)
	Calliphoridae	*Calliphora hortona* (Walker, 1849)
		*Calliphora quadrimaculata (*Swedarius, 1787*)*
		Calliphora stygia (Fabricius, 1794)
		Calliphora vicina Robineau-Desvoidy, 1830
		Lucilia sericata (Meigen, 1826)
		Pollenia pseudorudis (Rognes, 1985)
	Drosophylidae	*Drosophila sp.*
	Ephydridae	Unidentified species 1
	Empididae	Unidentified species 1
	Stratiomyidae	*Odontomyia atrovirens.*
	Muscidae	*Spilogona melas* (Schiner, 1868)
		Hydrotaea rostrata (Robineau-Desvoidy, 1830)
	Nematoceridae	Unidentified species
	Syrphidae	*Eristalis tenax* Linnaeus, 1758
		Melanostoma fasciatum (Macquart, 1850)
		Melangyna novaezelandiae (Macquart, 1855)
		Helophilus hochstetteri Nowicki, 1875
		Helophilus trilineatus
	Sarcophagidae	*Oxysarcodexia varia* (Walker, 1836)
	Tabanidae	*Scaptia sp.*
	Tachinidae	*Pales usitata* (Hutton, 1901)
		Pales marginata (Hutton, 1901)
		Protohystricia spp.
		Unidentified species
Coleoptera	Coccinelidae	*Coccinella undecimpunctata* (Linnaeus, 1758)
	Staphylinidae	Unidentified species
	Scarabaeidae	*Costelytra zealandica* (White)
Hemiptera	Pentatomidae	*Nezara viridula* Linneaus, 1758)
Lepidoptera	Pieridae	*Pieris rapae (*Linneaus, 1758*)*
	Crambidae	*Orocrambus sp.*
	Noctuidae	Unidentified species
	Nymphalidae	*Vanessa itea* (Fabricius, 1755)
Odonata	Coenagrionidae	*Xanthocnemis zealandica* (McLachlan, 1873)

Table 3.2: Median values per field per taxa to demonstrate spatial differences in four pollinator traits recorded in a single year of observation. Overall effectiveness calculated as per Rader *et al*. (2009). *Values to 4 sig figs.

Species	Stigmatic contact	Stigmatic pollen	Flower visits min^{-1}	* Rate of visitation	Overall effectiveness
Lincoln site 1					
Apis mellifera	0.9	99.5	27.46	0.01513	1339
Bombus terrestris	1	502	33.10	0.00044	265
Lasioglossum sordidum	0.3	14	12.87	0.00014	0.28
Leioproctus sp. 1	1	104	22.30	0.00021	17
Dilophus nigrostigma	0.5	22.5	5.29	0.00095	2
Eristalis tenax	1	50	23.62	0.00197	84
Melanostoma fasciatum	0.3	0.5	2.26	0.00033	0.004
Melangyna novaezelandiae	0.6	14	6.01	0.00146	2
Lincoln site 2					
Apis mellifera	0.9	127	21.70	0.00042	37
Bombus terrestris	1	49.5	17.50	0.00015	4
Lasioglossum sordidum	0.4	24.5	5.03	0.00001	0.01
Leioproctus sp. 1	1	247	16.13	0.00001	2
Dilophus nigrostigma	0.4	66	2.11	0.00005	0.1
Eristalis tenax	0.7	159	9.59	0.00025	10
Melanostoma fasciatum	0.05	14	5.07	0.00023	0.03
Melangyna novaezelandiae	0.3	35.5	5.61	0.00012	0.25
Gore site 1					
Apis mellifera	1	69	27.40	0.0026	177
Bombus terrestris	1	246	55.55	0.000083	41
Lasioglossum sordidum	0.3	34	8.08	0.000010	0.04
Leioproctus sp. 1	1	47	16.13	0.000069	2
Dilophus nigrostigma	0.4	18	2.16	0.0001	0.06
Eristalis tenax	0.95	71.5	5.45	0.0025	33
Melanostoma fasciatum	0.3	7	1.41	0.000041	0.004
Melangyna novaezelandiae	0.4	2	7.27	0.00047	0.1
Gore site 2					
Apis mellifera	0.9	107.5	50.85	0.0027	486
Bombus terrestris	1	336	43.40	0.000087	45
Lasioglossum sordidum	0.3	24.5	1.39	0.000014	0.005
Leioproctus sp. 1	1	104	1.31	0.000094	6
Dilophus nigrostigma	0.2	97	1.11	0.0001	0.08
Eristalis tenax	0.95	126	6.32	0.001	40
Melanostoma fasciatum	0.3	7	3.80	0.000051	0.01
Melangyna novaezelandiae	0.3	14	11.81	0.001	2

Table 3.3: Median values per field to demonstrate spatial and temporal differences in four pollinator traits between managed and unmanaged taxa. Stigmatic pollen loads, stigmatic contact and flower visits per minute recorded in a single year of observation. Rate of visitation recorded across four years of observation. Overall effectiveness calculated as in Rader et al. (2009).

Taxa	Stigmatic contact#	Stigmatic pollen	Flower visits per min#	Rate of visitation flower^{-1*} 10min^{-1}	Overall effectiveness per hour
Lincoln site 1					
Apis mellifera	*0.9*	*99.5*	*27.17*	*0.0154*	*13488*
Unmanaged taxa pooled	0.8	26	18.96	0.00150^	212
Lincoln site 2					
Apis mellifera	*0.9*	*127*	*16.33*	*0.000589*	*395*
Unmanaged taxa pooled	0.4	58	6.91	0.004^	230
Gore site 1					
Apis mellifera	*1*	*69*	*50.847*	*0.002*	*421*
Unmanaged taxa pooled	1	55	11.8	0.016^	3738
Gore site 2					
Apis mellifera	*0.9*	*107.5*	*27.39*	*0.002784*	*2655*
Unmanaged taxa pooled	0.4	126	29.17	0.013^	6880

Discussion

In this study I investigated spatial and temporal variation in pollinator assemblages and pollination services in a mass flowering *B. rapa* crop in New Zealand. I did this by examining flower-visiting taxa (i) spatially across four fields and (ii) temporally at two hourly intervals within days, and each year over a four year period. In total 43 taxa (including *A. mellifera*) were identified. Taxa were not equally abundant or frequent. For example, 36 unmanaged taxa provided pollination services in only one, two or three of the four years. Seven additional unmanaged taxa were shown to be frequent visitors across all fields over a four year period, thus providing consistent pollination services in space and over time.

Unmanaged taxa performed equally to the honeybee in two of the four components of overall pollinator effectiveness, stigmatic pollen loads and flower visits per minute. The capacity for unmanaged taxa to be as efficient as the honeybee is an important finding as stigmatic pollen loads can be a good indicator of pollinator effectiveness, even if rate of visitation is low. For example, Mayfield *et al.* (2001) demonstrated that long-tongued bumblebees deposited on average three-times as much outcross pollen per visit to virgin flowers, and elicited four-times as much seed production than hummingbirds, even though hummingbirds were the more frequent visitors.

Unmanaged pollinators were not however, as efficient as the honeybee in terms of stigmatic contact nor in the rate of flower visitation (abundance per available open flowers). Although unmanaged taxa were capable of efficient pollen transfer relative to the honeybee, the frequency in which contacts occurred was significantly lower in comparison to the honeybee. Nonetheless, some unmanaged taxa have been shown to contact stigmas as frequently as the honeybee (Rader et al. 2009) hence management to promote these taxa in particular could facilitate the consistency of unmanaged pollinator assemblages.

At the time of this study the *Varroa destructor* mite had not yet been recorded in the study area, hence, the expectation that *A. mellifera* would most likely be a consistent visitor at both spatial and temporal scales, was confirmed. However, unmanaged pollinator visitation rates (abundance per available open flowers) were significantly lower than the honeybee when observed across the four fields in one year, due to low abundances (Table 3.2). The nature of 'unmanaged' status may explain the lower and more variable visitation rates, since this variability most likely reflects the patchy presence of wild populations and their tendency to fluctuate with resource availability and environmental change (Cane and Payne, 1993; Cane and Tepedino, 2001; Roubik, 2001; Tylianakis, 2005).

The importance of the rate of visitation in determining overall pollinator effectiveness is a hotly debated topic due to the inconsistencies among studies (Alarcon; Mayfield *et al.*, 2001; Ivey *et al.*, 2003; Ghazoul, 2005b; Vazquez *et al.*, 2005). In some studies, the large variability in estimates of rate of visitation in time and space, (Mayfield *et al.*, 2001; Ivey *et al.*, 2003; Ghazoul, 2005b; Bos *et al.*, 2007) has minimized its utility as an index of overall effectiveness. Conversely, other studies have demonstrated the large contribution that visitation makes to overall pollinator effectiveness and that even less efficient taxa can become important if visits are frequent (Ivey *et al.*, 2003; Bloch *et al.*, 2005; Vazquez *et al.*, 2005; Sahli and Conner, 2007; Madjidian *et al.*, 2008).

Even though unmanaged taxa exhibited low visitation rates compared to the honeybee in one year at four fields, unmanaged pollinators provided equal visitation services to the managed honeybee over a four year time period (Figure 3.2). When unmanaged visitation services over this four year period were combined with median values of pollen transfer efficiency across different fields, unmanaged pollinators as a group differed little in overall effectiveness to the honeybee. This result highlights the novelty and importance of this study. The individual effectiveness of unmanaged taxa in crops has been demonstrated elsewhere in detail (e.g. reviewed in Klein *et al.*, 2008; Ricketts *et al.*, 2008; Rader *et al.*, 2009). Yet, the consistency of unmanaged visitation services to mass flowering crops over a period of years, has not yet been reported to our knowledge. Moreover, visitation rate (abundance per open available flowers) is dependent to a large extent upon the abundance of pollinators within a given interval of time and space (as well as the abundance of floral resources). I argue therefore that visits of individual pollinator taxa should not be treated separately in attempts to assess the overall effectiveness of unmanaged pollinators as an entity. Even though unmanaged taxa differ individually across traits within crops (Kremen *et al.*, 2002; Rader *et al.*, 2009), each individual unmanaged taxon visit occurs concurrently with other unmanaged taxa. A combined estimate of the pollination services provided by unmanaged taxa is therefore necessary in order to compare their effectiveness as a group, to the honeybee.

The potential provision of consistent inter-annual pollination services by a diverse unmanaged pollinator assemblage that is equal to those services provided by the managed honeybee, has important implications. First, these findings suggest that potential declines in honeybee abundance in association with colony collapse disorder and the *Varroa* sp. mite could be offset by alternative species that are unaffected by these conditions.

Second, the diversity of the assemblage may indicate a measure of resilience in pollination services, since individual population sizes in diverse species assemblages are unlikely to co-vary as a function of temporal environmental change (Yachi and Loreau, 1999; McCann, 2000; Elmqvist *et al.*, 2003), in support of the biological insurance hypothesis (Walker, 1992; Lawton and Brown, 1993; Naeem and Li, 1997; Naeem, 1998). This ensures some species are present when others are not (Kremen *et al.*, 2002; Ricketts, 2004). For example, Kremen *et al*. (2002) found that diversity was essential for sustaining the pollination services to watermelon crops. The authors demonstrate that year-to-year variation in community composition had the capacity to buffer crops against population fluctuations of any one given pollinator species. In this study, a majority of the 43 taxa also fluctuated in their visitation rates across the four year period yet visitation rates overall did not change from year to year or within a day.

Spatial and temporal differences in the rate of visitation can impact upon the ranking of pollinator importance (Fishbein and Venable, 1996; Hoehn *et al.*, 2008; Madjidian *et al.*, 2008; Olesen *et al.*, 2008) In this study however, the significant differences revealed in all four pollinator traits between field locations (Figure 3.1) did little to alter the ranking of pollinator importance (Table 3.2). This result may suggest that the relative differences in pollinator traits among taxa are consistent across fields, probably reflecting taxa-specific foraging behaviours (Thomson and Goodell, 2001; Goodell and Thomson, 2007; Jha and Vandermeer, 2009) such as response to floral resource availability (Jha and Vandermeer, 2009; Diekotter *et al.*, 2010) and/or the extent to which pollen is groomed from pollinator bodies (Rademaker *et al.*, 1997). Further, although the study landscape was highly intensified it is likely local resource heterogeneity exists and differs between crop fields (Tscharntke *et al.*, 2005; Rundlof *et al.*, 2008). In this study I lack the evidence to demonstrate the influence of local resources on variations in pollinator effectiveness, yet it is clear from other studies that resource heterogeneity can impact upon plant-insect interactions in highly modified systems (Isaia *et al.*, 2006; Tylianakis *et al.*, 2008). In our study area, floral resources such as weeds were present within field margins potentially providing the variation in local resources to impact upon the observed spatial variations in pollinator traits (Backman and Tiainen, 2002; Marshall *et al.*, 2006). Further research on taxa specific foraging behaviours is required to further elucidate the mechanisms driving the relative differences in pollen transfer efficiency.

In conclusion, this study demonstrates the potential for unmanaged pollinator taxa to provide efficient and consistent pollination services similar to those provided by the managed honeybee. Pollen transfer efficiency varied between field locations but this did not alter the ranking of unmanaged pollinator importance. As a group, the unmanaged component of pollinator services provided visitation rates consistent with that of the honeybee over a four year period. Coupled with equal services in two of the four pollinator traits determining overall effectiveness, unmanaged taxa have the potential to replace honeybee services. Future research should be directed toward (i) the determination of the specific resources required by unmanaged pollinators to facilitate populations of individual efficient taxa and (ii) a greater understanding of the mechanisms causing temporal and spatial variation in assemblages specifically in highly modified agricultural systems. This information is necessary in order to identify the best possible land management strategies to maintain or increase the population sizes of these species.

Chapter 4: Diverse assemblages enable all-day flower visitation: bee and fly taxa contribute to pollination services at different times of the day.

This chapter has been submitted:

Romina Rader, Will Edwards, Saul A. Cunningham, David A. Westcott and Bradley G. Howlett *Diversity and Distributions*

Abstract

The global decline in pollinator populations has drawn attention to the insurance value that diverse, multi-species assemblages may provide. Pollination insurance comes from functional complementarity between species, which can buffer adverse effects arising from declines in single species. I examine trait variation (differences in activity patterns and overall effectiveness) between bee and fly taxa that visit flowers, at two hourly intervals from 6am to 8pm in twelve mass flowering commercial *Brassica rapa* fields in New Zealand. Flies were most abundant and diverse in the early morning and afternoon while bees were most abundant at midday. The relative differences in visitation rates among taxa within a day did not reflect differences in overall pollinator effectiveness due to the wide variation in pollinator efficiency traits. Even though this resulted in flies being less efficient than bees in general, they visited flowers at times when the bees were in low abundance or absent, resulting in similar or higher rates of overall effectiveness to the bees at these times. Overall visitation rate did not differ significantly between sample periods, indicating that changing taxonomic composition in assemblage structure

was not accompanied by changes in potential pollination services. Understanding species complementarity will allow for a more accurate assessment of the possible consequences of declining populations of taxa that have been historically managed to provide pollination services. This will reduce the chance of the loss of pollination services in response to environmental change.

Introduction

The decline in global pollinator populations has highlighted the important role that multi-species assemblages can play in maintaining ecosystem function (Hooper *et al.*, 2005; Gross *et al.*, 2007). This is because diverse assemblages can increase the likelihood that an effective pollinator is present (i.e. sampling effect Tilman *et al.*, 1997) and because fluctuations in population size of any one individual species is buffered by the range of responses experienced across all species (Yachi and Loreau, 1999; McCann, 2000; Elmqvist *et al.*, 2003). Pollination services may thus be maintained even when fluctuations in environmental conditions cause differential responses in local population sizes of different species (e.g. Chesson and Huntley, 1997).

Combinations of different species with different functional traits can influence ecosystem properties and functions in several ways (Hooper *et al.*, 2005). For example, niche theory proposes that species coexist in a complementary way by their occupation of different niches, maximising resource use in time and space (Naeem *et al.*, 1994; Tilman *et al.*, 1997; Rosenfeld, 2002; Cardinale *et al.*, 2006; Fontaine *et al.*, 2006). Identification of functional complementarity therefore requires an understanding of the magnitude of trait variation that exists between all taxa that constitute the assemblage of interest, to determine the different niches occupied and whether this results in greater resource use.

Both bees and flies can act as pollinators in highly modified agricultural systems (Feldman, 2006; Klein *et al.*, 2007a; Jauker and Wolters, 2008; Rader *et al.*, 2009). The markedly different life histories, morphologies and behaviours of bees and flies make them good candidates to test for functional complementarity in a pollinator assemblage. Complementarity may be expected since trait variation (such as differences in activity patterns or pollen transfer efficiency) is likely to exist between taxonomic groups. For example, bees have high energy requirements in association with endothermic flight, nest building and offspring provisioning (Kearns, 1992). Bees therefore tend to avoid low temperatures when foraging (Cruden, 1972; Kearns, 1992). Conversely, flies have low energy needs and operate ectothermically (Arroyo *et al.*, 1982; Doucet *et al.*, 2009; Huey and Pascual, 2009; Richards *et al.*, 2009). Fly activity is therefore more likely when temperatures are not too hot (Huey and Pascual, 2009). If pollination services provided by bees and flies are functionally equivalent but temporally separated based on activity, total pollination services provided to crops will be greater when pollinator assemblages includes both groups than when either taxa alone attend flowers.

Implicit in the concept of functional complementarity is the positive relationship between diversity and productivity (Hooper *et al.*, 2005; Balvanera *et al.*, 2006; Cardinale *et al.*, 2006). Nevertheless, few field-based studies have investigated the possible value of different functional traits among species in pollinator assemblages and its affect upon productivity. This is especially true for the pollination of crop species (but see Hoehn *et al.*, 2008; Klein *et al.*, 2008). In the one study in which I am aware Hoehn *et al*. (2008) reported that assemblages comprising functionally complementary pollinator species differing in temporal activities according to their daily foraging behaviour, produced significantly higher seed set in pumpkin crops in Indonesia, compared to less diverse assemblages (Hoehn *et al.*, 2008).

In this study, I compare the visitation rates and overall effectiveness of bee and fly pollinator taxa to determine if functional complementarity plays a role in the provision of pollination services. I test this premise using a mass flowering food crop ("Pak Choi" *Brassica rapa)* which attracts a diverse assemblage of flower visitors (Rader *et al.*, 2009). Moreover, modified agricultural systems such as this do not present the high level of spatial heterogeneity in floral resources associated with more natural landscapes (Klein *et al.*, 2008; Tylianakis *et al.*, 2008) making them ideal to examine temporal differentiation in activity patterns in contrasting pollinator species free from possible taxa-specific floral requirements and preferences.

I compare visitation rates of a range of taxa at eight time intervals spanning every two hours from 6am to 8pm to ask.

1. Do bee and fly taxa exhibit functional trait differences (i.e. measured by activity patterns and overall effectiveness) which result in differences in the identity of the most effective visitor within a day?
2. Does the time of day influence total visitation rates (absolute number of visits across all species combined)?
3. Do unmanaged taxa (as a group) visit flowers at different rates or times of the day to the managed honeybee?
4. Does the presence of multiple taxa result in a higher visitation rate thereby supporting the concept of functional complementarity?

I examine these questions in light of their implications for what might be expected should single species (i.e. honeybee) population sizes decrease.

Materials and Methods

Overall pollinator effectiveness

I define overall pollinator effectiveness as the total contribution to pollination services provided by each taxa. This represents the product of pollen transfer efficiency at individual flowers and pollinator rate of visitation (Rader et al. 2009). I estimate pollen transfer efficiency at individual flowers using the product of stigmatic pollen load x proportion of stigmatic contact, and the first component of pollinator visitation rate (flower visits per unit time) using the methods and data outlined in Rader *et al*. 2009. This study concerns diurnal changes in the second component of pollinator rate of visitation (visitor abundance per number of open flowers; Rader et al. 2009). I calculate rate of visitation by observing flower visitors over an entire day to determine how overall pollinator effectiveness changes with varying rates of visitation.

Flower visitor observations

I observed flower visiting insects in 12 commercial *B. rapa* fields across the Canterbury region of New Zealand between December 5 2004 and March 20 2005. All fields were approximately rectangular and ranged in size from 0.75 to 2.0 ha. Observations were made at the time of peak flowering, defined as the period during which mature receptive female flower density exceeded 1000/m^2.

Five observation quadrats (10x10m) were established per field; one in the centre of each field and one near each of the four corners in four directions (NW, NE, SW, SE). Observations of floral visitors were made by walking along each of the four boundaries of the quadrat (i.e. 10m) and recording all insect species and their abundances within 1m of the boundary during a 10min time period. All five quadrats in each field were observed for a total period of 50min per time period for eight observation periods (8 x 50min) at two hourly intervals between 6am and 8pm. The order that fields, and quadrats-within-fields were examined was randomised across different survey events. All flower visitors were identified to species in the field where possible. Where species identity could not be determined at the time of observation, specimens were collected between observation periods and taken to the laboratory for identification. Data for all quadrats within each field were combined to estimate visitation rates as the mean number of visits per flower per 10min. All observations were made on sunny or partly cloudy days when the temperature was >16ºC and wind speed < 5 ms^{-1}*. S*ite characteristics and methodology are provided in more detail in Rader *et al*. 2009.

Data Analysis

To examine within-day change in activity patterns between taxa and over time, I used mixed effects models with REML (Restricted Maximum Likelihood; (Payne, 2005). First, I test the main effects and interaction of the terms “Taxa” (the six most frequent visitors i.e. “Individual taxa”) and “Time of day” (two hourly time intervals from 6am-8pm) with visitation rate as the response variable. Second, I use the same model as above with the exception of “Taxa”. In this second model, “Taxa” refers to comparisons between all pooled unmanaged taxa and the honeybee (i.e. “Grouped taxa”). Fields in which the observations were taken were considered a random factor to account for error due to spatial variation among taxa. Where applicable, all post-hoc pair-wise comparisons were performed using treatment contrasts with Bonferroni corrections to account for multiple comparisons. Statistical analyses were calculated with R Statistical Software (The R Foundation for Statistical Computing 2009).

Results

I observed 27 species of flower visitors making 9667 visits to *B. rapa* flowers over the field season (Table 4.1). The five most frequent visitors comprised two introduced bees, the honeybee, *A. mellifera* and Bumblebee, *Bombus terrestris*. and three flies, *Dilophus nigrostigma*, *Melangyna novaezealandiae* and *Eristalis tenax*. These taxa were all recorded in >400 observations in >6 time intervals and all calculations regarding visitation rates (below) refer to these taxa. I also include one other bee, *Leioproctus* sp. 1. This species was not considered a frequent visitor but its equivalence in efficiency to the honeybee in Rader *et al* (2009) warrants its inclusion to examine relative pollinator importance throughout a day. The remaining 20 species accounted for the remaining 24.5% of visits and comprised 14 additional Diptera, two additional hymenoptera, one coleoptera, one heteroptera and two lepidoptera taxa (Table 4.1).

Taxa, time and their interaction with visitation rate

There were differences in activity among the six visitors across time periods. The interaction between "individual taxa" and "time of day" was significant (individual taxa* time of day $F_{49,217} = 1.639$; $P = 0.009$; Figs 1 and 2) demonstrating differences in visitation rate between individual taxa depending on time within a day. Visitation rates also differed significantly between the six "frequent visitors ("individual taxa": $F_{7,217} = 15.4428$; $P < 0.0001$; Figure 4.3) and between the frequent visitors at different time intervals ("time of day": $F_{7,217} = 4.1497$; $P < 0.0003$).

For example, the three bees (*A. mellifera*, *B. terrestris* and *Leioproctus* sp. 1) and one fly *D. nigrostigma*, visited at a significantly higher rate in the middle of the day (i.e 10am, 12pm) than early in the morning at 6am (treatment contrasts: *A. mellifera* 6am and 10am: *P* = 0.009; 6am and 12pm: *P* = 0.001; *B. terrestris* 6am and 10am: *P* = 0.0023; *D. nigrostigma:* 8am and 12pm: *P* = 0.034 and *Leioproctus* sp. 1. 12pm and all other times except 10am: P<0.001) and late in the evening at 8pm (treatment contrasts: *A. mellifera* 10am and 8pm: *P* = 0.006; 12pm and 8pm: *P* = 0.001; *B. terrestris* 10am and 8pm: *P* = 0.003; *D. nigrostigma* 10am and 8pm: *P* = 0.022 Figs 1 and 2). In contrast, the remaining fly taxa (other than *D. nigrostigma*) visited flowers in greater numbers during the early morning and late in the afternoon/evening (Figure 4.3).

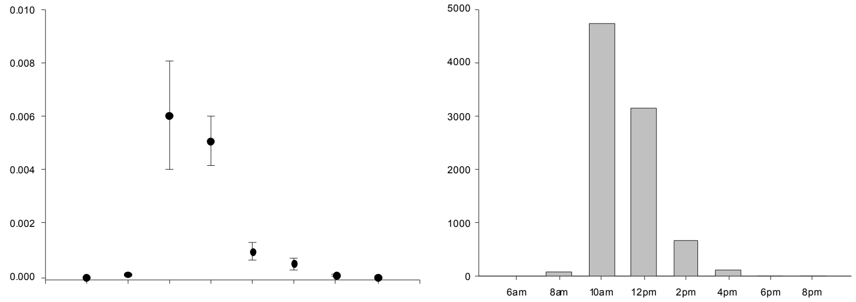
D. nigrostigma was the most frequent visitor at all time intervals except 10am and 12pm when it shared the most frequent pollinator position with *A. mellifera* (i.e. no significant difference in visitation rate between *A. mellifera* and *D. nigrostigma* at 10am: treatment contrasts: *P* = 0.214; 12pm: *P* = 0.103). *D. nigrostigma* was the most frequent visitor overall compared to the remaining five frequent visitors (treatment contrasts: *D. nigrostigma* and *A. mellifera, P* = 0.012; *D. nigrostigma* and *B. terrestris*, *P* = 0.0067; *D. nigrostigma* and *E. tenax*, *P* < 0.001; *D. nigrostigma* and *M. novaezelandiae*, *P* = 0.0031; *D. nigrostigma* and *Leioproctus sp.*, *P* < 0.001). *A. mellifera* visited at a significantly higher rate than *M. novaezelandiae, B. terrestris.,* *E. tenax*, *Leioproctus sp.* (treatment contrasts: *A. mellifera* and *M. novaezelandiae,* *B. terrestris*, *E. tenax* and *Leioproctus sp.*. *P* < 0.001).

Irrespective of its frequency of visitation however, *D. nigrostigma* was not the most effective pollinator overall, or at any time interval due its lower pollen transfer efficiency (Figure 4.2, Table 4.3). Further, *D. nigrostigma* was comparable in overall effectiveness with *Leioproctus* sp. 1 at 10am and 12pm, even though *Leioproctus* sp. 1 exhibited significantly lower visitation rates, the lowest of all common taxa recorded (Tables 4.2 and 4.3). This is due to *Leioproctus* sp. 1 being highly efficient at pollen transfer (Rader et al. 2009).

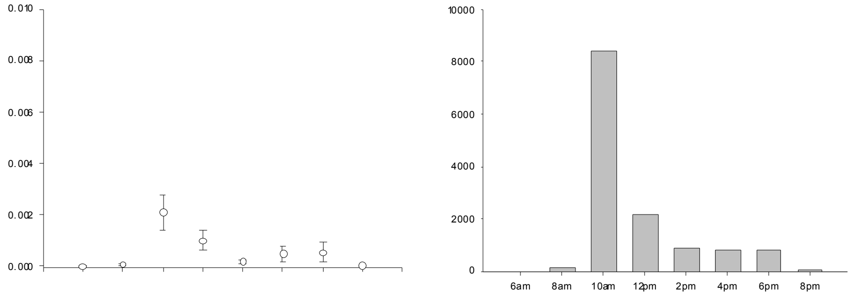
Overall pollinator effectiveness was also significantly influenced by pollen transfer efficiency in two other taxa, *B. terrestris* and *E. tenax*. These taxa visited flowers at significantly lower rates than *A. mellifera* and *D. nigrostigma*, yet this did not impact upon either of these taxa ranking as the most effective pollinators at 6am and 8am (*E. tenax*) and 2-8pm (*B. terrestris*). This finding is similar to that a study by Mayfield *et al*. (2001) which examined the pollinators visiting the plant I*pomopsis aggregate*, whereby the most effective pollinators overall (Bumblebees) were not the most frequent visitors.

There was strong evidence that differences in activity patterns combine to determine total pollination service. While individual taxa did visit flowers in different total abundances at different times of the day (above), total visitation rates (absolute numbers of visits across all species combined) did not differ between taxa (“grouped taxa” $F_{1,354} = 3.581$, $P = 0.0592$), or between time intervals (“time of day”: $F_{7,354} = 0.895$, $P = 0.509$; “grouped taxa * time of day: $F_{7,354} = 1.028$, $P = 0.4106$). This indicates that overall, rate of visitation was more or less equal at all time intervals and was independent of which individual taxa was most abundant at any given time.

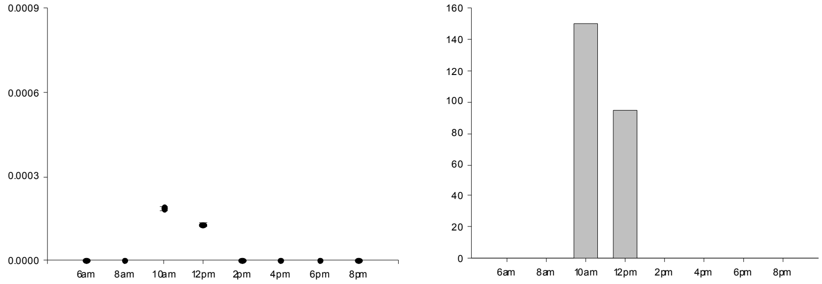
Rate of Visitation **Overall effectiveness**



Apis mellifera

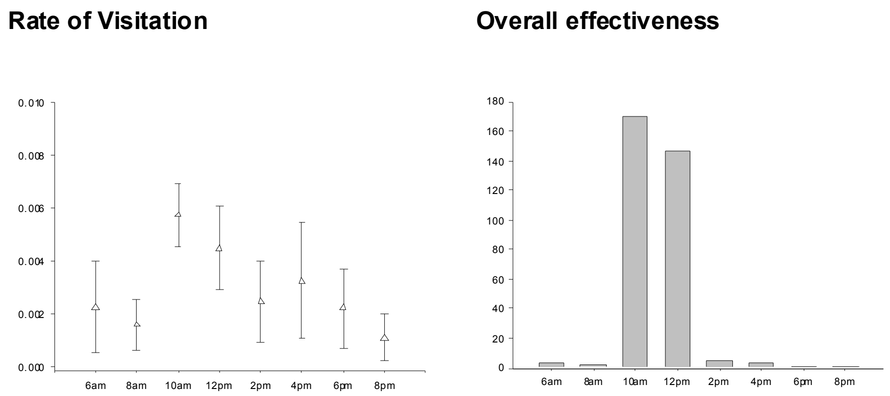


Bombus terrestris

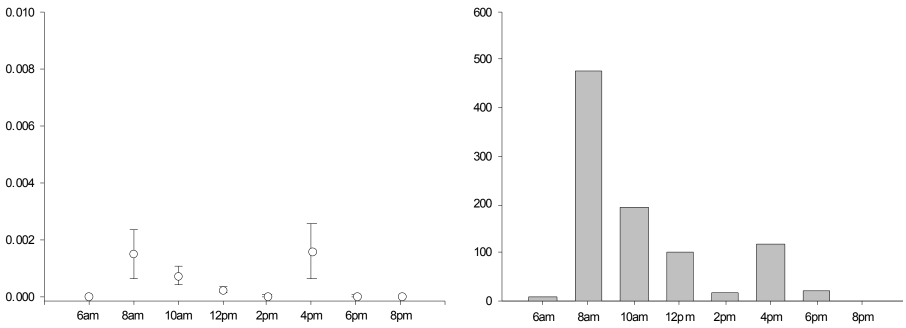


Leioproctus sp. 1

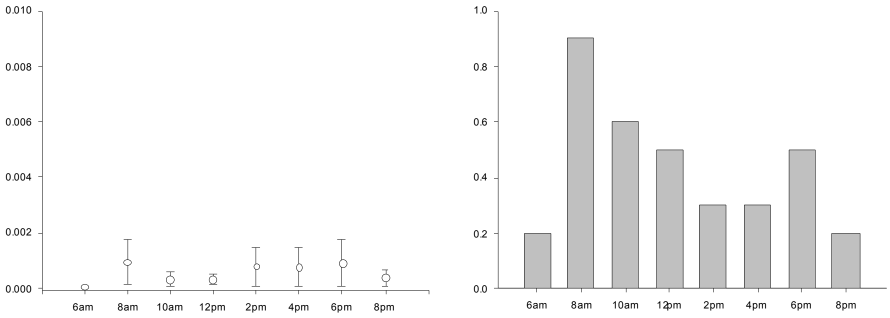
Figure 4.1: Diurnal visitation rates (visits per flower per 10min) and overall pollinator effectiveness of the three most frequent bee visitors to *Brassica rapa* fields in the Canterbury region, New Zealand.



Dilophus nigrostigma



Eristalis tenax



Melangyna novaezelandiae

Figure 4.2: Diurnal visitation rates (visits per flower per 10min) and overall pollinator effectiveness of the three most frequent fly visitors to *Brassica rapa* fields in the Canterbury region, New Zealand.

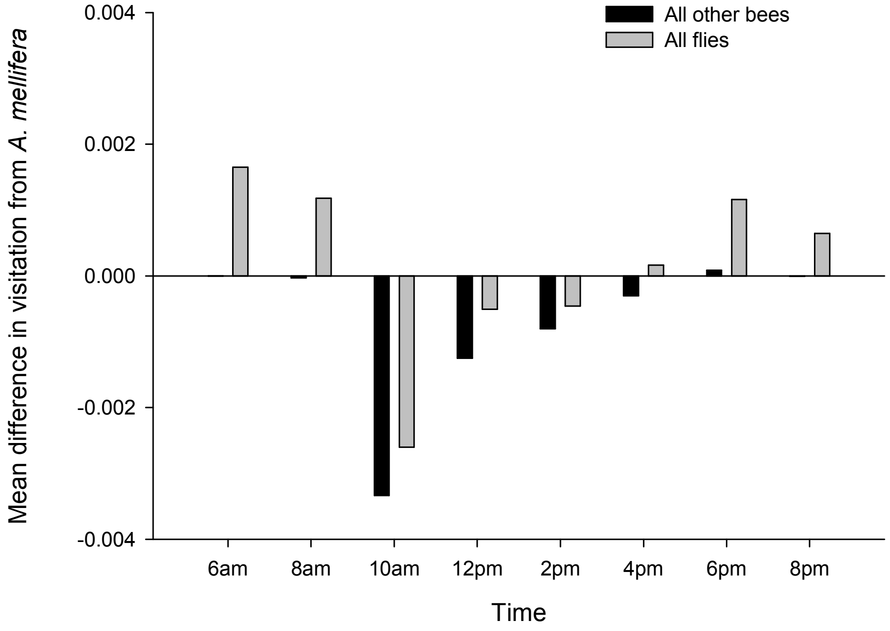


Figure 4.3: Mean differences in diurnal visitation rate (visits per flower per 10min) between *A. mellifera* and all other unmanaged bee and fly taxa visiting flowers in *Brassica rapa* fields, New Zealand.

Table 4.1: Taxa recorded visiting *Brassica rapa* flowers in Canterbury, New Zealand

Order	Family	Species
Hymenoptera	Apidae	*Apis mellifera* Linnaeus, 1758
		Bombus terrestris (Linnaeus, 1758)
	Halictidae	*Lasioglossum sordidum* (Smith, 1853)
	Colletidae	*Leioproctus* sp.1
	Ichneumonidae	Unidentified species
Diptera	Anthoymiidae	*Delia platura* (Meigen, 1826)
	Bibionidae	*Dilophus nigrostigma* (Walker, 1848)
	Calliphoridae	*Calliphora hortona* (Walker, 1849)
		Calliphora quadrimaculata (Swedarius 1787)
		Calliphora stygia (Fabricius, 1794)
		Calliphora vicina Robineau-Desvoidy, 1830
		Lucilia sericata (Meigen, 1826)
	Ephydridae	Unidentified species 1
	Stratiomyidae	*Odontomyia spp*.
	Muscidae	*Spilogona melas* Schiner 1868
	Syrphidae	*Eristalis tenax* Linnaeus, 1758
		Melanostoma fasciatum (Macquart, 1850)
		Melangyna novaezelandiae (Macquart, 1855)
		Helophilus hochstetteri Nowicki, 1875
	Sarcophagidae	*Oxysarcodexia varia* (Walker, 1836)
	Tabanidae	*Scaptia* sp.
	Tachinidae	*Pales usitata* (Hutton, 1901)
		Protohystricia spp.
Coleoptera	Coccinelidae	*Coccinella undecimpunctata* (Linnaeus, 1758)
Hemiptera	Pentatomidae	*Glaucias amyoi* (Dallas)
Lepidoptera	Pieridae	*Pieris rapae* (Linneaus, 1758)
	Nymphalidae	*Bassaris* sp.

Table 4.2a: Median values of pollen transfer efficiency across frequently visiting taxa (from Rader et al. 2009). Component 1 represents part of the calculation to determine overall pollinator effectiveness (i.e. Stigmatic contact x stigmatic pollen load x flower visits per hr); see Table 4.2b for overall effectiveness calculation.

Species	Stigmatic contact	Stigmatic pollen	Flower visits hr^{-1}	Component 1: Stigmatic contact x stigmatic pollen x flower visits hr^{-1}
Apis mellifera	0.9	89.5	1630	131331
Bombus terrestris	1	234	2590	606043
Leioproctus sp. 1	1	101	1274	128632
Dilophus nigrostigma	0.4	38.5	265	4070
Eristalis tenax	0.95	71.5	980	66599
Melangyna novaezelandiae	0.3	9	358	968

Table 4.2b: Component 2 of overall effectiveness calculation: median rate of visitation recorded in this study. This value is calculated as the number of visits per available open flowers per 10min time period (x 6 to calculate values per hour) for each of the 8 time intervals within a day from 6am to 8pm. Values to 3 sig figs.

Species	Rate of visitation							
	6am	8am	10am	12pm	2pm	4pm	6pm	8pm
Apis mellifera	0	6.59 x 10^{-4}	3.6 x 10^{-2}	2.4 x 10^{-2}	5.26 x 10^{-3}	1.1 x 10^{-3}	2.1 x 10^{-4}	1.16 x 10^{-4}
Bombus terrestris	6.0 x 10^{-6}	2.18 x 10^{-4}	1.38 x 10^{-2}	3.53 x 10^{-4}	1.4 x 10^{-3}	1.3 x 10^{-3}	1.36 x 10^{-3}	9.03 x 10^{-5}
Leioproctus sp. 1	0	0	1.94 x 10^{-4}	1.23 x 10^{-4}	0	0	0	0
Dilophus nigrostigma	8.6 x 10^{-4}	4.6 x 10^{-4}	4.2 x 10^{2}	3.6 x 10^{-2}	1.15 x 10^{-3}	9.77 x 10^{-4}	3.35 x 10^{-4}	3.22 x 10^{-4}
Eristalis tenax	1.37 x 10^{-4}	6.17 x 10^{-3}	2.89 x 10^{-3}	1.49 x 10^{-3}	2.55 x 10^{-4}	1.8 x 10^{-3}	2.93 x 10^{-4}	0
Melangyna novaezelandiae	2.19 x 10^{-4}	9.22 x 10^{-4}	5.81 x 10^{-4}	5.1 x 10^{-4}	3.13 x 10^{-4}	2.35 x 10^{-4}	5.5 x 10^{-4}	2.29 x 10^{-4}

Table 4.3: Overall effectiveness of each pollinator per hour is calculated as component 1 (Table 4.2a: stigmatic pollen load, contact and flower visits recorded in Rader et al. 2009) x component 2 (Table 4.2b; median visitation rates per hour recorded in this study).

Species	Overall effectiveness							
	6am	8am	10am	12pm	2pm	4pm	6pm	8pm
Apis mellifera	0	86	4728	3152	691	139	26	15
Bombus terrestris	3	132	8372	2142	852	816	822	55
Leioproctus sp. 1	0	0	150	95	0	0	0	0
Dilophus nigrostigma	4	2	170	147	5	4	1	1
Eristalis tenax	9	478	192	99	17	120	20	0
Melangyna novaezelandiae	0.2	0.9	0.6	0.5	0.3	0.3	0.5	0.2

Discussion

In this study I show that multiple taxa visited flowers in a mass flowering crop, but at different times throughout the day. Bee and fly trait variability resulted in differences in visitation rates between taxa. For example, bee activity patterns were generally highest in the middle of the day and lowest in the early morning and late afternoon/evening (Figure 4.3). One fly, *D. nigrostigma* visited flowers at similar time intervals to the bees (Figure 4.2). In contrast, the remaining flies demonstrated high visitation in the early morning and late afternoon/evening and low visitation in the middle of the day. These differences between taxa resulted in changes to the identity of the most effective pollinator at different time intervals.

The apparent segregation in the timing of fly and bee activity patterns most likely reflects expectations based on intrinsic and extrinsic factors that may regulate activity windows (Herrera, 1990; Roulston and Cane, 2000; Goulson and Darvill, 2004). Temperature in particular, is one of the most significant environmental influences on the biology of ectotherms (Wall *et al.*, 1992; Donovan *et al.*, 2006). For example, the tendency for *A. mellifera* and *B. terrestris* to be more active in the middle of the day is most likely associated with warmer temperatures and greater light intensity and is supported by much of the literature (Herrera, 1990; Corbet *et al.*, 1993; Herrera, 1995; Figueroa-Castro and Cano-Santana, 2004; Selvakumar *et al.*, 2006). Although some bees are capable of foraging at cooler temperatures, they are restricted in their activity windows by the high energy demands of endothermic flight, nest building and offspring provisioning (Kearns, 1992). In contrast, flies have lower energy needs and exhibit higher plasticity than bees in their thermal tolerances (Doucet *et al.*, 2009). Flies can regulate their core body temperature behaviourally and hence can regulate their activity times (Morgan and Heinrich, 1987; Graham *et al.*, 1997; Huey and Pascual, 2009). This behaviour may result in wider activity windows than bees, suggesting flies have a greater capacity to be active early in the morning and later in the evening when temperatures are much lower than during the middle of the day (McCall and Primack, 1992; Totland, 1993). This equivalence in function but partitioning by time suggests that pollination services may be extended throughout the hours of the day due to the trait variability exhibited between these taxa.

Visitation rates varied between individual taxa, yet there were no significant differences in overall visitation rate between time intervals. Although visitation rate did not appear to reflect overall effectiveness for the common taxa due to large differences in pollen transfer capacities among taxa, the combined service provided by a diverse range of taxa at all hours of the day will nonetheless improve the chance a flower will receive an efficient visit over time (Castro *et al.*, 2008). A consistent visitation rate throughout the day was a result of the combined effects of the presence of multiple floral visitors that differed in activity patterns. This is because there is a greater number of individuals providing the service (i.e. the higher visitation rate provided by many individual taxa combined, as opposed to few taxa).

In the absence of functional diversity, flower visitation would have been restricted to specific taxa and time intervals and not provided by a range of taxa all day, as in this assemblage. This ‘extra’ visitation provided by the diverse assemblage that resulted in increased effectiveness across an entire day (i.e. early morning and late evening), is likely a result of complementarity. I suggest complementarity is achieved via the functional equivalence of different taxa in providing visitation services (i.e. rate of visitation is similar over the course of a day), the significant contribution by many as opposed to few taxa, to provide this service (i.e. partitioning of activity windows), and the increased visitation provided at the end of a day.

Further, the contribution by flies to pollination across a day was unexpected. The literature to date concerning unmanaged pollinators has predominately focused on social native bees, which do not appear to perform well in highly intensive agricultural systems (Klein *et al.*, 2003; Brosi *et al.*, 2007; Ricketts *et al.*, 2008; Winfree *et al.*, 2009). The capacity for flies to perform as well or better than the honeybee in the company of two larger social bee species in an intensive agricultural system, highlights their potential as 'pollinator insurance' for the managed honeybee. The pooled visitation services provided by all fly taxa over an entire day contributed to the stable visitation rate throughout an entire day demonstrating the importance of the fly taxa in this assemblage. Further research is necessary to determine the pollen transfer efficiency of the remaining fly taxa in order to determine and compare their overall pollinator effectiveness.

In conclusion, this study highlights that the presence of multiple taxa differing in functional traits can result in a longer duration of flower visiting services (i.e. the addition of early morning and late evening flower visits) and more effective pollination services overall, since total possible pollination events are the result of the combined action of all species. I suggest that complementarity is the cause of this increased effectiveness. I recommend future studies further investigate functional trait diversity in pollinator assemblages in order to ascertain pollen transfer efficiency of other unmanaged taxa and their contribution to plant function (i.e. seed set). This will enable further understanding of the role of complementarity in diverse pollinator assemblages and allow for a more accurate assessment of the possible consequences of declining populations of taxa that have been historically managed to provide pollination services

Chapter 5: Pollen transport differs among taxa in a human-modified landscape: bees carry more viable pollen but flies travel further

This chapter has been submitted:

Romina Rader, Bradley G. Howlett, Saul A. Cunningham, David A. Westcott and Will Edwards. *Diversity and Distributions (submitted*)

Abstract

Dispersal distances of insect pollinators are critical in defining their contribution to landscape-wide pollen movement and ultimately gene flow in natural and agricultural systems. I ask if bee and fly pollinator taxa differ in their dispersal distances and transport of viable pollen in a human modified system in the Canterbury region of New Zealand. I captured pollen-carrying insects travelling outside of a model mass flowering agricultural crop, *Brassica rapa* using insect flight intercept traps at five distances (0, 100, 200, 300 and 400 m) from the pollen source. I examined pollen loads and pollen viability to determine whether pollen transport distance and viability differ between species. A total of 5453 insects were collected of which 717 individuals from 26 insect taxa were positively identified as dispersing pollen up to 400m from the source. These taxa consisted of four species from two bee families (Hymenoptera: Apidae and Halictidae), and eight species from four fly families (Diptera: Bibiondae, Stratiomyidae, Syrphidae and Tachinidae). Apidae generally carried higher pollen loads and more viable pollen than most fly taxa. Surprisingly however, taxa in the families Stratiomyidae and Syrphidae carried pollen further than both bee families. A diverse array of wild and managed flower visitors can transport

viable pollen from a pollen source to at least 400m. Knowledge of the differences in transport distances among generalist pollinators in human modified environments is crucial in order to fully understand the potential extent to which pollen transport can facilitate gene flow and the role of generalist insect pollinators in vectoring pollen between crops and related weeds that may lead to unwanted hybridisation.

Introduction

The potential for landscape-wide pollen movement by insects is a central issue in studies of the ecology and management of both natural and agricultural systems (Hayter and Cresswell, 2006; Hoyle *et al.*, 2007). Pollen transfer is a primary source of gene flow and has direct influence on the level of genetic exchange within and between plant populations (Ellstrand, 1992; Ennos, 1994; Burczyk *et al.*, 2004). Gene flow can be considered either beneficial or detrimental depending on the landscape context in which it occurs. For example, pollen-mediated gene flow between native plant populations is beneficial as it facilitates gene flow between remnants, including isolated trees existing within agricultural or urban land uses (Ellstrand, 1992; Richards, 2000; Volis *et al.*, 2005; Ottewell *et al.*, 2009). Conversely, pollen-mediated gene flow between weeds or crop cultivars in agricultural systems can be undesirable due to facilitation of weed invasion and/or reduced purity in seed crops (Lavigne *et al.*, 2002; Wilkinson *et al.*, 2003; Fenart *et al.*, 2007), particularly in relation to the potential hybridization of genetically modified crops (Scheffler *et al.*, 1993; Rieger *et al.*, 2002; Devaux *et al.*, 2008).

Optimal foraging theory predicts that plant reproductive success is negatively related to its isolation distance from a source population (Charnov, 1976; Pyke, 1979). This is because pollen transfer is hindered by fewer pollinator visits at farther distances and/or visits tend to be longer in duration, thus increasing the potential for self-pollen transfer (Lamont *et al.*, 1993).

This effect may however, be negated in two instances; First, isolated plant patches offering high value or abundant resources (i.e. such as mass-flowering trees) may be more attractive to flower visitors than those with few or low value resources, thus potentially sustaining a higher frequency of pollinator visits than expected (Oddou-Muratorio *et al.*, 2006; Ottewell *et al.*, 2009). Second, if highly mobile pollinator taxa are present (such as introduced honeybees or bird pollinators) the frequency of pollinator visitation may become independent of isolation distance, particularly in disturbed habitats (Dick, 2001; Ottewell *et al.*, 2009).

At present, our knowledge of the movement capabilities of insect pollinators is currently limited to a handful of iconic, large-bodied bees including the honeybee and bumblebees (Beekman and Ratnieks, 2000; Steffan-Dewenter and Kuhn, 2003; Knight *et al.*, 2005; Hoyle *et al.*, 2007; but see Pasquet *et al.*, 2008), native bees (Klein *et al.*, 2003; Kremen *et al.*, 2004; Blanche *et al.*, 2006; Greenleaf and Kremen, 2006b; Klein *et al.*, 2007b; Winfree *et al.*, 2007; Winfree *et al.*, 2008; Zurbuchen *et al.*, 2010a; Zurbuchen *et al.*, 2010b) and specialist and/or rare pollinators of conservation significance (Kwak *et al.*, 1998; Richards *et al.*, 1999; Schulke and Waser, 2001; Pauw, 2007; Albrecht *et al.*, 2009). Little is known however, of the movement patterns and pollen transport potential of other generalist insect pollinators such as flies that visit human modified environments (but see Jarlan *et al.*, 1997; Jauker and Wolters, 2008), any of which could set the upper range of isolation distances or provide dispersal to locations that might otherwise be unattainable (Westcott *et al.*, 2008).

Few studies in particular, have specifically contrasted the dispersal potential of well studied long distance pollinators (i.e. honeybee and bumblebee) to common and/or generalist insect assemblages in human modified systems, even though such landscapes are known to support diverse pollinator communities (Westphal *et al.*, 2003; Klein *et al.*, 2007a; Rader *et al.*, 2009). This comparison is necessary in order to (i) examine the relative potential for other insects to contribute to long distance pollen flow (Zurbuchen *et al.*, 2010b) (ii) quantify the extent of ecosystem services provided by native insects (iii) assess any potential risks of undesirable gene flow from agricultural crops to weedy relatives and (iv) to predict future distributions associated with climate and land use change (Engler and Guisan, 2009).

The lack of direct observations of pollinator-mediated transport is most likely because flight capabilities remain poorly resolved for most pollinating agents (Pasquet *et al.*, 2008) in addition to the difficulties associated with landscape scale studies of this dispersal mode (Steffan-Dewenter and Westphal, 2008). The complexity associated with studying whole-assemblage pollinator taxa has meant that baseline data documenting differences in movement distances and pollen transfer capabilities between species are not available for most systems. In this study, I investigate potential differences in pollen transfer between bee and fly taxa attending a model *Brassica rapa* var. *chinensis* (Brassicaceae) agricultural crop.

Specifically I asked the following questions:

1. Which insect species comprising the flower visiting assemblage travel outside the crop and hence are capable of pollen-mediated gene flow?
2. What proportion of this flower-visiting assemblage are involved in pollen transfer?
3. Does pollen viability (as a surrogate for potential gene transfer) differ between pollen carriers?

4. Do transport distances differ between bee and fly pollinators and is there a pattern in which the frequencies of insects (and hence the amount of pollen carried by an assemblage) change with distance from the source of pollen?

Methods

This study was carried out on the South Island of New Zealand using eight trial non-transgenic *B. rapa* fields each measuring 50m x 50m. Four fields were located in Lincoln, Canterbury (43˚ 38’ 24.91” S; 172˚ 29’ 03.01” E); and four fields were located in Gore, Otago (46˚ 06’ 51.77” S; 168˚ 54’ 51.90” E). The sites were chosen because of their isolated position with respect to other *B. rapa* seed crops in the district.

Sampling design and species collection

This study was conducted using an experimental crop as a mass flowering pollen source. I chose *B. rapa* as a model system because (i) it is a mass flowering crop with abundant floral resources per unit area that act as a large pollen source to examine long distance gene flow; (ii) *B. rapa* is a crop that is grown within a modified agricultural system. Spatial heterogeneity in floral resources is therefore lower than that associated with more natural landscapes (Klein *et al.*, 2008; Tylianakis *et al.*, 2008). This is ideal in order to examine differentiation in mobility patterns in contrasting pollinator species (iii) *B. rapa* attracts a diverse assemblage of insects (Feldman, 2006; Rader *et al.*, 2009), displays increased seed set in the presence of insect pollinators (Free, 1993) and is ubiquitous in most agricultural landscapes as a crop/environmental weed (Feldman, 2006; Sutherland *et al.*, 2006); (iv) it is capable of forming hybrids with other Brassica species including *B. napus and B. juncea*

(Scheffler and Dale, 1994; Hauser *et al.*, 1998; Stewart, 2002), which are among the first crops to be genetically modified for herbicide resistance (Hauser *et al.*, 1998; Rieger *et al.*, 2002; Allainguillaume *et al.*, 2006).

To estimate potential pollen transport distances and to compare these between taxa I captured insects using trap stations and hand nets. Two trap stations were positioned at each of five distances from the field boundary at 0, 100, 200, 300 and 400 m. Hand nets were used to capture insects by walking around and within 5m of each trap station for 10minutes, between 8.00-10.00, 12.00-14.00 and 16.00-18.00 h. Trap and hand net samples were pooled at each distance class for further analysis due to insufficient numbers.

Each trap station consisted of four traps; two replicates of each of two trap types. The trap types were “waratah” (W) traps and “flight intercept” (FI) traps.
The two trapping methods were used to maximize the diversity of insects captured. W traps consisted of a vertical yellow plastic board (1.6 x 0.6m) attached to star pickets. FI traps consisted of a yellow tray with two perpendicular perspex windows sitting within the tray. The location of each trap station was positioned 45-90 degrees from the previous distance to reduce the possibility that insects would be drawn out of the crop in a direct line. Tangle-Trap®, (a sticky paste to trap insects, The Tanglefoot® Company, Michigan USA) was applied to clear acetate sheets (22 x 30 cm) which were (i) Placed on the bottom of the tray in each FI and (ii) attached to the vertical plastic board on the W traps. Tangle-Trap was applied in a thin film to the entire surface area of each acetate sheet to ensure maximum insect capture. Application of Tangle-Trap as a thin film was designed such that pollen was retained

on insect bodies and not lost in drops of excess Tangle-Trap (preliminary trials were conducted in commercial *B. rapa* fields to perfect this method). Insect catches from both stations at each distance were pooled for analyses.

Although 400m was the maximum distance tested I do not exclude the possibility of dispersal events beyond this distance, particularly in the case of honeybees which have been recorded travelling long distances (i.e. > 9.5km Beekman and Ratnieks, 2000). I chose 400m as the maximum distance sampled for three reasons; First, pollen dispersal distance decreases markedly beyond the crop boundary (Morris *et al.*, 1994) and total pollen transfer is low beyond 100m (Scheffler *et al.*, 1993; Lavigne *et al.*, 1996) and is related to the size of the source crop (only 0.25ha in this study). Second, the maximum published distance estimate for pollen dispersal from a crop (by insects other than bees) was Syrphidae flies at 200m (Wratten *et al.*, 2003). Third, the logistics of the project design (there were many field barriers beyond 400m restricting trap placement beyond 400m; Wratten et al. 2003).

Insect movement of pollen over distance

Two methods were used to identify insects as potential pollen transporters. First, the entire 50m x 50m area in two Lincoln and two Gore fields (n = 4) were sprayed with a fluorescent liquid dye (SARDI, South Australia, as per Schellhorn *et al.*, 2004) to mark insects. Two remaining Lincoln and two remaining Gore fields were not sprayed (n = 4). All insects captured in traps associated with sprayed fields were examined for fluorescent dye.

Second, all insects captured in traps associated with untreated fields were examined for *B. rapa* pollen and all insect taxa identified with *B. rapa* pollen were considered pollen carriers. I am confident that all insects carrying *B. rapa* pollen attended flowers within our experimental crop fields since no other *Brassica sp*. crops were flowering within 5km of any of the field trials at the times of our experiment, and inspection of the surrounding fields and road verges did not reveal the presence of any flowering Brassicaceae weed species.

Insects were trapped over a 10 d period coinciding with peak flowering time at each location. Peak flowering at the Canterbury sites occurred between Dec 5-30, 2006 and at the Otago sites between Jan01-Feb10, 2007. Trapping was undertaken between 8am and 6pm each day. For the FI traps, all insects captured on acetate sheets were stored in liquid nitrogen for transporting and stored in a freezer (-80^{o}C) until processing. All insects were identified to family (sometimes species). Two taxa, *Protohystricia sp*. and *Pales marginata* (Tachinidae) were encountered in dyed samples but not in field samples used to assess pollen transport and pollen viability (see Table 5.2).

Pollen load comparisons between taxa

To assess potential pollinator status of individual taxa, the underside of each insect was dabbed onto a glass slide containing a drop of sucrose/Fluorescein diacetate solution (Kearns and Inouye, 1993). Pollen grains were observed under 10x power and the number of pollen grains on seven equally-spaced straight-line transects across the square coverslip (22x22mm) was recorded. When pollen grains were encountered I used high power magnification (40x) to ensure appropriate identification. I summed the pollen grain counts from all seven transects as an estimate of pollen carried per individual (Kearns and Inouye, 1993). This technique measures pollen on the underside of insects and does not include pollen contained within corbiculae which may contribute to total pollen load but are unlikely to be involved in pollination events (Thorp, 2000).

Pollen viability

Pollen viability was tested using the fluorochromatic method (Kearns and Inouye, 1993). Only *B. rapa* pollen was examined for viability.

Two fly families, Drosophylidae and Ephydridae, were difficult to process due to their small size. In order to assess whether the extra effort to include these taxa was warranted, I collected additional empirical data and counted pollen grains (using same methodology as current study) from 70 individuals of each of the above taxa. Only three (8%) and five (14%) individuals respectively, carried between one and three pollen grains and the remaining individuals did not carry pollen. These two families were therefore excluded from further analysis.

Data analyses

I found 26 insect taxa overall, but most taxa were represented by few individuals (see Results). To examine pollinator movement patterns between taxa, pollen loads and viable pollen loads, I group taxa at the family level for all analyses. The six families were: Apidae, Halictidae, Bibionidae, Syrphidae, Stratiomyidae and Tachinidae. All following methods and tests refer to these families only. Since our sampling also contained individuals that could not be positively identified as originating in the field (i.e. individuals without pollen or dye), I compared marked and unmarked insect distributions across all distances via Chi square tests.

I used three different models to test three subsets of the data. First, I investigated patterns in which the frequencies of all marked individuals differed between families and/or with distance from the crop edge. I used a mixed effects model using the nlme package (Pinheiro *et al.*, 2009) with “Taxa” and “distance” from the crop as fixed factors and “field” (the eight individual fields in which the data were collected) as a random effect. Violation of homoscedasticity required the log transformation of the dependent variable ‘number of individuals’. This model was chosen as it enabled the incorporation of random effects (Zuur *et al.*, 2009).

Second, I investigated how the total pollen carried differed between families and/or with distance from the crop edge. I used a generalized linear model (McCullagh and Nelder, 1989) with pollen counts as the response variable and “taxa” and “distance” from the crop as fixed factors. As the response variable consisted of counts, I employed a model based on a Poisson distribution and controlled for overdispersion by correcting the standard errors using a quasi-GLM model (Zuur *et al.*, 2009).

Third, I investigated how the proportion of viable pollen carried differed between families and/or with distance from the crop edge using a generalized linear mixed model with a binomial distribution using the lme4 package (Bates and Maechler, 2009).

Statistical analyses were calculated with R Statistical Software (The R Foundation for Statistical Computing 2009).

Results:

Insect movement outside of pollen over distance

A total of 5453 insects were collected across all trapping methods and locations. Of these, 717 were confirmed as originating from the trial crops, either because they were carrying *B. rapa* pollen (n=161), or because they were marked with fluorescent dye (n=556). In total, these 717 individuals represented 26 taxa from 15 families. Of the 717 individuals, only 675 occurred within six families (Hymenoptera: Apidae and Halictidae; Diptera: Bibionidae, Syrphidae, Stratiomyidae and Tachinidae) with sufficient numbers (>10 individuals) to be included in further analyses (Table 5.1). When considered independent of family identity, the total number of individuals across the five distances was unimodal and symmetrical. The highest number of individuals (39%) was recorded 200 m from the crop edge and the lowest numbers of individuals were captured at the crop edge at 0m (25%) and at 400m (8%). The overall pattern was unlike any negative exponential model describing decreased insect capture success at greater distance which might be expected based on area increasing as a function of the square of distance. The distributions of marked and unmarked individuals were not significantly different (χ^2_4 = 6.76, *P*=.0.149).

The mixed effects model revealed that the distribution of individuals differed between pollinator families ($F_{5,87}$ = 5.47, P = 0.0002). For example, the number of individuals in Apidae was significantly higher than Syrphidae (*P* = 0.01) and Tachinidae (*P* = 0.03). The number of individuals also differed between the distance classes ($F_{4,87}$ = 22.04, P < 0.0001). For example, the number of individuals was significantly higher at 200 m from the crop, than at 0 (*P* = 0.015), 300 (*P* = 0.0005) and 400 m (*P* = 0.00003) respectively.

Of the 161 individuals recorded as pollen carriers, 158 had sufficient numbers (>5) for analyses and consisted of representatives of four species in two Hymenoptera families (Hymenoptera: Apidae and Halictidae; Table 5.2), and eight species in four Diptera families (Diptera: Bibionidae, Stratiomyidae, Syrphidae and Tachinidae; Table 5.2).

Table 5. 1: All taxa recorded moving outside of flowering crop and marked with either dye or pollen (n =717). Taxa with asterisk were not included in final analyses.

Order	Family	Species	Common Name	N
Hymenoptera	Apidae	_Apis mellifera_ Linnaeus, 1758	Honeybee	112
		Bombus spp.	Bumblebee	22
	Halictidae	_Lasioglossum sordidum_ (Smith, 1853)	Native bee 1	131
		Leioproctus spp.	Native bee 2	27
	Ichneumonidae	_Netelia producta_ Brullé	Wasp 1	6
		Ichneumon promissorius (Erich)	Wasp 2	2
		*Unidentified sp. 3	Wasp 3	5
Diptera	Stratiomyidae	_Odontomyia atrovirens_ Bigot 1879	Soldier fly	86
	Syrphidae	_Eristalis tenax_ Linnaeus, 1758	Drone fly	22
		Melanostoma fasciatum (Macquart, 1850)	Orange hoverfly	45
		Melangyna novaezelandiae (Macquart, 1855)	Dark hoverfly	89
	Bibionidae	_Dilophus nigrostigma_ (Walker, 1848)	March fly	70
	Tachinidae	_Pales usitata_ (Hutton, 1901)	Grey-black tachinid	26
		Protohystricia spp.	Ginger bristlefly	14
		Pales marginata (Hutton)		31
	Ascilidae	*Unidentified sp. 1	Robber fly	2
	Anthomyiidae	_*Delia platura_ (Meigen, 1826)		4
		*Anthomyia punctipennis (Weideman, 1830)	Three spot fly	5
	Calliphoridae	_*Calliphora quadrimaculata_ (Swedarius, 1787)	New Zealand blue blowfly	1
		*Calliphora vicina Robineau-Desvoidy, 1830	European blue blowfly	2
		* Pollenia sp.	Bronze thorax fly	2
	Muscidae	_*Spilogona melas_ Schiner, 1868	Black triangle muscid	2
	Tipulidae	_Leptotarsus sp. 1_	Crane fly	3
	Sciaridae	_*Unidentified sp. 1_		2
Lepidoptera	Pieridae	_*Pieris rapae_ (Linneaus, 1758)	Cabbage moth	5
Neuroptera	Hemerobiidae	_*Unidentified sp. 1_	Brown lacewing	1

Table 5. 2: All taxa recorded carrying pollen (n =161). Taxa with asterisk were not included in final analyses. ^ represents maximum recorded distance for those individuals carrying pollen.

Order	Family	Species	Common name	Maximum recorded distance traveled (m) ^	N
Hymenoptera	Apidae	*Apis mellifera*	Honeybee	300	26
		Bombus spp.	Bumblebee	300	12
	Halictidae	*Lasioglossum sordidum*	Native bee	200	20
Diptera	Bibionidae	*Dilophus nigrostigma*	March fly	300	17
	Stratiomyidae	*Odontomyia atrovirens*	Soldier fly	400	25
	Syrphidae	*Eristalis tenax*	Drone fly	200	7
		Melanostoma fasciatum	Orange hoverfly	400	12
		Melangyna novaezelandiae	Dark hoverfly	400	27
	Tachinidae	*Pales usitata*	Grey-black tachinid	200	12
	Anthoymiidae	**Delia platura*		300	3

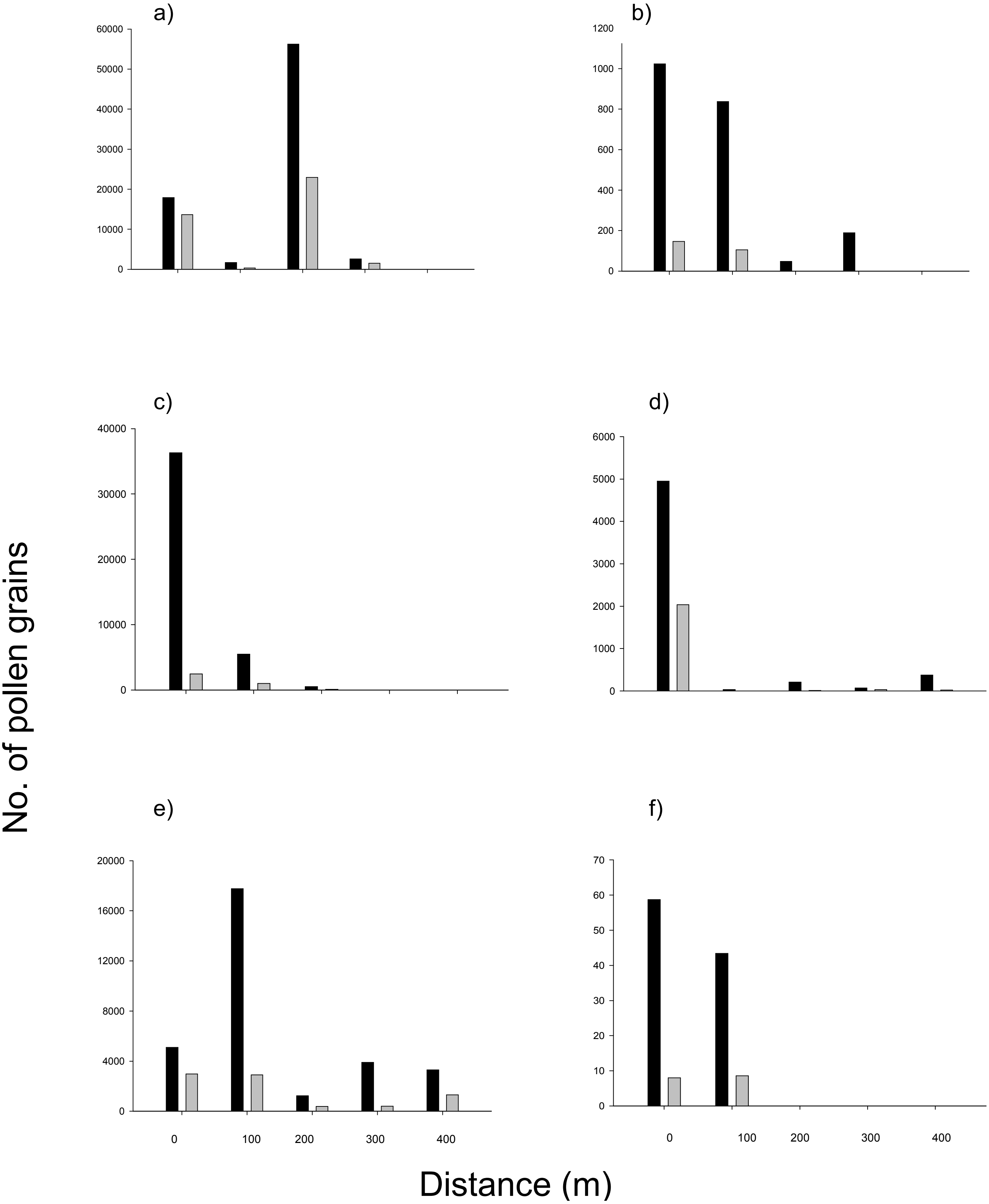


Figure 5.1a-f: Total pollen grains x frequencies of marked insects in each distance class. Black bars represent total number of pollen grains at each distance class. Grey bars represent viable pollen at each distance class; a: Apidae; b. bibionidae, c: Halictidae; d: Stratiomyidae; e: Syrphidae; f: Tachinidae.

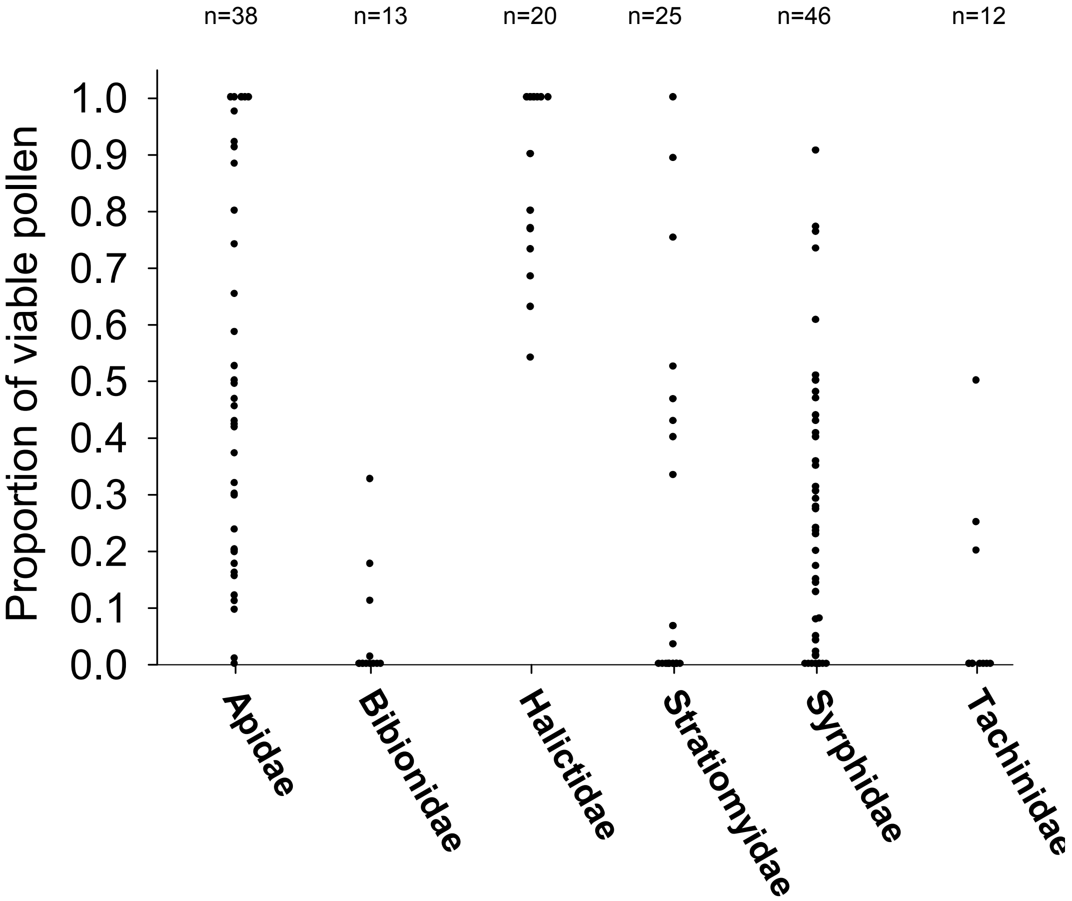


Figure 5. 2: Proportion of viable pollen carried among different insect families. Circles represent individual data points.

Pollen loads comparisons between taxa

The generalised linear model revealed that the amount of pollen carried differed between pollinator families (main effect family: $F_{5,145} = 14.42$, P < 0.0001). For example, Apidae carried the greatest number of pollen grains (Fig. 1a), significantly more than three families, Bibionidae ($P < 0.0001$; Fig. 1b), Syrphidae ($P = 0.007$; Fig. 1e), Tachinidae ($P < 0.0001$; Fig. 1f), and not different to Halictidae ($P = 0.05$; Fig. 1c) or Stratiomyidae ($P=0.425$; Fig. 1d).

Pollen was transported across all distances and total pollen number did not differ significantly between distance classes (main effect distance: $F_{4,145} = 1.22$, $P = 0$ 3065). Further, there was an interaction between family and distance (family x distance interaction: $F_{13,135} = 9.245$, $P < 0.0001$). Syrphidae and Stratiomyidae were the only taxa that carried pollen up to 400 m. Both bee families (Apidae and Halictidae) were recorded carrying pollen a maximum distance of 300m, and the fly family Tachinidae was recorded carrying pollen a maximum distance of 100m.

Pollen viability comparisons between taxa

Individuals from all six families carried viable *B. rapa* pollen outside the flowering Brassica crop (Fig. 2). Treatment contrasts resulting from the generalized linear model analysis revealed that the amount of viable pollen carried differed between families and distance but there was no significant interaction between family and distance. For example, Apidae carried a significantly higher proportion of viable pollen than Stratiomyidae (t = -2.960, P = 0.0037) and Bibionidae (t = -2.180, P = 0.036) but was not significantly different to Halictidae (t = -1.255, P = 0.211), Syrphidae (t = -1.1560, P = 0.249) or Tachinidae (t = -0.541, P = 0.589). The maximum distance over which viable pollen was recorded was 400m (Syrphidae). The total amount of viable pollen in total was significantly higher close to the crop margin (0m) than at distances 100 m and 200 m (0 and 100m; *P* = 0.04; 0 and 300m; *P*= 0.003) but was not different to 300 and 400 m (P > 0.05).

Discussion:

In this study I show that the potential for viable pollen to be transferred across a landscape is not confined to a single species, or even family of insects that may attend a flowering crop. Using a model mass flowering agricultural crop, I provide evidence insects can travel at least 400 m away from a pollen source and that all six families transport viable pollen as they move.

Different patterns of movement by different species indicate that at peak flowering, pollinating insects are capable of dispersal but not all taxa respond in the same way. The lower counts of insects at the crop edge, compared to frequencies at 200 and 300 m were contrary to our expectations based on optimal foraging theory. However, because this result was found in the frequency distribution of all other insects as well as those with either pollen or dye, I expect that this pattern was true phenomenon. One possible reason for this result is that adjacent to the crop, insects were more attracted to the larger, yellow signal provided by the crop itself rather than the traps, reducing trap effectiveness at close distances. As a result, frequencies were lower at close distances, relative to frequencies in traps which were far enough away from the crop to not interfere with the visual attraction of the yellow sticky boards. Alternatively, this pattern may be explained as a function of differences in foraging resource requirements or preferences between taxa. For example, in a study by Walther-Hellwig & Frankl (2000), *Bombus terrestris* gained a higher proportion of their food from distant temporary foraging habitats up to 1750 m away (from where they were marked), while *B. muscorum* visited food plants from permanent foraging habitat in close proximity to their origin. Hence the proximity to, and availability of specific floral resources (i.e. weeds and other crops) within and surrounding the crop may be impacting upon the patterns of movement and abundances of the taxa recorded.

This study demonstrates that all species (with the exception of the flies *Protohystricia spp.* and *Pales marginata*) recorded within the six families of generalist insects visiting a mass flowering agricultural resource, are capable of transferring pollen (Table 5.2), several up to 400m from its source. Of the Hymenopteran taxa, Apidae carried the greatest number of pollen grains (Fig. 1a).

This is not surprising considering honeybees and bumblebees are well known for their ability to carry large quantities of pollen (Free 1993). Surprisingly, two other taxa (Syrphidae and Halictidae) did not differ significantly to the honeybee in terms of their pollen carrying capacity or capacity to transport pollen. The Halictidae bees and Syrphidae flies are known to be effective pollinators (Rader *et al.*, 2009). Until this study however, their potential for pollen transport over distance was unknown. Although native bees are recognized as being effective pollinators in many systems (Kremen *et al.*, 2002; Klein *et al.*, 2003; Winfree *et al.*, 2007), pollen dispersal by flies, however, is less well known (but see Free, 1993; Jauker and Wolters, 2008).

Although mark-recapture studies have recorded flies traveling up to 400m (Dominiak *et al.*, 2003), little is known about their capacity to transport pollen over these distances. In this study, fly taxa in the families Stratiomyidae and Syrphidae carried pollen further than both bee families (Fig. 1).

The two bee families, however, carried both greater pollen loads and a greater amount of viable pollen than most the flies (Fig. 2) even though they travelled a shorter distance. I suggest two possible explanations for these differences in pollen viability between flies and bees; First pollen viability declines upon direct contact with some insects. For example, Richards, Stanley & Greg (2005) demonstrated that viability was significantly reduced when in contact with the proboscis of the moth *Helicoverpa armigera*. Second, variability in viability may occur as a result of differing pollinator behaviours, such as the extent and frequency of grooming (Harder, 1990; Harder and Wilson, 1998; Aizen and Harder, 2007). In contrast to the findings of this study, Kendall (1973) found little difference in viability among different pollinator taxa and suggested that the cleaning behaviour of the taxa concerned was sufficient to prevent the accumulation of non-viable pollen on the body hairs.

With the exception of the flies in Syrphidae (which retained viable pollen to 400 m), most families transported viable pollen to 200 m from the pollen source. Considering honeybees and bumblebees are capable of flying farther distances (i.e. at least 9.5km Beekman and Ratnieks, 2000), it is unlikely that this distance represents the tail end of the dispersal curve for these taxa. The paucity of data in the literature regarding the pollen carrying capacity of the remaining non-Apidae species combined with the low frequencies observed in this study make it difficult to predict the distance which would correspond to the tail end of the dispersal curve for these species.

Knowledge of the dispersal distances of generalist pollinators in human modified environments is crucial for our understanding of the future impacts of land use change. Fragmentation caused by global land use change has resulted in a mosaic of land use types (Saunders *et al.*, 1991; Harrison and Bruna, 1999; McKinney, 2002) many of which sustain different pollinator responses patterns which hinder or facilitate gene flow (Goulson *et al.*, 2002; Chapman *et al.*, 2003; Hirsch *et al.*, 2003).

Direct quantification of pollinator dispersal distances will assist in our evaluation of the ecosystem services provided by native and introduced pollinators in both natural and agricultural systems. Furthermore, it will serve to elucidate the potential role of generalist insect pollinators in vectoring pollen between crops and related weeds that may lead to unwanted hybridisation. In order to assess the contribution of different insects to gene flow, I suggest sampling entire assemblages more intensively to obtain higher frequencies and investigating the specific movements of each species over longer distances. This will allow a more detailed portrayal of the factors influencing landscape wide pollen movement and enable more accurate predictions for risk assessment.

Chapter 6: Synthesis

To investigate the utility of unmanaged taxa in providing pollination services to mass flowering crops in intensive agricultural landscapes, this thesis examined unmanaged pollinators in a mass flowering crop, *Brassica rapa*. Unmanaged and managed pollination services were directly compared using four pollinator traits. These were: the efficiency in which pollen is transferred (i.e. pollen transfer efficiency; Chapter 2), the frequency in which flowers are visited (pollinator visitation rate; Chapter 2), the consistency in which flowers are visited over time (pollinator consistency; Chapters 3 and 4) and the capacity to transfer viable pollen over distance (pollinator mobility; Chapter 5).

Chapter 2 identfied a range of potential pollinators from an assemblage of flower visitors and compared their services to the honeybee using two pollinator traits, pollen transfer efficiency and pollinator visitation rate. First, this study demonstrated that a diverse unmanaged component of the pollinator assemblage exists in *B. rapa* crops. In total 41 species were identified, seven of which were frequent visitors (Chapter 2). Second, the findings from this work resulted in the selection of three unmanaged pollinator candidates capable of providing pollination services equal to or better than the managed honeybee: *B. terrestris*, *Leioproctus* sp. and *E. tenax*. These three taxa did not differ from the honeybee in their capacity to transfer pollen and hence were equal to the honeybee in terms of pollinator efficiency. Unmanaged taxa also performed well in one of the two components of visitation rate. All three of the efficient unmanaged taxa visited the same, or a greater number of flowers per minute than the honeybee. In the second component of visitation rate, honeybees visited flowers at significantly higher frequencies than any of the other common visitors. This resulted in the honeybee being the most effective pollinator overall, due to its greater abundance overall. The result of this work demonstrates that unmanaged pollinators performed equally to the honeybee in terms of pollen transfer efficiency and the number of flowers visited per minute, yet larger population densities are required to achieve visitation rates per flower equal to the honeybee.

Achieving and maintaining higher densities of unmanaged pollinators will be challenging as little is known of most unmanaged pollinator species. Fundamental baseline research regarding the intrinsic biology and life history traits of these taxa will be required to understand what resources are needed to maintain stable populations in agricultural landscapes. This will require a change in land management practices to ensure year round refuge, feeding, nesting requirements are met. Nonetheless, Chapter 2 demonstrates that at least three unmanaged taxa may provide such services if higher densities can be achieved. Further research is required to gain a greater understanding of the resources needed to facilitate higher population sizes.

Chapter 2 demonstrated that in terms of pollinator efficiency and one component of pollinator visitation, several unmanaged taxa are comparable to the honeybee. For these taxa to be utilized in the longer term as pollinator’s in agricultural systems however, they need to be as reliable as the honeybee. This means they need to be present to provide pollination services all day and each year for several years. Chapter 3 investigates pollinator reliability by examining whether the same frequent visitors identified in a single season (Chapter 2), will return to provide pollination services over a four year period.

The results of this work suggest that six unmanaged taxa are present in sufficient densities to provide longer term pollination services and will return to crops year after year (Chapter 3). This means unmanaged taxa have the capacity to be consistent floral visitors in the longer term and hence populations have the potential to be of use to agricultural crops.

Two of these taxa, *E. tenax* and *B. terrestris*, were identified in Chapter 2 as potential candidates providing equal services to the honeybee in one season. The third taxon identified in Chapter 2, *Leioproctus* sp., was not present in sufficient numbers over the four year period to qualify as a reliable pollinator in the longer term. Chapter 3 also identified an additional four taxa as reliable visitors over time, three of which were mentioned in Chapter 2 as potential candidates by testing their pollen transfer efficiency and visitation rate. These candidates, *Dilophus nigrostigma*, *Melanostoma fasciatum*, *Melangyna novae-ze*landiae and *Lasioglossum sordidum* however, were not as efficient as the honeybee (Chapter 2). These findings demonstrate the need to incorporate a number of pollinator traits when evaluating the utility of unmanaged taxa in the pollination of mass flowering crops.

Chapter 4 extended the knowledge gained in Chapter 3 by further investigating unmanaged pollinator diversity as a potential mechanism facilitating reliability. Unmanaged pollinator diversity potentially facilitates reliability in unmanaged assemblages due to the increased chance that an effective pollinator is present and the potential range of responses experienced across all species (Yachi and Loreau, 1999; McCann, 2000; Elmqvist *et al.*, 2003). This chapter tested if the capacity for diverse assemblages to provide reliable pollination services over time was a result of the presence of many species as well as the presence of trait variation among taxa. This was achieved by examining the differences between bee and fly pollinator ‘activity windows’ across an entire day (6am to 8pm) to investigate functional complementarity between them. The longer time interval observed across a day in Chapter 4 enabled testing the findings of Chapter 3 (i.e. visitation of frequent visitors does not change between 10am and 2pm). Chapter 4 therefore examined if the same unmanaged taxa visited at similar rates between and outside of standard survey hours and to enable assessment of the contribution of any additional unmanaged taxa.

Chapter 4 demonstrated that unmanaged taxa visited at different rates throughout the day. All bees and one fly *D. nigrostigma*, were more active in the middle of the day. All other fly taxa were most active in the early morning and late afternoon. Overall visitation rate did not differ significantly between sample periods, indicating that changing taxonomic composition in assemblage structure was not accompanied by changes in potential pollination services. In addition, the contributions to visitation rate provided by fly taxa outside of standard survey hours resulted in a higher visitation rate than standard survey hours.

Chapter 4 demonstrated that unmanaged pollinators fluctuate in abundance throughout the day but provide a consistent visitation rate overall. This means that pollination services were stable across time. The provision of a range of taxa with different functional traits enabled all day visitation and the contribution of fly taxa outside of standard survey hours ultimately increased pollination services at the end of the day. Chapter 4 demonstrated that diverse pollinator assemblages can potentially reduce the chance of the loss of pollination services in response to environmental change.

Finally, Chapter 5 investigated the fourth trait used to evaluate the utility of unmanaged pollinators in mass flowering crops, pollinator mobility. The findings from this work demonstrated that unmanaged pollinators are highly mobile. The potential for viable pollen to be transferred across a landscape was not confined to a single species, or even family of insects attending a flowering crop. In addition to the honeybee, five of the seven unmanaged taxa identified in chapter 2 transported viable pollen outside of a mass flowering crop.

Alternative pollinators can transport viable pollen up to 300m (and non-viable pollen up to 400m), suggesting they are likely to be contributors to pollen flow in the context of the GM debate. These low frequency long distance dispersal events may prove to be highly relevant and have important consequences for risk assessment particularly when the organism is at a selective advantage compared to the residents (Lavigne *et al.*, 1996).

Limitations of this study and directions for future research

In this study I have shown a range of unmanaged bee and fly taxa can provide equivalent pollination services to the honeybee in a mass flowering crop. Other studies have demonstrated that unmanaged taxa can be used in other agricultural crops, including coffee (Klein *et al.*, 2003), watermelon (Winfree *et al.*, 2007) and pumpkin (Hoehn *et al.*, 2008). The utility of the unmanaged assemblage I investigated in thus study has thus only been demonstrated for a single crop species, *B. rapa*. It is likely that other crops offer different resources and hence may attract new assemblages. This methodology now needs to be tested on pollinator assemblages associated with a range of other crops and within a range of management intensities, climates and contexts (i.e. varying intensities, regions, land use types etc.).

In addition, the pollinator mobility trials (Chapter 5) were based on relatively small experimental fields of 0.25ha which is well below the average area cultivated for most crop plants. Considering the size of fields is positively related to the probability of detecting long distance dispersal (Scheffler *et al.*, 1993; Lavigne *et al.*, 1996; Lavigne *et al.*, 1998) I suggest that the data presented here underestimate the likelihood of long distance pollen dispersal.

The capacity for pollen movement by alternative insects in the assemblage is understated and is in need of further attention. In order to assess the contribution of different insects to gene flow, I suggest sampling the entire assemblage more intensively to obtain higher frequencies and investigating the specific movements of each species over longer distances. An investigation of the movement patterns of individual species is possible via fluorescent dyes (Schellhorn *et al.*, 2004), harmonic radar (Osborne *et al.*, 1999), marker genes (Hudson *et al.*, 2001) as well as other methods (Hagler and Jackson, 2001). This will allow a more detailed portrayal of the factors influencing landscape wide pollen movement and hence enable more accurate predictions for risk assessment.

In conclusion, alternative insect pollinator taxa can be as efficient, effective, consistent and mobile as the honeybee. Future research is necessary to elucidate what factors govern their abundance and distribution in a range of land use types and contexts to enable greater utilisation of their services.

Components of this study still in progress

This thesis outlines the findings of this PhD research conducted in New Zealand within mass flowering *B. rapa* crops. In addition to this study, several other studies were conducted that do not form part of this thesis due to time and resource constraints. These other research questions concern the impacts of land use intensity and modification on the functional diversity of wild and managed pollination services. Field work was conducted in both natural and human modified ecosystems along a gradient of land use intensity in both in Australia and New Zealand. The findings arising from this research will highlight differences in pollination services between natural and agricultural systems and will be published when analyses are complete.

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