The effects of isolation on mutualistic interaction networks

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Christie J. Webber

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Contents

Abstract

Global environmental changes, such as habitat fragmentation, are negatively impacting biodiversity and species interactions. The conservation of species and their interactions is essential to preserve the ecosystem system services they provide, such as pollination. This thesis examines how pollinators and their interactions with flowering plants respond to habitat isolation. In order to predict what will happen to mutualistic interactions in the face of global change, recent community- and metacommunity level studies have examined plant-animal mutualistic networks. I took a similar approach, by first examining changes in the pollinator community composition and the resulting consequences for pollination services, measured through seed set. This was followed by an investigation of plant-pollinator interactions using a network approach, specifically examining how the structure of plant visitation and pollen transport networks and interactions at the species level changed with increasing isolation.

To do this, I established a field experiment with plant patches at varying degrees of isolation on a sheep farm pasture in Oxford, New Zealand. Observations were conducted over a three-month period, during which all flower-visiting insects were collected to be identified and to have their body size measured and pollen load quantified. Each plant's seed set was also measured to calculate pollination success at patches.

My findings showed that isolation had a generally negative effect on pollinators, plants and their interactions. The body size of pollinators was smaller at isolated patches, as was the richness of the pollen they carried. The patterns were driven by a compositional shift of pollinator relative abundances, with a significant decline in the large generalist *Bombus* species being observed. These changes in species composition decreased one measure of the functioning of the system; plant seed set significantly declined with isolation for several of the patch plant species. Through analysis of plant interactions at the network level, I found that a decline in plant species seed set correlated with plant generality, and specialist plant species were more negatively affected by isolation than were generalist plant species. I also found that pollinator body size was positively correlated with the number of plant species or pollen grains with which a pollinator interacted. In addition, network connectance and nestedness declined with isolation. The trends observed could have important implications for the long-term stability and functioning of isolated plant communities, and their interactions with mutualists such as pollinators.

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Chapter 1: Introduction

1.1 Habitat fragmentation

Global demand for food and agricultural products is leading to rapid land-use change and the expansion and intensification of agricultural areas, including croplands, pastures and plantations (Foley *et al.* 2005). Habitat loss causes fragmentation and isolation of remaining habitat, which can negatively affect biodiversity through the removal of species (Kareiva 1987, Sala *et al.* 2000, Fahrig *et al.* 2003, Foley *et al.* 2005, Tylianakis *et al.* 2008a, Green *et al.* 2010, Tscharntke *et al.* 2012). Species loss and decreased biodiversity can subsequently threaten the stability and functioning of ecosystems and lead to the disruption of important ecosystem services, such as pollination (Kearns *et al.* 1998, Fahrig *et al.* 2003, Tylianakis *et al.* 2008a).

1.2 Insect pollination

The ecosystem service of pollination by insects is important on a global scale, both ecologically and economically (Klein *et al.* 2007, Potts *et al.* 2010). Pollinators are essential for the maintenance of plant biodiversity worldwide, contributing to the reproduction of an estimated 90% of flowering plants species (Ollerton *et al.* 2011). Human food security also depends on the pollination of crop plants by animals, which is estimated to be important for around 75% of the leading global food cops (Klein *et al.* 2007). However, bee populations are in decline globally and the pollination services they provide for crops and wild flowering plant species is under threat (Potts *et al.* 2010). Traditionally the honeybee (*Apis mellifera*) has been used in agricultural systems (Vanengelsdorp and Meixner 2010). But their now well documented decline highlights the inherent risks of relying on a single pollinator species (Potts *et al.* 2010). Bee species diversity is important for the stability and the effective pollination of plants (Hoehn *et al.* 2008). Managed bees can't be solely relied upon, and wild pollinators have been shown to contribute greatly and equally as efficiently to pollination as honeybees (Winfree *et al.* 2008, Garibaldo *et al.* 2013).

1.3 Mutualistic networks

In order to understand how habitat modification alters the mutualistic interactions between plants and pollinator, recent studies have examined plant-animal mutualistic interaction networks (Fortuna and Bascompte 2006, Aizen *et al.* 2012, Hagen *et al.* 2012). A mutualistic pollination network is made up of nodes, which can represent individual plants and animals, functional groups of animals and plants, or even local populations (Thébault and Fontaine 2010). These nodes are connected by links, which represent interactions (Thébault and Fontaine 2010). Aizen *et al.* (2012) examined mutualistic networks subjected to habitat loss and found that the underlying network structure influenced the persistence of mutualistic communities such that interactions were lost non-randomly (Aizen *et al.* 2012). Therefore, particular traits of plant-pollinator interactions increased their chance of disruption (Aizen *et al.* 2012). Although not specifically focused on habitat modification, Burkle *et al.* (2013) found similar results to those of Aizen *et al.* (2012), though they focused on traits of species themselves (and their likelihood of extinction) rather than their interactions. Fortuna and Bascompte (2006) also examined the effects of habitat loss, but they did so through a patch-model of plant-animal mutualistic communities. They found that, compared with randomly-connected communities, real communities start to decay sooner but persisted for longer (Fortuna and Bascompte 2006). The real community had a destruction threshold which, once passed, led to the collapse of the community (Fortuna and Bascompte 2006). Thus, the structure of mutualistic networks influences their response to habitat loss (Hagen *et al.* 2012), such that reduced habitats contain fewer links between plants and pollinators, and also reduced species richness (Larsen *et al.* 2005, Sabatino 2010).

1.4 Habitat isolation

Habitat isolation is known to affect species composition and genetic diversity (Leblois *et al.* 2006, Hendrickx *et al.* 2009). Isolation in an agricultural landscape was found to lead to a decrease in local species richness of poor-dispersing carabid beetles and an increase in species with high dispersal ability (Hendrickx *et al.* 2009). Thus, when investigating the effects of isolation, a simple measure such as species richness could obscure a change in species composition. Isolation of populations can also lead to the loss of genetic diversity, which in turn has implications for the fitness and long-term persistence of a population (Leblois *et al.* 2006). Rundölf (2008) suggested that fragmentation of foraging habitat will

affect pollinator species with medium sized foraging ranges the most, and that isolation decreases the number of links within pollination networks (Rundölf *et al.* 2008, Sabatino 2010). As isolated habitats interact over a landscape through dispersal, and because the structure of mutualistic networks are influenced by spatial processes, the effects of isolation should be examined within a spatial context (Morales and Vázquez 2008). This suggests that a useful approach for understanding responses of mutualistic networks to habitat fragmentation may be the examination of 'networks of networks', such as at a metacommunity scale (Dale and Fortin 2010). Sets of local communities, where species interact by affecting each other's demographic rates, connected by dispersal of multiple potentially interacting species make up a metacommunity (Leibold *et al.* 2004), and in this thesis I take a metacommunity approach to study pollination networks.

1.5 Body size as a driver of network structure

Recent studies have sought to understand what role species traits, such as body size and dispersal mode, play in driving local and metacommunity structure. De Bie *et al.* (2012) investigated the roles of body size and dispersal mode, of aquatic organisms, in shaping metacommunity structure (De Bie *et al.* 2012). Passively dispersing organisms with large body size displayed stronger spatial patterning within the metacommunity and increased dispersal limitation compared with small-bodied organisms (De Bie *et al.* 2012). Conversely, metacommunities with active dispersers, winged insects, showed a weaker imprint of dispersal limitation compared with passively dispersing groups of organisms of the same size (De Bie *et al.* 2012). Thus, body size had opposing effects for passive and active dispersers and body size is positively correlated with dispersal capacity of insects (Jenkins *et al.* 2007). A study of body size of birds in a fragmented Amazonian forest showed that larger birds were more capable of crossing long dispersal distances between patches and therefore were the most abundant species in isolated patches (Less and Peres 2009). McCann *et al.* (2005) found that larger animals play an important role in contributing to stability when in a heterogeneous and large spatial structure. Although this work focused on large mobile consumers, large-bodied mutualistic animals could play a similar role, particularly if they couple fragmented or island mutualistic webs.

Pollinators can be affected through a change in the availability of resources (Scheper *et al.* 2014). Loss of floral resources, in particular preferred floral resources, has been shown to

drive bee population declines (Scheper *et al.* 2014). Insect body size played an important role in the size of this response, with larger-bodied species being more affected by floral declines because they have larger pollen requirements than small species (Scheper *et al.* 2014). Larsen *et al.* (2005) also showed that habitat loss selected against large-bodied bees, and Benjamin *et al.* (2014) found that large bee species negatively responded to increasing agricultural cover at the landscape scale and field scale, while small bees responded slightly positively and negatively at the respective scales.

Thus, the extent to which isolation affects species may depend on species traits, such as body size, which is positively correlated with dispersal capacity of active dispersers (Larsen *et al.* 2005). Therefore, species traits may interact with landscape structure (i.e., patch isolation) to determine metacommunity structure in isolated patches (De Bie *et al.* 2012). In addition to affecting dispersal, traits such as body size may also determine the effectiveness of pollinators, such that a systematic bias on which species reach isolated communities may also influence ecosystem functioning by altering mean pollinator efficiency (Larsen *et al.* 2005, Hoehn *et al.* 2008).

1.6 Objectives and study outline

My research aims to determine the effects of habitat isolation on plant-pollinator communities and their mutualistic interaction networks. To investigate this, I set up a field experiment with flowering plant patches (Fig. 1.1) at varying degrees of isolation on a sheep farm pasture in Oxford, New Zealand. Observations were conducted over a three-month period, during which all flower-visiting insects (Fig. 1.2) were collected to be identified and have their body size measured and pollen load quantified. Each plant's seed-set was also measured to quantify pollination success at patches. These data were then used to study the effects of isolation.

Figure 1.1 Experimental plant community, fenced for protection from grazers, at study site in Oxford, Canterbury, New Zealand. Each patch contained a total of 60 plants of 10 different species.

Figure 1.2 *Bombus terrestris* (left) and the native *Lassioglossum sordidum* (right) are common pollinators in New Zealand, with *Bombus* being the largest, and *Lassioglossum* the smallest of New Zealand's bees. Species traits, such as body size, might have important implications for the effects of habitat change such as isolation on the mutualistic interactions between pollinators and plants.

The thesis is set out as two data chapters (Chapters 2 and 3) in standalone manuscript form, with an overall introduction (Chapter 1) and discussion (Chapter 4) to link them together. In Chapter Two, I examine the response of the pollinator community and individual species abundances to isolation, and the resulting consequences for pollination services, measured

through seed set. I also test how the species trait of body size interacts with isolation to influence the community composition of pollinators. This assessment of separate plant and pollinator responses sets the scene for the next chapter, which links the two together via their interactions.

In Chapter Three, I use a network approach to investigate the spatial structure of mutualistic communities. I examine how isolation influences plant-pollinator interactions at the network and species level. I also construct both plant visitation and pollen transport networks to assess changes to the community from both a plant and pollinator perspective.

I then summarise my findings, in Chapter Four, and discuss how the community measures relate to the network measures, and how these findings relate to the existing literature. I conclude by briefly discussing areas of potential interest for future research.

Chapter 2: Effects of isolation on pollinators and plant seed set

2.1 Introduction

Habitat loss, fragmentation, and resulting isolation, are global environmental changes that can have serious negative effects on biodiversity (Kareiva 1987, Fahrig 2003, Foley *et al.* 2005, Tylianakis *et al.* 2008a, Green *et al.* 2010, Tscharntke *et al.* 2012). Species loss threatens the stability and functioning of ecosystems and can lead to the disruption of important ecosystem services (Kearns *et al.* 1998, Fahrig *et al.* 2003, Hooper *et al.* 2005, Balvanera *et al.* 2006, Tylianakis *et al.* 2008a).

One such ecosystem service, which is threated by habitat change, is the pollination of plants by insects (Kearns *et al.* 1998, Winfree *et al.* 2009, Potts *et al.* 2010). This service is important on a global scale, both for maintaining biodiversity and for supporting human wellbeing through food production (Klein *et al.* 2007, Potts *et al.* 2010). Pollinating insects are essential for the pollination of many wild plants, and it is estimated that around 75% of the leading global food crops rely on pollination by animals (Klein *et al.* 2007).

Habitat loss, fragmentation and resulting isolation can disrupt pollination services and lead to the decline of plants and pollinators (Potts *et al.* 2010). The effects of habitat loss, fragmentation, and isolation on plants and pollinators are widespread and generally negative, reducing insect abundances, species richness, resulting pollination services and, in turn, plant reproductive success (Aguilar *et al.* 2006, Winfree *et al.* 2009).

However, these effects are not equal across all species; recent studies have shown the importance of body size in determining the response of pollinators to habitat reduction and increased agricultural cover, and concluded that larger-bodied pollinators were more negatively affected than smaller-bodied pollinators (Larsen *et al.* 2005, Benjamin *et al.* 2014, Scheper *et al.* 2014). In contrast to the effect of habitat loss, isolation caused by habitat fragmentation may select for larger-bodied pollinators, because body size is positively correlated with dispersal capacity of insects (Greenleaf *et al.* 2007, De Bie *et al.* 2012). In addition to affecting dispersal, traits such as body size may determine the effectiveness of pollinators (Larsen *et al.* 2005; Hoehn *et al.* 2008), such that a systematic bias on which

species reach isolated communities may also influence ecosystem functioning by altering mean pollinator efficiency. A study carried out by Steffan-Dewenter and Tscharntke (1999) found that the mean body size of pollinators increased with isolation of eight potted plants from a grassland habitat. However, there has been no investigation of how pollinator body size may interact with habitat isolation of large plant communities to determine pollinator species composition and plant reproductive success.

Finally, fragmented habitats do not exist in isolation, and dispersal among patches may determine the outcome of competitive interactions among species and thereby shape community composition. Such sets of communities connected by dispersal of multiple potentially interacting species are described as a metacommunity (Leibold *et al.* 2004), and changes to one metacommunity (e.g. pollinators) may have consequences for species with which they interact (e.g. plants). Therefore, studying such a system could lead to targeted conservation plans for pollinators that play an important role in connecting and pollinating isolated plant populations. It could also have implications for how far apart insect pollinated crops and plant restorations sites can be planted before pollinators become too scarce and inefficient.

In this chapter, I will examine the response of the pollinator community to isolation, and the resulting consequences for pollination services, measured through seed set. I will also test how the species trait of body size interacts with isolation to influence the community composition. To do this, I will control experimentally for confounding factors such as patch size and plant density and resource limitation. In particular, I will focus on three main hypotheses:

1) Isolation will alter properties of the pollinator community. Properties of the community will change across space in response to patch isolation. Patches closer in space will be more similar to each other due to dispersal between patches. As isolation increases, there may be a decline in pollinator richness, abundance, composition, and a reduction the pollen richness carried by pollinators.

2) Body size will interact with habitat isolation to influence the community composition. As isolation increases I expect to see a loss of smaller-bodied pollinators, which have smaller dispersal ranges than larger-bodied pollinators. Larger-bodied pollinators, such as bumble bees, will be present in all patches because they have high dispersal capabilities.

3) Seed set will respond to isolation, decreasing in isolated patches due to reduced pollinator diversity. However, this may be partially offset by isolated patches having larger-bodied pollinators, which may be more effective (Larsen *et al.* 2005; Hoehn *et al.* 2008).

2.2 Methods

2.2.1 Study site and experimental design

I conducted a manipulative field experiment on a sheep farm pasture in Oxford, North Canterbury, New Zealand, 43°19'21''S 172°12'25''E. Oxford is located in the Canterbury Plains, a highly modified landscape that supports a large agricultural industry. I selected the farm as the study site because it provided a 105 hectare space with naturally occurring insect populations and limited wild flowering plant cover, composed of gorse (*Ulex europaeus*), clover (*Trifolium repens*), haracium (*Hieracium* spp.), primrose (*Primula vulgaris*), broom (*Carmichaelia australis*), and yarrow (*Achillea millefolium*). This made it possible to set up experimental 'islands' of flowering plants at varying degrees of isolation.

On the farm I set up fourteen experimental plant patches at increasing distances, up to 1,483m, from each other. Patches were fenced off and rabbit proofed with gates and hex netting. The location coordinates of each patch were determined using Google Earth, and used for spatial analyses (see below). Nearby wild flowering plant cover was quantified for each patch as the mean percent cover in ten 1m x 1m quadrats placed within a 100m distance of patches. Quadrat placement was determined through the random generation of numbers, which related to 1m x 1m squares on grid maps of each patch area. Nearby stock dung cover was also quantified using the same methods.

Figure 2.1 Aerial view of study site. Patch locations marked with red dots.

2.2.2 Experimental plants

Each patch contained the same 10 plant species with equal numbers of potted individuals, totalling 60 plants per patch. Plant species were selected for their ability to effectively attract a range of pollinators. These were *Achillea millefolium* (yarrow), *Agastache foeniculum* (anise hyssop), *Brassica napus* (canola), *Coreopsis tinctoria* (coreopsis), *Echium vulgare* (blueweed), *Fagopyrum esculentum* (buckwheat), *Lobularia maritime* (alyssum), *Medicago sativa* (lucerne), *Nepeta grandiflora* (catnip), and *Trifolium repens* (clover). The plants were potted with slow release fertiliser and watered daily while out if the field, to control for potential resource limitation.

Trifolium repens was propagated from cuttings taken from the University of Canterbury grounds. I grew the remaining 9 plant species from seed. All plants were cultivated in the University of Canterbury glasshouses, timed to all begin flowering in early December.

2.2.3 Observation of flower-visiting insects

In order to determine the pollinator community at each patch, I made observations of flower visiting insects throughout the flowering season of the plants, December 2012 – February 2013. Six rounds of sampling were conducted under warm and dry weather conditions, in temperatures over 14°C, between 10:00 and 17:00 o'clock. At each patch I monitored flowers for one hour per round. All flower-visiting insects were captured and the plant species from which they were collected was noted. Each insect was stored individually to avoid pollen contamination and frozen.

I later identified all captured insects to species level with the use of identification keys (Dear 1985, Klimaszewski and Crosby 1997, Klimaszewski and Watt 1997, Dugdale 1998, Donovan 2007, Thompson 2008), or when identification keys did not exist I grouped insects into morphospecies (hereafter collectively referred to as 'species'), with the use of reference samples of insects which had previously been identified by a taxonomist (see Appendix 2 for full species list). A reference collection has been retained in the School of Biological Sciences, University of Canterbury invertebrate collection.

I also measured the inter-tegular span (IT span), i.e. the distance between wing bases, of all pollinators with digital calipers and used this measure as a proxy for body size so I could assess how pollinator body size changed with increasing isolation.

2.2.4 Pollen load

I examined the pollen load of all collected specimens to determine what pollen species individual insects were carrying. Pollen from the insects, excluding pollen carried in the corbiculae or scopae of bees (which is unlikely to be deposited on flowers), was removed with fuchsin gel and mounted on a microscope slide, using the method outlined in Dafni *et al.* (2005). I then systematically examined these pollen slides identifying the first twenty pollen grains on each slide to species level, with the use of reference pollen samples collected from the experimental plants and surrounding wild flowering plants, the latter will hereafter be referred to as matrix pollen.

2.2.5 Seed set

Plant reproductive success was measured through seed set in seven of the ten patch plant species; *Agastache foeniculum* (anise hyssop), *Brassica napus* (canola), *Coreopsis tinctoria* (calliopsis), *Fagopyrum esculentum* (buckwheat), *Medicago sativa* (lucerne), *Nepeta grandiflora* (border ballet), and *Trifolium* (clover). Due to damage caused by sheep grazing, seed set measures were not obtained for the remaining three patch plant species; *Achillea millefolium* (yarrow), *Echium vulgare* (blueweed), and *Lobularia maritime* (white alyssum). Five flowers of every experimental plant, which opened in the field, were tagged and monitored. Once flowers had closed and been given enough time to set seed, I collected the flower heads and counted the number of seeds produced by each flower.

2.2.6 Isolation metric

I used a distance based isolation metric to define isolation of patches. This was measured as the mean distance of the four nearest neighbours from a focal patch, which can better predict the isolation of a patch compared to the commonly used distance to single nearest neighbour metric (Bender *et al.* 2003, Tischendorf *et al.* 2003).

2.2.7 Pollinator analysis

To test if nearby patches had similar pollinator abundances, species richness, average pollinator body sizes, species composition, and abundances of individual species guilds, I used Mantel tests to compare a geographic distance matrix (Euclidean distance) to dissimilarity matrices of the above measures. I used two dissimilarity measures for the species composition matrix: Bray-Curtis distance (which incorporates both composition and relative abundance) and Jaccard dissimilarity (which only incorporates composition). These were calculated with the vegdist function from the vegan package (Oksanen *et al.* 2013) in R (R Core Team 2013), while the other (spatial) dissimilarity matrices were calculated using the dist function in the R Statistical Software base package (R Core Team 2013). The Mantel tests were carried out with the mantel function, set to 999 permutations, in the vegan package (Oksanen *et al.* 2013) for R (R Core Team 2013).

If there was significant spatial autocorrelation of a community measure, I then used correlograms to identify the spatial range over which the observed correlation occurred.

These were constructed with the correlog function in the ncf package (Ottar 2013) in R (R Core Team 2013), set to 999 permutations.

To account for spatial autocorrelation, I ran simultaneous autoregressive (SAR) models to test how measures of the pollinator community (pollinator abundance, species richness, and average pollinator body sizes) and also the abundances of individual species guilds responded to isolation and hostility of the surround matrix. Each test was run with a community measure as the response variable and isolation, nearby wild flowering plant cover and stock dung cover as the predictor variables. Autoregressive models are based on generalised least squares regression, and instead of assuming independent and identically distributed errors they assume a correlation function in the variance-covariance matrix based on spatial distance (Dormann *et al.* 2007). These models were run in the package SAM (Spatial Analysis in Macroecology) (Rangel *et al.* 2006). The maximal model was then simplified based on minimising the Akaike Information Criterion (AIC).

To determine if more isolated patches contained nested subsets of the species in less isolated patches and also whether species-poor patches contained nested subsets of the species in species-rich patches (i.e. whether species are lost sequentially), I ran permutation tests of whether my species by patch matrix was nested. This was conducted with the oecosimu function from the vegan package (Oksanen *et al.* 2013), set to 999 simulated communities, in R (R Core Team 2013), and nestedness was measured using the NODF algorithm (Almeida *et al.* 2008).

2.2.8 Pollen analysis

To test if nearby patches had similar pollen composition, I used a Mantel test to compare a geographic distance matrix (Euclidean distance) and a pollen species composition matrix (community dissimilarity), using two dissimilarity measures for the composition matrix (Bray-Curtis distance and Jaccard dissimilarity), as for the pollinator analyses above. The Mantel tests were carried out with the mantel function, set to 999 permutations, in the vegan package (Oksanen *et al.* 2013) for R (R Core Team 2013). Again, for significant spatial autocorrelation I used correlograms to identify the spatial range over which the observed correlation occurred.

I analysed two measures of pollen richness, the first was total pollen richness including both pollen from the patch plants and matrix pollen, and the second was the richness of patch plant pollen only (i.e. excluding matrix pollen). I tested for spatial autocorrelation with Mantel tests and then visualized the structure of the correlative relationship with a correlogram, using the same methods as above.

To see how pollen richness, for both of the pollen richness measures, responded to isolation and measures of the surrounding matrix hostility, and also how pollen richness responded to pollinator richness, abundance and body size I ran two simultaneous autoregressive models in SAM (Rangel *et al*. 2006). Pollen richness was the response variable in both cases, with abiotic variables (isolation, wild flowering plant cover, and stock dung) cover as predictor variables in one model and pollinator variables (pollinator richness, abundance and body size) as predictors in the other model. AIC was used to select the best fitting model.

Finally, to determine if any pollinator species carried more pollen species (i.e. higher pollen richness) I ran a linear mixed effects model, conducted in R (R Core Team 2013) with the lmer function from the lme4 (Bates *et al.* 2011) and lmerTest (Kuznetsova *et al.* 2014) packages. Pollen richness (of all plant species) was the response variable, with species identity as the fixed predictor variable and patch as the random effect.

2.2.9 Seed set analysis

I tested the seed set of the seven plant species for spatial autocorrelation with Mantel tests, using the same methods as above. For each plant species, I then ran a simultaneous autoregressive model testing the response of seed set to seven predictor variables; isolation, dung cover, plant cover, pollen richness, insect richness, insect abundance and pollinator body size. These models were run in the package SAM (Rangel et al 2006), and AIC was used to select the best fitting model

2.3 Results

2.3.1 Pollinators

A total of 1470 insects, from 73 species, were collected visiting patch flowers during the three month sampling period. The most common species sampled were large-bodied flies (from the families Sarcophagidae, Calliphoridae, Syrphidae, Tachinidae, Muscidae and

Stratiomyidae), solitary bees (Halactidae and Colletidae), and social bees (Apidae), making up 50%, 30%, and 11% of all flower visitors respectively. The remaining 9% of insects sampled were split across 21 families.

Spatial clustering in pollinator community composition

There was no correlation between the pairwise spatial distance of patches and their dissimilarity in pollinator species richness (Mantel test, r = 0.04, p = 0.354) or flower visitation frequency (r = 0.03, p = 0.347), as a measure of pollinator abundance. However, the relative abundances and identities of the species showed spatial clustering; pollinator composition was significantly spatially correlated, both considering relative abundances (Bray-Curtis: r = 0.45, p = 0.003) and comparing only presence/absence of species (Jaccard: r = 0.44, p = 0.002). A Mantel correlogram revealed that patches that were close together (less than and including 510m apart) were significantly more similar in their species composition, and this similarity declined with increasing distance (Fig. 2.2) to become greater than random dissimilarity at more distant sites, though this dissimilarity was only significant (p=0.002) in the 908m-1,302m distance class.
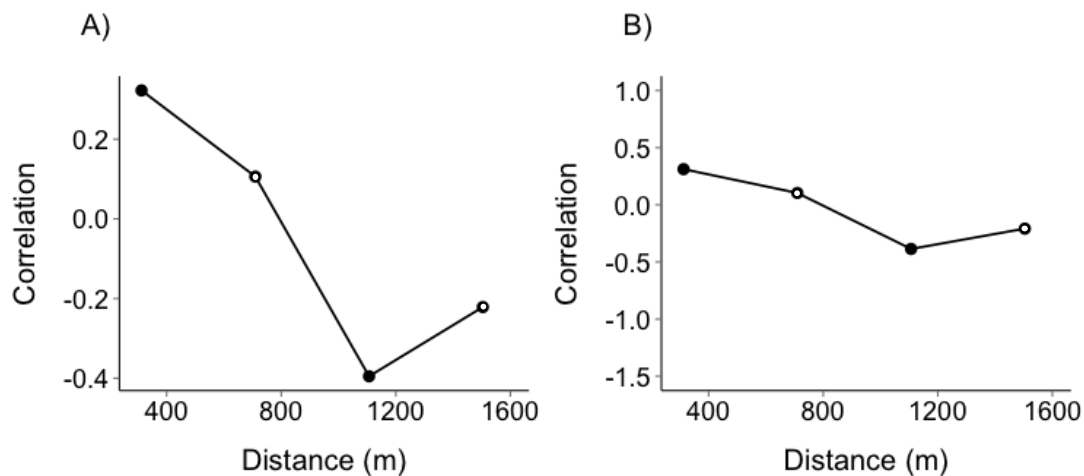
Figure 2.2 Correlation in pollinator composition between each class of distances using two dissimilarity measures, (A) Bray-Curtis and (B) Jaccard distance. Black points are statistically significant Mantel correlations at alpha = 0.05.

Both pollinator abundance and richness significantly increased with increasing wild flowering plant cover in the matrix (t = 2.36, p = 0.037; t = 2.66, p = 0.024). However, the abundance and richness of pollinators did not respond to patch isolation nor stock dung cover and both terms were removed from the models to improve AIC.

More-isolated patches (i.e. those with a large distance to the nearest other patches) contained a subset of the species observed in the less-isolated patches (permutation test on NODF with patches ranked by isolation, z = -2.04, p = 0.035). However, there was no consistent response of pollinator richness to patch isolation (t = 0.22, p = 0.083), and any tendency for patches with lower richness to contain nested subsets of the more species-rich patches was not significant (permutation test on NODF with patches ranked by richness, z = -1.92, p = 0.075).

Pollinator body size

Pollinator body size was significantly spatially correlated (r = 0.66, p < 0.001). The body size of pollinators at close and intermediate patches were significantly similar (p = 0.01, p = 0.007), but as distance between patches increased, body size became significantly dissimilar (p = 0.01, p = 0.001, Fig. 2.3). Pollinator body size also significantly declined with increasing isolation (t = -2.93, p = 0.013, Fig 2.4).
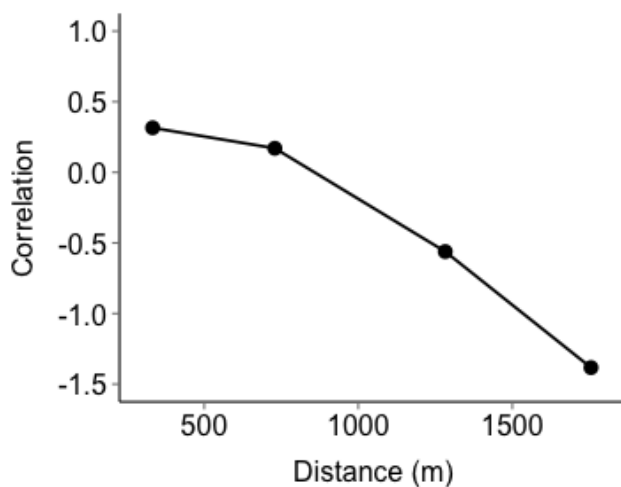
Figure 2.3 Correlation of mean pollinator body size at patches between each class of distances. Black points are statistically significant Mantel correlations at alpha = 0.05.
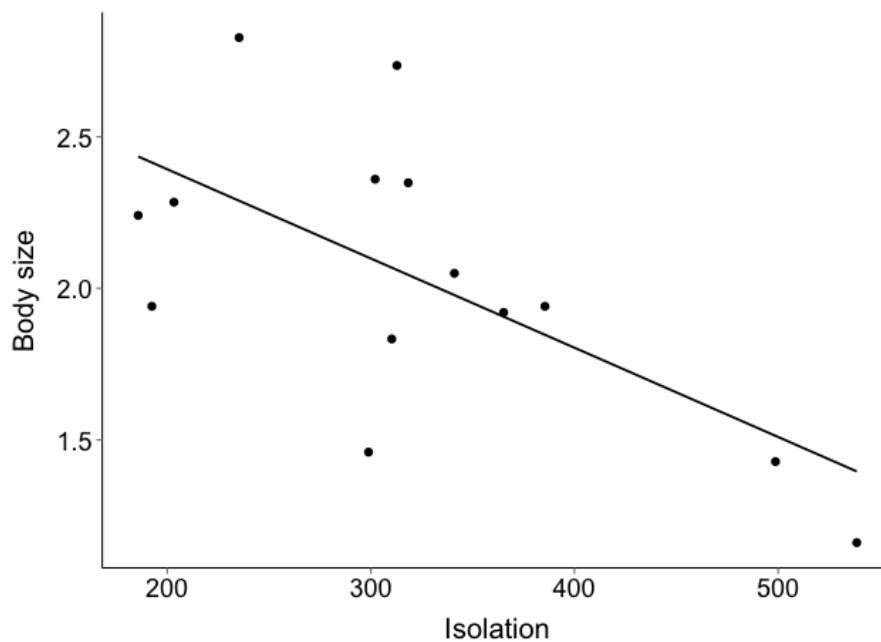
Figure 2.4 Pollinator body size over increasing habitat isolation (average distance from four nearest neighbours in meters).

Pollinator guilds

To understand the changes in species composition described above, I examined the responses of individual pollinator guilds. Two of the twelve pollinator guilds showed significant spatial correlation in their abundances; Solitary native bees (Mantel test: $r = 0.54$, $p = 0.041$), and parasitic wasps (Mantel test: $r = 0.28$, $p = 0.038$). Interestingly, the abundances of these guilds significantly increased with increasing isolation ($t = 3.19$, $p = 0.009$; $t = 2.35$, $p = 0.038$). While the abundances of bumbles bees and butterflies significantly declined with increasing isolation ($t = -2.20$, $p = 0.045$; $t = -2.5$, $p = 0.03$). The remaining eight guilds of pollinators did not respond to isolation (see Table 1). Although multiple testing increases the risk of a type I error, the probability of finding four tests out of twelve significant at an alpha of 0.041 can be calculated using a Bernoulli process (Moran 2003), and at $p = 0.001$, it is unlikely that these four tests would have been significant by chance alone.

Table 2. Flower visiting insects and their relationship to increasing isolation. Results of simultaneous autoregressive models are given.

Species guild	Number of individuals	Relationship	r^2	P
All bees (Hymenoptera: Apoidea)	612	Positive	0.366	0.022
Solitary bees (Colletidae & Halictidae)	447	Positive	0.507	0.009
Bumblebees (*Bombus* spp.)	121	Negative	0.31	0.05
Honeybees (*Apis mellifera*)	43	Not significant	–	–
Hover flies (Syrphidae)	148	Not significant	–	–
Flesh flies (Sarcophagidae)	254	Not significant	–	–
Blowflies (Calliphoridae)	180	Not significant	–	–
Flies (All other Diptera)	225	Not significant	–	–
Butterflies (Lepidoptera)	15	Negative	0.29	0.03
Bugs (Hemiptera)	8	Not significant	–	–
Beetles (Coleoptera)	8	Not significant	–	–
Parasitic wasps (Ichneumonidae)	13	Positive	0.28	0.038
Ants & wasps (Vespoidea)	7	Not significant	–	–
All flower visitors	1460	Not significant	–	–

2.3.2 Pollen

The overall composition of pollen across patches was not spatially correlated (Appendix 3). However, there was significant spatial autocorrelation of both pollen richness measures (Mantel test, all pollen: r = 0.79, p < 0.001; excluding matrix pollen: r = 0.74, p < 0.001). The pollen richness at close and intermediate patches was significantly similar (p < 0.001, p = 0.014; p = 0.001, p = 0.033), but as distance between patches increased, pollen richness became significantly dissimilar (p < 0.001, p < 0.001; p = 0.002, p = 0.001, Fig. 2.5).
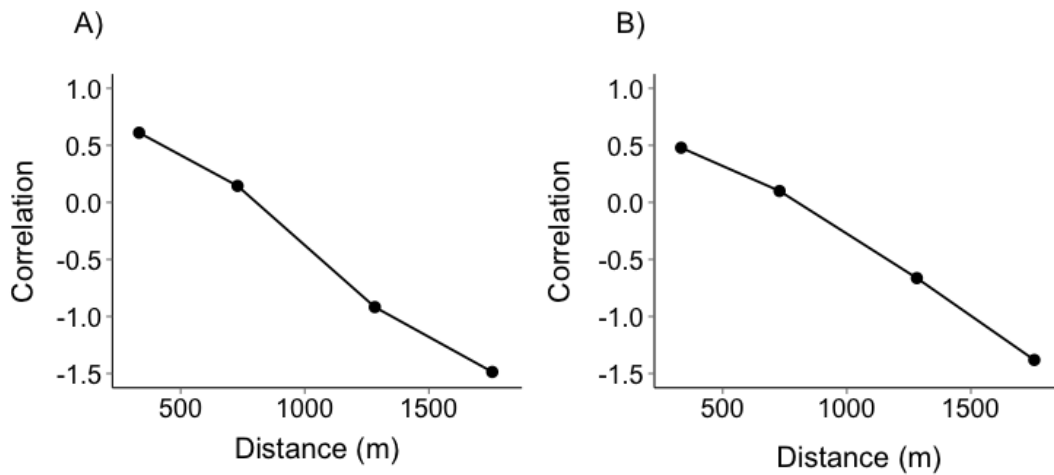
Figure 2.5 Correlation of pollen richness among patches of increasing distance, (A) all pollen species and (B) patch plant pollen species, excluding matrix pollen. Black points are statistically significant Mantel correlations at alpha = 0.05.

Again, for both measures, pollen richness significantly declined as patch isolation increased (t = -2.3, p = 0.044; t = -4.99, p < 0.001), and significantly increased with increasing pollinator body size (t = 3.01, p = 0.013; t = 2.41, p = 0.037). Neither wild flowering plant cover and stock dung cover nor insect richness and abundance significantly affected either of the pollen richness measures and AIC improved when these terms were removed from the models.
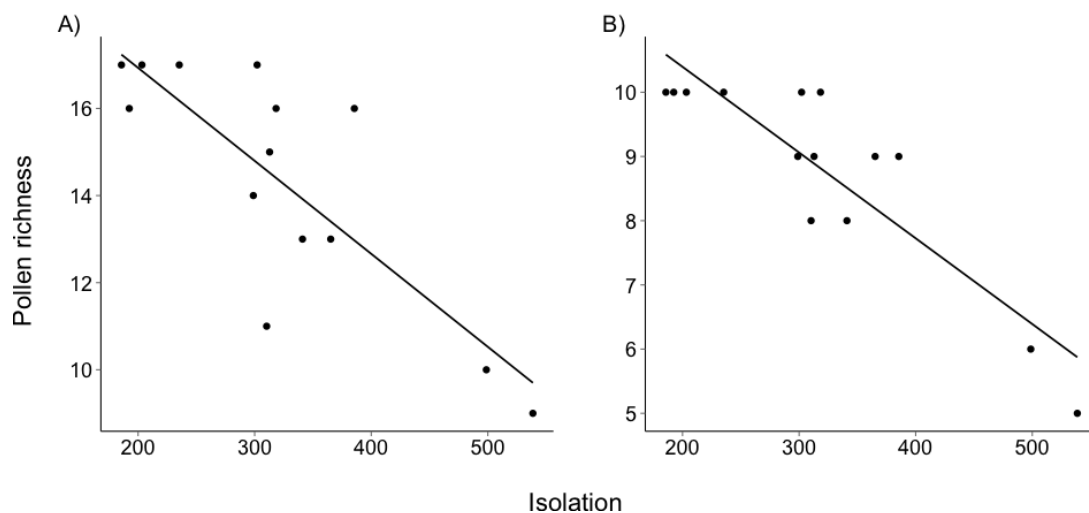
Figure 2.6 Pollen richness over increasing habitat isolation (average distance from four nearest neighbours in meters), (A) All pollen species and (B) Patch plant pollen species, excluding matrix pollen.

Pollen richness was similar across pollinator species, with the exception of three species that had significantly higher pollen richness; *Bombus terrestris* (z = 2.12, p = 0.033); *Lasioglossum sordidum* (z = 2.28, p = 0.022); and *Oxysarcodexia varia* (z = 2.22, p = 0.026).

2.3.3 Seed set

From the seven plant species tested, only anise hyssop and clover showed a significant correlation between the pairwise spatial distance of patches and their seed set (r = 0.70, p = 0.004 and r = 0.35, p = 0.012 respectively). However, this positive correlation was only present over short distances and declined rapidly thereafter, with the lack of a significant Mantel correlation at any specific distance class.
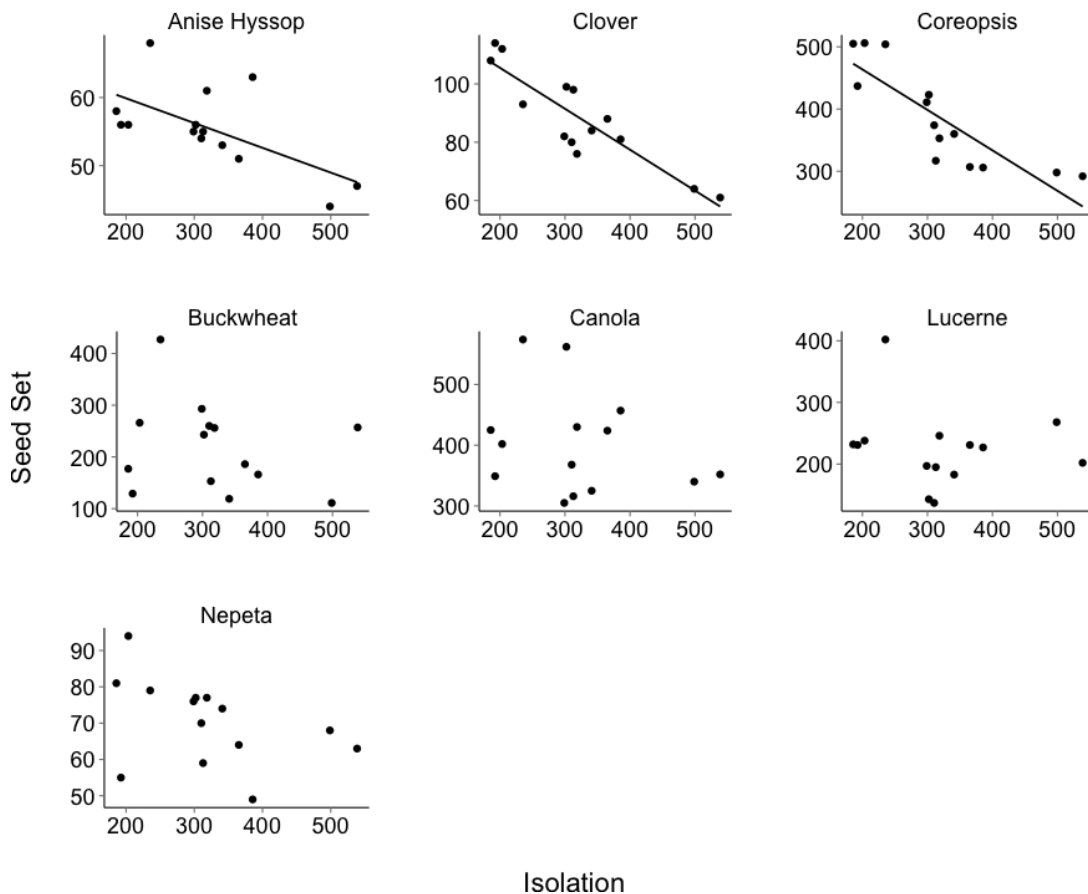
Figure 2.7 Seed set of plant species over increasing habitat isolation (average distance from four nearest neighbours in meters).

The seed set of three of the seven plant species significantly declined with increasing isolation; anise hyssop, clover and coreopsis (Figure 2.7, Appendix 4). While the seed set of three plant species significantly increased with increasing wild flowering plant cover in the matrix; coreopsis, buckwheat and nepeta (Appendix 4). Pollinator abundance a body size positively correlated with the seed set of two plant species, Canola and Lucerne (Appendix 4). Pollen richness, insect richness and stock dung cover did not significantly affect the seed set of any plants and were removed from all cases of the best fitting models. In all cases, AIC was lower for simultaneous autoregressive models than ordinary least squares models (OLS), demonstrating that the addition of spatial distance improved model fit and accounted for variation among patches that was not captured by the standalone variables in OLS regression.

2.4 Discussion

The plant-pollinator communities I observed were spatially structured, and as hypothesised, several measures of the community were significantly more similar at proximate patches and responded to isolation. Overall, pollinator abundance and richness were not affected by isolation, however pollinator composition was spatially correlated and the pollinator species present at isolated patches were a nested subset of species present at less isolated patches. Combined with previous work on the effects of fragment size (Aizen et al 2012), this suggests that both the habitat area and isolation components of habitat fragmentation select consistently for a subset of the species pool. Investigation of the abundances of individual species and species guilds across patches showed that the majority of pollinator species did not decline with isolation. However, there were some important changes in the abundances of bees. Contrary to my expectations, bumblebees (*Bombus* spp.) significantly declined with isolation and solitary native bees significantly increased. Bumblebees were the largest pollinator species in the community while the native bees, the most abundant species, were ranked in the bottom ten smallest species. The compositional shift of pollinators resulted in the significant decrease in mean pollinator body size with increasing isolation.

I hypothesised that larger bodied pollinators would be more abundant in isolated patches as flight range is positively related to body size (Greenleaf *et al.* 2007). However, larger bodied pollinators, such as bumblebees declined with isolation. Bumblebee flight ranges have been shown to be at least 1.5km, with 4km suggested to be about the maximum travel distance (Osborne *et al.* 2008). Therefore, dispersal to the isolated patches should have been

physically achievable. The response could have been behavioural, as when foraging for pollen (which is required for larvae rearing), social bees have been shown to be more selective in their choice of food plant (Goulson and Darvill 2004). So bumble bees may have remained in the area with the highest density of patches, as this provided the most abundant resources.

Alternatively, a response to agricultural cover could have been driving the observed patterns in bee size and large bee declines (Larsen et al 2005). Larsen *et al.* (2005) demonstrated that body size was positively correlated with the probability of loss of pollinators due to land-use change. Larger-bodied species are more affected by floral declines because they have larger pollen requirements than small species (Scheper *et al.* 2014). Therefore, the isolated plant communities may not have been adequate for large bodied pollinators, though it is highly unlikely that the scale of my experiment was sufficient to determine population dynamics within individual patches.

Ecological function can rapidly decline through the non-random loss of species (Larsen *et al.* 2005). Body size is correlated with pollination efficiencies for bee species (Larsen *et al.* 2005) and I found that pollinator body size was positively correlated with increased pollen richness of the pollen load carried by the pollinators. Large pollinators declined with isolation, and this change appeared to affect ecosystem functioning, as seed set of three of the seven plant species significantly declined.

Seed set of the seven plant species responded differently to habitat isolation (Figure 2.8). The seed set of three species significantly declined with isolation while the remaining four species responded to other measures of the pollinator community. This difference in species seed set responses could be due to differences in the plants' morphological and reproductive characteristics and differences in the traits of the pollinators they interact with.
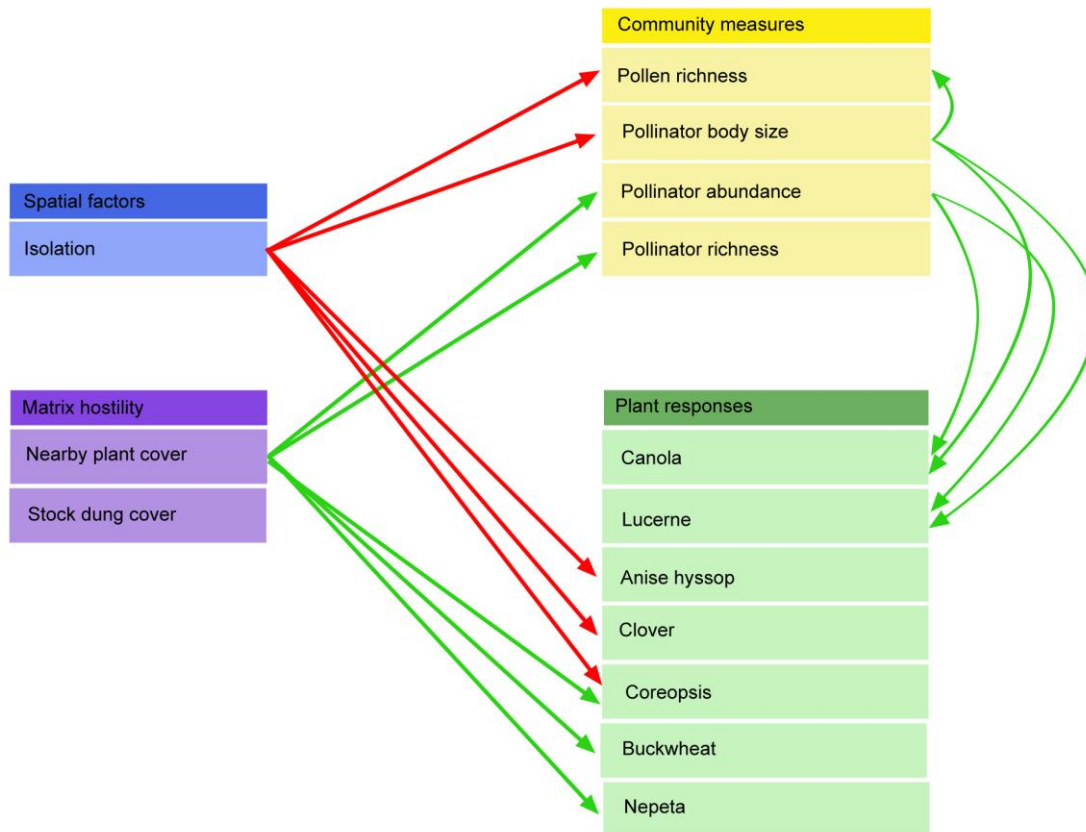
Figure 2.8 Summary of observed community responses. Red arrows indicate significant negative effects, green arrows are significant positive effects.

It is important to consider matrix composition when studying populations subjected to anthropogenic landscape modification (Watling *et al.* 2011). Matrix habitat influences ecological dynamics and a meta-analysis carried out by Watling *et al.* (2011) showed that studies which accounted for matrix composition, compared to those which didn't, were better able to detect changes in the abundance and occupancy of fragmented populations. I found bee richness and abundance at patches positively responded to increased nearby wild flower cover (Fig. 2.8). Wild flower cover and stock dung cover in the matrix could have worked to reduce the hostility of the matrix and increase its permeability. Therefore, surrounding land cover is also important for species in isolated habitats. This could have important implications for efforts to mitigate the negative effects of habitat isolation.

Habitat isolation disrupted ecosystem functioning and the ecosystem service of pollination by affecting species composition and individual species abundances. Non-random loss of species, in particular large bodied generalists, had consequences on the functioning of the system. The decline in pollination services for some species led to a reduction in plant seed-

set. Environmental drivers rarely work in isolation (Potts *et al.* 2010), and the combined effects of agricultural cover and habitat isolation may act to shape pollinator communities and thereby directly and indirectly impact pollination services in metacommunities.

Chapter 3. Plant-pollinator interaction networks and the effects of isolation

3.1 Introduction

Habitat modification is occurring on a global scale, with natural systems undergoing deforestation, agricultural conversion and urbanisation (Foley *et al.* 2005, Nelson 2005, Tilman *et al.* 2001, Pimm *et al.* 2014). These changes are causing habitat reduction, fragmentation and isolation of natural ecosystems and they pose a major threat to biodiversity and valuable ecosystem services, such as pollination (Nelson 2005, Krauss *et al.* 2010). Human wellbeing depends on pollination by mutualistic animals, as a substantial portion (estimated to be around 75%) of leading global food crops rely on pollination by animals (Klein *et al.* 2007). Therefore, it is important to understand how habitat change and isolation will affect mutualistic interactions such as pollination.

Interactions between species, such as those between pollinators and plants, can be viewed as a network whereby species are nodes and pollination interactions are links. The importance of this network perspective for quantifying changes to species interactions has been emphasised and incorporated into mainstream ecological theory (Bascompte 2009, Ings *et al.* 2009, Tylianakis *et al.* 2010). Measures of link occurrence, frequency, and distribution can be used to examine the underlying architectural structure of networks (Thébault and Fontaine 2010, Saavedra *et al.* 2011). For example, mutualistic networks are very heterogeneous (i.e. there are a few highly-connected species and many weakly-connected species; Fortuna and Bascompte 2006) and tend to be nested (specialists interact with a subset of the species that interact with generalists; Bascompte *et al.* 2003). Such architectural properties of mutualistic networks have been explored in the recent literature and found to promote stability (Fortuna and Bascompte 2006, Bascompte 2009, Bastolla *et al.* 2009, Thébault and Fontaine 2010, Saavedra *et al.* 2011). Interestingly, trophic and mutualistic networks have fundamentally different network architectures that favour stability (Thébault and Fontaine 2010); in mutualistic networks, highly connected and nested structures enhance stability, while in trophic networks compartmentalized and weakly-connected networks promote stability (Thébault and Fontaine 2010).

This new understanding of the network structure of mutualistic communities can enable assessment of the robustness of the network to perturbations such as habitat loss and species extinctions at a community level (Bascompte 2009). There is a growing base of literature examining plant-animal interaction networks of mutualistic communities and metacommunities that have been subjected to habitat loss, which assesses how the architecture of these networks changes with declining habitat area (Fortuna and Bascompte 2006, Sabatino 2010, Aizen *et al.* 2012). Although there is also some evidence of the effects of habitat isolation on network structure (Cadotte and Fukami 2005, Cadotte 2006, Driscoll *et al.* 2010, Åström and Bengtsson 2011), it remains relatively unexplored in mutualistic networks at the metacommunity level (Gilarranz and Bascompte 2012).

Recent work has found that interactions between generalists are the most robust to declining habitat area (Aizen *et al.* 2012), and generalist species form the core of the nested structure of pollination networks (Pauw 2007). However, although nestedness of mutualistic networks provides stability to the network as a whole, some species contribute very little to this nested architecture, while others are strong contributors (Saavedra *et al.* 2011). Unfortunately, the strong contributors are also more prone to extinction than nodes that contribute proportionally less to network persistence (Saavedra *et al.* 2011). This could be because of a fitness cost associated with linkage constraints, although the paradox is not entirely understood at present (Saavedra *et al.* 2011). It does, however, raise concerns over the persistence of mutualistic networks in the face of habitat modification, which disrupts links and removes nodes (Tylianakis *et al.* 2008a). Loss of these interactions has negative effects on plant sexual reproduction through pollen limitation and reduction of pollinator fitness through decrease in floral resources (Kearns *et al.* 1998, Fahrig *et al.* 2003, Tylianakis *et al.* 2008a). Moreover, the greater vulnerability of interactions to environmental changes suggests that their loss may precede species extinctions (Tylianakis *et al.* 2008a).

The role of body size in mutualistic networks is relatively unexplored compared with its known importance in food webs (Woodward *et al.* 2005), even though body size of pollinators is known to be an important trait for determining dispersal capacities and pollination success (Larsen *et al.* 2005, Hoehn *et al.* 2008). Work on mutualistic networks may benefit from insights into the role of species traits, such as body size and dispersal mode, for driving local and metacommunity structure in food webs. McCann *et al.* (2005) found that larger animals make an important contribution to stability by coupling multiple energy

channels, especially when in a heterogeneous and large spatial structure (McCann *et al.* 2005). Although this work focused on large mobile consumers, large-bodied mutualistic animals could play a similar role, particularly if they couple fragmented or isolated interaction networks. Larger consumers can transport more propagules over a greater distance, but they are often rarer than smaller species (Woodward *et al.* 2005). In a random removal scenario, the tolerance of a mutualistic network to species extinction is high (Östman *et al.* 2009). However, anthropogenic disturbances, such as habitat reduction do not randomly remove species from a networks, thus there is the potential to cause a sudden collapse of pollination networks (Östman *et al.* 2009). Therefore, body size may be an important determinant of the number and strength of links as well as the population size and extinction vulnerability (Larsen *et al.* 2005) of species in mutualistic webs, in much the same way as it is in 'traditional' food webs (Jacobson and Peres-Neto 2010).

Pollinator diversity affects pollination success and the stability of pollination in plants (Hoehn *et al.* 2008, Tylianakis *et al.* 2008b). A more diverse range of pollinators allows the potential for functional redundancy in the system, and therefore when a link is disrupted the loss in pollination efficiency can be mitigated by the presence of another pollinator that fills the same functional role (Yachi and Loreau 1998). Hoehn *et al.* 2008 showed a positive effect of pollinator diversity on the seed set of plants, and Tylianakis *et al.* 2008b found that this relationship can depend on spatial heterogeneity of flowering plants. However, these studies have focus on single plant species, and it is unclear whether that influence can scale up to whole communities, such that generalist plants (those species that are visited by many pollinators) would be able to maintain better pollination success in the face of habitat loss.

Current approaches to studying pollination networks have tended to take a plant-centric perspective, by watching plants to see which visitors come and interact with them (Bosch *et al.* 2009). However, Bosch *et al.* (2009) found that adding the pollinator's perspective, through examining pollen load of insects, unveiled a number of network interactions not found in the visitation network of the same system. Similarly, Lopezaraiza-Mikel *et al.* (2007) found that the importance of an invasive plant in a visitation network was low, but it dominated the pollen transport network. Therefore, the incorporation of both pollen and visitation networks could lead to a better understanding of how networks respond to changes such as habitat isolation.

In this chapter I will use the experimental pollinator metacommunity presented in Chapter 2 to examine the spatial structure of mutualistic networks. Specifically, I aim to answer the following questions:

1) How does network structure change with isolation and what implications could this have for the stability of isolated networks?

2) How does body size relate to pollinators' role (e.g. number of plant species a pollinator interacts with) within a network?

3) Are generalist plants (i.e. those species that interact with diverse pollinators) less susceptible to negative isolation effects on seed set than specialists?

4) Do pollen transport networks and plant visitation networks show the same response to isolation of the networks?

3.2 Methods

3.2.1 Experimental design

Plant visitation networks and pollen transport networks used for this study were constructed from samples I collected during a manipulative field experiment on a sheep farm pasture in Oxford, North Canterbury, New Zealand (see Chapter 2 methods). Fourteen patches of experimental plants, each with a total of 60 potted plants from 10 flowering plant species per patch, were set out at increasing distances from each other to establish identical plant communities over a gradient of isolation (see section 2.2.2 for experimental plant details). Wild flowering plant cover and stock dung (dung provides larval food for blowflies, which can be important pollinators in New Zealand) in the surrounding matrix were quantified to assess the effects of these potential pollinator resources.

3.2.2. Pollinator visitation and body size

I conducted observations at these patches over a three-month period from December 2012 to January 2013, during which pollinators were captured after visiting a flower within a patch (see section 2.2.3 for details on observations of flower-visiting insects). The flower species the pollinator visited before capture was noted, for construction of the plant visitation network. Collected specimens were then stored individually to prevent pollen contamination.

With the use of identification keys (Dear 1985, Klimaszewski and Crosby 1997, Klimaszewski and Watt 1997, Dugdale 1998, Donovan 2007, Thompson 2008), I identified all pollinators to species level where possible and when identification keys did not exist they were identified to morphospecies using reference specimen samples (hereafter collectively referred to as 'species').

To examine how body size related to pollinator interactions within the plant visitation and pollen transport networks I measured the inter-tegular span (IT span), i.e. the distance between wing bases, of all pollinators with digital calipers and used this measure as a proxy for body size.

3.2.3 Pollen load

I examined the pollen load of all collected specimens to determine which pollinator species carried which pollen species, and in what frequencies, for construction of the pollen transport network. Pollen from the insects, excluding pollen carried in the corbiculae or scopae of bees (which is unlikely to be deposited on flowers), was removed by dabbing the insect with fuchsin gel and mounted on a microscope slide, using the method outlined in Dafni *et al.* (2005). I then systematically examined these pollen slides identifying the first twenty pollen grains on each slide to species level, with the use of reference pollen samples collected from the experimental plants and wild flowering plants from the surrounding pasture matrix (hereafter referred to as 'matrix pollen').

3.2.4 Plant reproductive success

Plant reproductive success was measured through seed set in seven of the ten patch plant species; *Agastache foeniculum* (anise hyssop), *Brassica napus* (canola), *Coreopsis tinctoria* (calliopsis), *Fagopyrum esculentum* (buckwheat), *Medicago sativa* (lucerne), *Nepeta grandiflora* (border ballet), and *Trifolium* (clover). Due to damage caused by sheep grazing, seed set measures were not obtained for the remaining three patch plant species; *Achillea millefolium* (yarrow), *Echium vulgare* (blueweed), and *Lobularia maritime* (white alyssum). I counted the number of seeds produced from five flowers per plant of all seven experimental plants species listed above and summed the total number of seeds of each plant species at each patch (see section 2.2.5 for seed set details). Absolute seed set would not be comparable

across species due to genetic differences in plant fecundity. Therefore, to compare seed set rates across species, I standardized absolute seed set by scaling and centering (subtracting the mean and dividing by the standard deviation) seed set within each species. This meant that cross-species comparisons measured how the relative differences in seed set from different patches changed across species.

3.2.5 Network construction and properties

To analyse the effects of isolation on interactions between insects and plants I constructed plant visitation networks and pollen transport networks (Lopezaraiza-Mikel *et al.* 2007, Bosch *et al.* 2009) for the 14 patches and for the metacommunity as a whole. I also constructed a second pollen transport network that excluded matrix pollen species, in order to assess the role of matrix pollen in the network. I looked at both visitation and pollen transport networks to allow for a comparison of the two to see if their results and conclusions differed. The pollen transport networks were made using the identities of pollen grains carried by pollinators. An interaction (i.e. a network 'link') between a pollinator and plant species was defined by the presence of a plant species' pollen on any individual of an insect species. Thus, pollen transport networks provide a pollinator-centric view of interactions, which complements the traditional plant-centric sampling of visitation networks (Bosch *et al.* 2009).

Both networks were quantitative, in that interactions were weighted according to their frequencies. For pollen transport networks, this weighting was determined by the number of individuals of a pollinator species recorded to be carrying a pollen species. The visitation network links were weighted by the number of individuals of a pollinator species visiting a plant species.

Network-level and species-level properties of the pollen transport and visitation networks were calculated using the 'bipartite' package (Dormann *et al.* 2008) in R (R Core Team 2013). At the network level I selected connectance and interaction nestedness (measured using a nestedness metric based on overlap and decreasing fill, NODF, Almeida-Neto *et al.* 2009) as metrics for the assessment of community structure. Connectance is the realised proportion of possible links in the network and represents a measure of species specialisation within a patch (Dunne *et al.* 2002). While nestedness (the degree to which specialists interact with a subset of the species that interact with generalists) is important for network stability

and therefore any significant change in nestedness across the network could have implications to the long term persistence of the networks (Thébault and Fontaine 2010 and Bastolla *et al.* 2009). Network metrics were calculated using the bipartite package (Dormann *et al.* 2008) for the R environment (R Development Core Team 2011).

For species-level analysis I calculated the normalised degree of each insect species at each patch, which is a measure of the number of plant species an insect interacts with (Dormann *et al.* 2008). I also calculated plant degree at the meta-network level (i.e. all patches pooled together), as a measure of plant generality (Dormann *et al.* 2008). I pooled the networks for plants because plant degree was used as a predictor of their susceptibility to isolation effects, rather than as a patch-level response variable (as for pollinators). Therefore, pooling of the plant network data gave the best possible resolution on the number of potential pollinator species with which a plant species can interact.

3.2.6 Statistical analysis

To assess how pollen transport and visitation network structure changed with increasing isolation and changes in the surrounding matrix, I analysed the responses of connectance and nestedness to patch isolation and wild flowering plant and stock dung cover in the surrounding matrix. Metrics of empirical network structure can often be sensitive to the sampling intensity (Dunne *et al.* 2002). Because I used the same sample effort (time) at each patch, observed networks should be comparable representations of differences across patches. However, to determine whether any differences in the networks were simply due to differences in sample size across patches, I included the abundance of pollinators at each patch and the number of potential links (plant diversity x pollinator diversity) as covariates in the models testing for the response of connectance and nestedness to isolation. Plant diversity and abundance were experimentally fixed in all patches, so this was not included as a covariate. With each metric as a response variable and patch isolation, matrix plant cover, dung cover, insect abundance and species diversity as predictors, I ran simultaneous autoregressive (SAR) models in the package SAM (Rangel *et al.* 2006), after initial identification of spatial autocorrelation of the measures across patches. Each model was simplified to the best-fitting model by testing all subsets of predictors and choosing the best model based on the combination of predictor variables with the lowest Akaike Information Criterion (AIC).

I examined the relationship between pollinator body size and the number of species with which it interacted using a linear mixed effects model, conducted in R (R Core Team 2013) with the lmer function from the lme4 (Bates *et al.* 2011) and lmerTest (Kuznetsova *et al.* 2014) packages. Pollinator degree (normalised to be comparable across networks of different sizes) was the response variable, insect body size was the predictor and patch identity was the random effect (to control for the non-independence of species from the same patch network). This allowed me to assess the relationship between body size and species interactions across all pollinator species in the meta-network. I also ran a second model including pollinator species identity as a random factor, so that I could determine whether there was an effect of variation in body size within species.

To analyze how the effect of isolation on plant seed set related to a plant's generality, I ran a linear model using the lm function in the base package of R (R Core Team 2013), with the standardised mean seed set of all plants at each patch as the response variable and plant generality and isolation and their interaction as predictors. I was interested in their interaction effect because a positive significant relationship would indicate that the seed set of more generalist plants was less affected by isolation than specialist plants, while and negative interaction would indicate the opposite.

3.3 Results

3.3.1 Network level metrics

The best-fitting SAR models retained the spatial distance matrix (i.e. including information on spatial autocorrelation improved model fit), but even after accounting for spatial autocorrelation, there remained a significant effect of isolation in many cases. Connectance of the pollen transport networks significantly decreased as isolation of the patches increased ($t = -2.44$, $p = 0.038$), when sample size and the number of possible links were included as covariates in the model. Connectance responded to these covariates as expected, responding negatively ($t = -3.45$, $p = 0.007$) to the number of possible links between species (i.e. network size) and positively to the sample size of pollinators at each patch ($t = 3.75$, $p = 0.005$). In contrast, connectance of the plant visitation network was significantly positively correlated with an increase in isolation ($t = 2.80$, $p = 0.021$), while responding to the number of possible links and sample size in the same way as the pollen transport networks ($t = -4.21$, $p = 0.002$

and t = 2.57, p = 0.03). In both the pollen and visitation networks, surrounding wild flowering plant cover and stock dung cover had no significant effect and were removed from the best-fitting models.

Nestedness also significantly decreased in the pollen transport networks as isolation increased (t = -3.46, p < 0.007), whereas nestedness of the plant visitation networks did not change with isolation, and isolation was removed from the best-fitting model. Again, in both models nestedness responded to the covariates of sample size and diversity as expected, significantly increasing (pollen network t = 6.14, p < 0.001; visitation network t = 6.18, p < 0.001) and decreasing (t = -5.34, p < 0.001; t = -4.33, p = 0.001) respectively. Surrounding wild flowering plant cover and stock dung cover had no significant effect and were removed from the best-fitting models.

The observed changes in connectance and nestedness of the pollen transport network remained significant when pollen from the matrix plant species was removed (see Appendix 5 and 6).

3.3.2 Species level metrics

Body size and pollinator generality
When examining the full pollen transport network, the number of plant species a pollinator interacted with significantly increased with increasing body size, both within pollinator species (F = 4.24, p = 0.043) and across all pollinators (F = 6.09, p = 0.014). However, when matrix pollen species were excluded from the network, the number of plant species a pollinator interacted with significantly increased with increasing body size across pollinators (F = 3.77, p = 0.05), but there was no significant within-species effect (F = 2.55, p = 0.115). In the plant visitation network, the same correlations as in the reduced pollen network were observed, all pollinators (F = 3.73, p = 0.05), and within species (F = 0.33, p = 0.568).

Seed set and plant generality
The generality (normalised degree) of plants, in both the pollen and visitation meta-networks, significantly predicted the extent to which plant seed set was affected by isolation. Plant seed set positively responded to the interaction term between isolation and plant generality (pollen network t = 2.56, p = 0.012; visitation network t = 2.22, p = 0.029, Fig. 3.1). Therefore, as

plant generality increased, the negative effect of isolation on seed set (i.e. the slope of the relationship) significantly decreased (i.e. became less negative, showing that isolation had a stronger negative effect on the seed set of specialist plant species than more generalist plant species. This remained significant when matrix pollen species were removed from the pollination network (t = 2.56, p = 0.049).
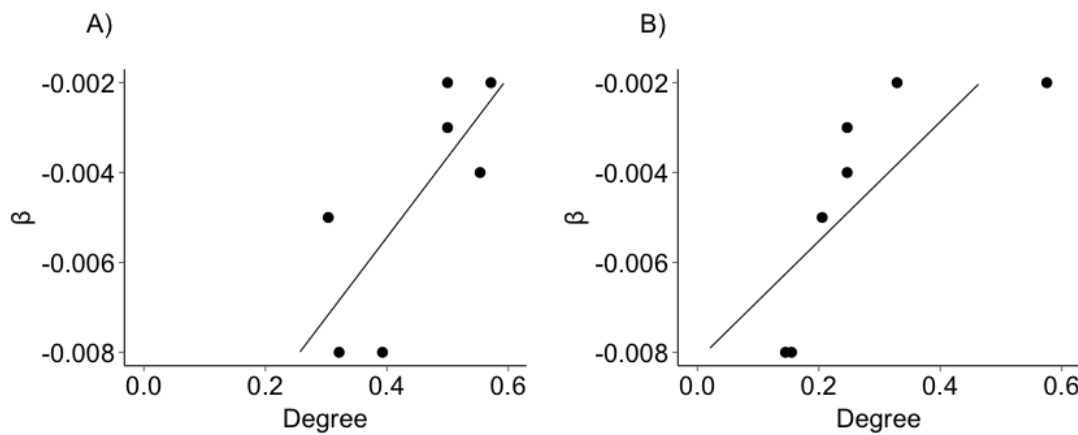
Figure 3.1 The analysis treated plant generality and isolation as predictors, with their interaction term being the effect of interest. Here, this interaction is presented as the change in the slope (β) of each species' response to isolation with increasing generality, (A) pollen transport network and (B) plant visitation network.

3.4 Discussion

Examination of interaction networks and their structure can provide a powerful tool for assessing properties of the system, which may not be apparent from simple community measures or the investigation of pairwise interactions alone (Tylianakis *et al.* 2008a, Bascompte 2009). I found that the structures of the mutualistic networks and their interactions changed as isolation of patches increased. Both connectance and nestedness of the networks were influenced by the spatial process of habitat isolation.

In mutualistic networks highly-connected and nested structures enhance stability and increase biodiversity (Bastolla *et al*. 2009, Thébault and Fontaine 2010). Isolation led to a decrease in the nestedness of the networks I observed, therefore decreasing the stability and in turn increasing the potential for further disruption of plant reproduction at the isolated patches. In

contrast, connectance of the plant-pollinator visitation network increased with isolation, indicating that pollinators interacted more frequently with plants in isolated patches. However, connectance in the pollen transport network showed the opposite pattern, which suggests that high connectance in flower visitation does not necessarily translate to high connectance in the pollen load carried by the visiting pollinators.

Body size plays an important role in structuring of food webs and through determining characteristics of interactions and species (Woodward *et al.* 2005). It is thought that the same patterns may apply to the structuring of mutualistic networks (Woodward *et al.* 2005). My results confirm the importance of body size in determining species interactions in plant-pollinator networks. Specifically, larger pollinators interact with a greater number of species than smaller pollinators. Therefore, large pollinators would be important for the maintenance of network connectance and they are also important contributors to the nested structure of pollination networks (Pauw 2007).

The relationship between pollinator body size and number of partners with which it interacts was significant across species for the different interaction networks I studied. However, only in the full pollination network with the inclusion of matrix pollen species was a within-species effect observed. This result shows the importance of trait variability within a species, and highlights the risks associated with using mean trait values (Ackerly and Cornwell 2007) to determine the influence of species and trait diversity on ecosystem functioning. However, this trend was not significant in the visitation network or the pollination network excluding matrix pollen. Therefore, this result could be driven by larger-bodied individuals within a species being better able to disperse into the matrix and utilise wild flower resources while smaller-bodied individuals were more confined to the plant patch area. Within a species, body size varied quite a bit, for example the smallest individual of *Lasioglossum sordidum* was less than half the size of the of the largest individual. This result gives an interesting insight into the interactions of pollinators with wild flowers in the matrix. The exclusion or not of matrix pollen from analyses showed that larger-bodied pollinators within a species used the matrix more, while smaller-bodied insects generally remained within the flower patch.

A meta-analysis on plant reproductive susceptibility to habitat fragmentation, conducted by Aguilar *et al.* (2006), found no consistent difference between the responses of generalist

versus specialist plants. However, they categorised plant species as either generalists or specialists based on the number of insect orders the plants interacted with (Aguilar *et al,* 2006). In contrast, I examined plant species generality as a continuous variable, through species degree in the meta-network, and found that seed set of specialist species was more negatively affected by habitat isolation than that of generalist plant species. The three plant species that showed significant decline in seed set with isolation were also the least generalist species.

Overall, results from the pollen transport network and the plant visitation network showed the same trends as each other. However, the trends in species connectance did differ. Connectance of the pollen transport networks significantly declined with isolation, while the connectance of the visitation networks increased. Because pollen is required for the reproduction of plants and not simply visits by insects, the results shown by the pollen transport network could give an important insight into the efficiency of the pollinators at isolated patches. When examining the relationship between body size of pollinators and the number of species they interacted with, the result obtained from the pollination network and visitation network differed slightly. However, these differences allow speculation into the behaviour of large-bodied pollinators, and how they may interact with flowers in the surrounding matrix. Therefore, the analysis and results of the visitation and pollination networks complement each other and future research could benefit from combing the two.

Chapter 4: Discussion

4.1 Effects of isolation on pollinators, plants and their interaction networks

Habitat modification, and the resulting isolation, of plant populations threatens the interactions between plants and pollinators (Kearns *et al.* 1998, Foley *et al.* 2005, Winfree *et al.* 2009, Potts *et al.* 2010). The conservation of these interactions is important for the maintenance of biodiversity and human wellbeing, as they provide us with the ecosystem service of pollination (Kearns *et al.* 1998, Fahrig *et al.* 2003, Hooper *et al.* 2005, Balvanera *et al.* 2006, Klein *et al.* 2007). The greater vulnerability of interactions to environmental changes suggests that their loss may precede species extinctions (Tylianakis *et al.* 2008a), and in order to conserve these species and their interactions we must first seek an understanding of how habitat change and isolation affect them.

In this thesis, I investigated the effects of habitat isolation on key properties of the pollinator communities, reproductive success of the plants involved, interactions between the two and the resulting network structures. Overall, I found that the impacts of habitat isolation were negative on both plant and pollinator populations and the structure of their interactions. The diversity of pollen being carried by pollinators significantly declined with isolation, along with the relative abundance of large-bodied pollinators (which presented itself as both a reduction in mean body size and a change in community composition) and the seed set of several plant species (Chapter 2). By investigating the observed shifts in community patterns with a network approach (Chapter 3), I was able to better understand the mechanisms behind the changes and their potential implications for the long-term persistence of isolated patches.

Nestedness of mutualistic networks provides stability to the network as a whole, however some species contribute very little to this nested architecture, while others are strong contributors (Saavedra *et al.* 2011). I found a significant decrease in nestedness as isolation increased and in the pollen transport networks there was also a significant decrease in the connectance of the networks. These changes in connectance and nestedness of species interactions could have been partially driven by behavioural changes in the pollinators at isolated patches, though it was most likely caused, at least in part, by the compositional shift in the pollinator species from generalist to specialist (Chapters 2 and 3). Larger-bodied

pollinators visited more plant species and carried more pollen species than smaller-bodied pollinators. Combined with the observation of declining pollinator body size and bumble bee abundances as isolation increased, the evidence points to a shift towards a more specialist pollinator community at isolated habitat patches. Generalist species form the core of the nested structure of pollination networks (Pauw 2007), so it is not surprising that I observed a decrease in nestedness with isolation. My results are therefore congruent with previous findings that the strong contributors to nestedness are also more prone to extinction than nodes that contribute proportionally less to network persistence (Saavedra *et al.* 2011).

Loss of these interactions has negative effects on plant sexual reproduction through pollen limitation and reduction of pollinator fitness through decrease in floral resources (Kearns *et al.* 1998, Fahrig *et al.* 2003). I observed a significant decrease in the seed set of three plant species as isolation increased (Chapter 2), which may have been caused by a disruption in the plants interactions with pollinators. However, not all plant species responded to isolation, with no change in the seed set of four of the species. With interaction network analysis, in Chapter 3, I was able to untangle potential reasons for the differing plant species responses.

Recent work has found that interactions between generalist plants and pollinators are the most robust to declining habitat area (Aizen *et al.* 2012). I also found that generalist plant species were the most robust to the effects of habitat isolation, although the same was not true for the pollinators. The disruption of interactions involving specialist plant species (i.e. those visited by few pollinator species) can have serious negative consequences for plant reproduction, because there is less functional redundancy of pollinators to ensure reproduction (Yachi and Loreau 1997). Normally the asymmetrical nature of plant-pollinator interactions, whereby specialist plant species interact with generalist pollinators, reduces the potential for the loss of interactions involving specialist species (Potts *et al.* 2010). However, I found that isolation was selecting against large-bodied generalist pollinators, such as bumble bees, which led to a more specialist pollinator composition in isolated patches. This could have led to the disruption of interactions between pollinators and specialist plant species, and therefore the reduced reproductive success of specialist plants in isolated habitats (Chapter 3).

4.2 The role of pollinator body size

The role of body size in structuring mutualistic metacommunities is not as well understood as the commonly studied importance of body size in food webs (Brose *et al.* 2005, Petchey *et al.* 2008). Body size is positively correlated with dispersal distance of insects (Larsen et al 2005, Greenleaf *et al.* 2007), thus I hypothesis that isolation would cause a loss of small-bodied pollinators. However, I observed the opposite trend and found that large-bodied pollinators declined with isolation. Through examination of the mutualistic interaction networks I also found that body size was positively correlated with the number of plant species a pollinator interacted with and the diversity of pollen carried by that insect. Therefore, in isolated patches, pollinator body size could be a potentially important response and effect trait (Lavorel and Garnier 2002, Suding *et al.* 2008), whereby body size determines functional contribution of a species through the amount and diversity of pollen it can carries and the number of partners it interacts with, but it is also sensitive to and declines with habitat isolation. Larsen *et al.* (2005) examined the importance of response and effect traits and the subsequent non-random removal of species, finding they lead to the rapid disruption of ecosystem functioning. In my study I found that the non-random selection against species, i.e. large bodied pollinators, correlated with the loss of ecosystem function, as in three of the seven plant species seed set significantly declined. Consequently, this could have important implications for the long-term persistence of plants in isolated habitats, and over time this may feed back to the population persistence or relative fitness of different pollinator species.

4.3 Future research

This research contributes to the understanding of how isolation affects mutualistic meta-community structure. In particular, it demonstrated how species traits can interact with landscape structure (i.e., patch isolation) to determine the meta-community structure in isolated patches. However, this was a short term experiment, which ran over a three month period and therefore did not allow for the measurement of multiple generations or involve self-sustaining populations. In this sense, my experiment was part of a metacommunity (communities connected by dispersal), rather than a self-sustaining metacommunity in its own right. Future research could be done to incorporate this kind of empirical data with a theoretical component, namely a metacommunity model, in order to look at the effects of isolation over a multi-generational scale, to predict which patches may become unoccupied

and which plants may be lost from certain networks as a result of the absence of their pollinators.

My results on the role of body size for determining species interactions within a species show that the fine scale examination of species traits (even within species) is important and can reveal properties of species interactions that would otherwise be missed if species averages were used. Therefore future research should aim to incorporate species trait information at the individual species level, and potentially link that to individual preferences of interaction partners.

A network approach can be applied in the planning and restoration of plant-pollinator networks through the identification of species of high conservation value, due to their role in determining network structure. Its potential to direct management decisions in the recovery of isolated habitat patches could be further explored at a metacommunity level.

In conclusion, habitat fragmentation, leading to habitat isolation, is an important force shaping the mutualistic interactions and altering pollination services in isolated habitats. I observed negative trends in pollinator community measures, plant seed set and interaction network structure as habitats became more isolated. Through the interplay of species traits and their interactions with other species the effects of isolation could determine the population viability and long term persistence of communities.

Appendix

Appendix 1. Characteristics of study patches including isolation metric (average distance to four nearest neighbours in meters), wild flowering plant and stock dung cover (average % cover in ten 1 x 1 m quadrats), and geographic location.

Patch	Isolation (m)	Wild flower cover (%)	Stock dung cover (%)	Latitude	Longitude
1	235.31	21	0	-43.32416	172.21045
2	318.36	14	1.24	-43.32316	172.20897
3	385.51	1	0.52	-43.32109	172.21175
4	192.44	1	0.73	-43.32473	172.21373
5	312.82	0	0.56	-43.32378	172.21553
6	203.29	8	1.75	-43.32573	172.21344
7	185.73	17	0.89	-43.32579	172.21168
8	302.12	15	0.84	-43.32737	172.21078
9	298.86	20	3.97	-43.32853	172.21689
10	341.03	9	5	-43.32704	172.21837
11	310.28	1	4.8	-43.32956	172.21989
12	365.23	1	1.62	-43.33109	172.21823
13	538.65	11	1.45	-43.33433	172.22232
14	498.73	7	1.43	-43.3319	172.22443

Appendix 2. Table of all insect taxa sampled, including total abundance of each taxon collected throughout the experiment, and the number of patches from which each taxon was collected (of a possible 14).

Species	Total abundance	Number of patches
Adalia bipunctata	1	1
Ancistrocerus gazella	2	2
Anthomyiidae sp.1	27	6
Anthomyiidae sp.2	7	4
Anthomyiidae sp.3	5	2
Apis mellifera	43	10
Asteiidae sp.1	1	1
Beris sp.1	1	1
Bombus hortorum	18	7
Bombus ruderatus	6	3
Bombus terrestris	97	13
Calliphora hilli	1	1
Calliphora stygia	4	4
Calliphora vicina	29	10
Chaetophthalmus bicolor	1	1
Chironomidae sp.1	1	1
Coccinella undecimpunctata	5	4
Eristalis tenax	66	13
Formicidae sp.1	1	1
Geometridae sp.1	1	1
Helcomyzidae sp.1	1	1
Helophilus hochstetteri	2	1
Hemiptera sp.1	5	3
Heteria sp.1	3	2
Heteria sp.2	1	1
Hylaeus asperithorax	5	3
Hylaeus capitosus	59	6
Hylaeus relegatus	2	2

Icumonidae sp.1	2	1
Icumonidae sp.2	3	1
Icumonidae sp.3	4	3
Icumonidae sp.4	2	1
Icumonidae sp.5	2	1
Lasioglossum sordidum	381	14
Lepidoptera sp.1	1	1
Lucilla cuprina	1	1
Lucilla sericata	92	12
Lycaena boldenarum	3	2
Lycaena feredayi	3	2
Melangyna novaezelandiae	26	12
Melanostoma fasciatum	39	6
Melyridae sp.1	1	1
Muscidae sp.1	26	10
Muscidae sp.2	8	4
Muscidae sp.3	2	2
Muscidae sp.4	1	1
Odontomyia sp.1	17	10
Orocrambus flexosellus	1	1
Oxysarcodexia varia	254	14
Periscelididae sp.1	14	5
Phasia campbelli	1	1
Philaenus spumarius	3	3
Phoridae spp.	1	1
Pieris rapae	4	3
Platycheirus spp.	16	6
Pollenia sp.1	51	14
Scaptia ricardoae	5	3
Scaptia sp.1	1	1
Spictostethus nitidus	3	1
Tachinidae sp.1	50	13
Tachinidae sp.2	29	10

Tachinidae sp.3	12	7
Tachinidae sp.4	3	2
Tachinidae sp.5	3	3
Tachinidae sp.6	1	1
Tachinidae sp.7	1	1
Tachinidae sp.8	1	1
Tachinidae sp.9	1	1
Vespula vulgaris	1	1
Xenocalliphora hortona	2	2
Zizina labradus	1	1
Zizina oxleyi	1	1
Zorion guttigerum	1	1

Appendix 3. Statistics for the pollen composition Mantel tests, including r values and P values. Community composition was calculated with two dissimilarity indices; Bray-Curtis and Jaccard.

Response	Dissimilarity measures	r value	P value
Pollen richness	Bray-Curtis	0.09	0.259
	Jaccard	0.09	0.261
Pollen richness	Bray-Curtis	0.18	0.136
excl. matrix	Jaccard	0.17	0.140
pollen			

Appendix 4. Final model statistics for seed set models; including the significant variables, the nature of the effect, the slope coefficient, t value and P value of the significant effects. The maximal model included isolation, pollen richness, insect richness, insect abundance, insect body size, stock dung cover and wild flower cover. AIC was used to select the best fitting model.

Species	Predictor(s)	Effect	β	t value	P value
Canola	Pollinator body size	Increase	113.75	3.469	0.006
	Pollinator abundance	Increase	1.349	3.096	0.011
Lucerne	Pollinator body size	Increase	1.211	3.447	0.006
	Pollinator abundance	Increase	66.059	2.67	0.023
Anise hyssop	Isolation	Decrease	-0.033	-2.32	0.041
Clover	Isolation	Decrease	-0.129	-5306	< 0.001
Coreopsis	Isolation	Decrease	-0.597	-7.032	< 0.001
	Wild flower cover	Increase	4.078	3.803	0.003
Buckwheat	Wild flower cover	Increase	7.072	3.167	0.009
Nepeta	Wild flower cover	Positive	1.018	3.3	0.007

Appendix 5. Statistics for the response of the pollen transport and plant visitation networks connectance to isolation, with insect abundance and number of possible links as covariates. Slope coefficients, t value and P values are given.

Connectance	Predictor(s)	β	t value	P value
Pollen network	Isolation	-0.058	-2.437	0.038
	Abundance	0.264	3.755	0.005
	No. of possible links	-0.09	-3.454	0.007
Pollen network, excl.	Isolation	-0.094	-3.36	0.008
matrix pollen	Abundance	0.374	4.414	0.002
	No. of possible links	-0.21	-3.894	0.004
Visitation network	Isolation	0.021	2.802	0.021
	Abundance	0.093	2.574	0.03
	No. of possible links	-0.091	-4.209	0.002

Appendix 6. Statistics for the response of the pollen transport and plant visitation networks nestedness to isolation, with insect abundance and number of possible links as covariates. Slope coefficients, t value and P values are given.

Nestedness	Variable	β	t value	P value
Pollen network	Isolation	-0.053	-3.463	0.007
	Insect abundance	0.45	6.14	< 0.001
	No. of possible links	-2.34	-5.342	< 0.001
Pollen network, excl.	Isolation	-0.089	-5.232	< 0.001
matrix pollen	Insect abundance	0.447	5.911	< 0.001
	No. of possible links	-2.394	-5.322	< 0.001
Visitation network	Insect abundance	0.234	6.177	< 0.001
	No. of possible links	-1.083	-4.331	0.001

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