Effects of the availability of floral resources on plant-pollinator interactions and the implications for the long-term survival of plant populations

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Declaration

All methods and analyses described in this thesis were performed by the author unless stated otherwise. No part of this thesis has been submitted for any other degree or qualification.

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Abstract

1. Insect pollinators have been shown to alter their foraging patterns in response to habitat and landscape composition, particularly in relation to changes in the availability of floral resources which provide essential pollen and nectar provisions. Changes to pollinator behaviour and community composition, may alter the distance, directness and frequency of pollen movement and thus, the compatibility and genetic relatedness of pollen transferred between plants. We still lack good understanding of how variation in the spatial and temporal availability of floral resources drives pollinator responses and in turn, affects the fitness of outcrossing plants. Knowledge in this area could contribute to improved management interventions to enhance pollination services for plant conservation.
2. Through a combination of habitat and landscape scale field experiments, I explored how the availability of floral resources at different spatial scales affected plant-pollinator interactions, pollen transfer and mating success in plant populations, particularly those isolated from conspecifics. This involved introducing different species of plants in experimental arrays across a range of study systems that varied in structure and floral availability. Over the course of the thesis, I measured the community composition and behaviour of pollinators visiting experimental arrays; focusing on traits considered important for pollen transfer (e.g. Inter-tegular ('IT') span). Pollen movement was quantified within and between populations (5-150m) and the resulting plant outcrossing rates were measured using different methods including paternity analysis and the use of a dominance inheritance system. In addition, the implications of variations in pollinator foraging and pollination services can be attributed to pollen and gene flow and subsequently the reproduction and fitness of plants were assessed as a means of predicting the impacts on longer-term plant survival.

3. Findings from this thesis demonstrate reductions in the activity density (the abundance of actively foraging pollinators) and richness of pollinators and thus, the potential for plant visitation in response to a high abundance of floral resources within a habitat. This led to disruptions in pollen transfer, illustrated through a lower incidence of intra and inter-population pollen movement, and ultimately, reduced plant outcrossing rates. In parallel, plant seed set and germination rates were also reduced in habitats with high resource availability. Changes to pollinator communities and pollination services varied with the spatial scale at which floral resources were measured. Pollinator communities (activity density, richness and IT span) were most affected by floral resource abundance at a local scale (1-50m), particularly within a 20m radius of a plant population. Intra-population pollen movement was similarly affected by floral resources at a local spatial scale (within a 1m radius of a plant population). In contrast, no effect was observed on pollinator communities, intra-population pollen movement or plant reproduction when floral resources were measured at a landscape scale (within a 100-1500m radius of a plant population). However, findings were variable across different experiments at the same scale of measurement. For instance, the availability of floral resources at a local scale did not always elicit an effect on plant reproduction. This reflects differences in plant species identity and the effects of breeding system and floral traits, illustrated through variations in visitation rates between plant species. Inconsistencies were further observed with pollinator activity density and richness, which were not related to floral resources at a habitat scale in one chapter.
4. This thesis highlights the importance of the availability of floral resources at a local scale on plant-pollinator interactions and pollination services to plants. Co-flowering plants within florally rich habitats compete for pollinators and subsequently, visitation and pollen transfer between individuals of low density plant populations is diluted rather than facilitated. This suggests that although pollinator abundance and

diversity may be enhanced through florally rich habitats (e.g. habitats implemented under the agri-environment scheme), pollination services are not automatically improved for plants which are present at low frequency in the landscape. This needs to be considered when designing and implementing management for threatened or isolated plants where plants may instead benefit from focused interventions. For instance, pollination services may be increased by efforts to maximise the facilitative effect of surrounding habitats, while increasing the ability of threatened or isolated plants to withstand competition from co-flowering plants.

Lay summary

Insect pollinators (including bees, butterflies and hoverflies) play a vital role in the sexual reproduction of flowering plants, inadvertently transferring pollen from male to female flowers, as they forage between plants in their search for an energy and nutrient rich reward of nectar and/or pollen. From an individual plant's perspective, successful sexual reproduction depends on pollen being delivered to or from another plant of the same species. How far a pollinator travels between two plants of the same species is also significant; greater distance travelled tends to lead to sexual reproduction between less closely related parent plants and therefore greater exchange of unique genes. However, pollinators are not trying to help plants; where, how far, how often and from which plants they choose to forage is based on a cost/benefit analysis of the energy they stand to gain compared to the energy they stand to lose during each foraging bout. These decisions are in turn shaped by the diversity and quantity of flowering plants within a particular patch of habitat, the distance to the next patch of flowering plants and the difficulty or danger a pollinator may face in flying in between them. While changes to the extent, distribution and diversity of wildflower habitats has been shown to have a profound impact on pollinator foraging behaviour, it is still relatively unclear however what the implications of these changes are for the reproductive success and long-term

survival of individual plant species. These implications may be particularly relevant for wildflower species which have become rare in nature and which may already be struggling to attract necessary pollinators.

In my research, I introduced different species of flowering plant, separated from each other by a range of different distances, into several farms across Oxfordshire, Buckinghamshire and Hertfordshire. The plants I introduced were not naturally present in the study area and were positioned in a way that would mimic a naturally rare wildflower species. I placed the introduced plants in fields which varied in the numbers of other wildflowers and wildflower species. I observed which and how many pollinator species visited the introduced species or were active within the vicinity and worked out how far pollinators were moving between individual plants by carrying out paternity analysis on the seeds produced by each plant. I also measured the reproductive success of individual plants based on the weight and number of the seed they produced and the percentage of seed that successfully germinated.

Results from my research showed that greater numbers of co-flowering plant species at a local scale (i.e. within a 50m radius of the introduced plants) led to fewer potential pollinator visitors at a plant level. The high number of co-flowering plant species meant that pollinators were less likely to successfully transfer pollen between two plants of the introduced species, particularly when separated by large distances. Ultimately, higher numbers of co-flowering plant species led to the introduced plants producing less seed with lower rates of germination. In contrast, in habitats with fewer co-flowering plants, the introduced species attracted a greater number of potential pollinator visitors, were more successful in moving pollen from one plant to another and produced more seeds with greater levels of germination. However, the quantity of florally rich habitat found within the larger landscape (e.g. a 100-1500m radius around a group of introduced plants) had no affect at all on the number of potential pollinators visiting the plant species, the amount of pollen movement, seed set or germination.

This thesis showed that the reproductive success of rare plant species can be negatively affected by a high abundance of co-flowering plant species found in the area immediately surrounding a given population. Even though greater numbers of wildflowers attract more pollinators into an area, higher numbers of co-flowering plants tend to outcompete rarer species which may either be overlooked or avoided by the visiting pollinators. Creation of wildflower habitats (e.g. the use of wildflower mixes in the agri-environment scheme) can increase the abundance and diversity of pollinator species and promote greater pollination services across the landscape, yet they may not automatically lead to an improved outcome for rare plant species. Rather, rare plants may require additional measures at the local scale that increase their ability to compete for pollinators and reproduce. This may include planting other wildflower species which, due to differences in their size, colour or structure, complement rather than compete with a target species, or it may involve directly increasing the size of the target species population through reintroduction planting, increasing the likelihood of pollen exchange between individuals.

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CHAPTER 1

Introduction

1.1 The value of plants and pollinators

Pollinating insects play an important role in the reproduction of flowers and agricultural crops and thus have a considerable environmental, economic and cultural value (Senapathi et al. 2015b). Indeed, pollinators have been shown to increase seed or fruit quality in 39 of 57 major crops worldwide (Klein et al. 2007), with contributions to global crop production valued at $235 billion-$577 billion United States dollars in 2015 (Potts et al. 2016a, Potts et al. 2016b). Along with the benefit to global food security, pollinating insects offer a valuable service to wider biodiversity by maintaining populations of wild and cultivated flowers which are important for ecosystem functioning (Hooper et al. 2005, Senapathi et al. 2015b, Potts et al. 2016b). These services in recent years have faced pressure from land-use change and management intensification; urbanisation; invasive alien species; the spread of pathogens and parasites and climate change (Potts et al. 2010a, Vanbergen et al. 2013, Potts et al. 2016b). The additive and synergistic effects of such pressures are likely to have culminated in reductions in the diversity and occurrence of pollinators at a global scale (Biesmeijer et al. 2006, Carvalheiro et al. 2013, Lebuhn et al. 2013, Ollerton et al. 2014, Senapathi et al. 2017). With approximately 90% of flowering plants characterised as reliant at least in part on pollinators for reproduction (Ollerton et al. 2011), plants are particularly vulnerable to reductions in pollinator availability (Burd 1994). Despite these acknowledged anthropogenic threats and evidence of declines, there remains a need to investigate the direct and indirect effects of anthropogenic changes to the structure and behaviour of pollinator communities and the implications for plant reproduction. Such research will provide understanding of how to maintain stable pollination services and mitigate the risks to them.

1.2 Plant-pollinator interactions

Plants and pollinators often exhibit a mutualistic relationship, where plants offer rewards in return for the transference of male gametes (pollen) between conspecific plants and thus, the fertilisation of a plant’s ovules (Proctor et al. 1996). Plant rewards predominantly come in the form of pollen and nectar which offer essential provisions (e.g. carbohydrates, proteins and amino acids); important for the sustenance and survival of pollinators (Proctor et al. 1996). Nectar, comprised of sugars (i.e. sucrose, fructose and glucose), water, and to a lesser extent, amino acids and lipids (Corbet 2003, González-Teuber and Heil 2009), is the main source of energy and the principal food requirement of winged adults (Proctor et al. 1996). Pollen, alternatively, provides an essential source of protein and nutrients necessary for production and utilisation of moulting hormones (Somme et al. 2015, Vaudo et al. 2016) and is thus important for larval development and growth (Behmer and Nes 2003). The nutritional value of these floral provisions is determined by the mixture of constituent carbohydrates, amino acids and micronutrients (Cnaani et al. 2006, González-Teuber and Heil 2009, Stevenson et al. 2017), which varies greatly between plant species and cultivars (González-Teuber and Heil 2009, Carruthers et al. 2017).

Pollinators differ in their metabolic needs (Sedivy et al. 2011) and nutritive requirements (Archer et al. 2014, Paoli et al. 2014, Stabler et al. 2015). For instance, some pollinators only require nectar while others require a combination of pollen and nectar (Proctor et al. 1996). These nutritive requirements can further vary depending on the insect’s life stage (Paoli et al. 2014, Stabler et al. 2015), with honeybees observed to shift from a diet of essential amino acids to one of primarily carbohydrates when transitioning from young bees to foragers (Paoli et al. 2014). Given this, pollinators have the capacity to discriminate between plants based on their nectar sugar concentration (Elisens and Freeman 1988) and pollen quality; adjusting their foraging decisions accordingly (Vaudo et al. 2016). Indeed, some bumblebee species preferentially visit plants comprising pollen with high amino acid concentration, while other bumblebee species can extend their diet breadth to plants comprising pollen with low amino acid concentration (Somme et al. 2015). Floral preferences may also be determined by adaptations to a plant’s physical structures, for example flowers with long corollas may only be visited by species possessing a long proboscis (Stang et al. 2006). Trait complementarity (i.e. the similarity between the reward that the plant offers and the reward that the pollinator requires; Santamaria and Rodriguez-Girones 2007) can lead to non-random relationships between pollinators and their preferred plant species (Brosi 2016). These relationships can lead to ecological, phenotypical or evolutionary specialisation, in which insects visit a small number of flowers or flower types (Armbruster 2017). Specialism is however recognised to be asymmetric in mutualistic networks (Vázquez and Aizen 2004), where specialist pollinator species tend to interact with a subset of the plant species network that interacts strongly with more generalised pollinator species (so called ‘nestedness’; James et al. 2012, Nicolson and Wright 2017). Although there remains much debate, at community scales this is thought to provide stability by decreasing the likelihood of cascades of species extinctions and the vulnerability of the network and pollination function to collapse (Thebault and Fontaine 2010, Ramos-Jiliberto et al. 2012, Rohr et al. 2014, Vieira and Almeida-Neto 2015, Valdovinos et al. 2016, Vanbergen et al. 2017).

The benefits of the mutualistic relationship between plants and pollinators can however be disrupted by exploitation from food deceptive plants (Internicola and Harder, 2011) or indeed, from nectar robbing pollinators, who bypass a plant’s reproductive parts (Leonard et al. 2013). Pollinator visitation therefore does not constitute effective pollen transfer. Instead, this varies considerably depending upon the composition and foraging behaviour of visiting pollinator communities (Barrios et al. 2016), which has been observed to change in response to ecological context (Ivey et al. 2003). These factors and the implications for pollen transfer are discussed throughout this chapter.

1.3 The effects of pollinator foraging behaviour on successful pollen transfer

1.3.1 Pollinator movement

The movement of pollinators between conspecific plants, and thus pollen transfer, depends upon the behaviour and dispersal distance of pollinators. Indeed, pollinator movement is governed by energetics whereby foraging is 'optimised' to maximise energy gain (Charnov 1976, Goulson 1999). This involves a process of non-random, systematic searching in order to enhance foraging efficiency by avoiding recently exploited flowers (Dreisig 1995), minimising flight distance (Carvell et al. 2012) and reducing search and handling times (Richards 1997, Goulson 1999, Spaethe et al. 2001). In some cases, foraging efficiency has further been demonstrated to be enhanced through collective foraging (Senior et al. 2016). Collective foraging refers to the ability of a pollinator to alter foraging decisions based on social interactions and positive feedback mechanisms. This is demonstrated in honeybees, where information regarding pollen and nectar sources is acquired through interactions between foragers (the waggle dance) (Biesmeijer and Seeley 2005). Collective foraging has further been shown to alter the probability of an organism visiting a resource, where visits increase non-linearly with the number of other organisms using that resource (Sumpter and Pratt 2009). This reduces search time and movement between nutritionally imbalanced foods (Senior et al. 2016). Moreover, efficient foraging behaviour will also affect the movement between resource patches. Indeed, the number of pollinators and the time spent within a patch has been observed to be proportional to a patch's productivity (Dreisig 1995) and optimal foraging theory predicts that pollinators will move from a patch when the advantage of leaving exceeds that of staying (marginal value theorem). Upon leaving, pollinators are predicted to travel large distances in order to locate subsequent optimal patches (Charnov 1976). Efficient foraging may thus affect dispersal distances and visitation rates to plant species.

Given that efficient foraging is expected to be dependent upon the availability of floral resources, pollinator movement and behaviour is recognised as being extremely plastic (Spaethe et al. 2001, Jha and Kremen 2013, Geslin et al. 2014). For instance, foraging distance has been shown to be affected by the spatial arrangement of floral resources (Lander et al. 2011, Lihoreau et al. 2012, Jha and Kremen 2013, Lander et al. 2013). This has been illustrated by *Bombus* spp., where shorter foraging trips were made in landscapes where coverage of semi natural habitats was high (Carvell et al. 2012, Redhead et al. 2016). This has resulted in variations in pollination probability over different habitats (Jha and Kremen 2013, Lander et al. 2013). Previous research looked to categorise changing pollinator movement in response to different landscape scenarios using a resource model (Lander et al. 2013). This hypothesises that pollinators will adapt their foraging path depending upon resource heterogeneity, choosing between straight line paths, weighted linear distances, least cost paths, or pair-wise resistance distances (Lander et al. 2013). When demonstrated experimentally, pollinators were observed to abide by weighted linear or straight line paths (i.e. Euclidean distance either accounting for the resistance value of a habitat or not respectively) where landscapes were comprised of resource rich habitats (Lander et al. 2013). Changes to pollinator foraging paths can affect the incidence and distance of pollen movement and in turn, alter gene flow between conspecific plant populations (Lihoreau et al. 2012). Moreover, pollinator foraging paths can affect the directness of pollen movement and thus, the compatibility of pollen transferred (discussed in section 1.4.1; Plant fitness).

Studies of pollen movement are widespread in the literature and offer an indirect means of analysing pollinator foraging behaviour. Methods span from the use of fluorescent dyes applied to pollen (Van Rossum and Triest 2010); examination of a pollinator’s load or pollen on a plant’s stigma (Schulke and Waser 2001); the use of a plant’s sexual or morphological traits (e.g. dominance of hilum colour; Bishop et al. 2016) and parentage analysis using genetic markers (Matter et al. 2013). Of the genetic methods, plant nuclear microsatellites are most commonly used (Barbara et al. 2007, Vanbergen et al. 2014b). Microsatellites are short tandem repeats in DNA sequences (2-5 base pairs, typically repeated 5-50 times) and have been discovered in high numbers throughout the genome (often in non-coding regions) of all organisms studied to date (Goldstein and Schlötterer 1999). They have high mutation rates (caused by slippage and point mutations; Goldstein and Schlötterer 1999). This results in a high degree of polymorphism between individuals at these points in the genome, thus allowing for intra-species discrimination (Goldstein and Schlötterer 1999). Such markers can provide a relatively cheap and highly effective method for population genetic analysis and use only small amounts of DNA (Ashley and Dow 1994). They have been used to inform paternity analysis (Dyer et al. 2004, Smouse and Robledo-Arnuncio 2005, Jones et al. 2008, Dyer et al. 2012, Jolivet et al. 2012) and mating analysis (i.e. the incidence of self-fertilisation; Vandepitte et al. 2010). These studies have progressed our knowledge of the likelihood and distance of pollen movement over changing landscapes (Jones et al. 2008) as well as the contribution of pollinator species to this pollen transfer (Matsuki et al. 2008).

1.3.2 Pollinator constancy

The transference of compatible pollen between conspecific plants is governed by a pollinator’s constancy. This is characterised as successive visits by a pollinator to individuals from the same plant species (Raine and Chittka 2007b); a behaviour which is frequently observed among pollinators (Waser 1986, Gegear and Laverty 2005, Fontaine et al. 2006). A pollinator’s ability to exhibit constancy has been argued to be driven by memory constraints and an inability to perceive multicomponent floral signals (Gegear 2005) and is explained by the ‘inference hypothesis’ and the ‘search image hypothesis’. The former assumes that pollinators are unable to hold two sensory stimuli in the brain simultaneously and therefore switching between plant species results in an initial increase in handling time. The latter alternatively assumes that retention of more than one image is neurologically unfeasible (Chittka et al.

1999, Goulson 1999). With this in mind, pollinators may be expected to demonstrate constancy based on flower complexity, only moving between plant species with similar floral structures (Darwin 1876). Although these implications have been demonstrated experimentally (Laverty 1994, Slaa et al. 1998), the inferred benefit of reduced handling time has been shown to be minimal (Gegear and Laverty 1995). Alternatively, it has been argued that pollinator constancy arises from a combination of learnt experiences and innate sensory biases, where visual, olfactory and mechanical cues are used to distinguish plants based on rewards (Raine and Chittka 2007a, Simcock et al. 2014, Ruedenauer et al. 2015). This was illustrated by controlled behavioural experiments, where *Bombus terrestris* was observed to exhibit an innate preference for violet flowers (Raine and Chittka 2007a). Furthermore, *Bombus* spp. demonstrated the ability to associate floral colour with rewards through learning (Gumbert 2000, Nicholls and de Ibarra 2014), even when colour associations represented different rewards (i.e. one colour for nectar and one for pollen; Muth et al. 2015).

Despite the learnt and innate associations between floral traits and rewards, pollinator constancy has been demonstrated to be a plastic foraging strategy (Kawaguchi et al. 2007), with pollinators observed to exhibit ‘minoring’ behaviour, whereby other plant species are sampled at low rates (Goulson 1999). Two of the main drivers of plasticity in pollinator constancy are changes in resource availability and interactions with other pollinators (Brosi 2016). Changes to resource availability can lead to a trade-off between reduced handling time and the bypassing of other potentially rewarding plant species (Sanderson et al. 2006, Cakmak et al. 2009, Gruter and Ratnieks 2011). Indeed, where preferred plant species are scarce, pollinators have been observed to expand their diet breadth to utilise other rewarding plant species within close range (Kunin and Iwasa 1996). This can be explained by the increased time taken for a pollinator to encounter a flower from the same plant species and the duration of the working memory (Marden and Waddington

1981, Chittka et al. 1997). For example, controlled behavioural experiments have demonstrated constancy among pollinators if a flower from the preferred plant species was encountered within a pollinator's working memory (<2 seconds); if however, a flower of the preferred plant species was not reached within this time period, the pollinator was likely to switch to an alternative plant species (Raine and Chittka 2007b). Therefore, as the distance between preferred flowers increases, the trade-off between reduced flight and handling time renders it advantageous to explore new floral resources (Goulson 1999). Similarly, as interactions between pollinator species change, so does the competition for resources and thus, the ability of a pollinator to exhibit constancy. This has been illustrated by reductions in constancy in response to increases in the density of conspecific pollinators (e.g. *Bombus terrestris*: Fontaine et al. 2008) as well as reductions in heterospecific pollinators (e.g. by removal of the most abundant *Bombus* spp.; Brosi and Briggs 2013), both of which affect the availability of resources. These changes in pollinator constancy will have considerable implications for the transference of pollen between conspecific plants, where reduced constancy may lead to a loss of conspecific pollen to co-flowering, heterospecific plants along with increased heterospecific pollen deposition (Morales and Traveset 2008) (discussed in section 1.41: Plant seed set).

1.3.3 Pollen transfer effectiveness

The capacity of a pollinator to make contact with a plant's anthers and stigmas is further recognised to influence the successful transference of pollen between conspecific plants. Pollen transfer can therefore vary in relation to characteristics of a flower (e.g. position of anthers and/or stigma) or of a pollinator (e.g. tongue length, foraging behaviour, body size and covering) (Campbell et al. 1991, Harder and Barrett 1993, Kobayashi et al. 1997, Adler and Irwin 2006, Willcox et al. 2017). Even if contact is possible, the transference of pollen can further vary depending upon the species-specific nature of pollen and nectar collection. For instance, many pollinators seek to optimise nectar collection efficiency during a visit which may reduce the probability for pollen transfer to conspecifics. This is illustrated by *Apis mellifera* workers which have been shown to exhibit ‘sideworking’ behaviour, where positioning of the head below the anthers and the proboscis directly into the filament bases to reach the nectary minimises contact with a plant’s sexual parts (Thomson and Goodell 2001). This will affect rates of pollen removal and deposition.

Pollen removal will vary between foraging visits, with pollinators unlikely to remove all of a flower’s resources. Instead, optimal foraging theory hypothesises that individuals will leave a flower when the rate of return falls below that of the cost of moving to another flower (Charnov 1976). The amount of pollen removed by a pollinator during a visit can differ markedly between pollinator species depending, in part, upon whether pollen is collected through passive or active means (Barrios et al. 2016). Passive pollen removal can involve electrostatic attraction, where a plant and insect exhibit an electric charge of opposite polarities (Jones and Little 1983). Upon contact with a flower, the force of attraction results in pollen detachment (Vaknin et al. 2001, Clarke et al. 2013). This can lead to enhanced pollen transfer (Vaknin et al. 2001). In contrast, active pollen removal can involve the intentional collection of pollen for sustenance using morphological adaptations such as hairs on the hind leg (scopa). By reducing the accessibility of pollen, this negatively affects pollen transfer (Thorp 2000). Pollen removal may further be affected by whether a pollinator is pollen or nectar collecting (Thomson and Goodell 2001) or by the physical attributes of a pollinator, such as the structure of body hairs (e.g. branching; Thorp 1979), body size (Larsen et al. 2005) and the width and length of a pollinator’s proboscis or tongue (Barrios et al. 2016). This will affect the surface area available for pollen collection and, by determining how deep within the flower structure pollinators are active, the likelihood of contact with a plant’s anthers (Simon-Porcar et al. 2014, Barrios et al. 2016).

High pollen removal however does not imply increased pollen transfer between conspecific plants. Instead, the fate of removed pollen, and thus successful pollen transfer, is determined, in part, by a pollinator's behaviour between flower visits. Many bees use morphological adaptations to groom pollen from their bodies during foraging bouts, resulting in pollen loss during flight (e.g. basitarsal combs in *Colletes* bees; Jander 1976). The end amount of pollen available for subsequent plant reproduction is thus influenced by the efficiency of grooming mechanisms and the way pollen is stored on the body (Freitas 1997, Thorp 2000). Indeed, pollen storage in specialised structures can reduce the rates of pollen deposition as well as the viability of removed pollen. For instance, the corbicula (pollen basket) found on the hind tarsi of bumblebees, can capture up to 50% of the pollen removed from a flower visit (Rademaker et al. 1997) and often involves the addition of nectar and salivary secretions (Keularts and Linskens 1968, Vaissiere et al. 1996). Given this, pollinator species can be characterised as efficient pollen removers but poor pollen depositors and vice versa (Young et al. 2007, Barrios et al. 2016). For instance, in some cases long tongued Lepidoptera are considered to be more efficient pollinators than short tongue Diptera (e.g. syrphids) because, although both remove small amounts of pollen, Lepidoptera deposit a large amount during flower visits (Simon-Porcar et al. 2014). Variations in the efficiency of pollen removal and deposition will affect pollen transfer and indeed, pollen carry-over, where pollen is deposited on successive flower visits (Thomson and Plowright 1980). This will have implications for the distance of dispersal and the genetic relatedness of pollen (discussed in section 1.4.1: Plant fitness).

1.4 The importance of pollen transfer for plants

1.4.1 Plant reproduction

1.4.1.1 The role of a plant's mating system

Plants exhibit a wide range of mating systems from asexual reproduction (where the need for fertilisation is overcome (e.g. apomixis)) to sexual reproduction (reproduction involving meiosis and the production and fusion of male and female gametes). Sexual reproduction is widespread in flowering plants and offers the benefit of allele exchange and thus the production of genetically varied offspring, together with an ability to purge deleterious alleles through selection (Smith and Maynard-Smith 1978). In sexually reproducing plants, some species have developed the ability to self-fertilise (referred to as self-compatible plants; Pannell 2001). However, this is largely dependent upon whether male and female reproductive structures occur on the same or separate plants (i.e. whether a plant has access to its own ("self") pollen (e.g. hermaphrodite plants)). Plants exhibiting a hermaphroditic system (the most widely adopted sex system in flowering plants; Silvertown and Charlesworth 2009) and self-compatibility can reproduce by self-fertilisation either from the transference of pollen within a flower (e.g. autogamy) or between flowers on the same plant (e.g. geitonogamy) (Richards 1997). Self-fertilisation is advantageous in terms of providing reproductive assurance at times of reduced pollinator availability, reducing expenditure on pollinator attraction which instead could be directed at ovule and seed production (Ornduff 1969), and allowing locally adapted genotypes to persist (Silvertown and Charlesworth 2009). In contrast, self-fertilisation has been associated with negative implications for plant fitness, both through an increased incidence of inbreeding depression (Barrett 1998) and pollen discounting, where pollen is used for self-fertilisation at the expense of outcrossing (Fishman 2000). In addition, as plants associated with self-fertilisation invest fewer resources in pollinator attraction, visitation may be lower and visiting pollinators may be less effective (Lloyd 1979).

Self-fertilisation is typically controlled by morphological, phenological or genetic mechanisms. Of these, morphological and phenological mechanisms include the separation of sexual parts, either in time (i.e. where anther dehiscence occurs before the stigma becomes receptive (protoandry); Sargent and Otto 2004), or space (e.g. where anthers and stigmas are separated on a flower (dicliny), or between individual plants (dioecy); Richards 1997). In response to these controls, plants can adopt self-fertilisation at different stages of stigma receptivity, which can lead to prior, competing or delayed selfing. Examples of this can be seen in species within the genus *Campanula,* where anthers are separated from stigmas in space and only fold over to contact stigmas in the absence of outcrossed pollen deposition (Willmer 2011). This delayed selfing is associated with increased reproductive efficiency by ensuring seed production during low pollinator availability, while reducing pollen discounting (Lloyd 1992). Other adaptations to prevent self-fertilisation are expressed at a genetic level and are characterised by self-incompatibility systems. These are adopted by 60% of flowering plant species and involve an ability to discriminate between self and non-self or related pollen, thus preventing self-fertilisation and mating between closely related individuals (Hiscock and McInnis 2003). This mechanism, in most species, is controlled for by a single multi-allelic locus known as the S-locus (Pannell 2001, Takayama and Isogai 2005). The self-incompatible response can occur at the stigma surface or in the style and is elicited when proteins from the male gamete and the female gamete come from the same S-haplotype (i.e. they share a common allele at the S locus). This system has evolved a number of different genetic forms (e.g. sporophytic and gametophytic self-incompatibility) which differ in the basis by which pollen is rejected (Richards 1997). In gametophytic systems (the most widely distributed self-incompatibility system), self-incompatibility is controlled by the haploid genome of the pollen (Hiscock and McInnis 2003). In this system, alleles at the S-locus are expressed co-dominantly in the pistil and pollen is rejected if the allele expressed in the male haplotype matches either of those expressed in the female haplotype (Silvertown and Charlesworth 2009). Conversely, in sporophytic systems, self-incompatibility is controlled by the diploid genome of the anther (Hiscock and McInnis 2003). In this system, a dominance structure exists in the pollen and pistil and therefore pollen will be rejected if the dominant allele expressed in the male haplotype matches the dominant allele expressed in the female haplotype (Hiscock and McInnis 2003). This can result in mating between plants which share a recessive allele, leading to progeny which are homozygous at the S-locus (i.e. recessive alleles are masked and therefore do not elicit a self-incompatibility response) (Silvertown and Charlesworth 2009). While break-downs in self-incompatibility do occur through ‘leaky’ systems or mutations, this is considered effective at reducing mating between related or closely related plants (Richards 1997). The compatibility of pollen and thus, the success of pollen transfer, will therefore vary depending upon a plant’s mating system.

1.4.1.2 Plant seed set

Depending upon whether male and female reproductive structures occur on the same or separate plant, or indeed flower, sexual reproductive output constitutes the number of seeds produced (maternal fitness), the number of seeds sired (paternal fitness), or a combination of both. In plants expressing both male and female reproductive structures (e.g. hermaphrodite or monoecious plants), reproduction is thus influenced by the allocation of resources to male and female gametes (pollen and ovules respectively) and traits that impact their fitness. To maximise reproductive output some plant species have the ability to alter resource allocation. For instance, *Aquilegia caerulea* plants have been shown to promote maternal success in early flowers and paternal success in late flowers to meet the requirements of their pollinator visitors (Brunet 1996).

A plant’s reproductive output is a function of the quantity of pollen received by a plant, where plants adopting sexual reproduction require a sufficient supply of compatible pollen (Bernhardt et al. 2008). Indeed, seed set has been demonstrated to exhibit a saturating positive relationship with pollen receipt (Shore and Barrett 1984, Spigler and Chang 2008, Briggs et al. 2016) and where pollen supply is limited, plants have been observed to rarely set seed (Groom 1998). Changes to a pollinator’s behaviour such as their constancy to a single plant species (see section 1.3.2. Constancy), can alter the quantity and quality of this pollen receipt. For instance, reductions in constancy can result in pollen loss to heterospecific plants (Morales and Traveset 2008). This will have negative implications for a plant’s paternal success through reductions in seed siring. Moreover, reductions in constancy can also lead to heterospecific pollen deposition. Indeed, the deposition of heterospecific pollen has been shown capable of clogging a plant’s stigma and style (Shore and Barrett 1984, Brown and Mitchell 2001) leading to premature closing of stigmas (Waser 1986). In addition, heterospecific pollen deposition can also lead to hybridisation (Brown and Brown 1996) and pollen alleleopathy (Murphy and Aarssen 1995), where heterospecific pollen releases growth inhibitors, leading to competition and repression of conspecific pollen growth (Char 1977). This can therefore have negative implications for a plant’s maternal fitness through reductions in seed set. The implications of heterospecific pollen deposition for seed production however are inconsistent (Tscheulin et al. 2009) and research has shown effects to be dependent upon the timing of incompatible relative to compatible pollen deposition, together with the amount of incompatible relative to conspecific pollen deposition (Shore and Barrett 1984).

1.4.1.3 Plant fitness

The fitness of a plant can be attributed to environmental and genetic conditions and the interaction between them (Walisch et al. 2012). Environmental conditions such as resource availability have been shown to affect plant development rates (Agren et al. 2012, Skalova et al. 2015) and seed production (Herrera 2000). This is due to trade-offs between the number of seeds produced and the size and quality of these seeds (Herrera 2000), together with the plasticity of allocation to vegetation and roots in response to resource limitation (Müller et al. 2000).

Genetic conditions alternatively can influence the compatibility of pollen, where genetic variation between parent plants is observed as having a positive relationship with plant fitness (Thiele et al. 2010). Reductions in genetic variation between parent plants caused by inbreeding, can occur as a result of self-fertilisation or through mating between closely related individuals and biparental inbreeding (Coutellec-Vreto et al. 1997). This has been shown to reduce allelic diversity, with self-fertilisation in particular observed to decrease the heterozygosity of alleles at a locus by 50% each generation (Richards 1997). Indeed, given that self-incompatible plants require variation at the S-locus for reproduction, a loss of genetic variation will result in a reduction in the effective population size (i.e. the number of compatible mates) (Byers and Meagher 1992). Moreover, inbreeding can lead to an increase in the expression of deleterious recessive alleles which would otherwise be selected against and is therefore considered generally detrimental to plant fitness (Silvertown and Charlesworth 2009, Eckert et al. 2010, Bellanger et al. 2015). The negative implications of inbreeding depression have been explained by the over dominance and the partial dominance hypotheses. While the former supposes that the reduction in fitness through inbreeding is a consequence of the superiority of heterozygotes, the latter attributes it to increases in homozygosity and the subsequent accumulation of deleterious recessive alleles (Charlesworth and Charlesworth 1987).

A plant’s fitness can be characterised by a number of traits (e.g. size, growth rate, number of flowers and plant biomass) expressed at different life stages (e.g. seed, seedling or adult) (Younginger et al. 2017). Indeed, inbreeding can be expressed in both early stage fitness traits (e.g. seed mass and germination rate) and late stage fitness traits (e.g. number of flowers on stalks) (Thiele et al. 2010, Walisch et al. 2012). This can vary with a plant’s mating system, where predominantly selfing plants tend to express inbreeding at later stages whereas outcrossing plants tend to express the effects of inbreeding throughout their life cycle (Husband and Schemske 1996). The effect of inbreeding on plants depends upon the functional trait affected and how it corresponds to plant fitness. For instance, inbreeding in *Echinacea angustifolia* was associated with reduced photosynthetic rates, which is correlated with the capture and use of resources and thus, plant performance and fitness (Kittelson et al. 2015).

Plants however have been shown to be capable of discriminating pollen compatibility based on genetic relatedness. This was illustrated by a study which observed faster pollen tube growth when recipient and donor plants are separated by a distance of 10m (i.e. inbred and outbred pollen will reach the ovules after optimal pollen) (Price and Waser 1979, Souto et al. 2002). In doing so, plants demonstrate the ability to optimise the genetic variation between parent plants and therefore the fitness of progeny. This is fundamental for a plant given the positive association with progeny fitness and the survival and reproduction of plants (Reed and Frankham 2003). Moreover, in some plants, the negative effects of inbreeding may be alleviated through purging, whereby deleterious recessive alleles are selectively removed (Ferriol et al. 2011, Lopez-Cortegano et al. 2016). Purging has been demonstrated as being particularly efficient at selecting against deleterious alleles, especially when plants are heterozygotes at these loci (Charlesworth and Charlesworth 1987, Husband and Schemske 1996). While this process is inconsistent and dependent upon a plant’s life history and the fitness trait to which the allele corresponds to, it implies a capacity of plants to recover or withstand inbreeding through selfing or mating between close relatives (Husband and Schemske 1996, Thiele et al. 2010).

1.4.2 Long-term plant population survival

Fitness in outcrossing plant populations is highly dependent upon gene dispersal and the exchange of novel alleles (Mannouris and Byers 2013). Plant populations commonly exhibit spatial autocorrelation (fine-scale spatial genetic structure), where relatedness declines with increasing distance (Loveless and Hamrick 1984) at a scale reflecting local mean pollen and seed dispersal as well as recruitment distances (Levin and Kerster 1974). Individuals that are closer together are thus more likely to be closely related. The spatial scale of pollinator movement may therefore alter the probability of mating between close relatives (Millar et al. 2014). Reductions in pollinator dispersal distance can lead to genetic divergence between plant populations as well as reduced genetic variation within populations (Silvertown and

Charlesworth 2009). These patterns are further exaggerated by the process of genetic drift (random fluctuations in allele frequencies over time; Silvertown and Charlesworth 2009). In the absence of the introduction of novel alleles into a population, common alleles can become more prevalent over time and are at an increased likelihood of becoming fixed (i.e. if an allele achieves a 100% frequency, other alleles will be lost from a population; Silvertown and Charlesworth 2009). This process is particularly exaggerated in small populations, where allelic diversity is typically low (Campbell 2007, Mannouris and Byers 2013). The fixation of alleles leads to a reduction of heterozygosity within a population and a subsequent departure from the Hardy-Weinberg principle (‘the Wahlund effect’; Silvertown and Charlesworth 2009). This in turn reduces polymorphism and subsequently leads to genetic uniformity within a population (Silvertown and Charlesworth 2009). Consequently, mate availability is reduced, particularly for self-incompatible plants which require genetic variation at the S-locus, resulting in a low effective population size. Once the effective population size falls below the threshold for a minimum viable population, plants face an Allee effect (i.e. a reduction in fitness with population size; Wagenius et al. 2007) and an elevated risk of extinction (Gilpin and Soule 1986, Brook et al. 2006, Schleuning and Matthies 2009, Silvertown and Charlesworth 2009).

1.5 Anthropogenic threats facing pollinators and insect-pollinated plants

1.5.1 Plant and pollinator declines

Since the mid-20th century the occurrence and diversity of pollinators has declined globally (Biesmeijer et al. 2006, Vanengelsdorp et al. 2008, Carvalheiro et al. 2013, Vanbergen et al. 2014a, Senapathi et al. 2015b, Potts et al. 2016b). These declines have affected honeybees (Potts et al. 2010b, Potts et al. 2016b), bumblebees, other wild bees, wasps (Biesmeijer et al. 2006, Goulson et al. 2008, Ollerton et al. 2014) and butterflies (Warren et al.

2001), though hoverflies, while declining at some local scales, have exhibited no consistent trend at a national scale (Keil et al. 2011). The declines in pollinators have been linked with parallel declines observed in the diversity and occurrence of plant species (Biesmeijer et al. 2006, Carvell et al. 2006, Carvalheiro et al. 2013, Albrecht et al. 2016). In Britain alone, 19% of all terrestrial and freshwater plant species are now classified as at risk of extinction under the IUCN Red List criteria (Burns et al. 2013), with those requiring insects for reproduction considered particularly threatened by range contractions and decreases in frequency (Biesmeijer et al. 2006, Vanbergen et al. 2014a). Patterns of decline vary between plant and pollinator species in relation to variations in key species traits. For instance, reduced dispersal capabilities (i.e. poor ability to migrate), longer development times (i.e. the duration from egg to adult) and specialised habitat and a narrow range of foraging requirements (e.g. oligolectic species) are all demonstrated to increase pollinator vulnerability (Biesmeijer et al. 2006). The loss of pollinator trait diversity at a community level can lead to functional homogenisation (Baude et al. 2016). In turn, plants that rely on pollinators for reproduction may become overly dependent on a few abundant pollinator species (Pradervand et al. 2014), with negative implications for plant species which require more rare pollinator species (e.g. long-tongued bees) (Fontaine et al. 2006, Vanbergen et al. 2014a).

1.5.2 The primary drivers of declines

Comprehensive literature reviews and international evidence assessments have identified the key threats driving pollinator declines, which include land-use change and management intensification; urbanisation; invasive alien species, the spread of pathogens and parasites, and climate change (Potts et al. 2010a, Vanbergen et al. 2013, Potts et al. 2016a, Potts et al. 2016b). Of these, land-use modification and the subsequent fragmentation and loss of habitats, has been implicated as one of the primary drivers (Aguirre-Gutierrez et al. 2015, Corlett 2016). Through altering the supply and diversity of floral and nesting resources (Scheper et al. 2014, Baude et al. 2016), this has led to malnutrition of individuals and colony stress (Vanbergen et al. 2013, Baude et al. 2016). Further threats include: pesticides and other agricultural chemicals, which lead to lethal and sub-lethal effects (Gill et al. 2012, Godfray et al. 2015); climate change, which imposes range shifts and changes to seasonal activity (Hegland et al. 2009b, Potts et al. 2016a); pests and pathogens, whose frequency are exacerbated by the large-scale commercial management and transportation of bees (Dohzono and Yokoyama 2010, Traveset and Richardson 2014, Potts et al. 2016a); and invasive species, which modify plant and pollinator networks (Morales and Traveset 2009, Dohzono and Yokoyama 2010, Potts et al. 2016a). Furthermore, concerns have been expressed about the potential for different anthropogenic pressures to combine, additively or synergistically, to elevate the threat to pollinators (Gonzalez-Varo et al. 2013, Vanbergen et al. 2013). Similarly, reviews combining spatially extensive monitoring networks, experimental work, remotely sensed data and historical records have identified key drivers of plant declines. From these, emphasis has been put on the contribution of climate change, which alters conditions such as water and nutrient availability and land-use change, which can alter plant population dynamics to plant declines (Franklin et al. 2016).

Plant and pollinator declines have been particularly severe within agricultural systems (Kovacs-Hostyanszki et al. 2017). Since the 1920's, improvements and enhancement in technology has led to the intensification of agriculture and a subsequent homogenisation of landscapes (Robinson and Sutherland 2002, Hodgson et al. 2005, Storkey et al. 2012). As a consequence, the yield of wind-pollinated crops has increased at the expense of pollinator-reliant crops (Deguines et al. 2014). This drive for greater yield in turn introduced pressures from increased use of agro-chemicals and the abandonment of marginal land, resulting in fewer floral and nesting resources (Storkey et al. 2012). This has directly been associated with declines in pollinators and indeed, plants, with 60% of plant species declines in the late 20th century associated with arable habitats (Preston et al. 2002a, Carvalheiro et al. 2013).

1.6 The implications of anthropogenic threats to pollen transfer

1.6.1 Pollination failure

The reproductive success of a plant is a function of the number of fruits produced (which is controlled by flower production), the number of seeds sired and the number of seeds produced (which is controlled by ovule production), together with subsequent seed recruitment (Stephenson 1981). For plants that rely on pollinating insects for pollen transfer, achieving their potential reproductive success requires sufficient pollinator activity to fertilise all ovules, together with adequate nutrients to ensure seed provisioning. In nature, plants have been shown to suffer a high degree of pollination failure (Larson and Barrett 2000, Cunningham 2000a, Wilcock and Neiland 2002, Ashman et al. 2004, Newman et al. 2013). This may occur at fertilisation stage (i.e. through low seed:ovule and flower:fruit ratios) or at the post-fertilisation stage (i.e. through low seedling survival) (Holland and Chamberlain 2007). Both proximate (e.g. pollen and resource limitation) and evolutionary (e.g. selection for addition flowers to increase pollinator attractiveness) factors have been implicated as drivers, though these are not mutually exclusive (Holland and Chamberlain 2007).

1.6.1.1 Proximate hypotheses

The *Pollen limitation hypothesis* has been argued to be one of the main causes of pollination failure. This supposes that failure of a plant to achieve its potential seed or fruit set is driven by an insufficient deposition of viable, compatible pollen, either in terms of quantity or quality (Waites and Agren 2004, Campbell 2007). This may be driven by changes to pollen availability or composition at the pre-dispersal (on the source flower), dispersal (in transit) and post-dispersal (at the destination flower) stage. Indeed, at the dispersal stage, the behaviour of pollinators can directly affect pollen quantity through grooming and pollen storage, along with pollen quality through visitation to non-compatible plants (increasing heterospecific pollen deposition). Furthermore, pollen age, which is influenced by the time taken for a pollinator to move between conspecific plants, affects both quality and quantity since pollen is generally short lived; maintaining viability up to 48-72 hours after anther dehiscence (Pacini et al. 1997, Wilcock and Neiland 2002). Supplementation experiments have demonstrated pollen limitation to be ubiquitous among plants and a primary contributor to reduced seed production (Ashman et al. 2004, Knight et al. 2005, Wagenius et al. 2007, Jakobsson et al. 2009).

It has however been argued that the pressures faced by the individual flower (i.e. insufficient pollen deposition) may not pair with those faced by the whole plant (Holland and Chamberlain 2007). The production of seeds is costly and in the case of hermaphrodite plants, the allocation of resources has to be divided between male (pollen) and female (ovule) functions as well as between flowers. This highlights the importance of resource availability (i.e. sufficient supplies of nutrients and water) for the maturation of all of a plant’s ovules and the subsequent maximisation of plant reproduction and survival (Stephenson 1981, Haig and Westoby 1988). This forms the basis of the *resource limitation hypothesis* (Ashman et al. 2004). If the supply of resources is inadequate, plants have been shown to release growth inhibitors which promote fruit abortion (Tamas et al. 1979); a condition observed frequently in plants (e.g. some species produce thousands of flowers for every fruit that they mature; Stephenson 1981). Indeed, Haig and Westoby (1988) argued that plants will evolve to be in equilibrium between resource and pollen limitation. If this equilibrium becomes unbalanced then the plant will shift its allocation towards seed production or seed siring accordingly (Haig and Westoby 1988). However, while there is growing consensus about the contribution of both pollen and resource limitation, the extent to which each drive pollination failure is still unclear (Holland and Chamberlain 2007). A greater understanding of why pollination failure occurs under different conditions will help decipher the underlying causes and could have applied implications for plant breeding, crop production and conservation management efforts.

1.6.1.2 Evolutionary hypotheses

Alternative hypotheses of pollination failure instead recognise low seed:ovule or fruit:flower ratios to be a by-product of evolutionary adaptations to increase the likelihood of reproduction. These include selection for: greater floral displays to enhance pollinator attraction; greater pollen reserves to heighten siring success; prezygotic or postzygotic selection based on the pollen source or the number of seeds in a fruit; lessening the effects of pre-dispersal seed predators or bet hedging (Holland and Chamberlain 2007). Of these, particular attention has been given to the theory of bet hedging, which suggests that plants have evolved to produce an excess number of ovules in response to stochasticity in the availability of pollinators and therefore, pollen receipt (Knight et al. 2005). Under this hypothesis, fertilisation of all of a plant's ovules is not expected and thus 'pollination failure' is destined. From an evolutionary perspective, this represents an advantageous strategy so long as the relative costs of flower production are low (Burd 1995). Any benefit associated with the production of surplus flowers and ovules must exceed the loss in reproductive potential associated with the wastage of resources on abscised flowers and fruits (Stephenson 1981). However, research into the role of evolutionary adaptations as drivers of pollination failure has demonstrated mean ovule production to not exceed mean pollen deposition, casting doubt on evolutionary hypotheses (i.e. if a plant was producing excess ovules as an evolutionary strategy, the ovule number would exceed pollen loads; Holland and Chamberlain 2007).

1.6.2 The extent of pollination failure

The extent of pollination failure experienced by a plant has been shown to vary depending upon intrinsic factors including a plant's mating system, life history and phenology (Vamosi et al. 2013). For example, plants exhibiting a perennial life cycle have a higher vulnerability to pollination failure (i.e. seed:ovule ratios are ~0.5 for perennial plants and ~0.85 for annual plants; Wiens 1984). In addition, extrinsic factors, including a plant's population dynamics (e.g. size, density and degree of isolation from conspecifics; Groom 1998) have similarly been shown to impact the extent of pollination failure faced by a plant. Each of these factors can be seen to impact the attractiveness of a plant, which affects the pollinator visitation and thus pollen deposition (Jakobsson et al. 2009).

Specifically, plant attractiveness depends upon population dynamics (e.g. population size and density) and phenological traits, together with the environmental context in which plants occurs, for instance as defined by the neighbourhood of conspecific and heterospecific plants in the local community. Neighbouring plants can facilitate pollinator visitation to conspecific plants (i.e. where conspecific plants receive higher visitation at no extra cost to the neighbouring plants) (Feldman et al. 2004). This can be a result of local floral communities supporting pollinator communities (i.e. by offering an abundance of floral resources) or through offering shared pollinators (Rathcke 1983). Facilitation may therefore be based on the sampling effect, where an increased pollinator community enhances the probability of pollinators showing preference for plant species at low frequency (Ghazoul 2006). This is argued to be particularly beneficial to rare plants, where the recruitment of pollinators is a function of the number of pollinator species in an area (Ghazoul 2006). Indeed, facilitation has been shown to be particularly effective when habitats are comprised of low-intermediate floral densities and flowers vary in colour morphs (Ghazoul 2006, Montero-Castano and Vila 2015). If, alternatively, flower densities increase beyond a low-intermediate density, neighbouring plants may instead compete with conspecific plants for pollinator visitation (i.e. where conspecific plants receive reduced visitation because of a preference for neighbouring plants; Rathcke 1983) (Ghazoul 2006, Montero-Castano and Vila 2015). This has been illustrated by greater heterospecific pollen deposition when the number of plant species within neighbouring communities increase (Bartomeus et al. 2008, Jakobsson et al. 2009, Arceo-Gomez and Ashman 2011). Indeed, facilitation and competition for pollinator recruitment are opposite ends of a continuum that is a function of the ecological context, such as the size, density or isolation of a population in relation to the neighbouring community (Rathcke 1983, Mitchell et al. 2009).

At a wider scale, plant attractiveness is also affected by landscape composition (i.e. regional plant diversity; Vamosi et al. 2013). This is illustrated by altered pollinator visitation in response to landscape composition and is explained by the landscape-moderated concentration and dilution hypothesis. This hypothesis proposes that, in landscapes characterised by an abundance of floral resources, plants will receive lower per 'floral unit' visitation (Tscharntke et al. 2012), despite higher pollinator densities (Root 1973, Totland and Matthews 1998). This can be a result of competition avoidance (i.e. pollinators choose habitats characterised by a scarcity of resources to avoid inter- and intra- species competition), or alternatively, because of a saturation effect where resources within florally rich landscapes are in surplus and therefore visitation is diluted (Wenninger et al. 2016). Indeed, effects on pollinator visitation can be interpreted differently depending upon the spatial scale at which it is recorded. For instance, visitation has been shown to exhibit a concentration effect at wider, landscape scales, whereas a dilution effect exists at fine, habitat scales (Veddeler et al. 2006, Hegland et al. 2009a). At these finer scales, landscape composition may also result in a spill-over effect (Tscharntke et al. 2012). This refers to movement from one distinct habitat to another and in doing so, reflects the permeability of the neighbouring matrix, which is recognised to be a function of its attributes (e.g. structural and functional features such as floral cover; Tischendorf 2001). This effect has been demonstrated with seed set decreasing at increasing distances from a nature reserve (Kohler et al. 2008). The drivers of pollination failure are often studied independently, but with research supporting impacts of intrinsic, extrinsic and landscape factors, it is more likely to be a complex of interacting, interdependent factors affecting plant attractiveness and thus, pollinator recruitment and behaviour.

1.7 Reversing negative anthropogenic effects in agricultural systems: Agri-environment schemes

Since the 1980s, policy in Europe has looked to reverse and mitigate the damaging effects of agricultural practises on biodiversity (Bignal 1998). These can be broadly characterised as ‘land sharing’ practices (e.g. organic and more extensive farming) and ‘land sparing’ practices (e.g. conservation, restoration and management of semi-natural habitats) (Albrecht et al. 2016). Both approaches have been supported by so-called Agri-environment schemes (AES) which pay compensation to landowners in return for modification of damaging farming practices and/or removal of land from agricultural production (Kleijn and Sutherland 2003). These schemes have been introduced throughout Europe, although the specific objectives vary depending upon a country’s priorities (e.g. reduction in chemical input, protection and enhancement of biodiversity and restoration of landscapes; Kleijn and Sutherland 2003).

Pollinator declines have been a focus of AES with the creation of flower rich habitats including pollen and nectar mixes, which are predominantly comprised of leguminous plant species (e.g. *Trifolium pratense, Lotus corniculatus*). The main target of this latter option has been the conservation of long-tongued bees, especially bumblebees, which depend on longer corolla flowers (Carvell et al. 2011). Experimentally these habitats have demonstrated high success rates in terms of increased diversity, abundance and reproductive rates of the pollinators they target (Pywell et al. 2006, Heard et al. 2007, Carvell et al. 2015). The success of AES however has been observed to depend, in part, on the ecological contrast of the surrounding landscape (Heard et al. 2007). For example, while the diversity of pollinators has been demonstrated to increase in sown patches within simple landscapes, the pattern in complex landscapes is less clear (Batary et al. 2011, Scheper et al. 2013). The success of AES has also depended upon the habitat at which the schemes have targed, where management of ‘out of production’ habitats (e.g. hedges) has demonstrated greater success at enhancing species diversity than management of productive habitats (e.g. grasslands) (Batary et al. 2015). Increases in pollinator abundance and diversity associated with these schemes however have been shown to apply to a small suite of pollinator species (e.g. the targeted long-tongued bumblebees) (Wood et al. 2015) which are shown to account for the majority of crop pollination (Kleijn et al. 2015). The diversity of other wild bees in contrast was not affected by AES (Kleijn et al. 2015). Although argued to not contribute significantly to pollination services to agricultural crops (Kleijn et al. 2015), supporting a diversity of bees is fundamental for ecosystem resilience and functional heterogeneity (Senapathi et al. 2015a). Therefore, reassessment of mixes is required to include more bee forage plant species in order to enhance ecosystem services whilst providing support for pollinator diversity (Scheper et al. 2015, Wood et al. 2015).

For plants, AES options have included conservation headlands (i.e. areas with a modified chemical regime) and uncropped, cultivated margins (i.e. uncropped margins where the chemical regime is minimal) (Byfield and Wilson 2005). These options, particularly uncropped, cultivated margins have been demonstrated to be particularly effective at increasing plant species richness and supporting a higher incidence of rare plants (Albrecht et al. 2016). This is illustrated by the occurrence of rare plants in 39% of AES margins (Walker et al. 2007, Romero et al. 2008, Fried et al. 2009). While we have an understanding of the effect of management interventions through AES on plants and pollinators, the effect on pollinator behaviour, and thus pollen transfer, has received little attention. Changes in plant-pollinator interactions are fundamental for the reproduction and long-term survival of insect-pollinated plant populations and thus require further investigation.

1.8 Project outline

1.8.1 Knowledge gaps

Changes to plant and pollinator interactions, and the mediators of these changes, have received a great deal of attention in recent years. Research in this field has highlighted a plant's attributes and population dynamics (e.g. size, density and isolation) as key drivers of changes to plant-pollinator interactions, and thus plant reproductive success (Brys et al. 2007, Spigler and Chang 2008, Nattero et al. 2011). However, in nature these factors are confounded with ecological context: a plant's population does not function independently from its surroundings. Ecological context can be influential at a local, habitat scale, through altering the balance between competition and facilitation (Ghazoul 2006) or at a wider scale, where landscapes can vary in their heterogeneity and thus, their attractiveness to pollinators (Steffan-Dewenter et al. 2002). The effect on pollinators has been observed to be a function of a species' dispersal capability, where pollinators with greater foraging ranges perceive ecological context at larger scales (Steffan-Dewenter et al. 2002). Given this, ecological context at different spatial scales is expected to govern the composition of pollinator communities (Bennett and Isaacs 2014, Cusser et al. 2016, Joshi et al. 2016, Senapathi et al. 2017). Moreover, optimal foraging theory hypothesises that pollinators will alter their movement and behaviour in response to ecological context in terms of forage availability (Charnov 1976), thus driving behavioural change (Jha and Kremen 2013). This has been studied in the context of pollen movement within specific landscape settings. Such work has illustrated the avoidance of pollinators to particular habitats, leading to disruptions to pollen transfer (Hadley and Betts 2009, Lander et al. 2011). However, by not quantifying the specific composition of a habitat (e.g. the availability of floral resources), this research has not demonstrated how pollinator behaviour, and thus pollen movement, varies in relation to the habitat type and landscape context.

Although research on pollinator communities and behaviour in response to local and landscape composition is an emerging field, the implications for pollen transfer and plant reproductive success have largely been neglected. Where the implications of changes to plant and pollinator interactions have been studied, research tends to focus on a plant's maternal success (e.g. seed set). In doing so, the important interactions between pollinator behaviour and a plant's paternal success are missed. A greater understanding of a plant's paternal success in relation to ecological context can be gained by studying pollen movement (Hopley et al. 2015). Moreover, studies often do not extend beyond direct reproductive measures (e.g. seed set) and thus fail to determine the short and long-term fitness implications of changes to plant-pollinator interactions. Given the positive association between pollen dispersal distance and the fitness of a plant's progeny (Mannouris and Byers 2013, Millar et al. 2014), an understanding of these interactions is essential when considering a long-term view of plant population persistence.

1.8.2 Study objective

The overarching objective of this thesis was to determine the effects of variations in the availability of floral resources at different spatial scales on plant-pollinator interactions and the implications for the long-term survival of plant populations. This was approached through two main research questions:

1. *How do variations in the availability of floral resources at different spatial scales alter pollinator foraging behaviour and what are the implications of this for pollen movement?* I approach this by exploring how various pollinator responses, including visitation at the plant level together with foraging behaviour (in terms of pollen transfer efficiency and constancy to a particular plant species) vary across habitats and landscapes. In addition, I explore intra- and inter-population pollen movement to infer changes in pollinator dispersal distance. This offers a greater understanding of the drivers of behavioural changes in pollinators. Given that pollinators are key vectors of pollen transfer and thus, essential for reproduction and outcrossing in the majority of plant species (Ollerton et al. 2011), this is

fundamental to decipher the mechanisms behind successful pollen transfer and gene exchange between plant populations. Moreover, it contributes to the evidence base informing policy on the restoration of plant-pollinator interactions under different circumstances.

2. *How do changes to pollinator behaviour at different spatial scales affect plant outcrossing, reproduction and fitness?* I approach this by exploring variations in outcrossing rates and reproductive success in multiple plant species (in terms of seed set and progeny fitness) when located within a variety of habitats and landscapes. In addition, long-term survival potential is inferred through measures of inter-population pollen movement, a function of plant population connectivity. This offers insights into the roles that habitat type and configuration play in determining the genetic diversity of plant populations. In an applied context this could help improve our understanding of the scale at which habitat management (e.g. through AES) most effectively promotes pollination.

1.8.3 Thesis structure

1.8.3.1 Chapter two

This chapter explores how the availability of floral resources at local (2m radius) and landscape (within a 1km² block) scales, together with a plant's population size, affects pollinator behaviour and the reproductive success of plant species. This was achieved by conducting field experiments where plants were introduced into habitats and landscapes, which varied in the availability of floral resources (Fig.1.I). Experimental arrays comprised two plant species; *Eschscholzia californica* and *Silene gallica*, which varied in mating systems and phenology. At each array, I measured visitation rates and the constancy (inferred through heterospecific pollen deposition) and pollen removal effectiveness (inferred through pollen removal) of pollinators. Furthermore, plant reproduction and progeny fitness were determined to develop an understanding of whether plants are compromised by changes to floral resources at local or landscape scales. It is assumed that plants with the ability to self will achieve reproductive assurance, where self-pollen is used in response to low pollinator activity (Eckert et al. 2010). I therefore explore whether there is a trade-off between reproductive assurance and fitness in such species. This enhances our understanding of how different plants are affected by management interventions at a habitat and landscape scale.

1.8.3.2 Chapter three

Building on chapter two, this chapter explores the fate of removed pollen in response to floral availability, using a greater range of semi-natural habitats and management interventions and a wider range of spatial scales (1-1500m radius) to account for variations in pollinator floral preferences and dispersal capabilities. Particular focus is put on small scales (1-100m radii) to reflect the tendency for localised pollinator foraging (Pasquet et al. 2008). This involved a multi-site field experiment where *Vicia faba* plants were introduced into habitats and landscapes which varied in floral availability (Fig.1.I). Intra-population pollen movement was tracked by variations in a progeny's hilum colour (a dominance inherited trait). This was related to the community composition of potential pollinator visitors, in terms of species richness, activity density and inter-tegular span (a proxy for body size). Further, I explored the implications for plant reproductive success through measures of reproduction rates and seed weight. This offers a greater understanding of the optimal scale at which habitat management should be targeted to positively enhance plant-pollinator relationships.

1.8.3.3 Chapter four

Following findings from chapter three, this chapter focuses on how floral availability at a habitat scale affects pollinator foraging behaviour, inferred through the incidence and distance of pollen movement (1-150m). This was achieved through a field experiment coupled with molecular analysis using microsatellite markers. Experimental arrays comprising a plant species with a low propensity to self (*E. californica)* were introduced into habitats of low or high floral availability (Fig.1.I). I measured the potential pollinator visitors (e.g. pollinator activity density and richness), from which I inferred visitation rates. In addition, I inferred pollinator foraging behaviour and dispersal distance through measures of inter-population pollen movement. In this chapter I focus not just on the implications for plant reproductive success, but also on the drivers of changes to reproductive success, including the incidence of pollen limitation and reductions in outcrossing rates. This offers a greater understanding of the dynamics of plant-pollinator relationships at a habitat scale and the implications for long-term, population persistence.

1.8.3.4 Chapter five

Chapter five expands on chapter four which looks at the effects of floral availability on pollen movement and instead looks to determine whether plant population connectivity at a local scale is a function of the floral community gradient. This involved two multi-plot field trials at the Park Grass Experiment, Rothamsted, where *Vicia faba* plants were introduced into plots varying in floral composition (Fig.1.I). I measured the incidence and distance of between- and within-plot pollen movement (5-15m). In addition, pollen movement was related to the potential pollinator visitors associated with each plot. The implications for plants were then quantified by measures of plant reproductive success in terms of reproductive rates and seed weight. This improves our understanding of the ability of habitats to impede or enhance pollen movement in relation to floral communities, and can inform policy on how best to promote pollen and gene flow between isolated conspecific plant populations.

1.8.3.5 Chapter six

In this final chapter, I draw together conclusions from chapters two, three, four and five, addressing how they contribute to the overarching research questions. I further consider the implications of findings on the effects of habitat and landscape composition on plants and pollinators for management and conservation efforts to enhance plant and pollinator relationships. I highlight key findings and areas which need further research.

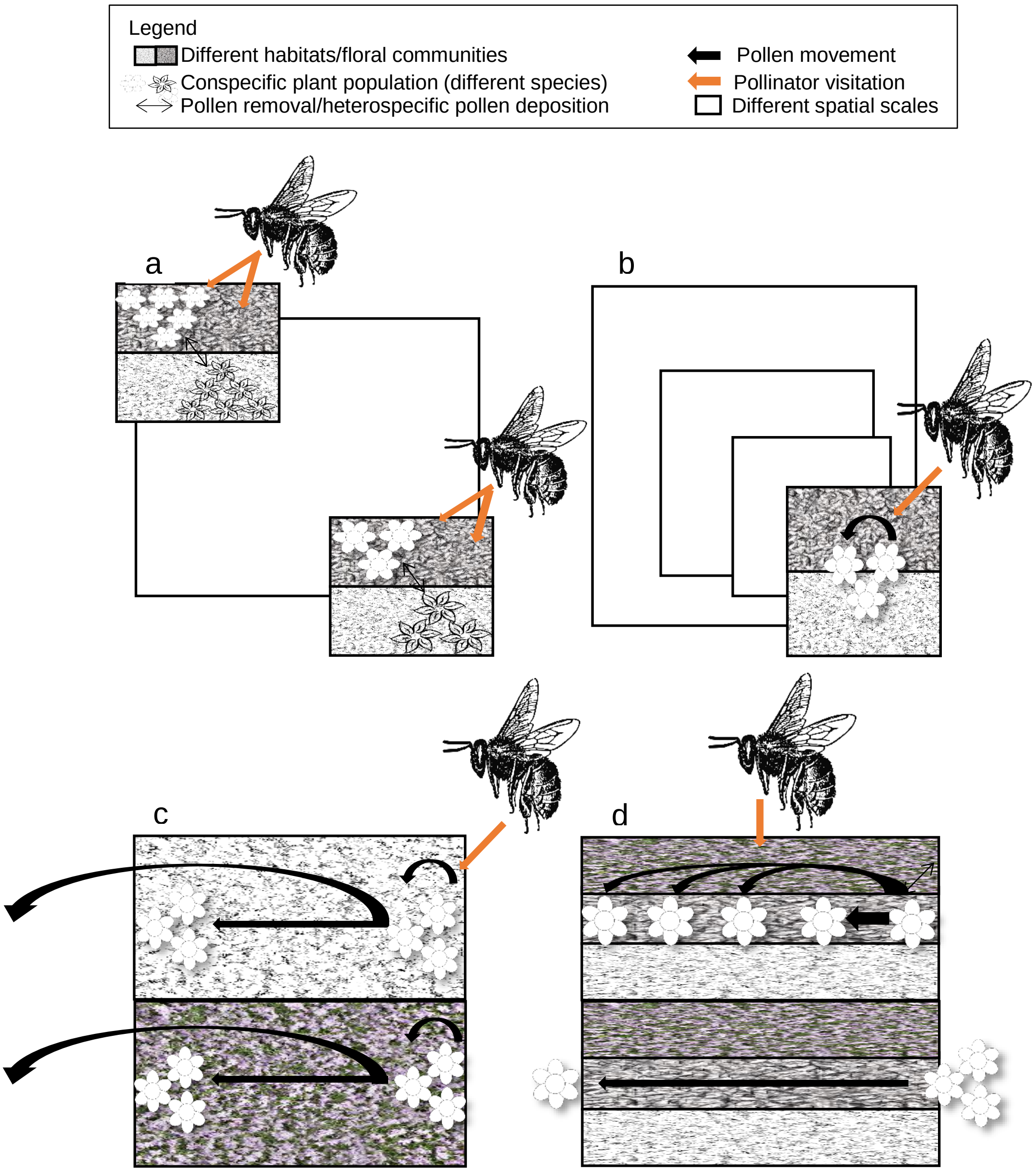

Figure 1.I A schematic of experiments conducted within each chapter, a. Chapter two, looks at the effects of floral resources at a habitat and landscape scale on pollinator foraging behaviour (visitation, constancy and pollen removal effectiveness) on two plant species differing in mating systems; b. Chapter three, looks at the effects of floral resources through a greater range of semi-natural habitats and management interventions and at a wider range of spatial scales on pollinator communities and intra-population pollen movement and the implications for a self-compatible plant; c. Chapter four, looks at floral composition at a habitat scale on intra- and inter-population pollen movement and the implications for a plant exhibiting a partially self-compatible plant and; d. Chapter five, uses two plot-level experiments to explore the effects of a gradient of floral communities on the movement of pollen both between plots and at different distances within a plot.

CHAPTER 2

The effects of the availability of floral resources at a local and landscape scale and plant population size on plant-pollinator interactions

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2.1 Abstract

1. The relationship between plants and pollinators is often of a mutualistic nature. In return for provisions, pollinators transfer pollen between plants and thus play a major role in the reproduction and fitness of outcrossing plants. However, the quantity and compatibility of pollen transfer is influenced by a pollinator's behaviour, including visitation and foraging patterns (e.g. a pollinator's pollen removal and transfer effectiveness and constancy to a preferred plant species). These behaviours are affected by the attractiveness and suitability of an area for pollinators, determined in part by the availability and composition of floral resources. It is however unclear to what extent changes to floral resources, brought about by habitat and landscape modification, affect pollinator behaviour and, consequently, the compatibility and quantity of pollen transfer.
2. I conducted a large-scale field experiment to investigate how the availability of floral resources at a local (floral abundance and diversity within a 2m radius of a plant population) and landscape (the proportion of florally rich habitats within a 1km² square) scale affected pollinator behaviour and in turn, plant reproductive success. In addition, I explored how plant population size affected the response. Experimental arrays comprising two plant species (*Eschscholzia californica* and *Silene gallica*) which differed in breeding system were introduced into habitats and landscapes which varied in floral availability. Pollinator behaviour was measured through visitation rates, constancy to a single plant species and pollen removal effectiveness (inferred through heterospecific pollen deposition and pollen removal rates respectively). I then measured plant reproductive success by determining the seed set and fitness traits of progeny.
3. Findings here show visitation rates to be two times greater to large experimental arrays (12 plants), when compared to small arrays (three plants). Furthermore, *E. californica* received a greater richness and

abundance of pollinator visitors when compared to S.gallica. No effect however was observed between the availability of floral resources at any spatial scale and either visitation or heterospecific pollen deposition. In contrast the removal of pollen was positively related to floral resources at a landscape scale (1km² square), though this effect varied between the two plant species. Despite this, no discernible effects were measured on the reproductive success of either plant species. The fitness of *Silene gallica* progeny (a self-compatible plant species) was marginally greater when experimental arrays were small, though this was restricted to seed weight, an 'early lifecycle' fitness trait, and did not translate to germination success. Given that seed weight was observed to be greater in outcrossed progeny of *S. gallica*, these results could reflect a higher incidence of outcrossing within small populations.

4. This study, while highlighting the importance of a high availability of floral resources in the wider landscape for facilitating pollen removal, recognises the benefit of plant attractiveness in providing a competitive advantage against co-flowering plants. Both landscape context and plant population size are thus demonstrated to be important in plant-pollinator relationships. However, given the negligible effects on plants, the fate of removed pollen and the mechanisms influencing plant reproductive success need further investigation. This is of particular importance in order to implement effective management in arable systems at a time when plants and pollinators are facing declines.

2.2 Introduction

A plant's reproductive success comprises its reproductive output (the production and siring of seeds), weighted by the fitness and dispersal of its progeny (Richards 1997). This is affected by a plant's mating system and can involve asexual reproduction whereby fertilisation is bypassed (i.e. apomixis) or sexual reproduction. In plants adopting sexual reproduction, outcrossing can be characterised as obligate (self-incompatible) or facultative (self-compatible), where the propensity to outcross is influenced by molecular, morphological or phenological adaptations preventing self-fertilisation (Pannell 2001). Outcrossing in such plants is predominantly conducted by external means including abiotic (e.g. wind) or biotic (i.e. animal) vectors; approximately 90% of the world's flowering plant species rely upon pollinating animals (Ollerton et al. 2011). By effecting pollen transfer, pollinators influence the paternal success of a plant through seed siring, together with the maternal success of a plant through the fertilisation of ovules and consequently seed production (Devlin et al. 1992). Furthermore, pollinators with large dispersal capabilities have the capacity to move pollen between genetically distant individuals, thus mediating the exchange of novel alleles (Carvell et al. 2012, Redhead et al. 2016). In doing so, pollinators affect the fitness of progeny and subsequently, the long-term survival of plant populations (Busch 2005, Eckert et al. 2010, Mannouris and Byers 2013).

Mutualistic interactions between plants that provide pollen and nectar rewards and pollinators which offer pollination services are integral to a plant's reproductive success (Thomson 2003, Bascompte and Jordano 2007, Mitchell et al. 2009). The energy expenditure allocated to the production of rewards is a function of a plant's dependency on these interactions. Given that pollinator species differ in their nutritional requirements, the characteristics of the rewards offered (i.e. the chemical composition of pollen and nectar; Vaudo et al. 2016, Stabler et al. 2015), together with a plant's floral traits (e.g. deep or shallow corolla) can affect which pollinators visit a plant species (Pleasants 1980, Elisens and Freeman 1988, Proctor et al. 1996). Indeed, pollinator species have been demonstrated to vary in the number of flowers visited and time spent on a flower during a foraging bout (Silva et al. 2013); their effectiveness at pollen removal and deposition (Matsuki et al. 2008); their specificity to a particular flower species (i.e. constancy; Van Rossum and Triest 2010) and; their dispersal distance (Matsuki et al. 2008). The composition of local pollinator communities may thus affect the dynamics of plant-population interactions underpinning conspecific pollen transfer.

In insect-pollinated plant species, reproductive success is determined by the quantity and compatibility of pollen received according to the frequency and effectiveness of pollinator visitation (Iwaizumi and Takahashi 2012, Saez et al. 2014) (i.e. multiple insect visits are often required for a plant to attain sufficient pollen to fertilise all ovules; Bernhardt et al. 2008). Therefore, failure of a plant to attract high numbers of effective pollinators can lead to an inconsistent or insufficient supply of compatible pollen (Engel and Irwin 2003). As a consequence, plants can suffer from either a reduction in reproductive output (Wagenius et al. 2007) or, in the case of self-compatible plants, increased self-fertilisation (Kalisz et al. 2004). The compatibility of this deposited pollen is affected by the constancy and dispersal distance of a pollinator (Glaettli et al. 2006, Ashman and Arceo-Gomez 2013). For instance, the degree of pollinator constancy to a particular plant species will affect the incidence of interspecific pollen transfer. Interspecific pollen transfer can lead to conspecific pollen being lost during visitation to co-flowering heterospecific plants. Moreover, the physical or chemical interference from heterospecific pollen deposition on the stigma/style junction of plants (a symptom of inter-specific pollen transfer) can cause a reduction in seed set (Arceo-Gomez and Ashman 2014). Pollinator dispersal distance alternatively will influence the genetic relatedness of pollen receipt (Kenta and Nakashizuka 2003). Given that plant populations have been shown to exhibit genetic structure, whereby relatedness declines with distance between plants (Loveless and Hamrick 1984), increases or decreases in the dispersal distance of pollen could result in progeny exhibiting outbreeding or inbreeding depression (Zhao et al. 2009). This is attributable either to the introduction of maladapted alleles into a population or to increased homozygosity and a subsequent rise in the fixation of recessive deleterious alleles (Pannell 2001), both of which have negative implications for progeny fitness (Busch 2005). A sufficient supply of compatible pollen is thus essential for plant reproductive success and the maintenance of a minimum viable population. Given this, a greater understanding is needed of the underlying mechanisms influencing plant and pollinator relationships in order to ensure the long-term survival of plant populations.

The reproductive success of plants has been shown to vary considerably and is, in part, attributable to a plant’s phenology and mating system (Knight et al. 2005, Vamosi et al. 2013). However, a plant’s failure to achieve its full potential seed set has been observed under a multitude of conditions (Campbell 2007). This has been demonstrated to be context specific and affected by the dynamics of a plant population (e.g. population size, plant density and the degree of isolation; Knight 2003, Waites and Agren 2004) together with the composition of surrounding habitats (Jakobsson et al. 2009). These factors all contribute to the attractiveness of an individual plant and its ability to compete for pollinator visitors. For instance, the extent and distribution of floral resources within a pollinator’s foraging range is recognised to positively affect the attractiveness of an area and thus, the abundance of potential pollinator visitors (Steffan-Dewenter et al. 2002, Heard et al. 2007, Venjakob et al. 2016). Whether a plant population however benefits from the increased pollinator activity depends upon the extent of rewards offered and whether a competitive advantage is achieved over co-flowering heterospecific floral communities (Goodell and Parker 2017). This is determined in part by the phenology of the focal plant together with the size and density of a population (Brys et al. 2007, Sun et al. 2010). Indeed, competitive advantage can further be affected by the composition of co-flowering floral communities. Co-flowering heterospecific plants can introduce competition for pollinators, thereby reducing potential visitors (Ghazoul 2006). Alternatively, if floral communities are characterised as displaying complementary plant species (e.g. species comprising different flower colour forms; Ghazoul 2006, van der Kooi et al. 2016) at an intermediate abundance, co-flowering plants can offer a facilitative effect, thereby increasing potential visitors (Ghazoul 2006, Mesgaran et al. 2017). Therefore, by affecting the availability and type of resources for pollinators, local floral communities are thus expected to alter a pollinator’s constancy (Fontaine et al. 2008) and foraging distance (Carvell et al. 2012). The extent to which floral resources at different spatial scales disrupt plant-pollinator interactions and therefore affect plant reproductive success however is unclear.

Landscape modification and fragmentation have been shown to threaten plant-pollinator relationships by altering pollinator communities and pollinator foraging behaviour (Steffan-Dewenter et al. 2002, Carvell et al. 2012, Redhead et al. 2016). For instance, at larger scales the availability of resources determines the carrying capacity, while at local levels the relative abundance of resources determines pollinator behavioural responses (Meyer et al. 2017, Moquet et al. 2017, Senapathi et al. 2017). Here, I used a large-scale field experiment to explore how pollinator foraging behaviour changes in response to the availability of floral resources at a local (2m radius) and landscape ($1km^2$) scale and variations in plant population size. More specifically, I measured the effects on the reproductive success of two plant species with dissimilar mating systems. I hypothesise:

i) The richness and abundance of pollinator visitors is positively correlated with the size of the experimental array and the availability of floral resources at the landscape scale ($1km^2$). Visitation is also greater to *E. californica* plants, which exhibit a lower propensity to self-fertilise than *S. gallica* plants; there is however a negative relationship between pollinator visitation and the abundance and diversity of floral resources at a local scale (2m radius). This reflects patterns of pollen removal;

ii) Heterospecific pollen deposition (a measure of interspecific pollen transfer), exhibits no relationship with the availability of floral resources at a landscape scale ($1km^2$) though is negatively correlated with the abundance and diversity of floral resources at a local scale (2m radius); heterospecific pollen deposition similarly exhibits a negative relationship with the size of the experimental array;

iii) A plant’s seed set is positively related to the size of the experimental array and the availability of floral resources at a landscape scale ($1km^2$). In contrast, the abundance and diversity of floral resources at a local scale (2m radius) has a negative effect on the seed set of *E. californica* plants (a self-incompatible species);

iv) Progeny fitness (seed weight and germination rates) exhibits a positive relationship with the size of the experimental array and the availability of floral resources at a landscape scale (1km^2); the relationship between fitness and the abundance and diversity of floral resources is however negative at a local scale (2m radius), reflecting a higher incidence of self-fertilisation in plants.

2.3 Methods and Materials

2.3.1 Experimental site and study system

The experiment was conducted on the 900ha Hillesden estate in Buckinghamshire, UK (1º00'01''W, 51º57'16''N: Fig.2.I). Hillesden is situated on heavy clay soils with a relatively flat topography and is characterized by large homogeneous arable fields (10–20 ha), cropped under a simple rotation of autumn-sown wheat followed by either oilseed rape or field bean crops. Under compliance with the English agri-environment scheme (AES), a proportion of the arable land within the estate has been converted to wildlife friendly habitat. Overall, these wildlife habitats comprised ~4% of the total area and include pollen and nectar mixes and wildflower areas.

To test pollinator-mediated effects of floral resources at a local and landscape scale on plants exhibiting different mating systems I introduced two plant species; the small flowered catchfly, *Silene gallica* L. (Caryophyllaceae) and the Californian poppy, *Eschscholzia californica* Cham. (Papaveraceae). Plants were selected from a list of candidates which were previously distributed within the locality and thus, would likely have access to suitable pollinators. The chosen species met the desired criteria and represented model organisms by having an availability of genetic data and simple flower structures. Both plants were also absent from the landscape (confirmed by ground-truthing), preventing additional conspecific pollen sources affecting plant reproduction.

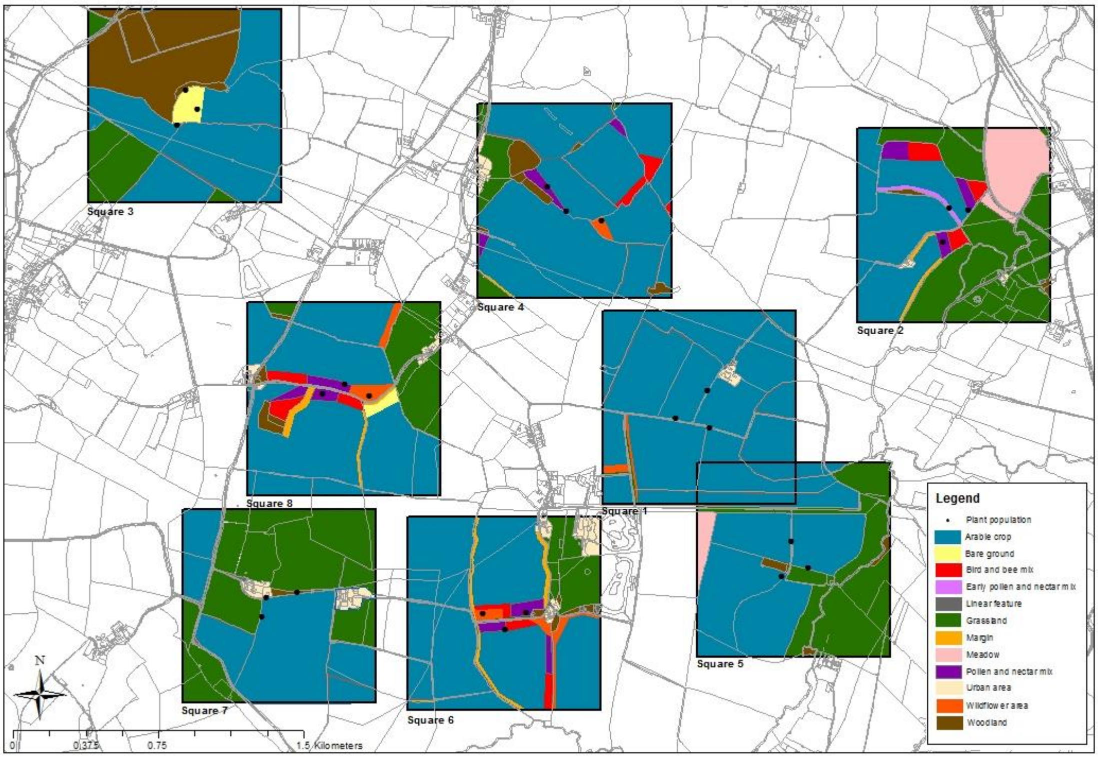

Figure 2.I Experimental set-up at Hillesden estate, Buckinghamshire, UK. Florally rich habitats refer to: Bird and bee mixes, Early pollen and nectar mixes, Margins, Meadows, Pollen and nectar mixes and Wildflower areas.

Silene gallica is a self-compatible wildflower (Desfeux et al. 1996). It has small, pink flowers (mean ± SE flower diameter 0.98 ± 0.03cm (n = 10); Fig.2.II) and large floral displays (mean ± SE flower number 36.7 ± 2.56 (n = 10)) and is visited by insects within the order Diptera (Gibson et al. 2006). It rewards visiting pollinators with nectar comprising 38.8% sucrose (Witt et al. 2013). In contrast, *E. californica* exhibits a partially self-compatible mating system with a low propensity to self-fertilise (Wright 1979) and thus is characterised as an obligate outcrosser (Becker et al. 2005). It produces large, yellow flowers (mean ± SE flower diameter 6.64 ± 0.46cm (n = 10); Fig.2.II) in small floral displays (mean ± SE flower number 5.3 ± 0.42 (n = 10)) and although it is characterised as nectarless, it produces an abundance of pollen. It is visited by a diversity of insects within the orders Hymenoptera, Diptera, Lepidoptera and Coleoptera (Cook 1962).

Plants were grown from seed (seed source: Chiltern seeds Ltd, Wallingford, UK (*E. californica*) and Herbiseed, Berkshire, UK (*S. gallica*)) in compost-filled seed trays under glasshouse conditions (20°C during the day, 16°C at night, with 12 hours of light and 12 hours of dark) and were transferred to 1L pots when at the seedling stage. In June 2014, when plants were at reproductive maturity, three experimental arrays of each plant species were introduced into the centre of each of eight large (1km^2) parcels (squares) of cropped land (Fig.2.I). Within each square, arrays were separated by at least 150m (mean ± SE 173.99 ± 10.44m) to minimise inter-array pollen movement (Matter et al. 2013). To test the impact of floral resources at a local scale on pollination, arrays in four squares were placed within florally 'poor' habitats (grass margins) (Fig.2.III), while arrays in the remaining four squares were placed within florally 'rich' habitats (a combination of wildflower corners and pollen and nectar strips; AES options: EF1 and EF4 respectively: Natural England 2010; Fig.2.III). Floral resources at the local scale were characterised by the abundance and diversity (Shannon diversity index (H)) of floral units (defined as discrete floral entities, thus one composite flower head of species like *Taraxacum officinale* agg. represented one floral unit) measured within a 2m

Figure 2.II The flowers of plant species used in this study, from left to right; 1. Eschscholzia californica, 2. Silene gallica

radius surrounding each array (Table 2.I and Appendix 1: Plant list). To quantify the effect of the availability of floral resources at a landscape scale on pollination, I measured the proportion of land within squares which comprised of florally rich habitats. This varied systematically across the squares from 0 to 19.48% (estimated using ArcGIS) (Table 2.I). Each square was separated by at least 500m to reduce the likelihood of inter-square pollen transfer by larger pollinators such as *Bombus* spp. (Carvell et al. 2012, Danner et al. 2016).

Plants were introduced into pre-marked locations immediately prior to flower opening (late bud stage) and the three focal plants were placed within 0.5 x 0.5m cages to protect against herbivory (prior testing comparing visitation demonstrated that cages to not impede pollinator visitation). In each array, plants were arranged in a linear formation and were separated by approximately 10cm, to limit pollination via contact but to enable ease of movement between plants. Plants remained in the field for six days to ensure full anthesis (Becker et al. 2005). To test how plant-pollinator relationships varied with plant population size, two experiments were conducted in two consecutive weeks. In the first, arrays were characterised as small and comprised of three plants and in the second, arrays were characterised as large and comprised of 12 plants. For practical reasons the manipulation of population size was made sequentially although both were introduced within a 12-day window to avoid any effects of compositional changes in the pollinator communities or surrounding floral resources.

Table 2.I Quantification of the floral resources at a local and landscape scale. The abundance and diversity of floral resources was measured as the total number of floral units in a 2m radius surrounding the experimental array. The availability of floral resources at a landscape scale was measured by calculating the percentage of land within a 1km² square which comprised of florally rich habitats.

Square	Experimental array	The abundance of floral resources at a local scale (2m radius)	The diversity (Shannon diversity index H) of floral resources at a local scale (2m radius)	The availability of floral resources at a landscape scale (1km²) (%)
1	1	0	0	0.74
	2	20	0	0.74
	3	10	0	0.74
2	1	623	0.72	19.48
	2	1660	1.75	19.48
	3	1261	1.40	19.48
3	1	100	0	0
	2	75	0.8	0
	3	2	0	0
4	1	1015	1.37	4.52
	2	1480	1.15	4.52
	3	1300	0.85	4.52
5	1	52	0.19	2.21
	2	153	1.17	2.21
	3	0	0	2.21
6	1	1315	1.34	8.16
	2	735	0.78	8.16
	3	2042	1.39	8.16
7	1	1	0	0
	2	120	0.45	0
	3	51	0.10	0
8	1	381	1.26	9.29
	2	1379	0.92	9.29
	3	1202	1.21	9.29

Figure 2.III Experimental arrays with focal plants placed in a metal cage to protect from herbivory, from left to right; 1. A large array (experiment 2) located within a florally poor habitat (grass margin), 2. A small array experiment 1) located within a florally rich habitat (pollen and nectar mix).

2.3.2 Pollinator behaviour

2.3.2.1 Visitation rates

To measure the abundance and richness of pollinators visiting *S. gallica* and *E. californica* plants under field conditions I conducted timed visitation observations of experimental arrays. Each array was surveyed between 09.30 and 17.00 across three consecutive days and surveys were systematically randomised to ensure a balance of morning and afternoon observations across all squares and arrays. Observation periods lasted for 15 minutes, during which all insect pollination activity (contacting an anther or stigma) on *S. gallica* or *E. californica* plants were recorded and identified according to recognisable taxonomic units (RTUs) (*Bombus* spp., *Apis mellifera*, solitary bee (small, medium and large), Syrphidae (small, medium and large), Lepidoptera, Coleoptera and non-syrphid Diptera). RTUs have been used in previous studies to measure pollinator visitation patterns (Lortie et al. 2012, Vanbergen et al. 2014b) and while simplifying estimates, they offer an effective method of identification in the field (Oliver and Beattie 1996).

2.3.2.2 Pollen removal

To ascertain a measure of the pollen removal effectiveness of local pollinator communities, I quantified the amount of pollen removed from pollinator exposed *S. gallica* and *E. californica* flowers. To measure this, two flowers (late bud stage) were tagged on each plant prior to the start of the experiment. Of these, one flower remained unmanipulated (henceforth ‘pollinator exposed’) in order to measure the quantity of pollen removed when flowers were exposed to pollinators and the other flower was covered in a fine muslin to provide a control when access to pollinators was restricted (henceforth ‘pollinator excluded’). After six days, when plants were collected in, dissecting forceps were used to remove three anthers from pollinator excluded flowers and three anthers from pollinator exposed flowers. Each anther was stored separately in an Eppendorf containing 100µl of 70% ethanol.

To measure the number of pollen grains remaining on anthers, Eppendorfs were sonicated and three 30µl sub-samples were individually removed by a pipette from each anther sample and examined under a haemocytometer. The number of conspecific pollen grains on the four diagonal 1mm^2 squares on the haemocytometer grid was calculated for each sub-sample. From this I calculated an average number of pollen grains in a 1mm^2 grid square (over the three replicates of four squares) and multiplied the average by a factor of ten to get the total number of pollen grains in 1µl (as: length;1mm x width; 1mm x height; 0.1mm = volume; 0.1µl). I then multiplied the number of pollen grains in 1 µl by the total volume of ethanol in the Eppendorf to determine the average number of pollen grains per anther. Pollen removal was expressed as a ratio between the amount of pollen on a pollinator exposed flower and the amount of pollen on a pollinator excluded flower (a measure of pollen removed due to pollinator activity). Where the quantity of pollen was greater on pollinator exposed anthers or where no pollen was recorded on either sample, the proportion of pollen removed was recorded as 0 (*E. californica*: n = 7 flowers; *S. gallica*: n = 98 flowers).

2.3.2.3 Heterospecific pollen deposition

To ascertain the incidence of heterospecific pollen deposition, a measure of pollinator constancy, I quantified the number of heterospecific pollen grains being deposited on a stigma. To do this, a further two flowers were tagged on each plant prior to the start of the experiment; as before, one was labelled ‘pollinator exposed’ and one was covered with a fine muslin and labelled ‘pollinator excluded’. After six days, when plants were collected in, dissecting tweezers were used to remove stigmas from pollinator exposed and pollinator excluded flowers. Each stigma was stored separately in an Eppendorf containing 50µl of 70% ethanol.

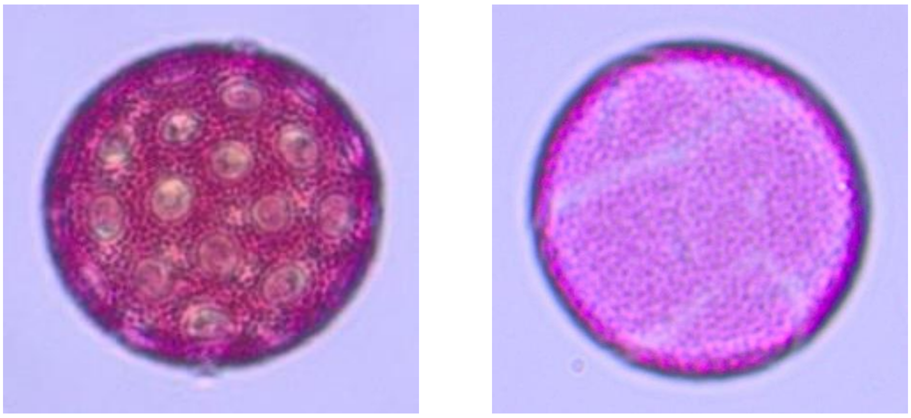

Figure 2.IV Conspecific pollen grains stained with Calberla's solution and viewed at X20 magnification, from left to right; 1. Silene gallica, 2. Eschscholzia californica.

Prior to measuring heterospecific pollen deposition, ethanol was left to evaporate from the Eppendorf. Following this, three drops of Calberla's solution (5ml glycerine, 10ml 95% ethanol, 15ml distilled water and 2-5 drops of fuchsin mix (50:50 distilled water and fuchsin crystals) (Dafni et al. 2005)) was added to each Eppendorf in order to stain the pollen and aid identification. The Eppendorf was then sonicated and the solution was mounted on a slide using a pipette and sealed with a coverslip. Slides were examined under a compound microscope at X20 magnification and the number of heterospecific pollen grains were counted. Additionally, I measured the number of conspecific pollen grains (Fig.2.IV) in order to verify the suitability of the method.

2.3.3 Plant reproductive success

2.3.3.1 Seed set

To determine seed set, a further two flowers were tagged on each plant prior to the start of the experiment; as before, one was labelled 'pollinator exposed' and one was covered with a fine muslin and labelled 'pollinator excluded'. After six days, when fruit was developed, the muslin was removed and plants were collected in and stored under controlled glasshouse conditions (day: night = 20ºC:15ºC photoperiod light: dark = 12:12hr). If fruit had not formed, muslin was left on or was subsequently applied to flowers prior to transference to the glass house and was kept on until fruit had formed. Upon maturation, fruit was collected and seeds were counted. Furthermore, the seed set of any fruit produced by flowers tagged for the analysis of pollen removal and deposition were additionally counted.

2.3.3.2 Plant fitness

Given the high propensity of *S. gallica* to reproduce by self-fertilisation along with an absence of seeds produced by control flowers in *E. californica*, early stage fitness measures were quantified for *S. gallica* progeny only. Although the intention was to relate fitness traits to the incidence of outcrossing, this was precluded given problems with identifying molecular markers necessary to conduct mating system analysis (Appendix 2: Primer note).

To determine the effect of habitat and landscape context on early stage fitness, I quantified the seed weight from field exposed *S. gallica* plants. Prior to weighing, seeds were stored in paper envelopes and were oven dried at 50ºC for seven days in order to obtain a standardised dry mass. Subsequently, a sub-sample of ten seeds (given the low weight of seeds) from a pollinator excluded fruit and ten seeds from a pollinator exposed fruit were weighed from each plant (using fruits tagged for measures of seed set). Where fruit produced fewer than ten seeds, the total seed set was weighed and this was divided by the number of seeds and multiplied by ten to obtain the weight of ten seeds. Following this, the germination rates of these seeds were quantified. This involved taking a sub-sample of six seeds from a pollinator excluded fruit and six seeds from a pollinator exposed fruit from each plant. Seeds were then sown into individual 50x48mm wells within compost-filled seed trays. Seed dormancy was broken by placing seed trays in a chiller, set to 4°C, for seven days to simulate winter. Seed trays were then arranged under glasshouse conditions (as above). Germination was recorded over a 30-day period and any seeds which failed to germinate after 90 days were recorded as inviable.

2.3.4 Statistical analysis

Pollinator behaviour and plant reproductive success parameters were analysed using generalised linear mixed effects models (GLMMs). The richness (RTU's) and abundance of pollinator visitors were respectively modelled with a Poisson and Negative Binomial error distribution (n = 96). Similarly, heterospecific pollen deposition (the number of heterospecific pollen grains deposited on stigmas (n = 144 for each plant species)) was analysed with a Negative Binomial error distribution (both for models for pollinator exposed flowers and when comparing pollinator excluded and exposed flowers). Pollen removal (n = 144 for each plant species) alternatively was analysed with a Binomial error distribution using a combination of logit and probit links depending upon the best fit. Plant seed set (n = 148 and n = 226 for *E. californica* and *S. gallica* respectively (following variations in abortion rates)) and the germination rate (n = 288) and seed weight (n = 288) of *S. gallica* progeny were respectively modelled with Poisson (log), Binomial (logit) Gaussian (log) error distributions (both for models for pollinator exposed flowers and when comparing pollinator excluded and exposed flowers). The difference in *E. californica* seed set between pollinator exposed and pollinator excluded flowers however was modelled with a Negative Binomial error distribution. Models were conducted using the lme4 package (Bates et al. 2015) in R (version x64; R Core Team 2013), with the exception of: the abundance of pollinator visitors; heterospecific pollen deposition and the difference in seed set between pollination treatments, which were modelled using the glmmADMB package (Fournier et al. 2012, Skaug et al. 2012) in R (version x64; R Core Team 2013) to allow for zero-inflation.

In all models, fixed effects comprised: i) size of experimental array, ii) the abundance and Shannon diversity (*H'*) of floral resources at a local scale and iii) the availability of floral resources at a landscape scale. An exception was models comparing pollinator exposed and pollinator excluded flowers where fixed effects were confined to pollination treatment (pollinator exposure). Experimental array nested within experimental square was included as a random effect in all models to account for the nested spatial structure of the experimental design. An additional random effect of plant identity was used with pollen removal, heterospecific pollen deposition and plant reproductive success models to account for natural variation between plants. Where present, over dispersion in the data (e.g. variation between fruit) was controlled for by fitting an observational level parameter to the random effects (Harrison 2014). To account for differences in physiology, *S. gallica* and *E. californica* were analysed separately in all models with the exception of pollinator visitation models.

Prior to analysis, variables were tested for collinearity. AIC stepwise selection was used to find the minimum adequate model (Burnham and Anderson 2003) and all models were analysed using Laplace approximation (given that each model comprised of <3 random effects; Bolker et al. 2009). The significance of the final models was analysed by comparison to a null model with the same random effects structure using an ANOVA.

2.4 Results

2.4.1 Pollinator behaviour

2.4.1.1 Visitation rates

The total abundance of pollinators visiting *S. gallica* and *E. californica* arrays (3 focal plants) within the field was two times greater when experimental arrays were large (mean ± SE large arrays (12 plants) = 6.46 ± 1.36; small arrays (3 plants) = 3.25 ± 0.8; glmmADMB z = -2.86, $p < 0.001$; Fig.2.V). Of these, five times more pollinators visited *E. californica* than *S. gallica* (mean ± SE *E. californica* = 8.21 ± 1.43; *S. gallica* = 1.5 ± 0.31; glmmADMB z = -7.56, $p < 0.0001$; Fig.2.V).

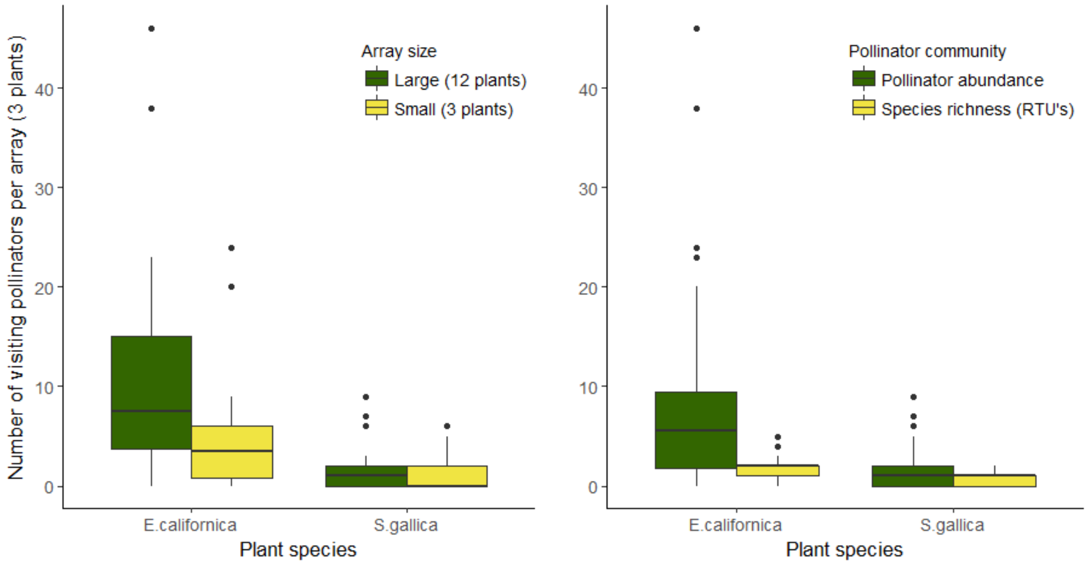

Figure 2.V From left to right: 1. The abundance of pollinating insects visiting small (3 plants) and large (12 plants) arrays of E. californica and S. gallica plants; 2. The abundance and species richness (RTU's) of pollinating insects visiting arrays of E. californica and S. gallica plants. Boxes represent the upper quartile range (Q3), the median value (Q2) and the lower quartile range (Q1) accordingly. Whiskers represent data that is +/- 1.5x the interquartile range (Q3-Q1) and data exceeding this are characterised as outliers and marked with circles (this representation is similar in all following graphs).

Similarly, the richness of visitors (RTU's) was two times greater to *E. californica* arrays compared to *S. gallica* arrays (mean ± SE *E. californica* = 1.54 ± 0.17; *S. gallica* = 0.60 ± 0.09 GLMM z = -4.276, df = 92, p < 0.0001; Fig.2.V). These pollinators were predominantly from the order Coleoptera (55% of all visits) followed by insects from the Syrphidae family (Diptera) (42% of visits; especially those within the medium size category: 37% of visits). The abundance and diversity (Shannon H') of floral resources at the local and landscape scale however, had no effect on the abundance (p = 0.95, p = 0.42 and p = 0.26 respectively) or species richness (RTU's) (p = 0.62, p = 0.69 and p = 0.16 respectively) of pollinating visitors.

2.4.1.2 Pollen removal

The proportion of pollen removed from 'pollinator exposed' flowers (inferred from the average of three anthers per flower) was variable between plant species (mean ± SE *E. californica* 0.8 ± 0.02 (n = 144); *S. gallica* 0.27 ± 0.04

(n = 144)). In *E. californica*, this pollen removal was positively correlated with the availability of floral resources at the landscape scale (the proportion of land within a 1km² square which comprised of florally rich habitats) (GLMM z = -2.26, df = 138, p = 0.02, R^2c=0.18; Fig.2.VI).

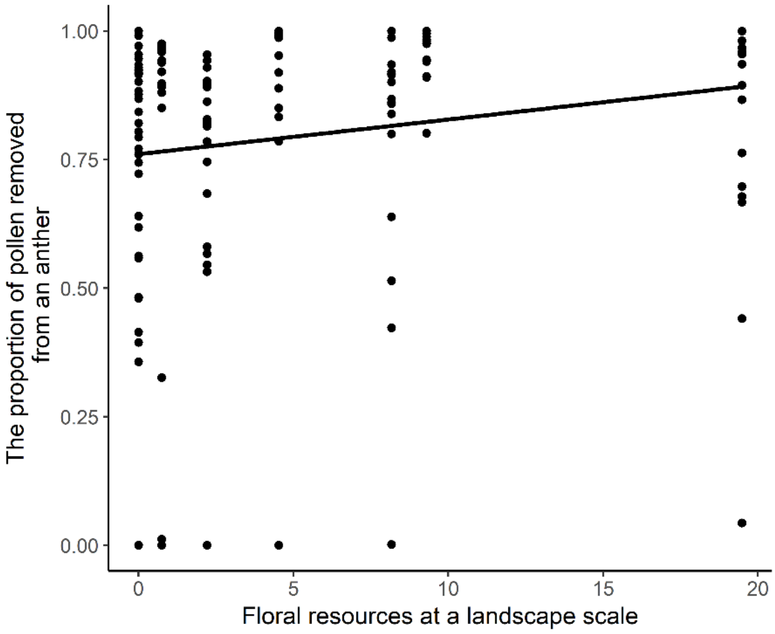

Figure 2.VI The proportion of pollen removed from an E. californica flower (average of three anthers) in relation to floral resources at a landscape scale (the percentage of land within a 1km² square comprising florally rich habitats)

No relationship was observed however between pollen removal and floral resources at a local scale (p = 0.08 and p = 0.22 for floral abundance and diversity respectively) or the size of the experimental array (p = 0.36). Pollen removal in *S. gallica* was not affected by floral resources either at a local (p = 0.65 and p = 0.92 for floral abundance and diversity respectively) or landscape scale (p = 0.8), or indeed, by the size of the array (p = 0.74).

2.4.1.3 Heterospecific pollen deposition

The quantity of heterospecific pollen grains deposited on ‘pollinator exposed’ flowers (pollinator constancy) was low and varied depending upon the plant species (mean ± SE Number of heterospecific pollen grains per plant: *E. californica* = 0.97 ± 0.37 (n = 144), *S. gallica* = 1.15 ± 0.18 (n = 144); Proportion of total pollen deposition: *E. californica* = 0.006 ± 0.003 (n = 144), *S. gallica* = 0.07 ± 0.02 (n = 144)). Unsurprisingly therefore, for both plant species the incidence of heterospecific pollen deposition and hence, pollinator constancy, exhibited no relationship with floral resources at either a local (Floral abundance p = 0.93 and p = 0.3 for *E. californica* and *S. gallica* respectively; Floral diversity p = 0.89 and p = 0.41 for E. californica and S. gallica respectively) or landscape scale (p = 0.96 and p = 0.34 for *E. californica* and *S. gallica* respectively) or indeed the size of the array (p = 0.44 and p = 0.71 for *E. californica* and *S. gallica* respectively).

2.4.2 Plant reproductive success

2.4.2.1 Seed production

In *E. californica* the total number of seeds produced in a fruit was greater in ‘pollinator exposed’ flowers when compared to ‘pollinator excluded’ flowers (mean ± SE Pollinator exposed = 56.17 ± 3.02 (n = 148), Pollinator excluded = 2.16 ± 0.91 (n = 114); glmmADMB z = 15.14, p < 0.001). In contrast, in *S. gallica* plants, the total number of seeds produced in a fruit was greater in ‘pollinator excluded’ flowers when compared to ‘pollinator exposed’ flowers (mean ± SE Pollinator exposed = 37.32 ± 0.88 (n = 226), Pollinator excluded = 40.36 ± 0.86 (n = 180); GLMM z = -2.62, df = 400, p < 0.01), suggesting a negative effect of pollinator exposure due to removal of pollen otherwise available for self-fertilisation. For ‘pollinator exposed’ flowers, the number of fruit produced by tagged flowers per plant was variable (mean ± SE *E. californica* = 1.97 ± 0.07, *S. gallica* = 2.01 ± 0.05). For both plant species, however, the seed set of these fruits was not affected by floral resources at either a local (Floral abundance p = 0.93 and p = 0.6 for *E. californica* and *S. gallica* respectively; Floral diversity p = 0.55 and p = 0.86 for *E. californica* and *S. gallica* respectively) or landscape scale (p = 0.34 and p = 0.5 for *E.*

californica and *S. gallica* respectively) or indeed the size of the array (p = 0.09 and p = 0.12 for *E. californica* and *S. gallica* respectively).

2.4.2.2 Plant fitness

The seed weight from the fruits of surviving field exposed *S. gallica* plants (ten seeds) was significantly greater in fruits from 'pollinator exposed' plants when compared to 'pollinator excluded' plants (mean ± SE (mg) Pollinator exposed = 3.72 ± 0.05 (n = 135); Pollinator excluded = 3.35 ± 0.04 (n = 140); GLMM t = -6.55, df = 269, p < 0.001; Fig.2.VII). In addition, seed weight was marginally greater when experimental arrays were small (3 plants) when compared to when arrays were large (12 plants) (mean ± SE (mg) Large arrays = 3.66 ± 0.07 (n = 71); Small arrays = 3.79 ± 0.06 (n = 64); GLMM t = 1.91, df = 129, p < 0.06; Fig.2.VII). The weight of *S. gallica* seeds however, exhibited no relationship with floral resources at either a local (p = 0.91 and p = 0.88 for floral abundance and diversity respectively) or landscape scale (p = 0.4).

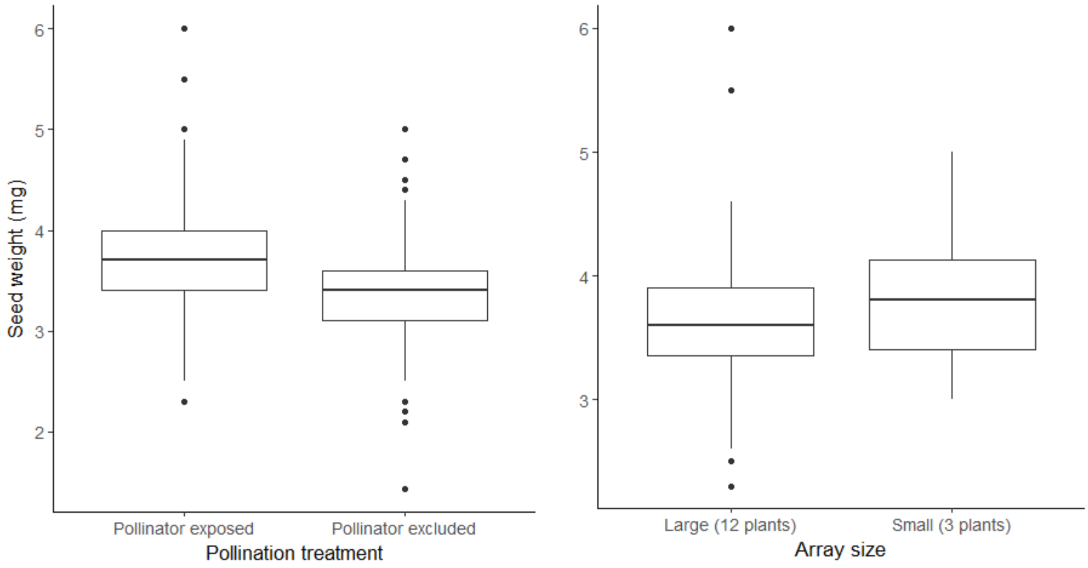

Figure 2.VII From left to right: 1. The average weight (mg) of seeds (10 seeds) produced by field exposed S. gallica plants across squares and experiments when flowers were exposed to or excluded from pollinators; 2. The average weight (mg) of seeds across squares produced from pollinator exposed S. gallica plants placed in large (12 plants) and small (3 plants) experimental arrays.

The germination rates of *S. gallica* seeds did not differ between pollination treatments (mean ± SE Pollinator exposed = 0.89 ± 0.01 (n = 135), Pollinator excluded=0.87 ± 0.02 (n = 140) (p = 0.54)). Furthermore, the germination of 'pollinator exposed' *S. gallica* seeds similarly exhibited no relationship with floral resources at either a local (p = 0.78 and p = 0.96 for floral abundance and diversity respectively) or landscape scale (p = 0.76), or indeed, by the size of the array (p = 0.17).

2.5 Discussion

In this study, findings demonstrate no relationship between the abundance and composition of pollinator visitors and floral resources at a local or indeed, a landscape scale. Visitation to plants however was affected by array size, where large experimental arrays and *E. californica* plants received a greater number and richness (in the latter) of pollinator visitors (though differences in richness may be due to differences in the abundance of pollinator visitors). The behaviour of visiting pollinators, measured through pollen removal and heterospecific pollen transfer, was unaffected by both floral resources at a local and landscape scale and the size of the experimental array. An exception was pollen removal, which in contrast, was positively related to floral resources at a landscape scale. This had no discernible effect on the reproduction of *E. californica* or *S. gallica* plants, which was similarly unaffected by both floral resources at a local and landscape scale and the size of the array. The fitness of *S. gallica* progeny however, while unaffected by floral resources both at a local and landscape scale, was reduced in pollinator excluded flowers and in large populations, albeit marginally.

2.5.1 The effects of floral resources at a local and landscape scale on plant and pollinator interactions

Pollinators have been shown to respond to floral resources at a number of scales, from a local, habitat scale (Masters and Emery 2015) to a landscape scale (Steffan-Dewenter et al. 2002). This is illustrated by a greater abundance and diversity of pollinators and indeed, higher visitation rates in habitats and landscapes comprising an abundant and diverse floral community (Steffan-Dewenter et al. 2002, Heard et al. 2007, Woodcock et al. 2013, Orford et al. 2016, Pisanty et al. 2016). Findings here, in contrast to previous research, suggest that floral resources both in the immediate vicinity and within the wider landscape (1km^2 square) did not facilitate visitation to *E. californica* or *S. gallica* plants (Carvalheiro et al. 2012). Indeed, visitation rates were low in this study, either due to the reduced attractiveness of experimental arrays or reflecting the survey effort. These low visitation rates could provide reasons for the inconsistencies between studies. Alternatively, inconsistencies may highlight pollinator specific responses to local and landscape context (Woodcock et al. 2013, Pisanty et al. 2016) and the importance of species comprising floral communities for attracting pollinators and facilitating visitation (Wood et al. 2015). For instance, in one study, while visitation by honeybees to individual plants was negatively related to the coverage of semi-natural habitat at the landscape scale, visitation by solitary bees displayed the opposite trend (Steffan-Dewenter et al. 2002). This reflects the differences in pollinator requirements and floral preference. Indeed, the density and diversity of bumblebees has been shown to increase within habitats associated with agri-environment schemes (e.g. pollen and nectar mixes), given the predominance of leguminous species such as *Trifolium repens* (Heard et al. 2007, Carvell et al. 2011). Research has however indicated that the benefits of habitats associated with agri-environment schemes are limited to a small suite of pollinator species (Wood et al. 2015). In this study, few bumblebees were observed in visitor observation surveys. This could either be because bumblebees were too specialised to visit phytometer plants (Weiner et al. 2011), or alternatively, it could be an indication that small populations of plants which produce limited rewards, are not attractive to bumblebees. The low level of visitation by bumblebees (the dominant pollinator species associated with these mixes), together with the predominance of species from the orders Coleoptera and Diptera (which vary considerably in their life history and foraging behaviour) may therefore provide reasons for the lack of variation in abundance and diversity of visitors in response to increases in floral resources at a local and landscape scale.

Pollinator visitation has been shown to exhibit a positive relationship with pollen removal (Rush et al. 1995). In this study, despite there being no effect of floral resources on visitation, findings demonstrate a positive relationship between pollen removal and floral resources at a landscape scale (1km^2). The poor model fit (R^2=0.18) however suggests that this trend may be driven by outliers in the data as a result of an unbalanced experimental design and low replication of landscapes with high floral abundance. This raises doubt that these findings are indeed ecologically significant and further hypothesis testing is needed to verify results. This said, results do suggest an increased pollen removal effectiveness of pollinators where floral resources are abundant at a landscape scale. This pattern was however only seen in *E. californica* plants where pollen was copious and the likelihood of contact between pollinators and anthers was high given floral traits (Harder 1990). Pollen removal was not however affected by floral resources at a local scale, reflecting the presence of a shared pollinator community within habitats (Potts et al. 2003, Klein 2009). Indeed, pollen removal was measured as the additional number of pollen grains removed due to pollinator exposure, thereby giving an indication of pollinator activity. In *S. gallica* plants, low pollen removal therefore indicates a high level of pollen use for self-fertilisation. The differences in pollen removal between plant species may additionally reflect the variation in visitation between plant species and the species comprising these pollinator visitors. Indeed, pollinator species have been shown to differ in the amount of pollen removed and deposited and hence their transfer effectiveness (Thomson and Goodell 2001), where some species are characterised as high removers and low depositors and vice versa (Young et al. 2007). Pollinators visiting *E. californica* plants were diverse and included insects from the orders Hymenoptera, Lepidoptera, Diptera and Coleoptera, whereas those visiting *S. gallica* plants only comprised insects from the family Syrphidae (Diptera). In one study, Syrphids were characterised as low pollen removers given that visitation was shown to have no effect on pollen removal (Rush et al. 1995). This offers support to the reductions in pollen removal due to pollinator activity observed in *S. gallica* within this study. Pollen removal is an indication of the fitness of a flower’s male function and, by contributing to the number of seeds sired, is therefore an important measure of plant reproductive success. The fate of this pollen will influence genetic exchange and thereby contribute to the fitness of a plant (Mannouris and Byers 2013). In order to gain an understanding of the absolute transfer efficiency of pollinator communities however it is important to relate pollen removal with subsequent deposition (Galen and Stanton 1989).

While the fate of removed pollen was not directly explored within this study, the deposition of heterospecific pollen was measured to better understand the foraging behaviour of pollinators and thus, the destination of conspecific pollen. Heterospecific pollen deposition is a measure of pollinator constancy to a particular forage plant species (Dafni et al. 2005). This is a consequence of the relaxation of forage preferences in the face of resource depletion, caused by altered landscapes (Kunin and Iwasa 1996) or competition with other pollinators (Fontaine et al. 2008). The incidence of heterospecific pollen deposition is however complex and previous research has alternatively demonstrated an increase with the density of heterospecific flowers within the neighbouring community (Jakobsson et al. 2009, though see Feinsinger et al. 1986). Findings here however demonstrate no relationship between the deposition of heterospecific pollen and floral resources at a local or landscape scale. Indeed, the incidence of heterospecific pollen deposition was low in this study, reflecting the low visitation rates observed (Morales and Traveset 2008). This offers an explanation for the inconsistencies between findings. Alternatively, the low incidence of such deposition may indicate a sufficient supply of floral resources within the wider landscape or indeed, the specialist nature of pollinator visitors. This is illustrated by a greater incidence of heterospecific pollen deposition in *S. gallica* plants, which were visited only by

Syrphids, of which some species are considered generalists (Pontin et al. 2006). Heterospecific pollen deposition is an important measure for plants by affecting reproductive success through disruptions at the fertilisation stage, either through physical (e.g. blocking the stigma or style; Scribailo and Barrett 1994, Holland and Chamberlain 2007) or chemical (e.g. allelopathy; Murphy 2009) interference. Furthermore, it offers an indication of the fate of conspecific pollen (Morales and Traveset 2008). For instance, where heterospecific pollen deposition is low, as observed here, it suggests a high rate of direct pollen transfer.

2.5.2 The effects of plant population size on plant and pollinator interactions

The relationship between plants and pollinators is governed in part by the attractiveness of a plant population and the level of rewards offered (Weber et al. 2012). In this study, pollinator visitation was positively related to the size of the experimental array. This is consistent with previous studies (Bernhardt et al. 2008) and suggests that small populations have fewer resources to attract and sustain pollinator communities (Phillips et al. 2014), particularly when competitive co-flowering plants are abundant (Johnson et al. 2012, Tscheulin and Petanidou 2013). As populations increase in size they become more attractive and the competitive effect of co-flowering heterospecific plants is switched to a facilitative effect (Ghazoul 2006). This competitive advantage attributed to large population size however may be plant species dependent. Here, *E. californica* plants attracted a greater abundance and richness of pollinator visitors when compared to *S. gallica* plants. This further illustrates the importance of a plant’s rewards. *Eschscholzia californica* is reliant on pollinators and therefore invests greater energy expenditure in pollen provisions and floral displays. It is noteworthy however that, although *S. gallica* has been shown to be pollinated by Syrphids (Gibson et al. 2006), nocturnal Lepidoptera have been recognised to be important pollinators of *Silene* spp. (Young 2002), suggesting that visitation to this species may have been underestimated by visitation observations. Given differences in visitation rates between species however, it is likely that *E. californica* in this study outcompeted *S. gallica* for pollinator visitors. This was not however reflected in the levels of heterospecific pollen deposition, which exhibited no relationship with the size of the experimental array in either plant species. This contradicts previous observations of an increased incidence of interspecific pollen transfer in small plant populations driven by a greater potential for shared pollinators (Ghazoul 2006). Of the two plant species, *S. gallica* received slightly higher heterospecific pollen deposition when compared to *E. californica*. This is surprising given the restricted nature of flowers, which has been negatively related to heterospecific pollen deposition (Montgomery and Rathcke 2012) and the lower rates of pollen removal due to pollinator activity. The absence of a relationship between the size of the experimental array and both pollen removal and heterospecific pollen deposition may instead be due either to populations being too small, with differences between small and large arrays insufficient to detect an effect, or indeed, due to the experimental design. Faced with impracticalities during experimental set-up, population size was confounded within experiment. This raises doubt as to whether the effects attributed to size of experimental array may instead be driven by variations in experimental conditions. Such variations were however expected to be minimal given that both experiments were conducted within a two-week period.

2.5.3 The implications of local and landscape context on plants

Based on previous research, plant seed set is expected to increase in response to a plant’s population size (Bernhardt et al. 2008, Duffy et al. 2013, Anic et al. 2015, though see: Johnson et al. 2012) and floral resources at a local (Orford et al. 2016) and landscape (Valdes and Garcia 2011) scale. However, greater pollinator visitation and pollen removal (in response to increases in the size of the experimental array and floral resources at a landscape scale respectively), did not translate into an effect on plant seed set in this study. This is surprising given that previous research has demonstrated a requirement of multiple pollinator visits for a plant to achieve its full potential seed set (Bernhardt et al. 2008). Given the positive relationship between pollinator visitation, pollen receipt and subsequently seed set in self-incompatible plants (Spigler and Chang 2008), this suggests visitation to be a poor predictor of pollination (Snow 1982, Rymer et al. 2005, King et al. 2013). Instead, pollinator effectiveness has been argued to be a superior predictor of pollination success (Lazaro et al. 2015). This was illustrated by research into the efficiency of butterflies at pollen transfer, where individuals were observed to collect 10% of available pollen when visiting a flower and then deposit only 10-17% of this pollen on the subsequent flower (Richards 1997). Ineffective pollen transfer may alternatively be driven by low mate availability. Indeed, the small size of arrays in this study may have resulted in a limited pollen pool and thus, a reduction in the availability of compatible pollen (de Waal et al. 2015). Given that neither the source of the conspecific pollen deposited (i.e. whether it was self or outcrossed pollen) nor the fate of removed pollen was measured, the relationship between pollinator visitation and pollination effectiveness could not be deciphered. However, the low variance in seed set suggests that plants were not limited by pollen availability. This suggests that when visitors comprise effective pollinators, low visitation rates may be adequate for plants (Saez et al. 2014). The perceived absence of pollen limitation in *S. gallica* plants can be explained by the capacity of this species to use self-pollen to offer reproductive assurance during low pollinator availability. Indeed, in *S. gallica*, seed set was in fact greater in pollinator excluded plants, thereby suggesting a negative effect of pollinator exposure due to removal of pollen otherwise available for self-fertilisation.

The effect of self-fertilisation on plant fitness is complex and has been demonstrated to vary between plants depending, in part, on their mating system (Wright et al. 2013). Plant fitness is fundamental for reproductive success and thus the maintenance of a minimum viable population and long-term survival (Schleuning and Matthies 2009). In this study, early stage fitness was measured in order to gain an understanding of these effects. Given that arrays were not expected to exhibit genetic structure in this study (due to their random positioning in the field, coupled with the low propensity of *E. californica* to reproduce by self-fertilisation), fitness (seed weight and germination success) was only measured in *S. gallica* plants. In support of previous research, findings here demonstrate a reduction in seed weight from self-compatible plants due to self-fertilisation (Lowry 2007), though this had no effect on seed germination rates. Indeed, research deciphering the effect of seed weight on zygote fitness and later seedling development is inconsistent (Houssard and Escarre 1991) and has been shown to be species and context specific (Marshall 1986, Kromer and Gross 1987). Furthermore, the negative effects of self-fertilisation can be expressed at different life stages and previous research has demonstrated a plant’s ability to recover from inbreeding expressed in early fitness traits (Angeloni et al. 2011). The absence of a relationship between self-fertilisation and seed germination rates within this study, despite reduced seed weight, therefore supports this recovery hypothesis and demonstrates a negligible effect of seed weight on the survival of a plant’s progeny (Schaal 1980). Seed weight was additionally, albeit marginally, demonstrated to exhibit a negative relationship with the size of the experimental array, suggesting a higher incidence of self-fertilisation in large plant populations. This is unexpected and contradicts previous research which reports an increase in the rates of self-fertilisation within small plant populations (Routley et al. 1999). While this may indicate higher rates of geitonogamous pollen movement and thus, reduced pollen carry-over in large populations, it may alternatively be an artefact of the experimental set-up, where the size of the array was confounded with experiment. It is noteworthy however that whether these effects are attributed to increased self-fertilisation in large arrays cannot be verified given technical issues with primer development in *S. gallica* plants (Appendix 2: Primer note).

2.5.4 The management and conservation of plant populations

Management interventions such as those implemented under the English agri-environment scheme have resulted in conflicting trends. Indeed, benefits to pollinators have been demonstrated to be dependent upon landscape complexity and the ecological contrast with introduced habitats (Batary et al. 2011, Scheper et al. 2013, Hammers et al. 2015). Moreover, the magnitude of effects will vary between pollinator species. For instance, while the density and diversity of pollinators has been shown to be enhanced by wildlife friendly habitats (Jonsson et al. 2015, Campbell et al. 2017), this pattern is limited to targeted pollinator genera (Wood et al. 2015, Wood et al. 2017). Furthermore, when comparing farms adopting agri-environment schemes with those complying to standard practises, Wood et al. (2015) demonstrated that the presence of florally rich habitats had no effect on the diversity of species comprising local pollinator communities. This was argued to be driven by the high abundance of a small number of plant species within wildlife friendly habitats associated with agri-environment schemes, thereby reducing the attractiveness and suitability of an area to a larger pool of pollinator species (Wood et al. 2015). Indeed, a diverse pollinator community provides functional complementation, which is important for providing pollination services to a diverse plant community (Pisanty et al. 2016). In this study, the implementation of wildlife friendly habitat had no effect on plants at a local scale, though exhibited an effect on pollen removal where a large proportion of the wider landscape comprised such habitats. This suggests a benefit of agri-environment schemes on plant-pollinator interactions. The absence of a benefit of local floral resources on plant-pollinator interactions however points towards a failure of wildlife friendly habitats to facilitate pollinator visitation to plants present at low frequency. Visitation has instead been shown to be facilitated by linear features such as hedgerows (Cranmer et al. 2011). This, coupled with findings here demonstrating benefits of increased plant attractiveness, indicates a need for further research into how habitats and landscapes can be managed and used to enhance the facilitation of generalist pollinators to plant populations.

2.5.5 Summary

Findings from this study indicate that pollinator visitation was not affected by floral resources at a local or landscape scale. Instead plants benefited from the increased attractiveness created by large populations and the high rewards offered by obligate outcrossing plants. The removal of pollen and heterospecific pollen deposition were not impacted by local or landscape context, with the exception of pollen removal in self-incompatible plants, which was positively related to floral resources at a landscape scale. Changes in pollinator behaviour, in terms of visitation and pollen removal, did not translate to changes in plant reproduction, which was comparable across arrays irrespective of local and landscape context. The fitness of progeny was however marginally reduced in large *S. gallica* arrays. Given the negative implications of self-fertilisation shown here on the weight of *S. gallica* seeds, this indicates a higher incidence of self-fertilisation in large arrays. Despite reduced seed weight in response to self-fertilisation, the comparable germination rates between pollinator exposed and pollinator excluded progeny points towards an ability to recover from inbreeding expressed in early stage fitness traits. This suggests a minimal impact of self-fertilisation on the survival of self-compatible plants. This study therefore highlights the complexity of the underlying mechanisms governing plant and pollinator relationships.

With pollinators facing declines in abundance (Scheper et al. 2015) and functional diversity (Forrest et al. 2015) in arable systems (Marini et al. 2014) and the widespread implementation of agri-environment schemes with the purpose of enhancing pollinator diversity (Batary et al. 2015), it is important to gain an understanding of the changes to plant and pollinator relationships in these systems. Here, local and landscape context was shown to have a negligible impact on the maternal reproductive success of plants when populations comprise less than 12 plants. Given however that a positive effect of floral resources at a landscape scale was observed on pollen removal, research should now focus on the destination of this pollen and the scale at which floral availability has the greatest effect on pollen movement. By determining the fate of pollen, a greater understanding of the implications for paternal reproductive success, through seed siring, together with maternal reproductive success, through genetic diversity, will be attained.

CHAPTER 3

Spatially-dependent effects of floral resource availability on pollinator communities and pollination services to plants

3.1 Abstract

1. Pollinator species alter their foraging behaviour according to the extent and distribution of floral and nesting resources at a spatial scale related to their dispersal capability. Landscape changes (e.g. agricultural intensification and agri-environment planting) modify the spatial and temporal availability of floral resources and thus affect the accessibility of forage for pollinators. It is important to understand how such changes, particularly within scales reflecting typical foraging distances, affect pollinator community composition and foraging behaviour (e.g. dispersal distance). This is expected to have implications for pollen transfer and thus, the outcrossing, reproduction and fitness of insect-dependent plants.
2. This study used a multi-site field experiment to explore how floral resource availability (using semi-natural habitat cover as a proxy) at habitat and landscape (1.5km radius) scales influenced pollinator communities and pollination services to plants. I measured activity density (the abundance of actively foraging pollinators), species richness, and community-weighted mean inter-tegular span ('IT' span) of potential pollinator visitors. In addition, I introduced experimental arrays of a self-compatible focal plant, *Vicia faba*, to sites to assess intra-population pollen movement (as an indicator of pollinator behaviour) and plant reproductive success. Furthermore, this experiment tested the magnitude of effects on pollinators and the focal plant at more localised spatial scales (1-100m radius).
3. Relationships between floral resource availability and both pollinator communities and intra-population pollen movement differed at habitat and landscape scales. At a habitat scale, pollinator activity density and species richness were related negatively and community weighted mean IT span positively to greater availability of floral resources. However, there were no apparent effects on pollination services or the reproduction or progeny fitness of focal plants. At a landscape scale,

floral resource availability did not affect pollinator communities, pollination services or the reproduction and fitness of focal plants. When measuring the magnitude of effects at different spatial scales, pollinator communities and intra-population pollen movement were negatively affected by floral resources at local scales (1-50m and 1m from experimental arrays respectively), with pollinators most affected by floral availability within a 20m radius.

4. This study indicates that while landscape context has been highlighted elsewhere as important for pollinator attraction, interactions between plants and pollinators are affected more by floral resources at a habitat scale. Here, reductions in pollinator activity density and thus, potential visitation, in response to high floral availability are paralleled by disruptions to pollen transfer. This suggests that plant outcrossing will be reduced within populations comprising low frequency when surrounded by competitive co-flowering plant species. While reproduction and fitness were not affected in this self-compatible plant species, this may not hold true for obligate outcrossing plants that rely on pollen transfer for long-term fitness and survival.

3.2 Introduction

Pollinating insects require an adequate supply of floral resources and provisions for shelter and breeding in order to maintain stable populations (Torne-Noguera et al. 2014). These resource requirements vary between pollinators in relation to a species' nutritive demands, floral preferences and life history strategies (Goulson 2003, Gegear and Laverty 2005, Potts et al. 2005, Fontaine et al. 2006, Kremen et al. 2007, Torne-Noguera et al. 2014). Habitat composition can therefore play a fundamental role in governing pollinator communities. For instance, semi-natural habitats, by comprising a large variety of floral resources and provisions for shelter and breeding, have been recognised to support rich, abundant and productive pollinator communities (Steffan-Dewenter and Tscharntke 2001, Steffan-Dewenter et al. 2002, Potts et al. 2003, Hines and Hendrix 2005, Williams and Kremen 2007, Roulston and Goodell 2011, Rollin et al. 2013). Indeed, the extent and distribution of floral resources is expected to influence pollinator community structure, foraging behaviour and have far-reaching implications for pollen movement, subsequent reproduction and longer-term survival of plants. Increasing pressure predominantly from agricultural intensification, however, is leading to habitat fragmentation and is thus threatening the availability of these resources across landscapes (Osborne et al. 2001). While effects have been studied individually, often at a single scale, knowledge is incomplete of the interplay of floral resource availability at different scales on plant reproduction and fitness mediated by changes in pollinator communities.

The availability and diversity of floral resources within a habitat determines its attractiveness to pollinators (Essenberg 2012, Montero-Castano and Vila 2012) and indeed, a positive relationship has been observed between pollinator abundance and floral resources, especially when such resources are limited at the landscape scale (Heard et al. 2007). Floral resources across landscapes vary both temporally (in relation to flowering periods) and spatially (Devoto et al. 2014). The accessibility of floral resources throughout a pollinator’s foraging season will therefore vary between species depending upon their seasonal activity (Cane and Payne 1993) and foraging range (Steffan-Dewenter et al. 2002). Indeed, foraging range varies considerably between pollinator species, with solitary bees observed to travel short distances of 100-300m (Gathmann and Tscharntke 2002), compared to honeybees which can travel up to 3000m on a foraging bout (Hagler et al. 2011). Given this, the attractiveness of a landscape to pollinators is recognised to be a function of foraging capacity (Steffan-Dewenter et al. 2002, Westphal et al. 2006, Redhead et al. 2016). For example, *Bombus* spp. and *Apis mellifera* are central place foragers, exhibiting high mobility and considerable nutritive demands (Paoli et al. 2014, Stabler et al. 2015). Recruitment of these more mobile species has been shown to be affected by changes in the composition of resources across larger spatial scales (e.g. <3000m) compared to less mobile central place foragers, such as solitary bees, which are more sensitive to changes in resource availability at more local spatial scales (e.g. <750m) (Holling 1992, Steffan-Dewenter et al. 2002, Westphal et al. 2006, Carvell et al. 2012).

Landscape context, while important in governing pollinator attraction, has also been shown to be an influential driver of pollinator foraging behaviour. Pollinator foraging behaviour is characterised by systematic, non-random movements (Dreisig 1995, Brosi 2016). These movements are governed by energetics and are expected to reflect a trade-off between the cost of movement and the energy gained at the destination (i.e. through provisions at the subsequent flower) (Charnov 1976). This is reflected by the tendency of pollinators to forage locally (Matter et al. 2013, da Silva et al. 2015), only leaving a habitat when the cost of staying (e.g. resource depletion) exceeds that of leaving (e.g. energy loss) (Charnov 1976, Jha et al. 2013). This trade-off is therefore influenced by the distance between suitable floral resources as well as perceived barriers to pollinator movement (e.g. habitat edges and the risk of desiccation and predation) (Osborne et al. 2001). For example, in landscapes characterised by a continuous availability of floral resources, pollinators have exhibited larger foraging distances (Jha and Vandermeer 2009) than in landscapes low in floral resources, where greater time is typically spent within patches or on individual plants (Cresswell and Osborne 2004). To further minimise energy expended during foraging pollinators have been shown to make repeated visits to patches through the use of ‘trap-lining’ behaviour (Thomson et al. 1982, Osborne et al. 1999, Osborne et al. 2001, Ohashi and Thomson 2009). Such constancy to specific plants or patches is recognised to be a plastic foraging strategy (Fontaine et al. 2008) with return visits occurring at a greater frequency in areas where floral resources are abundant (Kunin and Iwasa 1996). In contrast, where floral resources are scarce, competition-led resource depletion is expected to reduce a pollinator’s floral constancy (Fontaine et al. 2008).

Changes to pollinator visitation and foraging behaviour are expected to have considerable implications for pollen transfer, which is essential for seed production and genetic exchange in most flowering plants (Ollerton et al. 2011). The recruitment of a diverse and abundant pollinator community can provide diurnal and functional complementarity in foraging activity and thus, increase the potential for pollinator visitation (Albrecht et al. 2012). While dependent upon the species and characteristics (e.g. body size) of visiting pollinators, visitation has been demonstrated to exhibit a positive relationship with pollen deposition at the plant level (Engel and Irwin 2003, Larsen et al. 2005, Bernhardt et al. 2008). The deposition of pollen is fundamental for seed production. It is the quality of this pollen, however, that ultimately influences seed viability and plant reproductive success. This is partly determined by a pollinator’s foraging behaviour, where large pollinator dispersal distances and a high incidence of pollinator constancy to a specific plant species increase the deposition of compatible, outcrossed pollen. Indeed, reductions in the constancy of a pollinator can introduce competition between plant species for pollinator visitors leading to a higher incidence of interspecific pollen transfer (Veddeler et al. 2006, Jha and Vandermeer 2009, Wenninger et al. 2016). Interspecific pollen transfer disrupts movement between conspecifics, leading to loss of pollen to heterospecific plants (Morales and Traveset 2008). Furthermore, interspecific pollen transfer can result in an increased incidence of incompatible, heterospecific pollen deposition (Holland and Chamberlain 2007). While the effects of heterospecific pollen deposition are inconsistent, such deposition has been observed to clog a plant’s stigma and style and thus reduce plant reproduction (Brown and Mitchell 2001). A combination of high levels of inter-specific pollen transfer and localised pollen movement can lead to reductions in a plant’s outcrossing rates and subsequently increases in the incidence of self-fertilisation and geitonogamous pollen transfer (fertilisation of a flower by pollen from another flower on the same plant) (Kwak et al. 1998, Silvertown and Charlesworth 2009). These changes can negatively affect the genetic diversity of a population, elevating the risk of inbreeding depression and subsequently reducing plant fitness and long-term survival (Charlesworth and Charlesworth 1987, Richards 1997, Barrett 1998).

Although much research has been done on how floral resources affect pollinator behaviour, there are still large gaps on how this is manifested at different scales, from the local to landscape level. An additional knowledge gap includes how these scale-dependent changes in behaviour also go on to affect plant-pollinator interactions and the reproductive success of plant species. In this study, I explore how the availability of floral resources at a habitat and landscape (1.5km radius) scale moderates pollinator communities and pollination services to plants. Furthermore, I assess the magnitude of these effects when the availability of floral resources is measured at different spatial scales (1-100m). Using a multi-site field experiment, I introduced experimental arrays of a self-compatible plant which benefits from pollinator triggered pollen release (*Vicia faba)* (Aouar-Sadli et al. 2008). At each array, I determined the potential pollinator visitors and measured intra-population pollen movement and plant reproductive success. I hypothesise:

i) The activity density and species richness of potential pollinator visitors is inversely related to the availability of floral resources at a habitat scale as available pollinators are effectively ‘diluted’. No relationship however is observed at a landscape scale (1.5km radius). The relationship with pollinator activity density and richness is strongest where floral resources are measured at scales of 1-50m;
ii) The community weighted mean IT span of potential pollinator visitors is positively related to the availability of floral resources at a habitat scale given the nature of plant species comprising wildflower mixes. No relationship however is observed at a landscape (1.5km radius) scale as plant heterogeneity increases. The relationship with IT span is strongest where floral resources are measured at scales of 1-50m;
iii) Intra-population pollen movement is inversely related to the availability of floral resources at a habitat scale. No relationship however is observed at a landscape (1.5km radius) scale. This relationship

parallels changes to potential pollinator visitors and is strongest where floral resources are measured at scales of 1-50m;

iv) There is no relationship between the number of seeds produced and the availability of floral resources at a habitat or landscape (1.5km radius) scale, or indeed, when floral resources are measured within 1-100m radii given the self-compatibility system of *V. faba*. Driven by a negative relationship between self-fertilisation and fitness, the weight of seeds (both mean seed weight and reproductive biomass) however, is inversely related to the availability of floral resources at a habitat scale, though no relationship is observed at a landscape (1.5km radius) scale. This relationship with seed weight is strongest where floral resources are measured at scales of 1-50m, in line with changes to intra-population pollen movement.

3.3 Methods and materials

3.3.1 Experimental site and study system

The experiment was conducted across four sites in Oxfordshire and Buckinghamshire, UK, separated by a maximum distance of 26 miles: Widmere farm, Marlow (0°48′2.15″W, 51°35′44.51″N); The Waddesdon estate, Waddesdon (0°55′54.52″W, 51°50′45.9″N); The Earth trust, Little Wittenham (1°11′20.69″W, 51°37′50.16″N) and The Hillesden estate, Hillesden (1°00′01″W, 51°57′16″N); Fig.3.I). Sites were situated on a mixture of acid loam, clay and chalk soils with relatively low topography (Cranfield University 2017). The predominant land use across all sites was arable agriculture characterised by a cropping rotation of winter cereal followed by either oil seed rape or field beans. Each site included areas of semi-natural habitat (defined as: grassland; woodland and any ‘wildlife friendly’ habitat implemented under the English agri-environment scheme). These semi-natural habitats often comprise relatively good cover of mixed flowers, so the extent of semi-natural habitat at a landscape scale was used as a proxy for the availability of floral resources. This was measured using ArcGIS by creating a 1.5km radius from the centre of each site to allow for the maximum foraging range of the majority pollinators (Osborne et al. 1999, Carvell et al. 2012). Sites were then ground-truthed and the total area of land within the radius comprising semi-natural habitat was calculated. Sites were selected based on initial scoping to identify locations which varied in landscape composition; two sites had a relatively high proportion of semi-natural habitats (Widmere Farm = 0.547; Waddesdon Estate = 0.530) while the other two sites had half this amount; (Earth Trust= 0.290; Hillesden Estate = 0.224; t = 4.82, df = 3, p = 0.017; Fig.3.I).

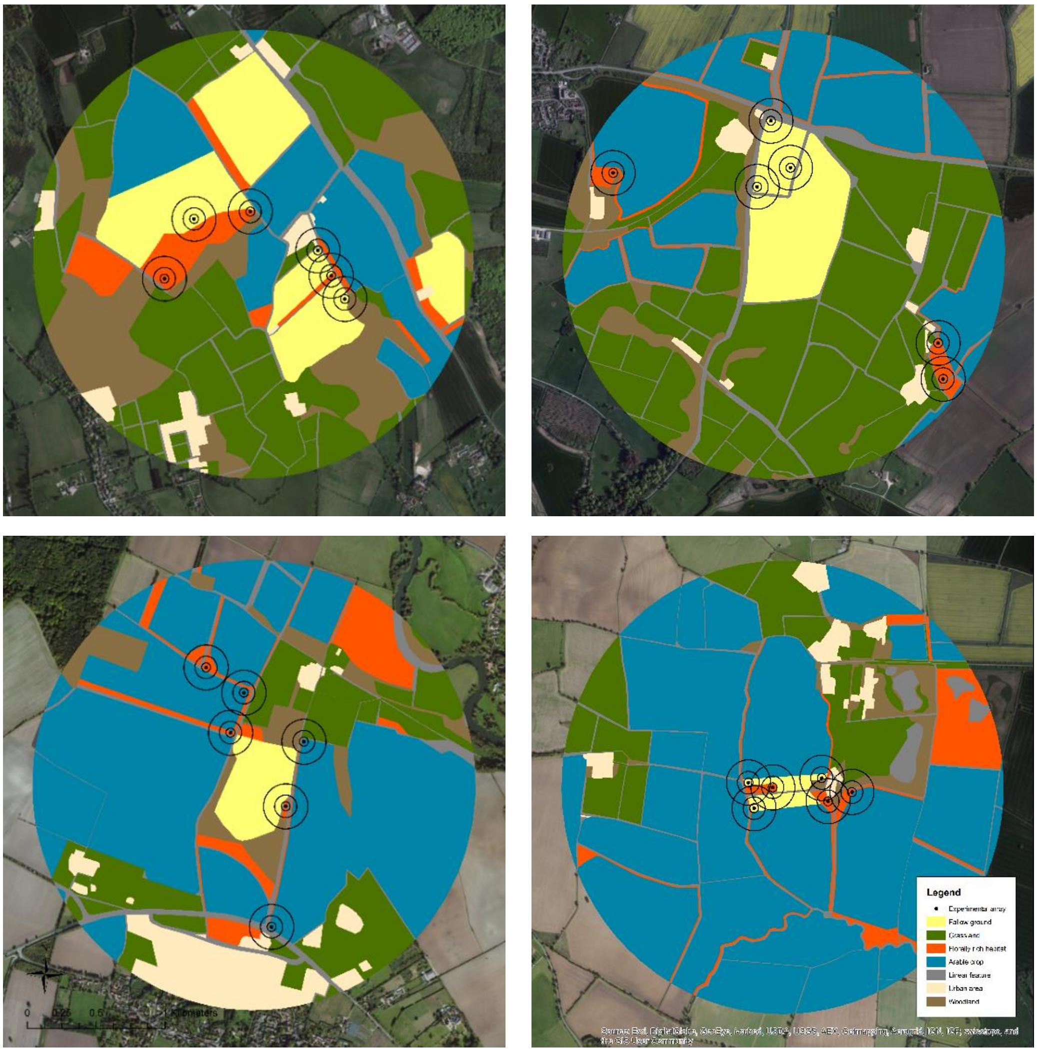

Figure 3.I The landscape composition of the four sites used for this study, with a 20m, 50m and 100m radius surrounding each experimental array illustrated. From top left to bottom right (in decreasing order of semi-natural habitat cover at a landscape scale); Widmere farm, The Waddesdon estate, The Earth trust and The Hillesden estate. Semi-natural habitats comprised: Grassland (green), Florally rich habitats (orange) and woodland (brown).

To test the effects of habitat and landscape composition on pollinator and plant dynamics *Vicia faba* L. (Fabaceae) plants were introduced to each of the experimental sites. Two cultivars displaying comparable phenology were used: Arthur and Wizard. The use of Arthur and Wizard cultivars enabled discrimination of pollen movement through a dominance inheritance of hilum (seed connection scar) colour. Wizard plants produce seeds with a white hilum (homozygous recessive) and Arthur plants produce seeds with a black hilum (homozygous dominant) (Holden and Bond 1960, Bishop et al. 2016). Given that hilum colour is maternal, any seeds produced by Wizard plants which exhibit black hilum (expressed in second generation seeds) will therefore be the product of outcrossing with Arthur plants (Bishop et al. 2016). *Vicia faba* is a nectar producing plant characterised by small, white flowers (Fig.3.II). It exhibits a self-compatible mating system, making it capable of self-fertilisation, though the importance of pollinator visitors has been highlighted for both triggering pollen release and enabling outcrossing (Aouar-Sadli et al. 2008). Outcrossing rates in *V. faba* are variable and have been demonstrated to account for 29.5-69.8% of mating events under field conditions (Holden and Bond 1960). Pollinators include species from the families, Apidae, Megachilidae, Halictidae and Syrphidae (Aouar-Sadli et al. 2008, Garratt et al. 2014); though of these, *Bombus spp.* are recognised to be the most efficient pollinators of *V. faba* (Garratt et al. 2014).

Plants were grown from seed (seed source: Aberystwyth University) in compost-filled seed trays under glasshouse conditions (20°C during the day, 16°C at night, with 12 hours of light and 12 hours of dark) and were transferred to 1L pots when at the seedling stage. In June 2016, *V. faba* plants were introduced to form experimental arrays across each site where they were left for a 16-day period to allow for multiple pollination events (flowers remain open for three days; Osborne et al. 1997). Arrays comprised of six plants, separated by 1m and arranged in a triangle, with three central Wizard plants and three outer Arthur plants (Fig.3.II). These arrays were introduced in two different habitats embedded within each site; a florally rich habitat (a standardised sown wildflower mix) and a habitat devoid of flowers (either a cereal crop (within tramlines) or on tilled, fallow ground) (Fig.3.II). The wildflower habitats, typically introduced as part of the English agri-environment scheme (Carvell et al. 2007), comprised a common mix of approximately 25 species which included key species like *Trifolium pratense*, *Centaurea nigra* and *Leucanthemum vulgare*. Each array was positioned 15m from any habitat edge to standardise the proximity to the neighbouring habitat. The experiment was spatially replicated, with arrays positioned in three locations within both habitat types in each of the four sites. All arrays were positioned in separate habitats where possible or separated by a distance of over 100m to minimise inter-array movement (Matter et al. 2013). Care was taken to ensure that a distance exceeding 500m was maintained between arrays and any field bean crop.

Figure 3.II. From top left to bottom right; (1). A Vicia faba flower and (2) an experimental array comprising six V. faba plants (inner three plants constituted Wizard plants and outer three plants constituted Arthur plants) introduced on (a) tilled fallow ground and (b) within a wildflower mix. Red crosses demonstrate where quadrats were dropped at each experimental array when quantifying local floral abundance

The availability of floral resources immediately surrounding each experimental array was measured at the start of the experiment (Table 3.I and Appendix 1: Plant list) and was calculated as the total number of floral units (e.g. one *Taraxacum officinale agg.* flower head represented one floral unit) averaged across three 0.5m² quadrats, dropped each side of the triangular array (Fig.3.II) (henceforth '1m radius'). The availability of floral resources was then measured at different 'local' spatial scales (20-100m) following protocols used to determine landscape composition but instead by specifying different radii surrounding the experimental arrays (Table 3.I).

Table 3.I The availability of floral resources when measured at different radii from experimental arrays, averaged across sites and between habitat types. Floral resources at a 1m scale are measured as the average number of floral units (Flower heads) in 0.5m² Comparably, floral resources within 20-100m radii are measured as the proportion of land within the radius comprising semi-natural habitats.

Spatial scale	Site	Habitat	The availability of floral resources (mean ± SE)
1 m	Widmere farm	Florally rich	29.44 ± 5.62
		Florally poor	0.00 ± 0.00
	The Waddesdon estate	Florally rich	23.22 ± 9.59
		Florally poor	0.00 ± 0.00
	The Earth trust	Florally rich	20.44 ± 6.60
		Florally poor	0.00 ± 0.00
	The Hillesden estate	Florally rich	51.44 ± 24.49
		Florally poor	0.00 ± 0.00
20m	Widmere farm	Florally rich	0.80 ± 0.11
		Florally poor	0.04 ± 0.04
	The Waddesdon estate	Florally rich	0.90 ± 0.04
		Florally poor	0.00 ± 0.00
	The Earth trust	Florally rich	0.67 ± 0.08
		Florally poor	0.08 ± 0.04
	The Hillesden estate	Florally rich	0.98 ± 0.01
		Florally poor	0.06 ± 0.03
50m	Widmere farm	Florally rich	0.60 ± 0.16
		Florally poor	0.20 ± 0.05
	The Waddesdon estate	Florally rich	0.68 ± 0.12
		Florally poor	0.03 ± 0.02
	The Earth trust	Florally rich	0.35 ± 0.08
		Florally poor	0.25 ± 0.12
	The Hillesden estate	Florally rich	0.59 ± 0.13
		Florally poor	0.20 ± 0.05
100m	Widmere farm	Florally rich	0.52 ± 0.22
		Florally poor	0.20 ± 0.03
	The Waddesdon estate	Florally rich	0.53 ± 0.09
		Florally poor	0.15 ± 0.08
	The Earth trust	Florally rich	0.23 ± 0.04
		Florally poor	0.34 ± 0.12
	The Hillesden estate	Florally rich	0.40 ± 0.13
		Florally poor	0.22 ± 0.05

3.3.2 Pollinator communities

Potential pollinator visitors within the vicinity of *V. faba* arrays were quantified using pan traps (Westphal et al. 2008). Pan traps have been used to describe pollinator species richness and activity density (Westphal et al. 2008) as well as providing a surrogate measure of visitation (Ricketts et al. 2008). However, this survey method has been recognised to exhibit bias (Roulston et al. 2007) with the attractiveness of pan traps depending upon habitat and landscape context (Baum and Wallen 2011). Pollinators are less likely to encounter traps when floral resources are abundant and more likely to encounter traps when floral resources are scarce i.e. capture rates are proportional to visitation rates per unit flower area (Veddeler et al. 2006). I exploited this phenomenon to measure the attractiveness and pollinator activity density at the experimentally rare plant arrays located within different habitats.

Traps comprised three water-filled circular plastic bowls (80x200mm) painted with non-toxic fluorescent paint (1 yellow, 1 blue and 1 white; UV Gear, UK) placed in the centre of each experimental array. Traps were deployed on two occasions during the study (total traps = 48) and were placed out in the four sites in a randomised order, between 0930 and 1700 over a two-day period (i.e. two sites per day). After traps were deployed for 48 hours, the insect catch was strained through fine muslin and stored in 70% ethanol until sorting and identification. Insects from groups known to pollinate *V. faba* (Hymenoptera: Apoidea and Diptera: Syrphidae) were counted and identified to species level. Given the positive relationship between body mass and both foraging distance (Greenleaf et al. 2007) and pollen deposition rate in Apoidea (Larsen et al. 2005), the distance between the wing bases (Inter-tegular span, henceforth 'IT' span), which provides a proxy for dry body mass in Apoidea (Cane 1987), was determined. This relationship is less clear in Lepidoptera and Syrphidae, however inter-tegular span has previously been used as a measure of dry body mass in the latter given observed correlations (Folkö 2014). I therefore adopted this measure to maintain consistency, but it is important to note the potential inaccuracy in estimations due to differences between pollinator groups. The IT span (mm) was therefore measured for up to five insects from each species of pollinator within groups observed to visit *V. faba* plants (depending upon the number caught) using digital callipers, From this, an average IT span (body size) was calculated for each species observed.

3.3.3 Intra-population pollen movement

Upon reaching reproductive maturity (i.e. flower production), all open flowers on Wizard plants were removed and one bud on each plant was covered in a fine muslin to measure the incidence of auto-pollination. Plants were subsequently introduced into pre-marked locations over a two-day period (two sites per day). Following the 16-day study period, Wizard plants were collected in (Arthur plants were discarded) and all unopened buds were removed to avoid confusion with flowers subjected to field conditions. Plants were then stored under glasshouse conditions (as above) until fruit maturation. Following the maturation of fruit, a sub sample of three seeds from up to three pollinator exposed fruit from each plant (depending on the number of fruits produced) were sown and were stored under glasshouse conditions (as above). When at reproductive maturity, flowers from second generation plants were continually ‘tripped’ (agitated) to encourage self-fertilisation (given that hilum colour is maternally expressed). Upon maturation, one fruit from each second generation plant was then examined (given that all fruit produced by a maternal plant will produce seeds exhibiting the same hilum colour). For each experimental array the number of the second generation plants comprising black hilum and the number comprising white hilum were then recorded. This system precludes discrimination of Wizard to Arthur movements as well as Wizard to Wizard or Arthur to Arthur movements (Bishop et al. 2016) and thus, here, measures levels of detected intra-population pollen movement.

3.3.4 Plant reproductive success

The number of viable seeds was counted for each fruit produced by field exposed Wizard plants (described above) as an indicator of the implications of the availability of floral resources at habitat and landscape scales on the reproduction of self-compatible plants. Given that self-fertilisation has been associated with reductions in plant fitness, seed weight was determined from each fruit to provide a proxy for plant fitness (Tremayne and Richards 2000). Seeds were placed in a sealed paper envelope and oven dried at 80°C for seven days to obtain a standardised desiccation state (Bishop et al. 2016). Upon drying, the total seed set of each fruit was weighed to obtain the biomass of reproductive output and then divided by the total number of seeds to obtain mean seed weight.

3.3.5 Statistical analysis

A combination of generalised linear mixed effects models (GLMMs) and linear regression models were used to analyse the effects of the availability of floral resources at different spatial scales on pollinator communities, intra-population pollen movement and plant reproductive success. When analysing pollinator communities, the activity density (n = 48) of pollinators was modelled using a GLMM with a Poisson error distribution. In contrast, the community weighted mean IT span (the mean IT span for each experimental array: n = 24) and the species richness of pollinators (n = 24) were modelled using linear regression models with Log-normal (to account for non-integers) and Gaussian error distributions respectively, given that no variance was observed in the random effects. In the latter model, data was pooled across trapping dates to avoid overestimation of species richness and was standardised (subtracting the mean and dividing by the standard deviation; z-scores) prior to analysis to account for differences in pollinator catch. Intra-population pollen movement (n = 22 given that progeny from two arrays produced no fruit) was modelled using a GLMM with a Binomial error distribution, where the incidence of intra-population pollen movement was expressed as a ratio between the number of intra-population movements and the total number of progeny examined from each array. Plant reproductive success variables were similarly analysed using GLMMs. Seed set (n = 207) was modelled with a Binomial error distribution where the number of seeds produced by plants subjected to field conditions was modelled against the maximum seed set produced within a fruit during the experiment (given that seed set was constrained at low numbers). Seed weight (n = 207) together with reproductive biomass (n = 207) were modelled with Log-normal error distributions (for reasons mentioned above). The relationship between intra-population pollen movement and pollinator activity density, species richness and community weighted mean IT span (body size) was modelled separately using a generalised linear model, with Binomial error distributions (as above).

Fixed effects comprised of habitat type (florally rich and florally poor) and the availability of floral resources at a landscape scale (1.5km radius). Models then explored the availability of floral resources at different spatial scales (1- 100m radii). Response variables were modelled against each fixed effect individually (given their interdependence). Array (nested within site) was fitted as a random effect in all GLMMs to account for the spatial structure of the experimental design. In addition, plant (nested within array) was included as a random effect when analysing plant reproductive success models to account for between plant variation. Where present, over-dispersion in the models was controlled for by fitting an observational level parameter to the random effects (Harrison 2014). The minimum adequate model was then determined using Laplace approximation (suitable for <3 random effects; (Bolker et al. 2009)) and was compared with a null model (containing an identical random effects structure) using an ANOVA. Likelihood ratio test statistics (LRT) were calculated to provide an indication of the importance of floral resources at different spatial scales on each response variable.

Model residuals were then analysed using Moran’s I test with an inverse distance weighting to determine whether models had sufficiently accounted for any spatial autocorrelation within the data. Low values were obtained in all cases ($-0.006 > I < -0.01$) and no models exhibited spatial autocorrelation ($0.33 > p < 0.92$). This indicates that data was independent and not correlated with the distance between sites. All analyses were conducted in R computer software (version x64; R Core Team 2013) using the following packages: lme4 (Bates et al. 2015), ape (Paradis et al. 2004) and ggplot (Wickham 2009).

3.4 Results

3.4.1 Pollinator communities

A total of 1203 potential *V. faba* pollinators comprising 53 identified species were recorded across all surveys (Potential pollinators included groups observed to visit *V.faba* (Apoidea and Syrphidae) but visiting species were not verified by visitor observations and so may not all represent *V.faba* pollinators; Appendix 3: Pollinator list). Of these, pollinator communities (per unit area) associated with each experimental array varied in their degree of similarity (ranging from 0-66.67%), with variations predominantly driven by habitat type rather than site location (Fig.3.III). The activity density of pollinators was affected by habitat type, where fewer pollinators were caught within pan traps located in florally rich habitats (mean ± SE Florally rich habitat = 5.29 ± 1.03, Florally poor habitat = 44.83 ± 9.58 GLMM z = -6.854, df = 43, p < 0.001). Of these pollinators, fewer species were observed in florally rich habitats (mean ± SE Florally rich habitat = 6 ± 1.02, Florally poor habitat = 10.75 ± 1.060 GLMM t = -3.237, df = 22, p = 0.004). The activity density and richness of pollinators however were not affected by the availability of floral resources at a landscape scale (1.5km radius) (p = 0.755 and p = 0.958 respectively). When analysing the effects of floral resources at different spatial scales the activity density of pollinators was negatively correlated with floral resources at a 1m radius (GLMM z = -4.72, df = 43, p < 0.001), a 20m radius (GLMM z = -5.80, df = 43, p < 0.001) and a 50m radius (GLMM z = -2.21, df = 43, p = 0.027) from the survey location (Fig.3.IV). Similarly, the species richness of potential *V. faba* pollinators was negatively correlated with floral resources at 1m radius (LM t = -2.95, df = 22, p = 0.007) and a 20m radius from the survey location (LM t = -2.96, df = 22, p = 0.007) (Fig.3.V).

The community weighted mean IT span (body size) of pollinators within the vicinity of experimental arrays was higher in communities located within florally rich habitats (mean ± SE (mm) Florally rich habitat = 3.87 ± 0.25, Florally poor habitat = 2.53 ± 0.06 GLMM $t = -6.158$, $df = 19$, $p < 0.001$). This was not however affected by floral resources at a landscape scale ($p = 0.409$). When measuring the effect of floral resources at different spatial scales, a positive relationship was observed between the pollinator community weighted mean IT span (body size) and floral resources at a 1m radius (LM $t = 3.08$, $df = 22$, $p = 0.005$, $R^2c=0.29$), a 20m radius (LM $t = 5.02$, $df = 22$, $p < 0.001$, $R^2c=0.52$) and a 50m radius (LM $t = 2.31$, $df = 22$, $p = 0.031$, $R^2c=0.19$) from the survey location (Fig.3.VI). The availability of floral resources within a 20m radius from the pan trap had the greatest effect on pollinator activity density and community weighted mean IT span, though all variables affected pollinator richness to a similar degree (inferred by the LRT statistic; Table.3.II). No effect was observed in any of the response variables when analysing floral resources at a 100m radius from the survey locations (Table.3.II).

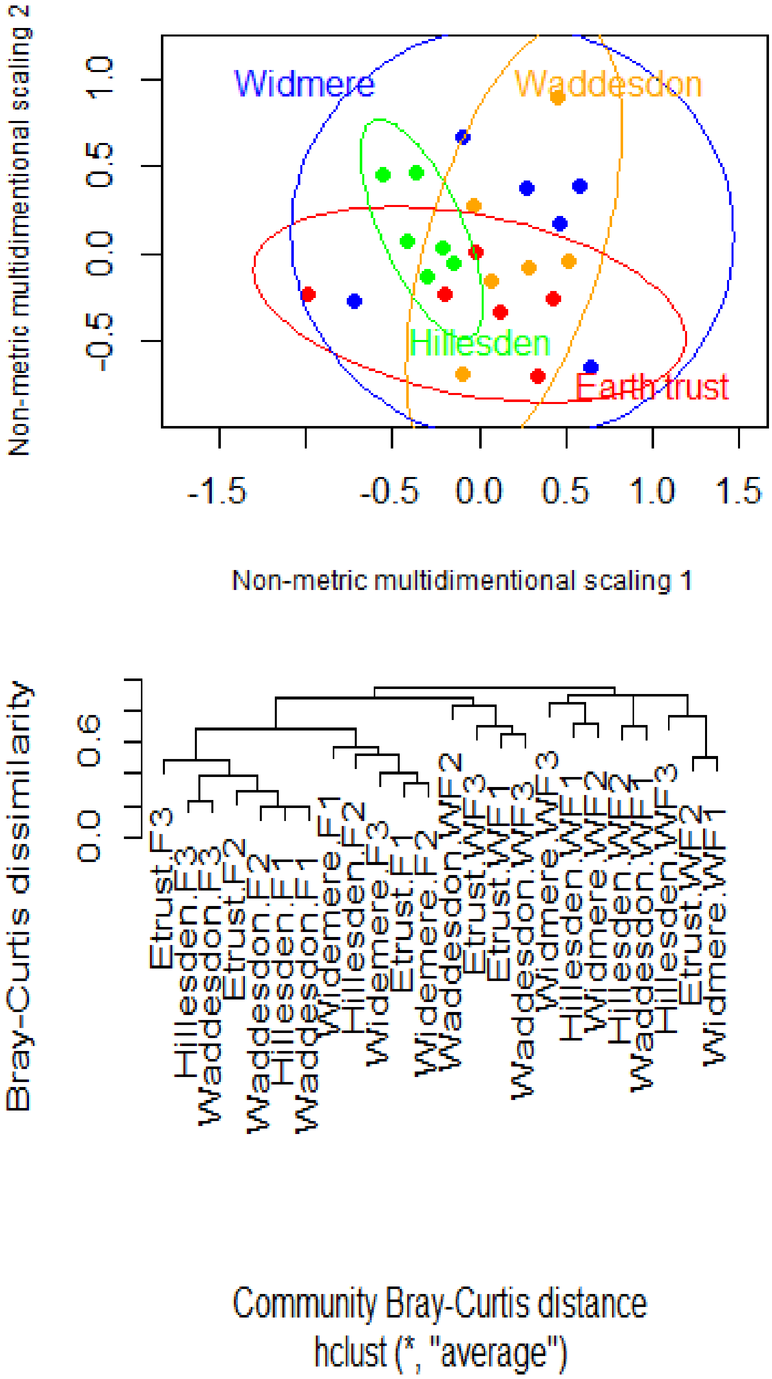

Figure 3.III. The composition of pollinator communities at each experimental array within each of the four sites, using Bray-Curtis dissimilarity index. From top to bottom; 1. An ordination plot using non-metric multidimensional scaling to illustrate dissimilarity in pollinator communities and 2. A cluster dendrogram using an agglomerative hierarchical clustering algorithm, where communities with high similarity are positioned closer together (Labels F1, F2 and F3 represent arrays located within fallow ground whereas labels WF1, WF2 and WF3 represent arrays located within wildflower patches).

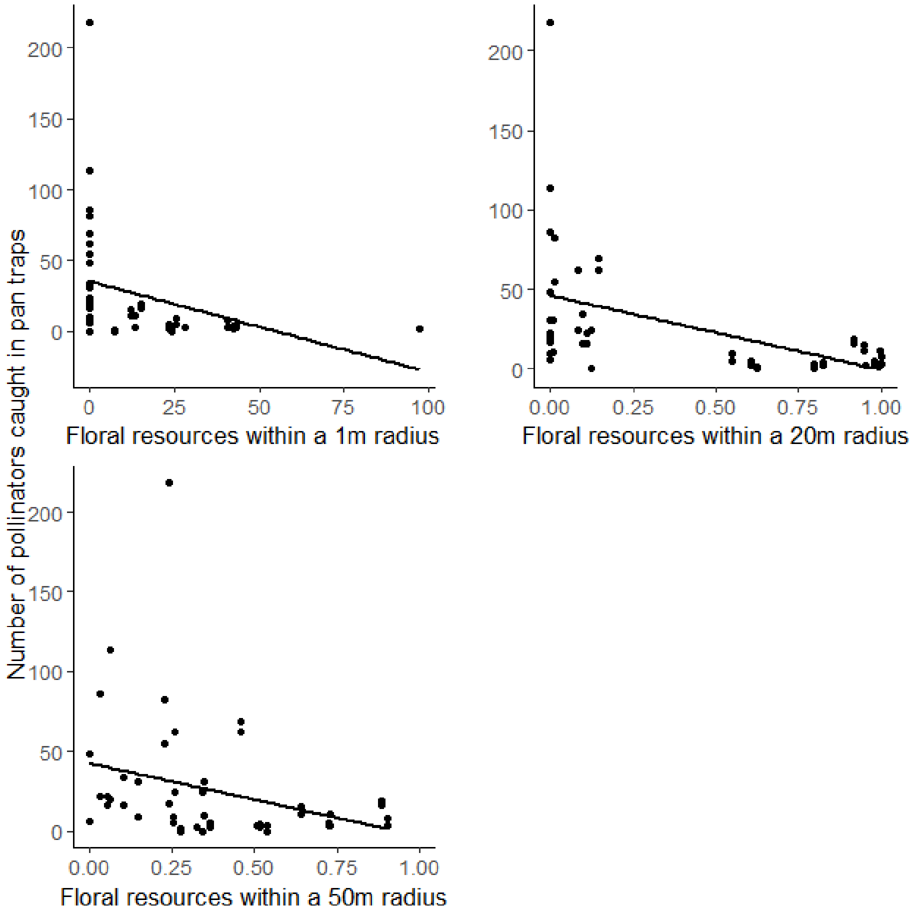

Figure 3.IV. From top left to bottom right; the total number of pollinators caught in pan traps alongside each experimental array in relation to floral resources at a 1m (the number of floral units), a 20m and a 50m (the proportion of florally rich habitats) radius from the survey locations.

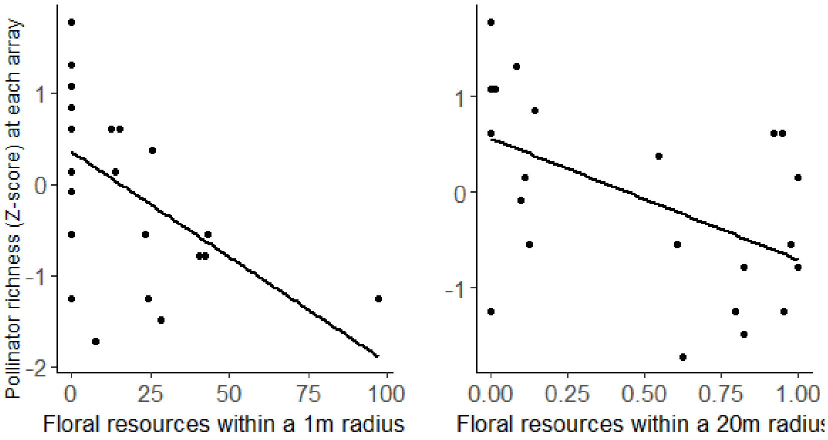

Figure 3.V. From top left to bottom right; The number of pollinator species (standardised to account for differences in catch) recorded in pan traps at each experimental array in relation to floral resources at a 1m (the number of floral units) and a 20m (the proportion of florally rich

habitats) radius from survey locations. Points represent the cumulative standardised species richness across the two trapping periods.

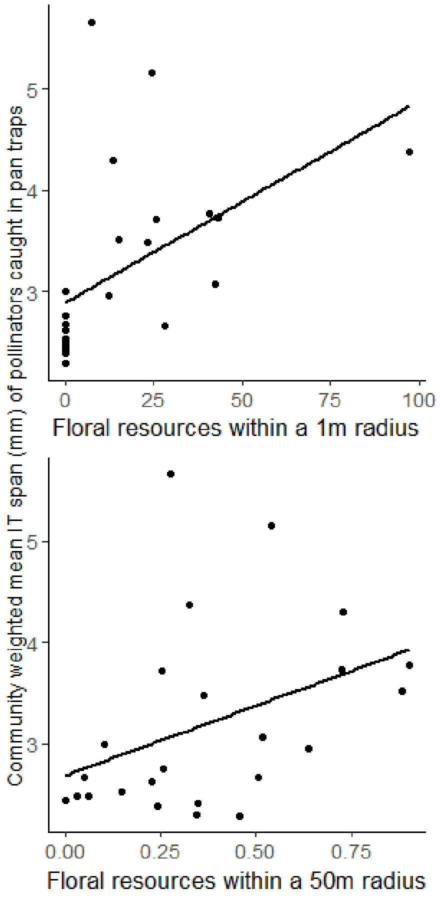

Figure 3.VI From top left to bottom right; The mean community inter-tegular span (body mass) of pollinators caught in pan traps at each experimental array in relation to floral resources at a 1m (the number of floral units), a 20m and a 50m (the proportion of florally rich habitats) radius from the survey locations.

3.4.2 Intra-population pollen movement

Detected intra-population pollen movement was high in this study, with movement between conspecific plants within an array accounting for 40.95% of seed paternity. Rates were however variable across experimental arrays (range: 0-100% of progeny examined; Appendix 4). When analysed as a ratio, intra-population pollen movement was negatively correlated with the availability of floral resources within a 1m radius (GLMM z = -1.97, df = 18, p = 0.049, $R^2C=0.07$; Fig.3.VII). However, this relationship was not significant when floral resources were measured at a habitat (p = 0.908) or landscape (p

= 0.870) scale or indeed, within 20-100m radii from the experimental array (Table 3.II). Although no significant relationship was observed between intra-population pollen movement and the richness or community weighted mean IT span (body size) of pollinators (p = 0.101 and p = 0.354 respectively), there was a weak positive trend between intra-population pollen movement and the activity density of pollinators (p = 0.089).

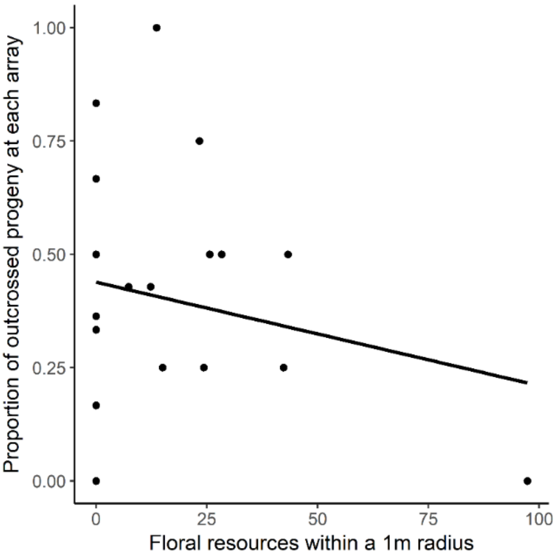

Figure 3.VII The relationship between the availability of floral resources within a 1m (the number of floral units) radius from experimental arrays and intra-population pollen movement at each experimental array, when analysed as a ratio between detected intra-population pollen movement and the total number of progeny analysed.

3.4.3 Reproductive success

The number of seeds produced by field exposed *V. faba* plants did not vary considerably between fruit (mean ± SE 2.85 ± 0.08, range 1-5). This was reflected by the absence of a relationship between seed set and both habitat type and the availability of floral resources at a landscape scale (p = 0.362 and p = 0.133 respectively). Similarly, when analysing the effect of floral resources at different spatial scales, seed set was not affected by floral resources at 1-100m radii from the experimental arrays (Table 3.II). Seed weight and reproductive biomass, similarly, were not affected by habitat type (p = 0.811 and p = 0.483 respectively) or the availability of floral resources at a landscape scale (p = 0.787 and p = 0.471 respectively). This was consistent when analysing floral resources at 1-100m radii from the experimental arrays (Table.3.II). The estimation of auto-pollination was precluded given the high abortion rate of pollinator excluded *V. faba* flowers. Given that *V. faba* plants have been shown to be capable of auto-pollination, this suggests a possible bag effect which should be born in mind when conducting controls in the future.

Table 3.II. Model outputs for each response variable analysed against the availability of floral resources at different spatial scales from experimental array's (1-100m radii).

Response variable	Spatial scale at which floral resources were measured	AIC	LRT statistic (χ^2/ F)	P-value	Slope	Upper 95% confidence interval	Lower 95% confidence interval
Pollinator richness	1m	65.08	8.72	0.01*	-0.02	-0.01	-0.04
	20m	65.06	8.74	0.01*	-1.27	-0.38	-2.16
	50m	72.01	1.01	0.33	-0.80	0.85	-2.44
	100m	73.08	0.01	0.94	0.08	2.18	-2.02
Pollinator activity density	1m	382.29	17.48	<0.001*	-0.04	-0.06	-0.03
	20m	378.35	21.42	<0.001*	-2.38	-1.53	-3.24
	50m	393.75	6.02	0.03*	-2.09	-0.15	-4.04
	100m	397.78	1.99	0.55	-0.79	1.90	-3.47
Pollinator 'IT' span	1m	0.16	9.48	0.01*	0.01	0.01	0.002
	20m	-9.55	25.18	<0.001*	0.46	0.64	0.27
	50m	3.56	5.32	0.03*	0.44	0.83	0.04
	100m	6.64	2.03	0.17	0.36	0.89	-0.16
Intra-population pollen movement	1m	70.28	4.19	0.05*	-0.023	-0.001	-0.05
	20m	74.26	0.21	0.64	-0.32	1.20	-1.66
	50m	74.37	0.10	0.75	0.38	3.06	-2.03
	100m	73.44	1.03	0.31	1.78	5.70	-1.75
Plant seed set	1m	624.55	0.44	0.5	-0.002	0.01	-0.01
	20m	623.25	1.74	0.17	-0.26	0.14	-0.65
	50m	639.69	1.97	0.14	-0.43	0.19	-1.03
	100m	622.21	2.79	0.08	-0.7	0.13	-1.50
Reproductive Biomass	1m	492.51	1.34	0.47	-0.51	0.6	-1.54
	20m	492.04	1.24	0.31	-0.18	0.15	-0.52
	50m	491.74	1.54	0.26	-0.31	0.2	-0.85
	100m	492.00	1.28	0.3	-0.39	0.32	-1.14
Seed weight	1m	300.96	0.3	0.63	-0.001	0.003	-0.005
	20m	301.08	0.18	0.72	-0.03	0.15	-0.24
	50m	300.77	0.49	0.54	-0.09	0.19	-0.41
	100m	301.19	0.08	0.81	-0.05	0.34	-0.48

3.5 Discussion

3.5.1 The effects of the availability of floral resources at different spatial scales on pollinator communities

Findings from this study demonstrate reductions in the activity density and richness of pollinators (per unit area) in response to local floral availability, particularly within a 20m radius from survey locations. This adds a more nuanced understanding to existing research showing a positive relationship between the availability of floral resources and the abundance, richness and productivity of visiting pollinators (Steffan-Dewenter et al. 2002, Kremen et al. 2004, Hines and Hendrix 2005, Ricketts et al. 2008, Couvillon et al. 2014, Jonsson et al. 2015, Sarospataki et al. 2016). These studies have similarly used semi-natural habitat as a proxy for floral cover and therefore inconsistencies are not expected to be based on the measurement of floral availability. Instead, inconsistencies between studies can be seen to arguably be driven by the spatial scale at which the landscape was parameterised. Previous studies have focused on the effects of floral resources at radii of 250-3000m from a plant population (Steffan-Dewenter et al. 2002). Indeed, in this study, I focus on floral resources within 1-1500m radii from experimental arrays to reflect the range of pollinator foraging distances and the tendency for localised pollinator movement. This suggests that the availability of floral resources switches from having a negative effect on pollinator activity density to having a positive effect at spatial scales between 50m and 250m from survey locations. This pattern has been demonstrated in a previous study (Jha and Vandermeer 2009) and reflects a behavioural concentration and dilution effect operating at different spatial scales. Indeed, where the landscape comprises a high availability of floral resources at greater spatial scales (<250m), the attractiveness of an area is enhanced, resulting in increased pollinator activity density and subsequently, visitation at the individual plant level. In this study however, consistent with previous research, findings suggest that as floral resources increase at smaller, habitat scales (<50m radius from the survey location) a saturation point is reached where pollinator communities are insufficient to exploit all available resources (Veddeler et al. 2006, Sjodin 2007). Pollinator visits per plant are thus effectively ‘diluted’ (Tscharntke et al. 2012). Lower pollinator availability coupled with competition from co-flowering plants will, in turn, result in lower ‘per floral unit’ visitation at a local scale (Ghazoul 2006). It is worth noting that the concentration-dilution effect observed in this study could indeed be an artefact of the attractiveness of pan traps being dependent upon habitat and landscape context. To the same end, this would imply that the attractiveness of a plant would also be dependent on habitat and landscape context and thus it can be argued that the patterns discussed would apply to isolated plant populations.

Pollinators vary in their response to floral resources and studies which have discriminated between pollinator taxa (e.g. honeybees, bumblebees and solitary bees) have indeed observed large differences in their response to the availability of floral resources (Steffan-Dewenter et al. 2002, Carre et al. 2009). While *Apis mellifera*, *Bombus* spp. and solitary bees have all been shown to respond positively to a high availability of floral resources (Steffan-Dewenter et al. 2002, Jauker et al. 2009), syrphids have contrastingly been shown to increase in abundance with distance from habitats of high floral cover (Jauker et al. 2009). In relation to spatial scale, *Apis mellifera* and *Bombus* spp., which are central place foragers and highly mobile, have been shown to respond to landscape composition at far greater scales than solitary bees, which are less mobile (Steffan-Dewenter et al. 2002, Westphal et al. 2003, Hines and Hendrix 2005, Jha and Vandermeer 2009, Sarospataki et al. 2016). This may provide explanation for inconsistencies between findings. In this study, I was however interested in the general response of all potential pollinators, discriminating instead between pollinator community function in order to make inferences on local pollen movement. Pollinator body size, through positive associations with pollen deposition and foraging distance, provides a measure of the function of pollinator communities (Larsen et al. 2005, Greenleaf et al. 2007). In this study, findings indicate an effect of habitat type on the mean IT span (a proxy for body size) of a pollinator community, where the availability of floral resources at a 20m radius from survey locations had the greatest effect on the size of visiting pollinators. This suggests that larger pollinators (e.g. *Bombus* spp.) may increase in abundance in relation to the availability of floral resources at a local scale. Given that *Bombus* spp. have been shown to be the most efficient pollinator of *V. faba* (Garratt et al. 2014), these higher numbers in relation to a high availability of floral resources, may supersede the advantage of high activity densities of more generalist taxa, such as syrphids (which dominate pollinator communities in this study) in habitats of low floral resource availability.

3.5.2 The effects of the availability of floral resources at different spatial scales on pollinator foraging behaviour

Findings here indicate a negative relationship between intra-population pollen movement and floral resources at a local scale (1m radius from experimental arrays). By demonstrating the tendency of pollinators to exploit nearby flowers when resources are scarce, these findings reflect energy efficient foraging behaviour. It is however worth drawing attention to the poor model fit (R^2=0.07; Table.3.II) and the importance of outliers in driving these conclusions, which, in this case was the result of a particularly high floral abundance in a 1 metre radius from one experimental array. Given that outliers represented real data in this instance, they were left in the analysis. While it is advised that habitats of high floral abundance be replicated further to verify findings, these results are consistent with previous research observing pollinators to forage in a manner which reduces the energy loss associated with movement (Heinrich 1979). When foraging within fragmented landscapes, pollinators increased both the time spent and the number of flowers visited within a patch (Goverde et al. 2002), often reverting back into patches (Osborne and Williams 2001) and revisiting flowers (Cresswell 2000). In this study, this led to increased pollinator activity density and pollen transfer between plants when surrounded by fewer competing flowering plant species. Conversely, reductions in foraging distance and increased localised pollen movement have similarly been observed when the availability of floral resources is high and less fragmented at landscape scales (Danner et al. 2016, Redhead et al. 2016). Consequently, we might expect the availability of floral resources at a larger spatial scale to drive local pollen movement. In this study, however, no relationship was detected between intra-population pollen movement and the availability of floral resources at a landscape scale (1.5km radius from experimental arrays), suggesting that local pollen movement is mediated by competition between plants for pollinators and pollinator constancy.

Constancy is an energy efficient foraging strategy thought to reduce handling costs associated with switching between plant species (Chittka et al. 1999). Indeed, pollinators have been shown to exhibit constancy to flower species (Fontaine et al. 2006) as well as floral patches (Osborne and Williams 2001, Dorchin et al. 2013), though this varies with the generalist nature of species (Ranta and Lundberg 1981) and the sex of visiting pollinators (Carvell et al. 2007). Pollinator foraging does however display density dependence (Rathcke 1983). In habitats comprising a scarcity of flowers, pollinators are expected to expand their diet and increase visitation to other plant species (Kwak et al. 1998). Indeed, this is supported by previous research measuring a reduction in both inter-plant flight distance and constancy as rewards diminish (Thomson et al. 1982). In this study, a higher incidence of intra-population pollen movement within arrays surrounded by a low availability of floral resources suggests a reduction in constancy in response to low forage availability and resource depletion. Given the low number of co-flowering plants within florally poor habitats, this is expected to have resulted in direct intra-population pollen movement in this study. Reduced constancy can have negative implications for plant populations leading to the loss of conspecific pollen and the subsequent deposition of heterospecific pollen on conspecific plants (Brown and Mitchell 2001, Morales and Traveset 2008). Low constancy, however, does not prevent outcrossing and, in fact, pollen carry over between plant visits is often observed (Feldman et al. 2004). A combination of reduced constancy and pollen carry-over can therefore be argued to be driving increased local pollen movement in habitats comprising a low availability of floral resources.

3.5.3 The effects of pollinator communities and pollen movement on plant reproductive success

Variations in pollinator communities and pollen movement in response to local floral availability did not reflect measures of plant reproductive success, which wasn’t affected by the availability of floral resources at any spatial scale. However, in plants comprising a self-incompatibility system, pollen deposition and indeed, seed set has exhibited a positive relationship with the availability of floral resources, both at a habitat scale and within the wider landscape (Kremen et al. 2004, Albrecht et al. 2007, Taki et al. 2010, Martins et al. 2015). Inconsistencies in responses between plants with different mating systems most likely reflects compensation by the self-compatibility system through the use of self-pollen. *Vicia faba* has demonstrated a high capacity to reproduce by self-fertilisation (Holden and Bond 1960) and indeed, previous studies have observed seed production in *V. faba* to be equivalent between plants that were exposed to and excluded from pollinators (Garratt et al. 2014). Similarities in seed set between arrays can therefore be argued to not indicate similarities in pollen transfer rates but instead reflect differences in the incidence of self-fertilisation in response to reduced pollinator activity density and intra-population pollen movement within habitats comprising high floral availability.

Self-fertilisation has been associated with detrimental effects on plant fitness, driven by increases in inbreeding depression observed through reductions in allelic diversity and heterozygosity (Barrett 1998, Goverde et al. 2002). These effects on plant fitness can be illustrated through changes to seed weight, germination rates and plant growth among others (De Clercq et al. 2003, Teixeira et al. 2009, Ferriol et al. 2011). Seed weight, by determining the availability of resources needed for the growth and development of an embryo, is particularly important for plant emergence (Kalisz 1989) and fitness (Black 1958, Silvertown and Charlesworth 2009). Here, findings indicate no effect of potential self-fertilisation on seed weight. This may reflect the nature of the *V. faba* plants used (two inbreeding lines), where cultivars have been artificially selected for high fitness in response to self-fertilisation and have thus been purged of deleterious mutations. Given this, *V. faba* plants may not be expected to show any variation in fitness irrespective of their parentage. Alternatively, this could be the result of low levels of self-fertilisation and instead, mating between Wizard plants. Previous studies exploring differences in the fitness between selfed and outcrossed *V. faba* plants have however consistently demonstrated no negative effect of self-fertilisation (Garratt et al. 2014). The effect of self-fertilisation on plants is however complex and has not produced a consistent pattern across studies (Baskin and Baskin 2015). Inconsistencies between studies may be driven by the life stage at which plant fitness is assessed. Indeed, inbreeding has been shown to be expressed a number of life stages in plants (i.e. at early stages (e.g. seed weight and germination rate), or indeed, at late stages (e.g. number of flowers on stalks); Thiele et al. 2010). Most self-pollinating species have been shown to express inbreeding at later stages whereas outcrossers express it throughout their life cycle (Husband and Schemske 1996). Further research is necessary to assess the effects of self-fertilisation on *V. faba* plants through observing both early and late stage fitness traits in multiple generations.

3.5.4 The implications for landscape management

The management of habitats to enhance plants and pollinators is often targeted at a landscape scale (e.g. the implementation of agri-environment schemes across farms). At these wider scales, the presence of resources can enhance the attractiveness of a habitat to pollinators and thus, positively affect visitation rates and plant reproduction (Steffan-Dewenter et al. 2002). Findings here, however, demonstrate a contrasting effect of floral resources at more local scales on pollinator communities and pollen movement (though only at a 1m radius in the latter). While this displayed no measurable effect on the reproductive success of a self-compatible plant, an abundant, diverse pollinator community and local pollen movement is essential for pollen transfer and thus, plant outcrossing rates. This highlights the importance of factoring local context (within a 50m radius from the plant population) into the design of management options aimed at ensuring the long-term survival of plant populations. Focus should be placed on increasing the attractiveness of plant populations, ensuring isolated plants are better able to compete with competitive co-flowering plants. This can be achieved by: enhancing the size and density of plant populations (Mayer et al. 2012); introducing co-flowering plants with complementary phenotypes so to add a facilitatory effect (Ghazoul 2006) and, if the focus is on re-introductions, populations should be positioned where competition from co-flowering plants is minimised (e.g. where floral resources are low within a 50m radius of the population) (Baskett et al. 2011). Furthermore, this study emphases the importance of accounting for spatial scale in monitoring the success of management schemes in order to gain an accurate account of the response of different pollinator taxa. This is of particular importance when plants rely on specialist pollinators.

3.5.5 Summary

Findings from this study illustrate a negative effect of floral resource availability on pollinator communities and pollen movement. The activity density and richness of potential pollinator visitors is reduced in response to a high abundance of floral resources at local spatial scales (<50m), driven by a dilution effect. Under these conditions, pollinators are expected to exhibit a high degree of constancy, thus reducing ‘per floral unit’ visitation rates and pollen transfer between conspecific plants when present at low frequency. Disruptions to pollen transfer can be seen by parallel reductions in intra-population pollen movement in relation to a high availability of floral resources. The absence of an effect on the reproductive success of *V. faba* plants however suggests minimal implications at the plant level. Further studies are needed to determine the effects of reductions in potential pollinator visitors and pollen movement on plants unable to offer reproductive assurance and over a greater range of fitness traits.

This study highlights the importance of incorporating local spatial scales into interpretations of the effects of floral resources on pollinator foraging behaviour and pollen movement. However, with intra-population pollen movement only accounting for 40.95% of pollen movement, the destination of 59.05% of pollen in relation to local context is not known. Future studies should therefore look to determine the importance of variations in floral resources on long-distance pollen movement in order to enhance our understanding of the long-term survival potential of plant populations in relation to habitat context.

CHAPTER 4

How does habitat floral composition affect the pollination and reproduction of isolated plants?

4.1 Abstract

1. Pollinators are observed to forage optimally, moving short distances between plants and only leaving a habitat when resources are depleted. The diversity, abundance and spatial configuration of floral resources at a habitat scale is therefore expected to drive pollinator foraging behaviour (e.g. visitation and dispersal distance). The implications of changes to pollinator foraging behaviour on the incidence, distance and directness of pollen movement and ultimately, the reproductive success of insect pollinated plants however remains unclear, especially for species which are rare or isolated in the landscape.
2. I used a landscape-scale experiment, coupled with microsatellite genotyping, to explore how the floral composition of habitats affected pollinator behaviour and pollination effectiveness. Small arrays of the partially self-compatible plant California poppy (*Eschscholzia californica),* were introduced across a landscape gradient to simulate rare, spatially-isolated populations. I measured the effects on pollinator activity density (the abundance of actively foraging pollinators) and richness and inter-population pollen movement, along with the implications for plant outcrossing and plant reproduction.
3. In florally rich habitats, reduced pollen movement between plants was observed, leading to fewer inter-population pollination events, lower plant outcrossing and a higher incidence of pollen limitation. This pattern indicates a potential reduction in pollinator visitation, as suggested by the lower activity density and richness of pollinators observed within florally rich habitats. In addition, seed production reduced by a factor of 1.8 in plants within florally rich habitats and progeny germination reduced by a factor of 1.2. I show this to be a consequence of self-fertilisation within *E. californica*.
4. These findings indicate that locally rare or isolated plants are at a competitive disadvantage within florally rich habitats because co-

flowering plant species disrupt conspecific mating by co-opting pollinators. Ultimately, this Allee effect may play an important role in determining the long-term persistence of rarer plants in the landscape, both in terms of seed production and viability. Community context therefore requires consideration when designing and implementing conservation management for plants which are comparatively rare in the landscape.

4.2 Introduction

Changes to the availability and diversity of floral resources through altered land use, including increased landscape fragmentation and simplification, can have considerable impacts on the structure, abundance and diversity of pollinator communities (Vanbergen et al. 2013, Senapathi et al. 2015b, Potts et al. 2016b). With an estimated 87.5% of flowering plant species worldwide at least partly reliant upon pollinators for reproductive success and long-term survival, this will have direct implications for plants (Ollerton et al. 2011). By transferring conspecific pollen between plant individuals, pollinators not only facilitate seed production but have important effects on fitness and population genetic diversity by increasing outcrossing and the exchange of novel alleles (Levin and Kerster 1974, Frankham 2005, Mannouris and Byers 2013).

Plant-pollinator interactions vary with plant population size, density and habitat context (Essenberg 2012, Mayer et al. 2012). Habitats supporting a high abundance and species richness of flowering plants may either enhance or disrupt the transference of pollen to plants (Blaauw and Isaacs 2014, Vanbergen et al. 2014b). The outcome depends on pollinator visitation patterns, which are determined, in part, by the demography and characteristics of a species' population relative to heterospecific co-flowering plants (Essenberg 2012). For instance, when at low floral densities, co-flowering heterospecific plants can facilitate pollinator visitation to a plant population by enhancing the overall attractiveness of a floral patch (Rathcke 1983). At high floral densities, co-flowering heterospecific plants may result in inter-specific competition for pollinators, which can reduce ‘per floral unit’ visitation to a plant population, resulting in an insufficient supply of pollen that limits potential seed set (Ghazoul 2006). Alternatively, although pollinators may prefer foraging on particular plant species (Waser 1986, Chittka et al. 1999, Gegear and Laverty 2005), such fidelity may be relaxed with increasing conspecific pollinator density or low floral richness, introducing the potential for inter-specific pollen transfer (Kunin and Iwasa 1996, Fontaine et al. 2008). This has potential negative implications for plant reproduction. The supply of conspecific pollen to a plant can be reduced if it is lost during visitation to heterospecific plants (Wilcock and Neiland 2002); moreover, the deposition of heterospecific pollen, by clogging the stigma and style of conspecific plants, can inhibit pollination (Holland and Chamberlain 2007). Both lead to reduced pollination effectiveness and ultimately a reduction in plant seed set, which could be detrimental for annual plants as well as perennial plants if continued across subsequent years.

Pollinators face a metabolic trade-off when foraging for pollen and nectar (Charnov 1976, Vaudo et al. 2016) and optimal foraging theory predicts that they will maximise gain and minimise loss of energy (Charnov 1976). Thus pollinators may forage slowly through habitats rich in floral resources, minimising travel distances between flower visits, and either avoid or promptly traverse florally-poor habitats (Pasquet et al. 2008, Lander et al. 2011). Moreover, pollinator foraging distances have been shown to exhibit an inverse relationship with the proportion of available foraging habitat (Carvell et al. 2012). Pollinator sensitivity to the dispersion of floral resources at different spatial scales is partly influenced by traits, such as body size, that predict their mobility and capacity to forage and disperse pollen (Greenleaf et al. 2007, Redhead et al. 2016). Given the capacity of pollinators to mediate plant gene flow, changes in foraging behaviour or pollinator community composition (e.g. body size distributions) in response to variation in habitat floral resources may profoundly affect plant fitness (Ward et al. 2005, Vanbergen et al. 2014b). This may be particularly important for spatially isolated populations of uncommon plant species because increases in floral diversity might lead to greater inter-specific plant competition for pollinators (Ghazoul 2006) and reduce the probability of long distance pollen dispersal (Eckert et al. 2010).

One approach to understanding the interaction between floral community diversity and pollinator-mediated gene flow in locally rare plant populations is to analyse plant mating patterns using highly variable molecular markers (microsatellites). This permits inference, and even direct observation, of patterns of gene movement and mating (Ashley and Dow 1994), enabling the quantification of relatedness between plants (Ashley and Dow 1994). The use of such molecular methods has revealed that plant populations often exhibit spatial genetic structure, where relatedness declines with distance between individuals (Loveless and Hamrick 1984). Increased frequency of selfing and mating between close relatives within plant populations can lead to inbreeding, resulting in reduced allelic diversity and greater homozygosity, which has been linked to a reduction in the fitness and long-term survival of plants (Byers and Waller 1999). Low allelic diversity is particularly detrimental for self-incompatible plants whose reproduction requires allelic variation at a single locus (the ‘S-locus’; Byers and Meagher 1992). Although mutations can cause self-incompatibility systems to break down, resulting in partial self-compatibility, self-fertilisation and mating between close relatives in these plants is typically prevented (Richards 1997). As S-alleles are frequently lost through genetic drift, plant populations could face a reduction in compatible mates with negative implications for the effective population size and thus, plant reproduction (Wagenius et al. 2007). Self-incompatibility coupled with spatially structured populations may therefore render some plant species vulnerable to reductions in gene flow due to altered pollinator foraging behaviour.

In this study, I investigate how genetic connectivity and the reproductive success of a locally rare and partially self-compatible plant species is a function of habitat composition and the activity and richness of potential pollinator visitors. To simulate a species occurring at low frequency, small arrays of Californian poppy (*Eschscholzia californica*) were deployed into a landscape-scale field experiment where floral composition had been manipulated through agri-environment planting of wildflower patches. In these experimental arrays, I measured pollinator activity, insect-vectored pollen movement using microsatellite genotyping, seed set and progeny viability. Based on previous observations of altered pollinator behaviour in response to floral cover (Heard et al. 2007), I hypothesise that:

i) Habitats supporting high floral availability increase activity density and species richness of pollinators in the vicinity of experimental arrays of a partially self-compatible plant (*E. californica*);

ii) The inter-tegular span (a proxy for body size) of pollinators is greater in florally rich habitats, reflecting the preference of *Bombus* spp. to plant species within sown wildflower patches (Carvell et al. 2007);

iii) Pollen movement between introduced experimental arrays of *E. californica* is reduced in florally rich habitats, leading to pollen limitation, lower outcrossing rates, and fewer inter-population pollination events;

iv) The reproductive success (seed set and progeny viability) of *Eschscholzia californica* is reduced in florally rich habitats, reflecting a higher incidence of self-fertilisation, which has negative implications for the fitness of *E. californica* plants.

4.3 Materials and Methods

4.3.1 Experimental site and study system

The experiment was conducted on the Hillesden estate in Buckinghamshire, UK (1º00’01’’W, 51º57’16’’N), an intensive arable farm (~1000ha) situated on heavy clay soils with a relatively flat topography. Since 2005 a number of experimental landscape management ‘treatments’ have been established and managed across the estate within a randomised block design. These treatments, applied to 50-60 ha replicated land parcels, comprise varying proportions (0-8% of land out of production) of a range of wildlife habitat restoration options (including pollen and nectar rich flower margins and wildflower patches for pollinators) under compliance with the English agri-environment scheme (AES) (Pywell et al. 2015). Overall, these wildlife habitats comprised ~4% of the total area (Fig.4.I).

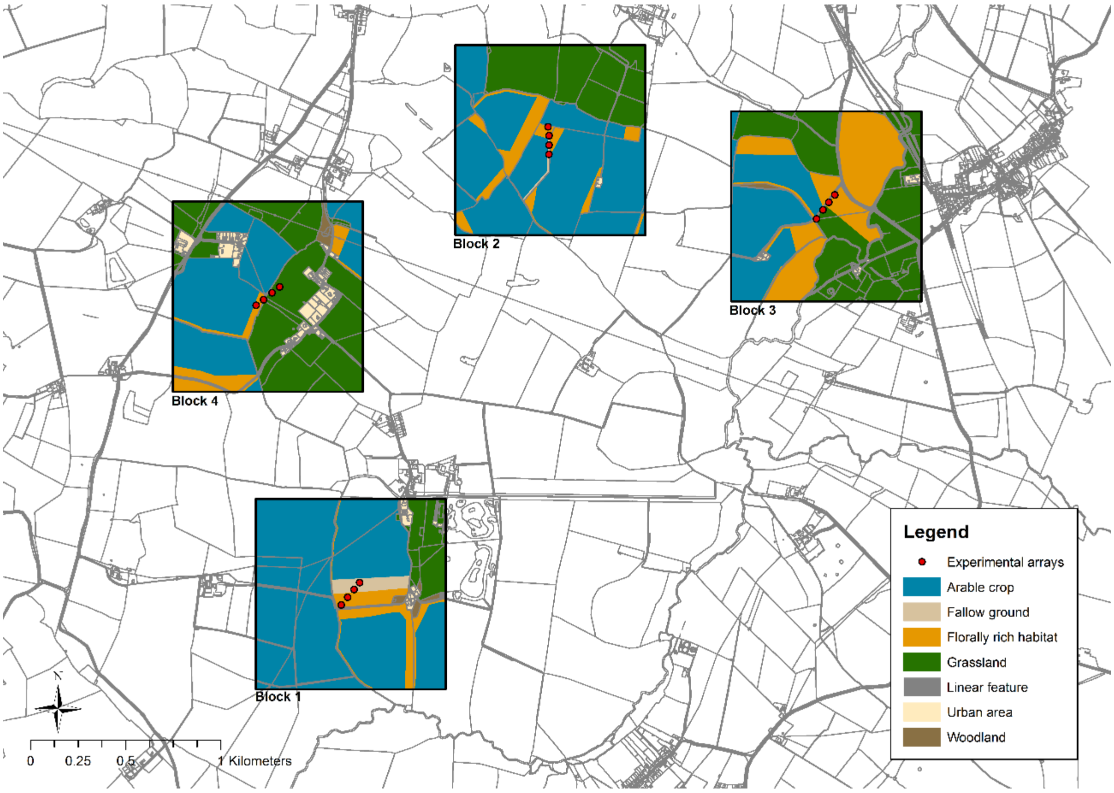

Figure 4.I The experimental set-up at the Hillesden estate, Buckinghamshire, UK. Blocks are denoted by boxes and are labelled blocks 1-4. Flower rich habitats represent all wildlife habitat options implemented under the English Agri-environment scheme.

To test hypotheses, I introduced the Californian poppy, *Eschscholzia californica* Cham., (Papaveraceae). Although considered naturalised in the UK (Preston et al. 2002b), *E. californica* was locally absent, allowing me to unequivocally ascribe paternity in mating events. *Eschscholzia californica* is a diploid species, with a partially self-compatible mating system, characterised by a low propensity to self-fertilise (Wright 1979), and thus predominantly requires insects for pollen transfer (Becker et al. 2005). It possesses large, open flowers (Fig.4.II) and is visited by a variety of insects from the orders: Diptera, Hymenoptera and Coleoptera (summarised in Cook 1962).

Figure 4.II Eschscholzia californica flower at the final developmental stage: characterised by stigma receptivity and dehiscing anthers.

Plants were grown from seed (seed source: Chiltern seeds Ltd, Wallingford, UK) in compost-filled seed trays under glasshouse conditions (20°C during the day, 16°C at night, with 12 hours of light and 12 hours of dark) and were transferred to 1L pots when at the seedling stage. In early June 2015, groups of three potted *E. californica* plants were positioned in a triangular experimental array to simulate a locally rare plant population. Plants were separated by 1m to prevent fertilisation by direct neighbour contact. A total of sixteen arrays were introduced for a 16-day period across four 1km² replicate blocks (four arrays per block) separated by >500m to minimise between block movement of insect pollinators (Fig.4.I). At the centre of each block, four experimental arrays were placed at 50m intervals along a 150m transect laid symmetrically across the boundary between an established wildflower patch (henceforth ‘florally rich’ habitat) and bare, fallow ground (henceforth ‘florally poor’ habitat) (Fig.4.1). This ensured the first two arrays on a transect were located within the florally rich habitat, and the second two arrays within the florally poor habitat. The use of AES wildflower patches, sown with a common mix of approximately 25 species including *Trifolium pratense*, *Centaurea nigra* and *Leucanthemum vulgare* at a rate of 37 kg ha−1 (Carvell et al. 2007), allowed for the standardisation of florally rich treatments across the four blocks. To ensure the habitat classification was accurate, prior to the start of the experiment the local floral abundance (mean ± SE flowers m^{-2}, florally rich = 235.25 ± 42.15; florally poor = 26.25 ± 14.08) and plant diversity (Shannon mean ± SE florally rich = 0.83 ± 0.17; florally poor = 0.28 ± 0.15) was established by recording all floral units within a 1m radius surrounding each experimental array (Appendix 1: Plant list).

4.3.2 Pollinator activity and species richness

Pan traps are typically deployed to describe pollinator species richness and activity density (Westphal et al. 2008). They have also been used to provide a surrogate measure of visitation, allowing for longer periods than standard observation methods (Ricketts et al. 2008). However, this survey method has been recognised to exhibit bias (Roulston et al. 2007) because the attractiveness of pan traps depends upon habitat and landscape context (Baum and Wallen 2011). Pollinators are less likely to encounter traps when floral resources are abundant and more likely to encounter traps when floral resources are scarce i.e. capture rates are proportional to visitation rates per unit flower area (Veddeler et al. 2006). I exploited this phenomenon to measure the attractiveness and pollinator activity density at the experimentally rare plant arrays located within different habitats.

Pan traps comprised three water-filled circular plastic bowls (80 x 200 mm) painted with non-toxic fluorescent paint (1 yellow, 1 blue and 1 white; UV Gear, UK) placed in the centre of each array. Traps were deployed for 24 hours at each of the 16 arrays on the same day, twice weekly over the 16-day study period (totalling four surveys). Each survey was done in randomised order, between 0930 and 1700. Emptied traps were left *in situ* to maintain the same levels of visual attractiveness to foraging insects throughout the experiment. All insects from the main pollinator groups (Hymenoptera: Apoidea, Diptera: Syrphidae and Lepidoptera) were counted and identified to species level. In addition, given that Apoidea body mass has been shown to correlate with foraging range (Greenleaf et al. 2007) and to a lesser extent, pollen deposition (Larsen et al. 2005), I measured the inter-tegular span (the distance between the wing bases) of each insect from the main pollinator groups using digital callipers (given the relationship between inter-tegular span and body mass in Apoidea; Cane 1987) to determine the body size distribution of pollinator communities. This relationship is less clear in Lepidoptera and Syrphidae, however inter-tegular span has previously been used as a measure of dry body mass in the latter given observed correlations (Folkö 2014). I therefore adopted this measure to maintain consistency, but it is important to note the potential inaccuracy in estimations due to differences between pollinator groups.

To ensure pollinators caught within pan traps could be used as a proxy for visitation, these data were calibrated by direct visitor observations on the *E. californica* plants. Pollinator visitor observations were conducted for each experimental array between 09.30 and 17.00 over four surveying days (two per week). Observations lasted for 15 minutes, during which every insect foraging (contacting an anther or stigma) was recorded and identified to a broad pollinator group as above.

4.3.3 Genotype analysis

Eschscholzia californica was grown in compost under glasshouse conditions (day: night = 20ºC:15ºC photoperiod light: dark = 12:12hr). Once at seedling stage, 50mg of fresh leaf material was removed from 95 plants and DNA was extracted from each sample following the Qiagen DNeasy 96 plant kit protocol

(QIAGEN Ltd., Manchester, UK). The concentration of DNA was quantified on a spectrometer (ND8000) and subsequently diluted to 10ng/µl. Polymerase chain reaction (PCR) was conducted using seven non-overlapping microsatellite markers (Veliz *et al*. 2012) with fluorescent dyes attached to the forward primer (DS-33 dye set, Applied Biosystems™, California, USA). Separate PCRs were conducted for each primer set, with the exception of two primers (Ecalifdi11 and Ecalifdi1), which were successful in a multiplex PCR.

The PCR program settings were: 95°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 55°C (or 56°C depending upon loci) for 60 seconds, 72°C for 30 seconds, followed by a final elongation phase of 72°C for 10 minutes. Standard reaction conditions were as follows: 10ng of DNA, 0.1µl of reverse primer (20µM) and DS-33 attached forward primer (20µM), 0.08µl dNTPs (100µM), 0.1µl BSA, 1µl Buffer and 0.1µl Taq polymerase in a 10µl reaction. The PCR products were combined and visualised on a 2% agarose gel. Fragment analysis was then performed on an ABI3730 under the following conditions: 0.3µl Liz 500 size standard, 8.7µl HiDi formamide and 1µl PCR product. Alleles at all seven loci were manually scored on Genemarker V1.95 and ambiguous alleles were cloned and sequenced using TOPO® TA cloning kit® (Invitrogen™, California, USA) to verify that they were true alleles. Following this, I selected 48 plants with distinct genotypes to be deployed at pre-determined locations across the landscape (Fig.4.I). Where possible, plants were selected so that the three individual plants within each array were homozygous with the same allele at a selected locus. Whereas, each experimental array (a triplet of plant individuals) within a block was homozygous for a different allele at this locus. This allele structure in the design allowed for verification of inter-population pollen movement (i.e. the presence of a novel allele at the selected locus was indicative of the array from which the pollen was sourced). During initial assessments, the selected plants were shown to be polymorphic at the seven studied loci (7 loci: Number of alleles, A = 2-8; Observed heterozygosity, Ho = 0.083-0.75). This points towards a high diversity of S-alleles in the base population, indicating cross-compatibility between parent plants.

4.3.4 Pollen movement

To detect pollination events approximately ten progeny per plant from each of the 48 field exposed plants (mean ± SE = 9.52 ± 0.39) were genotyped using 50mg of fresh leaf material and following protocols as above. The incidence of self-fertilisation in plants from each habitat was calculated manually by individually comparing each successfully amplified progeny against their maternal plant. If, at each of the seven loci, the progeny was a complete match for the maternal genotype, or was homozygote for one of the maternal plants alleles, it was scored as selfed. Alternatively, if any novel alleles were observed in the progeny that were not present in the maternal plant, the plant was classified as outcrossed. Paternity was determined using Cervus 3.0.7 (Kalinowski et al. 2007), where each progeny sample was listed detailing alleles at the seven microsatellite loci, specifying the known maternal sample as well as the potential paternal samples. Here all progeny from within a block were analysed against all potential parents within that block. I accounted for self-fertilisation and selected for the most likely paternal parent based on a derivative of likelihood ratios; the delta score (Δ), which is the difference between the likelihood score of the most likely parent and the second most likely parent (Marshall et al. 1998). Only assignments with a trio Δ confidence (the likelihood score of a mother-father-offspring match) above 95%, which is classified as high confidence, were included in the analysis (Marshall et al. 1998). For all paternal assignments, I recorded which habitat, if any, the pollen had crossed together with the distance travelled.

4.3.5 Plant fitness components: seed production, germination rates and progeny traits

All open flowers were removed from the 48 genotyped *E. californica* plants, prior to their placement in pre-marked locations across the landscape. They remained in the field for 16 days to ensure full anthesis of new flowers (which takes 3-4 days; Becker et al. 2005) and to allow for multiple pollination events. After this period, all fruit were tagged to ensure that only fruit development arising from the period of the field experiment were included in analyses. Plants were then collected and stored under controlled glasshouse conditions (as above) until fruit maturation. Upon maturation, tagged fruit were collected and the numbers of filled seeds per fruit were counted to quantify seed set per plant.

To determine whether field exposed plants were limited by pollen, I supplemented a flower from each of the 48 plants with outcrossed pollen. This involved methodically wiping four dehiscing anthers from a donor plant onto the receptive stigma of a field exposed plant with dissecting tweezers. Supplemented flowers were then covered with fine muslin to protect against accidental windborne transfer of pollen from the glasshouse air-conditioning system. Once matured, fruit were collected and the number of seeds per fruit was counted to determine maximum seed set. The degree of pollen limitation was expressed as a ratio between the actual seed set (field exposed plants) and the potential seed set (supplemented) in each of the 48 field exposed plants.

To measure the viability of progeny from field exposed plants, 20 seeds from each of the 48 plants were sown into compost and kept under glasshouse conditions (as above). Germination was recorded daily over a 30-day period and any seeds which had not germinated after 90 days were recorded as inviable. The germination success was expressed as a ratio between the number of seeds which successfully germinated against the number of seeds which failed to germinate in each of the 48 field exposed plants. Indeed, some species and populations of *E. californica* can exhibit seed dormancy (Cook 1962), though this was found to be absent within experimental plants (personal observation).

To further assess how reproduction by self-fertilisation affects the viability and growth traits of a partially self-compatible plant I performed a glasshouse experiment using 40 artificially crossed plants. On each plant, two flowers were emasculated and supplemented; the first with outcrossed pollen and the second with self-pollen. This involved methodically wiping two dehiscing anthers from a donor plant or the focal plant onto the receptive stigma with dissecting tweezers, before covering it in fine muslin. From each supplemented plant, a seed was sown from the outcrossed fruit and from the selfed fruit (given that selfed fruits predominantly only produced one seed) into 1L pots. These were then stored under glasshouse conditions (as above). I recorded the following fitness traits; the germination rate, the duration from germination to reproductive maturity (time of first flower), together with the height (cm) and the number of buds at reproductive maturity (biomass).

4.3.6 Statistical analysis

Pollinator activity density (a proxy for visitation) and the cumulative counts of pollinator species recorded at each experimental array were modelled using generalised linear mixed models (GLMMs) with a Poisson error distribution. When analysing the IT span of pollinator species caught within pan traps however, a Log-normal error distribution was instead used to account for non-integers. Plant fitness components were similarly analysed using GLMMs with a combination of Poisson (seed production per plant) and Binomial (pollen limitation of each plant and the germination success of progeny) error distributions.

Within the models, fixed effects comprised of habitat type (florally rich/florally poor). Experimental block (Fig.4.I) was fitted as a random effect to account for the spatial structure of the experimental design. For pollinator activity models, additional random effects were included to account for survey date and the pollinator species, when analysing the activity density (64 surveys) and IT span (203 pollinators) of pollinators respectively. Additional random effects for models of plant fitness components were ‘plant identity’ for pollen limitation (42 surviving plants) and germination success (48 plants) and ‘fruit nested within plant’ for seed production (n = 618) to account for variation between plants and fruit. Where present, over-dispersion in the data was controlled for by fitting an observational level parameter to the random effects (Harrison 2014). AIC stepwise selection was then used to find the minimum adequate model (Burnham and Anderson 2003) and all models were analysed using Laplace approximation. The significance of the final models were analysed by comparison to a null model with the same random effects structure using an ANOVA. All analyses were conducted with R (version x64; R Core Team 2013) using the lme4 package (Bates et al. 2015).

When analysing the effects of self-fertilisation on plant fitness traits (e.g. height) I used a combination of chi-square contingency tables (the germination of selfed and outcrossed seeds), generalised linear models (GLMs) with a Poisson error distribution (plant height at reproductive maturity) and ANOVAs (duration to reproductive maturity and plant biomass at reproductive maturity). In both GLMs and ANOVAS the fitness trait measured was modelled against the mating system (outcrossed or selfed) for all surviving germinated seeds (n = 56).

When analysing pollen movement parameters I used a combination of chi-square contingency tables (the incidence of self-fertilisation modelled against the number of outcrossing events) and binomial proportion tests (the distance of pollination events, the movement of pollen across habitats of different floral covers and the movement of pollen to and from habitats of different floral covers). For the distance of pollination events, the cumulative number of inter-population pollination events at each distance (50, 100 and 150m) was analysed against the total number of inter-population (50-150m) pollination events (n = 34). For the movement of pollen across habitats all 50m movements where the intervening habitat varied (i.e. florally poor, a mixture of florally poor and florally rich and florally rich) were analysed against the total number of 50m pollination events (n = 22). The movement of pollen to and from each habitat was similarly analysed by comparing the cumulative counts of inter-population pollination events (50-150m) leaving or entering a habitat against the total number of inter-population (50-150m) pollination events (n = 34). For all models of pollen movement I used cumulative counts across all blocks. The relationship between the number of selfing incidents and the total number of inter-population pollen movements (50-150m) to and from each array was then analysed against the abundance of pollinators caught in pan traps using generalised linear models with a Poisson error distribution.

4.4 Results

4.4.1 Pollinator activity and species richness

Considering insect taxa generally thought to be the most effective pollinators (i.e. Apoidea, Syrphidae and to a lesser extent Lepidoptera), greater numbers were caught in pan traps centred on the experimental plant arrays in florally poor habitats (mean ± SE Florally rich = 7.63 ± 0.96; Florally poor = 17.75 ± 3.87; GLMM z = -3.85, df = 59, p < 0.0001; Fig.4.III). Furthermore, the species richness of these main pollinator groups was similarly higher in traps centred on plant arrays in florally poor habitats (mean ± SE Florally Poor = 9.25±1.31; Florally rich = 5.5±0.57; GLMM z = -2.74, df = 13, p = 0.006; Fig.4.III) (Appendix 3: Pollinator list). However, the IT span of visiting pollinators was not significantly different between florally poor and florally rich habitats (mean ± SE Florally rich = 2.97 ± 0.13; Florally poor = 2.60 ± 0.07; p = 0.427).

The activity density of the main pollinator groups was mirrored by the overall catches of all potential pollinators (including non-Syrphid Diptera and Coleoptera). Twice as many pollinating insects were recorded in pan traps centred on the experimental plant arrays in florally poor habitats (mean ± SE 672.5 ± 103.14) compared to florally rich habitats (mean ± SE 318.5 ± 56.83) (GLMM z = -4.68, df = 59, p < 0.0001). Non-syrphid Diptera and Coleoptera comprised the greatest proportion of flower visiting taxa in both habitats

(Florally poor = 0.97, Florally rich = 0.98) reflecting their typically greater abundance, although their efficacy as pollinators is largely unknown for most plant species (but see Orford *et al*. 2015).

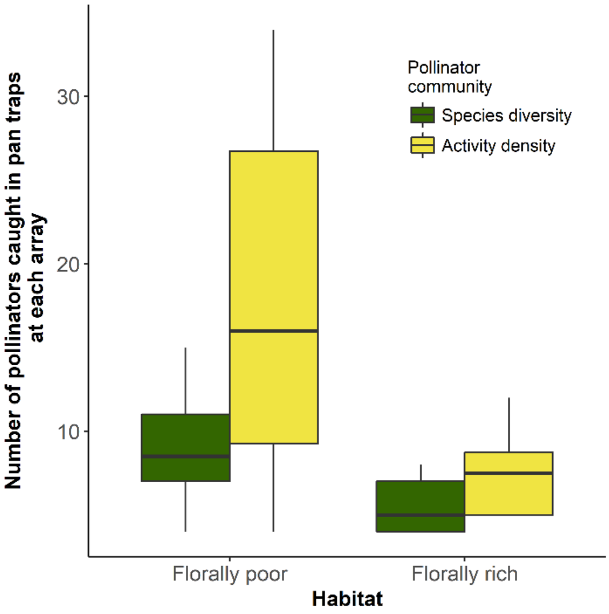

Figure 4.III The activity density (yellow boxes) and species richness (green boxes) of insects within main pollinator groups caught in pan traps within habitats differing in floral composition. Box plots represent the cumulative counts of all trapping periods, with counts averaged across each experimental array within florally poor and florally rich habitats. Bars summarise the median value (50th percentile), with boxes illustrating the upper and lower quartiles (25th and 75th percentile). Whiskers illustrate the minimum and maximum count.

The catches of pollinators within pan traps (from the main pollinator groups: Apoidea, Syrphidae and Lepidoptera) resembled the proportions of pollinator groups observed to actively visit *E. californica* (Fig.4.IV). Although only 3 out of the 5 groups were observed during observations, this justified the use of activity density from pan traps as a proxy for actual plant visitation. Statistical analysis of these direct observations of pollinator visitation was however precluded by the sparseness of this data (total pollinators observed (main pollinators groups) = 8 individuals).

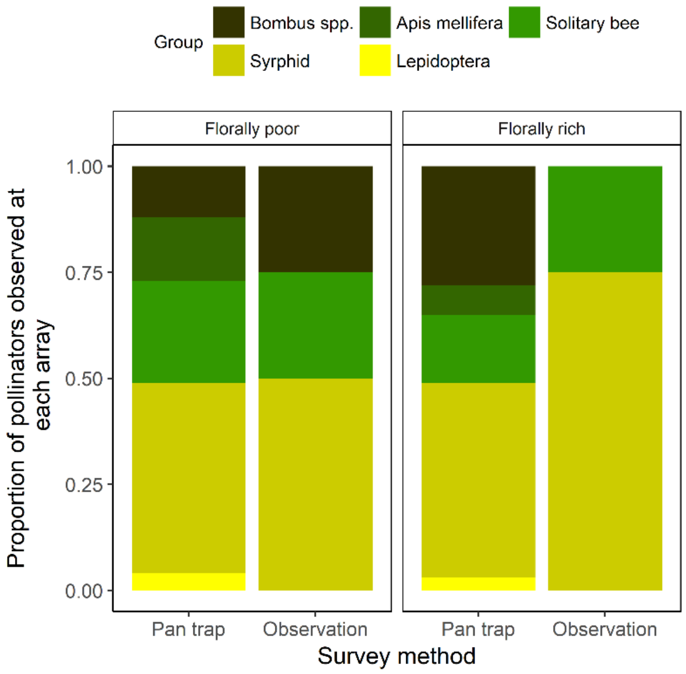

Figure 4.IV The proportion of insects within main pollinator groups observed during direct visitor observations of E. californica plants and those caught in pan traps within habitats differing in floral cover.

4.4.2 Pollen movement

As expected for a partially self-compatible species, levels of selfing were low in field exposed plants. However, the proportion of progeny that were produced by self-fertilisation was marginally greater from plants within florally rich habitats (Florally rich = 15%; Florally poor = 9%; $\chi^2 = 3.69$, df = 1, p = 0.055). The incidence of selfing was not however correlated with pollinator activity density (p = 0.097).

Paternal assignments were achieved for 300 out of the 457 amplified samples, with the remainder (n = 157) disregarded (trio Δ confidence score of below 95%). The greatest proportion of pollination events comprised intra-population pollen movements, representing short distance pollen movement (1m = 72%; Fig.4.V). A number of long distance inter-population pollen movements were observed (n = 34 (11% of all movements) and of these, a significantly greater proportion travelled 50m (65%), with fewer movements between 100 (24%)

and 150m (12%) ($\chi^2 = 23.65$, df = 2, $p < 0.001$). These long distance inter-population pollen movements (50-150m) were significantly more frequent both to (Florally rich = 32%; Florally poor = 68%; $\chi^2 = 7.12$, df = 1, p = 0.008) and from (Florally rich = 29%; Florally poor = 71%; $\chi^2 = 9.94$, df = 1, p = 0.002) arrays within florally poor habitats. The movement of pollen between experimental arrays was affected by the floral richness of the intervening habitat. Regarding the total number of 50m pollination events across all blocks, pollen movement was greatest between two arrays positioned within florally poor habitats i.e. where the intervening habitat had low floral cover (Florally poor cover = 73%, a mixture of both florally poor and florally rich cover = 14% and florally rich cover = 14%; $\chi^2 = 23.05$, df = 2, $p < 0.001$; Fig.4.VI). Furthermore, the total number of long distance inter-population pollen movements (50-150m) to and from each array was positively correlated with pollinator activity density (GLM z = 2.06, df = 15, p = 0.036).

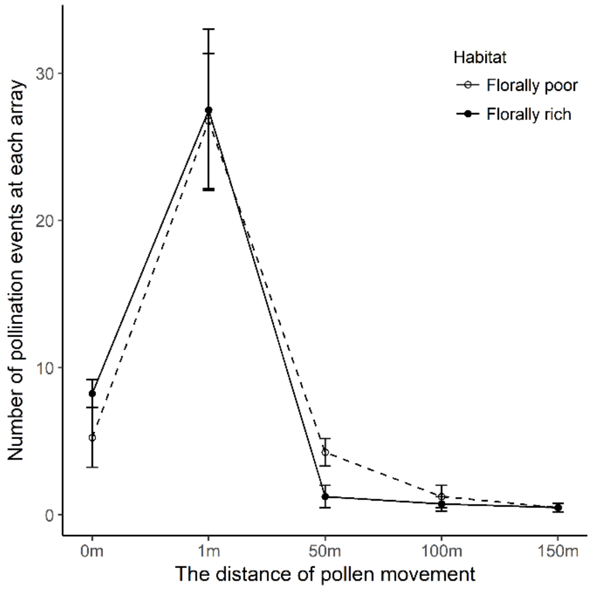

Figure 4.V The distance of pollen movement, averaged across all blocks, from experimental arrays located within habitats differing in floral composition (self-fertilisation is denoted by 0m). Dashed lines with open circles represents pollen movement from florally poor habitats and solid lines with filled circles represents pollen movement from florally rich habitats.

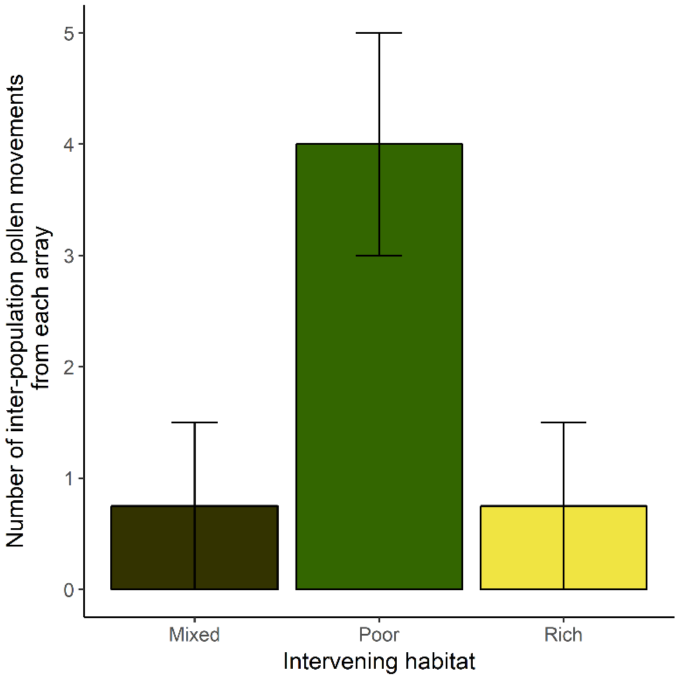

Figure 4.VI The incidence of inter-population pollen movement (50m), averaged across all blocks, over habitats differing in floral composition. Mixed habitat denotes when the intervening habitat comprised of 25m of florally rich habitat and 25m of florally poor habitat; poor habitat denotes where the intervening habitat is comprised of 50m of florally poor habitat and rich habitat denotes where the intervening habitat is comprised of 50m of florally rich habitat.

4.4.3 Plant fitness components: seed production, germination rates and progeny traits

The number of fruits and seeds produced per plant were highly variable (fruit range = 4-23, seed range = 0-589). However, total seed set in arrays within florally poor habitats was 1.8 fold greater than in those within florally rich habitats (GLMM z = -1.980, df = 613, p = 0.048; Fig.4.VII). Furthermore, the number of additional seeds produced by pollen supplementation was greater in florally rich habitats (GLMM z = 2.396, df = 38, p = 0.017; Fig.4.VII), indicating that plants were more pollen limited in florally rich habitats.

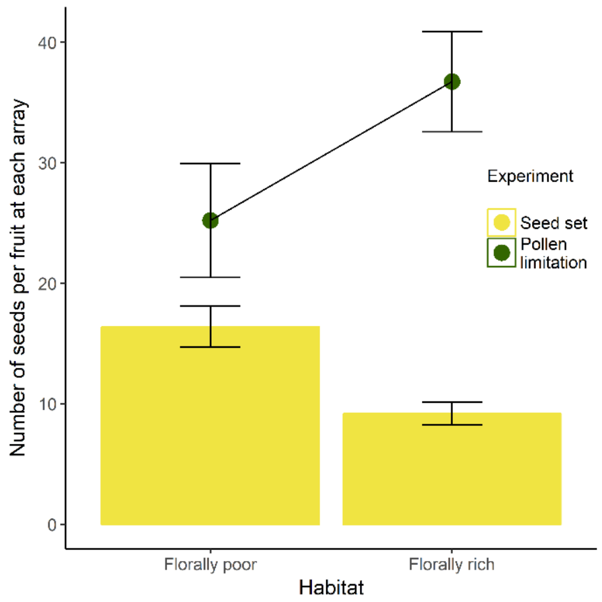

Figure 4.VII The mean number of seeds (denoted by open bars) per fruit produced by plants within habitats comprising different floral composition, together with the mean degree of pollen limitation (denoted by filled points) of these plants. Pollen limitation is illustrated here as the number of additional seeds produced by a plant after pollen supplementation (when compared to the number of seeds produced by the same plant under field conditions).

Germination rates of progeny arising from plants located in florally rich habitats was reduced, albeit marginally (mean ± SE Florally rich = 10.67 ± 0.85; Florally poor = 12.96 ± 0.87, GLMM z = -1.940, df = 44, p = 0.052). The glasshouse viability trial to quantify the implications of selfing on progeny viability showed that a lower proportion of seeds germinated when produced by self-fertilisation, compared to seeds which were a product of outcrossing (outcrossed seeds = 0.8 (n = 33); selfed seeds = 0.6 (n = 24); $\chi^2 = 3.91$, df = 1, p = 0.048, phi = 0.25). However, no effect of self-fertilisation was found in *E. californica* on later stage fitness traits (time to reproductive maturity (first flower) p = 0.210; height at reproductive maturity GLMM p = 0.078; biomass at reproductive maturity p = 0.143). The negative implications of self-fertilisation were thus limited to reduced germination.

4.5 Discussion

4.5.1 Habitat effects on pollinator visitation

Consistent with previous work (Veddeler et al. 2006) I show a negative association between florally rich habitats and the activity density and species richness of pollinators. Elsewhere, the abundance and richness of pollinators has been observed to increase with floral cover (Williams et al. 2015), especially where this cover is limited within the wider landscape (Heard et al. 2007). However, these results suggest that despite the increased aggregation of pollinators in habitats providing abundant, diverse floral resources, pollinator visitation is effectively 'diluted', which may result in lower 'per floral unit' visitation and greater interspecific competition for pollination (Veddeler et al. 2006, Sjodin 2007). Consequently, when embedded within a diverse community of co-flowering heterospecific plants offering a variety of floral pollen and nectar, rare plant species may be unable to co-opt pollinators (Ghazoul 2006). In contrast, where co-flowering, heterospecific competitors were scarce, findings suggest that available pollinators would become concentrated, leading to potential increases in 'per floral unit' visitation rates at the individual plant level (Veddeler et al. 2006, Tscharntke et al. 2012). It should be noted however, this study did not take the wider landscape into consideration. Given that the experiment was conducted in the same locality, landscape composition may have been a driver of the low visitation rates across both habitats and thus, the patterns observed. In future experiments, the wider landscape should be considered in order to verify that these patterns aren't context specific.

A diverse community of pollinators can provide niche complementarity (Pisanty et al. 2016), often leading to enhanced pollen deposition (Larsen et al. 2005) and seed production (Martins et al. 2015). Alternatively, a high diversity of pollinators visiting diverse plant assemblages can result in an increase in heterospecific pollen deposition, which can interfere with conspecific pollination by stigma clogging (Holland and Chamberlain 2007). The extent to which the diversity of pollinator species provides a benefit to plants is determined by the functional diversity and pollination effectiveness of communities (Perfectti et al. 2009). Indeed, pollinator species vary in their specialisation, pollen carrying behaviour and daily activity preferences, all of which affect pollination effectiveness (Rader et al. 2011, Martins et al. 2015). Furthermore, pollination effectiveness has been associated with body size, where larger pollinator species can travel greater distances (Greenleaf et al. 2007) and deposit a larger amount of pollen per visit (Larsen et al. 2005). In this study, however, no difference was found in the size distribution (IT span) of pollinators between florally rich and florally poor habitats, indicating that by this measure there was no difference in the trait structure of pollinator communities between habitats with different floral cover that could alter pollination effectiveness. Instead, pollination effectiveness may be driven by changes to the foraging behaviour of pollinator communities.

4.5.2 Habitat effects on pollen movement

Consistent with previous studies, findings indicate that pollen movement between local populations was strongly affected by the floral composition of a habitat (Lander et al. 2011, Dyer et al. 2012). Pollen movement between experimental arrays (50m) was greater when the surrounding and intervening habitat comprised livestock grazed grassland or fallow ground with low richness of floral resources. In addition, very few pollination events were observed between arrays separated by habitats of high floral cover or those with heterogeneous intervening habitats (i.e. a mixture of habitats comprising high and low floral cover). These results are consistent with the hypothesis that the foraging behaviour of pollinator communities is highly determined by habitat composition. This higher level of pollen movement between populations in florally poor habitats supports research which shows pollinators to conform to the weighted line foraging principle when encountering heterogeneous landscapes (Lander et al. 2013). This principle assumes that pollinators will occupy optimal foraging habitat until resources are depleted, thus making short, energy efficient, movements between flowers. Conversely, pollinators are expected under this principle to move greater distances within habitats that are nutritionally sub-optimal (Lander et al. 2013). By altering the insect-mediated pollen movement between plant populations, the weighted line foraging strategy will have implications for genetic exchange and the genetic diversity of rare plant populations.

The floral cover of the surrounding habitat greatly affected the distance of pollen movement with plants in florally poor habitats subject to more long distance inter-population pollination events than those in florally rich habitats. Furthermore, this is shown to be positively correlated with activity density of pollinators. This suggests that pollinators were following optimal foraging expectations, where movement reflects energy efficient behaviour. Indeed, findings indicate that in both habitats the majority of pollen movement was localised (1m). Of the long-distance inter-population pollination events, a greater proportion were between plants separated by 50m, with fewer between distances of 50-150m. This pattern is consistent with a wealth of research indicating that although capable of travelling large distances (Hagler et al. 2011), pollinators predominantly travel considerably shorter distances (Rader et al. 2011), remaining in localised resource patches (Pasquet et al. 2008). This results in a distance decay distribution of pollen movement (Matter et al. 2013), suggesting that between block movement (>500m) in this experiment would be minimal. In spatially genetically structured plant populations, reduced long distance inter-population pollination events, particularly in florally rich habitats, will result in a higher frequency of mating between close relatives. As a consequence, self-incompatible and partially self-compatible plants will suffer from increased biparental inbreeding and a reduction in compatible mates (Turner et al. 1982). This will negatively impact plant seed set and viability (Ward et al. 2005), together with the adaptive potential and consequently, the long-term survival of rare plant populations (Etterson 2004).

4.5.3 Implications for plant reproductive success

Reductions in the activity density and richness of pollinator species in florally rich habitats reflect the increased pollen limitation and reduced individual plant reproduction observed within experimental arrays located in florally rich habitats. Pollen limitation has been related to competition for pollinator visitation, with similar results observed in response to an increase in diversity (Vamosi et al. 2013) or density (Jakobsson et al. 2009) of co-flowering plants. Low pollen receipt, a cause of pollen limitation, can result either in an increase in self-fertilisation (Kalisz et al. 2004), or in the case of self-incompatible or partially self-compatible plants, where it is particularly detrimental, a direct reduction in seed production (Wagenius et al. 2007). Given the limited duration of stigma receptiveness the ability of a plant to attract pollinators is therefore important for both pollen receipt and seed production (Bernhardt et al. 2008).

As well as the supply of pollen, the quality of pollen is also critical to plant reproduction and fitness. Pollen quality refers to both the deposition of heterospecific pollen, which can result in physical or chemical inhibition of seed set (Kanchan and Jayachandra 1980, Holland and Chamberlain 2007) and to the genetic relatedness of pollen, which can lead to inbreeding depression (Fischer et al. 2003). Findings indicate that, through alterations to pollinator visitation and subsequent reductions in pollen receipt, florally rich habitats can promote higher levels of self-fertilisation. Further, given reduced germination rates in progeny from plants in florally rich habitats and the negative relationship observed between germination and self-fertilisation, results are indicative of higher rates of self-fertilisation then detected by microsatellite analysis. Reproduction by selfing in self-incompatible or partially self-compatible plants can have a negative impact on the fitness of progeny, shown in this study through a reduction in germination rates. These findings are consistent with previous research where self-fertilisation in self-incompatible plants resulted in inbreeding depression with negative implications for plant fitness (Bellanger et al. 2015). However, in contrast to previous studies (Thiele et al. 2010) reductions in germination did not translate into negative impacts on late fitness traits (e.g. time to reproductive maturity) of surviving plants. This suggests that the immediate effects on population persistence would be due more to changes in vital rates than trait differentiation.

4.5.4 Implications for the conservation of rare plants

Rarity in plants is frequent and can be driven by biological or anthropogenic factors. It is often characterised by populations comprising low genetic variation together with restrictions in size, local abundance, geographical range and/or habitat specificity (Espeland and Emam 2011). In this study, by simulating anthropogenically rare plant populations, I show that restrictions in the population size of a naturally abundant plant, over the longer term, could lead to an Allee effect, whereby increases in mating between close relatives, coupled with higher self-fertilisation rates further reduces genetic variation and ultimately, increases the risk of local extinction (Etterson 2004). Conservation efforts for plants facing conditions associated with anthropogenic-induced rarity may benefit from focus on enhancing visitation and movement of pollinators between conspecifics. This could be achieved through a combination of: i) increasing the competitive advantage of plant populations (e.g. increasing a plant’s population size; Mayer et al. 2012), ii) managing surrounding habitats to enhance facilitation of pollinators to plant populations (e.g. introducing co-flowering species which have complementary phenotypes; Ghazoul 2006), and iii) reducing the distance between conspecific populations (Van Rossum and Triest 2010).

4.5.5 Summary

Findings from this study show that habitat context mediates plant–pollinator interactions and alters the reproduction of anthropogenically rare plant populations. In florally rich habitats, isolated plant populations are at a competitive disadvantage for pollinator visitation when faced with more abundant co-flowering heterospecific plants. Consequently, anthropogenically rare plant populations in these habitats suffer from increased rates of self-fertilisation, limited pollen movement, and reduced reproductive success. The implication is that plant populations dependent on insect pollinators may become less connected and more genetically depauperate when located in florally rich habitats, increasing the risk of genetic drift and extinction. Such an effect may hold for not only anthropogenically rare plants but also plants that are widespread but occur at low frequency within the environment.

This study highlights the importance of floral availability at a habitat scale on pollen transfer to isolated plants, illustrated through changes to plant outcrossing and long distance, inter-population pollen movement. Given differences in a pollinator's floral preferences and specialism to plant species, these effects are however expected to differ given variations in floral communities. Future studies should therefore focus on how these interactions vary with a gradient of floral communities within the intervening habitat between conspecific populations. This will enhance our understanding of the conditions under which facilitation for pollinator visitation is enhanced at a habitat scale.

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CHAPTER 5

Is the connectivity of conspecific plant populations a function of the floral communities within a habitat?

5.1 Abstract

1. Connectivity is a function of the interactions between plant populations and therefore reflects the level of pollen movement between conspecifics. Inter-population pollen movement is fundamental for maintaining genetic exchange and consequently, enhancing plant fitness and long-term survival potential. For isolated, insect-pollinated plants, interactions between populations is determined by the ability of pollinators to navigate across a variety of habitats. However, our understanding of how inter-population pollen movement between plants separated by different distances is affected by a gradient of floral resources is unclear.
2. This study explored the effects of variations in floral communities at local scales on pollen movement by insect pollinator communities between conspecific plant populations. Individuals of the self-compatible plant *Vicia faba* were introduced into plots representing a gradient of floral communities. I measured pollen movement at different distances through a plot and between adjacent plots and explored the relationship with the activity density (the abundance of actively foraging pollinators) and functional traits of pollinator communities. The implications for plants was then determined through measures of heterospecific pollen deposition and plant reproductive success.
3. The incidence of both between- and within-plot pollen movement was low in this study. Although this precluded formal analysis, findings demonstrated patterns of reduced pollen movement in relation to a high abundance of floral resources. This was illustrated by a greater incidence of between-plot pollen movement where the abundance of floral resources within the intervening plot was classed as intermediate (5-25 floral units/0.5m^2). This reflects the negative relationship observed between the abundance of floral resources within a plot and the activity density of potential *V. faba* pollinators. Despite this, the abundance of floral resources had no measured effect on the incidence of

heterospecific pollen transfer or the reproductive success of *V. faba* plants.

4. Findings from this study suggest that floral resources at a local scale, when at a low abundance, can benefit isolated plants by facilitating visitation and thus, pollen transfer. As floral resources increase, competition for pollinators can disrupt interactions between isolated plant populations by reducing visitation constancy. While needing further research to draw concrete conclusions, this highlights the importance of maintaining a low level of floral resources between isolated plant populations in order to enhance pollen and gene flow.

5.2 Introduction

Connectivity is a measure of the extent to which a landscape impedes or facilitates the movement of organisms between patches or populations (Tischendorf and Fahring 2000). The movement of organisms may be affected by landscape structure (e.g. the linkage of habitats by linear features; Cranmer et al. 2011), or by elements within the landscape which are of relevance to an organism (e.g. floral resources) and thus, affect behavioural responses (Tischendorf and Fahring 2000). A high degree of inter-population movement is particularly important for outcrossing species, which rely on pollen transfer in order to achieve mating between genetically-unrelated individuals and thus, enhance long-term survival potential (Weidema et al. 2000, Lennartsson 2002, Bartlett et al. 2016, Gomez-Fernandez et al. 2016). For pollinator-dependent plants however, the successful transfer of pollen between conspecific plant populations depends upon the ability of pollinators to move across landscapes of varying composition (Taylor et al. 1993). This is governed both by the distance between conspecific plant populations (Gustafson and Gardner 1996, Moilanen and Hanski 2001, Murphy and Lovett-Doust 2004) and the attributes of the intervening habitat (Taylor et al. 1993, Dileo et al. 2014). Our current understanding of the effects of habitat composition on pollinator foraging behaviour and thus the movement of pollen between plant populations is limited and would provide valuable insight into the implications of habitat modification and management interventions on plants.

Landscapes were once often considered to be a binary mosaic of habitat considered suitable for organisms and a background matrix (MacArthur and Wilson 1967). The background matrix was considered equally inhospitable and impervious to organisms and was thus expected to restrict movement between optimal habitats or patches (Wright 1943, MacArthur and Wilson 1967, Murphy and Lovett-Doust 2004). This has since been challenged and the permeability of a habitat to the movement of organisms has been recognised to be a function of its structural (e.g. hedges or corridors; Epps et al. 2007) and functional (e.g. floral resources; Lander et al. 2011) attributes (Tischendorf 2001), although these are not always synonymous (Tischendorf and Fahring 2000, Aavik et al. 2014). For instance, while trees can enhance the movement of organisms by providing sustenance and protection from predation and desiccation (Dick et al. 2003); to some organisms they can act as barriers (Rocha and Aguilar 2001, Zeller et al. 2012). This highlights the importance of assessing landscape composition from a species' viewpoint, incorporating structural components of particular importance to an organism, along with the scale of relevance and specific behavioural responses (Tischendorf and Fahring 2000). For pollinators, the availability of preferred plant species within a habitat is particularly important (Rosa et al. 2015) and where these resources are scarce, pollinators have been observed to avoid habitats (Hadley and Betts 2009). From a plant's perspective, however, this habitat avoidance can result in increases in long distance and direct pollen movements. For instance, a higher incidence of inter-population pollen movement has been observed over resource poor habitats (e.g. pine plantations) when compared to habitats rich in floral resources (e.g. clearfell woodland) (Lander et al. 2011).

The movement of pollinators is expected to reflect optimal foraging behaviour, where decisions are made to minimise the physiological costs associated with searching and handling (Charnov 1976). This is illustrated by a high degree of constancy among pollinators to single plant species (Chittka et al. 1999, Gegear and Laverty 2005) along with a tendency for localised foraging, where movement has been shown to be greatest between plants separated by shorter distances (Ghazoul et al. 1998). Pollinator foraging behaviour is therefore expected to be largely influenced by the extent and distribution of floral resources across the landscape. Indeed, changes to floral communities can alter pollinator constancy. For instance, where floral resources are scarce, pollinators have been shown to relax preferences in order to use all available flowers and thus reduce energy expended through dispersal (Levins and MacArthur 1969, Kunin 1993). Changes to floral communities can similarly affect pollinator dispersal distance. Depending on its life history (Jauker et al. 2009) and characteristics such as body mass (Greenleaf et al. 2007), a pollinator is capable of travelling large distances (Osborne et al. 1999, Gathmann and Tscharntke 2002, Hagler et al. 2011). However, these distances often do not reflect typical pollinator foraging behaviour (Fahrig 2001, Rader et al. 2011). Instead, pollinator dispersal distance appears to be a function of the extent and spatial distribution of floral resources, where foraging distance is inversely related to floral cover (Danner et al. 2016, Redhead et al. 2016).

Depending on the distance of separation between plants, changes to foraging behaviour, both in terms of the dispersal distance and constancy of visiting pollinators, will have considerable implications for pollen transfer (Murphy and Lovett-Doust 2004, Dileo et al. 2014). Reproductive potential is influenced by the quality of intraspecific pollen deposition (e.g. the genetic relatedness). Plant populations typically exhibit spatial genetic structure, where relatedness declines with distance between individuals (Loveless and Hamrick 1984) as a function of seed dispersal capability. Consequently, the genetic relatedness of pollen received by a plant is driven by pollinator dispersal distance (Matsuki et al. 2008). For instance, localised pollen movement results in a greater incidence of mating between close relatives and an increased likelihood of self-fertilisation through geitonogamous pollen transfer (Ellstrand and Elam 1993). This can elevate the risk of inbreeding depression (Turner et al. 1982), which is characterised by increases in homozygosity and reductions in allelic diversity (Byers and Waller 1999). Furthermore, population genetic theory says that increased inbreeding, coupled with genetic drift, will culminate in a build-up of recessive deleterious alleles (Charlesworth and Charlesworth 1987); an effect that will be particularly severe in small, isolated populations (Ellstrand and Elam 1993). This said, localised pollen movement and self-fertilisation can be seen to negatively impact plant fitness and adaptive potential (Richards 1997, Grindeland 2008). However, fitness has also been shown to be reduced when outcrossed pollen is sourced from plants separated by large distances (e.g. outbreeding depression) (Ruane et al. 2015) and indeed, a benefit of shorter dispersal distances has been observed (Grindeland 2008). A plant's reproductive potential is further affected by inter-specific pollen transfer, which can be affected by changes to pollinator constancy (Bell et al. 2005). For instance, as pollinators move more readily between different plant species, the incidence of heterospecific pollen transfer increases, introducing potential for clogging at the stigma or style level (Shore and Barrett 1984, Brown and Mitchell 2001). Depending upon a plant's mating system and given the positive relationship between compatible pollen deposition and seed production (Shore and Barrett 1984, Bernhardt et al. 2008, Briggs et al. 2016), such changes can have negative implications for a plant's reproductive potential.

Driven by landscape change, plants are facing an increased risk of isolation from conspecifics. Under these conditions, ensuring inter-population pollen movement is essential in order to maintain genetic exchange and thus, long-term population survival (Steffan-Dewenter and Tscharntke 1999). Previous research has however demonstrated reductions in inter-population pollen movement in response to high floral availability between conspecific plant populations (Evans et al. 2017). In this chapter I expand on this research and focus on the effects of a gradient of floral communities between conspecific plant populations on plant-pollinator interactions. More specifically, I explore how variations in the floral resources within a plot affects the incidence and distance of inter-population pollen movement and the relationship with pollinator communities and plant reproductive success. This is approached through two temporally separated experiments using a self-compatible plant, *Vicia faba,* which benefits from pollinator-triggered pollen release (Aouar-Sadli et al. 2008). Plants were introduced into plots varying in floral composition and were separated by different distances. Pollen movement was measured both between plots (15m) and at different distances within plots (5-8m) and the relationships between pollen movement and pollinator activity density and inter-tegular span ('IT' span) were determined. In addition, the implications for *V. faba* plants were explored through measures of heterospecific pollen deposition and plant reproductive success. I hypothesise:

i) The incidence of pollen movement, both between and at different distances within a plot are inversely related to the abundance and diversity of heterospecific floral resources within a plot;

ii) The activity density of potential *V. faba* pollinators is negatively correlated with the abundance and diversity of floral resources within a plot; this is positively related to the incidence of between- and within-plot pollen movement;

iii) The IT span (a proxy for body mass) of potential *V. faba* pollinators is positively related to the incidence of between- and within-plot pollen movement. In contrast, pollinator IT span is not related to the abundance or diversity of floral resources within a plot;

iv) The deposition of heterospecific pollen is negatively correlated with the abundance and diversity of floral resources within a plot;

v) Reproductive activity in *V. faba* plants is not related to the abundance or diversity of floral resources within a plot. However, the mean seed weight and total biomass of seed production, is negatively correlated with the abundance and diversity of floral resources within a plot.

5.3 Methods and materials

5.3.1 Experimental site and study system

The two experiments were conducted on the long-term Park Grass Experiment at Rothamsted Research, Hertfordshire, UK (0° 21' 22.76"W, 51° 48' 34.4448"N; Fig.5.I); an area characterised by flat topography and a temperate climate. Park Grass was set up in 1856 (Silvertown et al. 2006) and is the oldest ecological experiment in existence. It was originally set up to understand the effects of different fertilisers on the yield of hay meadows (Lawes and Gilbert 1859) but in recent times has proved invaluable in exploring the long-term dynamics of plant populations. The site comprises 97 adjoining plots spanning over 2.8ha (Fig.5.I) (Silvertown et al. 2006). Plots vary in size (~0.013ha to ~0.05ha) and, driven by the historic differences in fertiliser regimes, each comprises a distinct floral community (Silvertown et al. 2006). Modern species numbers vary from virtual monocultures to 44 species per 200 m^2 among the plots (Silvertown et al. 2006).

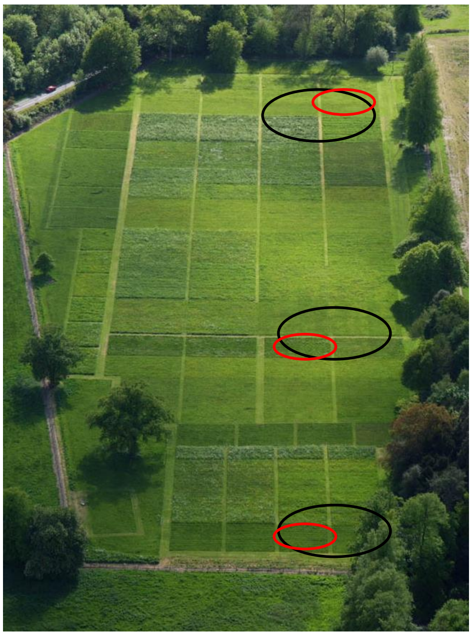

Figure 5.I The layout of the Park Grass experiment at Rothamsted Research, Harpenden, UK (Courtesy of Rothamsted Research). Black circles illustrate the 12 plots used in experiment one and red circles illustrate the three plots used in experiment two

To test the effects of variations in floral resources at local scales on the incidence and distance of pollen movement, *Vicia faba* L. (Fabaceae) plants (two cultivars with comparable phenology; Wizard and Arthur) were introduced into experimental plots. *Vicia faba* is a nectar producing plant characterised by small, white flowers (Fig.5.II). Whilst it is self-compatible, pollinators have been demonstrated to trigger pollen release and enable outcrossing (Aouar-Sadli et al. 2008). Outcrossing rates in *V. faba* are variable and have been demonstrated to account for 29.5-69.8% of mating events under field conditions (Holden and Bond 1960). Pollinators include species from the families Apidae, Megachilidae, Halictidae and Syrphidae (Aouar-Sadli et al. 2008, Garratt et al. 2014) though of these, *Bombus spp.* are recognised to be the most efficient (Garratt et al. 2014). The use of *V. faba* var. Arthur and *V. faba* var. Wizard enabled discrimination of pollen movement through a dominance of hilum (seed connection scar) colour. Wizard plants produce seeds with a white hilum (homozygous recessive) and Arthur plants produce seeds with a black hilum (homozygous dominant) (Holden and Bond 1960, Bishop et al. 2016). Given that hilum colour is maternal, any seeds from the Wizard progeny which exhibit black hilum (expressed in second generation seeds) will therefore be the product of outcrossing with Arthur plants (Bishop et al. 2016). Plants were grown from seed (seed source: Aberystwyth University) in compost-filled seed trays under glasshouse conditions (20°C during the day, 16°C at night, with 12 hours of light and 12 hours of dark) and were transferred to 1L pots when at the seedling stage.

The study consisted of two experiments (Fig 5.III). Experiment one looks at the effects of a gradient of floral communities at a local scale on between-plot pollen movement. Experiment two instead focuses on the effects of a gradient of floral communities at a local scale on plant-pollinator interactions within a plot, through measuring the distance and directness (through heterospecific pollen deposition) of within-plot pollen movement.

Figure 5.II From left to bottom right: A Vicia faba flower on a plant raceme and: V. faba seeds exhibiting black hilum and white hilum (photos taken from Khamassi et al. 2014).

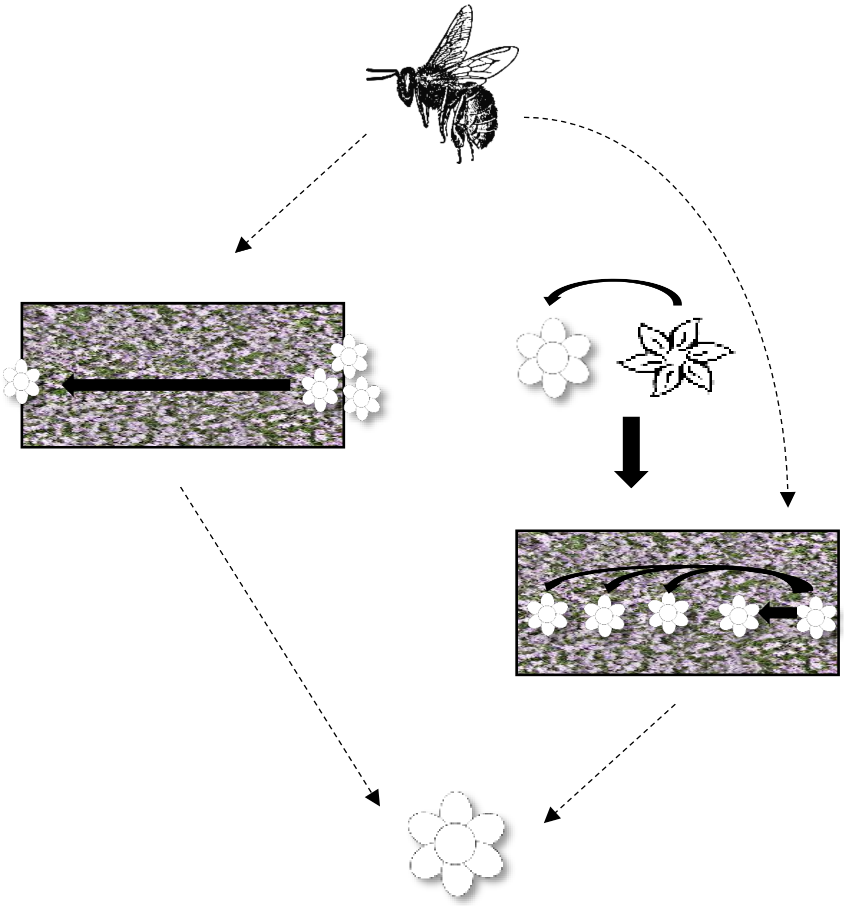

Figure 5.III A schematic illustrating experiments one and two which explore the effects of a floral gradient on plant-pollinator relationships. Experiment one (left) measures between plot pollen movement and the relationship with pollinator activity density along with the implications for plant reproductive success. Experiment two (right) alternatively measures the distance and movement of pollen within a plot and the incidence of inter-species pollen transfer (through heterospecific pollen deposition). Here again I explore the relationship between pollen movement and pollinator activity density along with the implications for plant reproductive success

Experiment one:

In May 2016, *V. faba* plants were introduced at the junction between four Park Grass plots to form experimental arrays. Arrays comprised of four plants, separated by 0.5m and arranged in a triangular formation with a central Wizard plant and three outer Arthur plants (henceforth ‘central array’: Fig.5.IV). Four additional arrays were then positioned along a plot edge at a 15m distance from the central array; each was separated from the central array by a different intervening plot (forming a cross formation; Fig.5.IV). These arrays consisted of three Wizard plants, separated by 0.5m and arranged in a triangular formation (henceforth ‘radiating array’). This set-up was replicated in three locations across the experimental site; each separated by a distance greater than 100m in order to restrict pollen movement between spatial replicates (Evans et al. 2017) (Fig.5.I). Plants remained in the field for seven days and the experiment was repeated temporally over four consecutive weeks. The abundance and diversity of floral resources within each of the 12 plots was measured weekly over the duration of the experiment and was calculated as the total number of floral units (e.g. one *Taraxacum officinale agg.* flower head represented one floral unit) averaged across five 0.5m² quadrats which were randomly dropped within the plot (Appendix 1: Plant list). Floral communities differed across spatial and temporal replicates (Wilcox Abundance: $V = 820$, $df = 47$, $p< 0.001$; Diversity: $V = 820$, $df = 47$, $p< 0.001$; Fig.5.V). Plots were then divided into classes of floral abundance using the Braun-Blanquet scale to assess the effect of habitat (Braun-Blanquet, 1932) (Number of floral units/0.5m² habitat class 1 = 0, habitat class 2 = 1-5, habitat class 3 = 5-25, habitat class 4 = 25-50, habitat class 5 = 50-75, habitat class 6 = <75).

Figure 5.IIV From top left to bottom right: A central Vicia faba array in the junction of four plots; the three spatial replicates of the experimental set-up in experiment one. Arrows illustrate the intervening plot between central and radiating arrays.

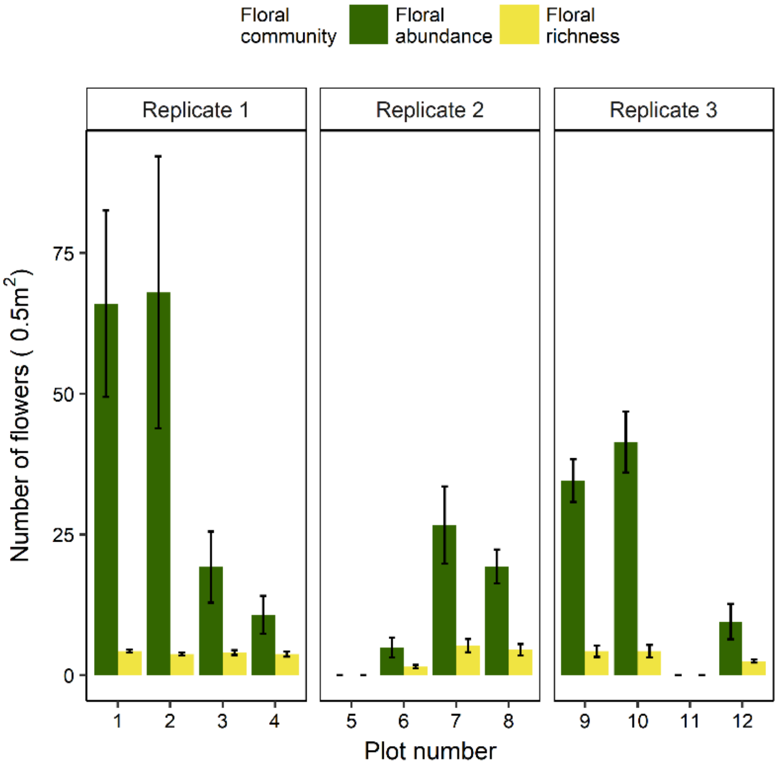

Figure 5.V The abundance and richness of floral resources in each of the 12 plots across the three spatial replicates (measured using five 0.5m² quadrats), averaged over the four temporal replicates.

Experiment 2

In August 2016, central arrays of *V. faba* (as in experiment one: Fig.5.VI) were introduced into three of the plots used in experiment one (ensuring a gradient of floral resources) (Fig.5.I). Eight Wizard plants were then positioned at different distances (2x5m, 2x6m, 2x7m, 2x8m) from the central array (forming a circular formation; Fig.5.VI). As before, this set-up was repeated in three locations across the experimental site; each separated by a distance greater

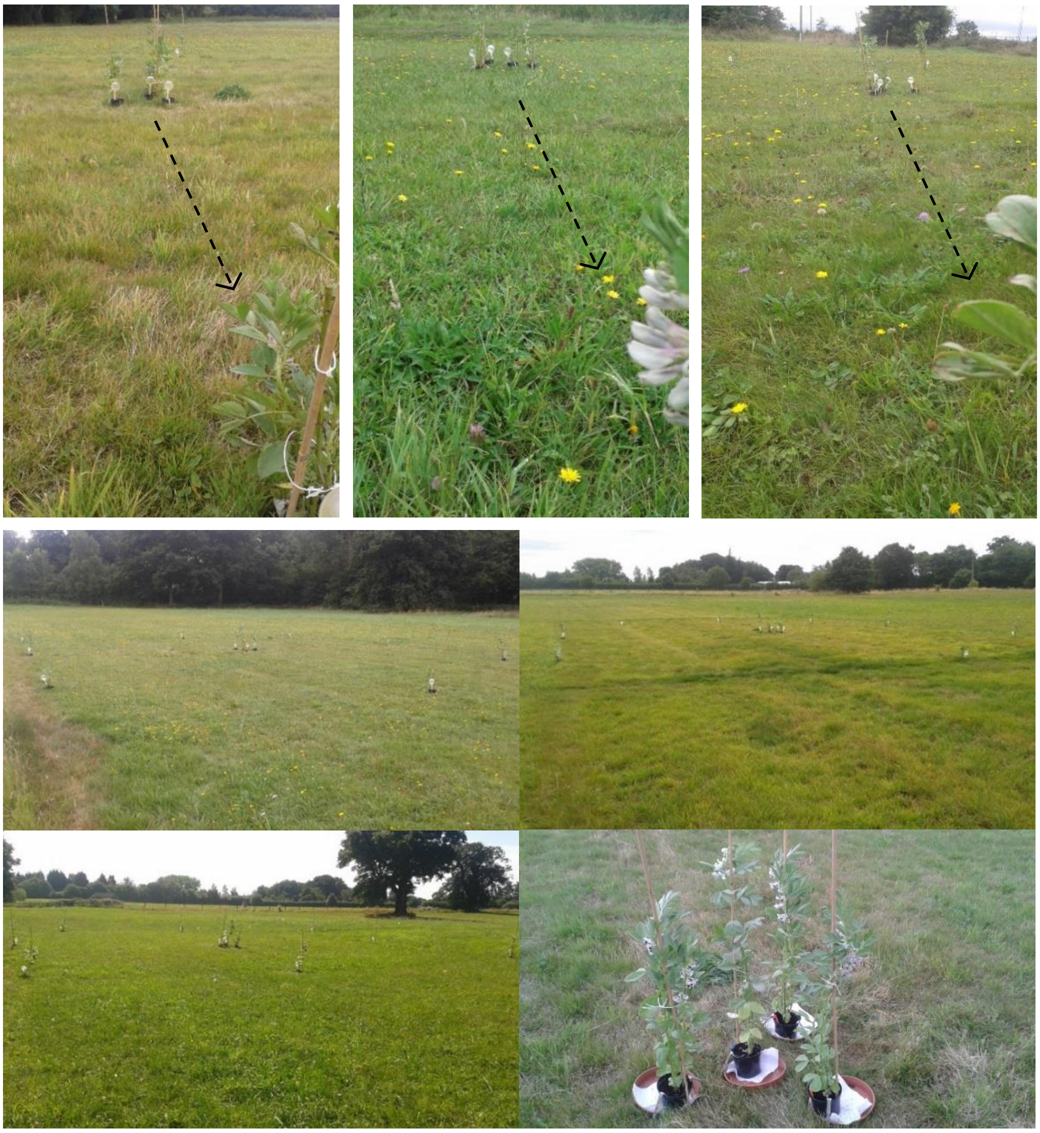

Figure 5.VI From top left to bottom right: The three plots in experiment two. Arrows illustrate the intervening plot between the central array and radiating Wizard plants; the three plots, illustrating the central array and the eight radiating Wizard plants at different distances and the central V. faba array.

than 100m. Plants remained in the field for seven days and the experiment was repeated temporally over four consecutive weeks (one replicate was however destroyed by inclement weather leaving only three replicates). The abundance and diversity of floral resources was measured (as in experiment one) (Appendix 1: Plant list) and similarly differed across spatial and temporal replicates (t test Abundance: t = .54, df = 8, p = 0.008; Wilcox Diversity: V = 45, df = 8, p < 0.009; Fig.5.VII).

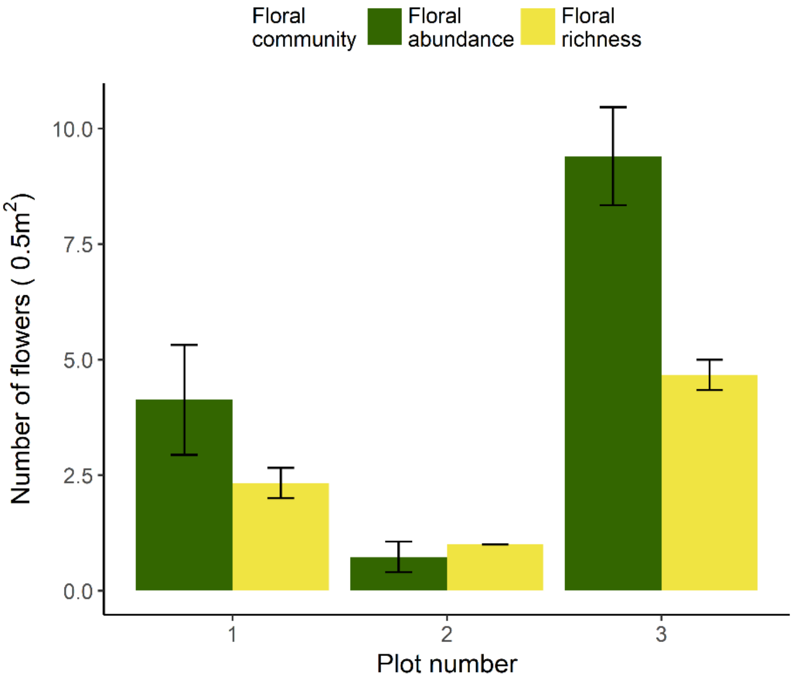

Figure 5.VII The abundance and richness of flower resources in each of the three plots (measured using five 0.5m² quadrats), averaged over the three temporal replicates.

5.3.2 Pollen movement

Upon reaching reproductive maturity (i.e. flower production), all open flowers on Wizard plants were removed and one flower on each plant was covered in a fine muslin to measure the incidence of auto-pollination in the absence of pollinator availability. Plants were subsequently introduced into pre-marked locations. Following the seven-day study period Wizard plants were collected in (Arthur plants were discarded) and all unopened buds were removed to avoid confusion with flowers subjected to field conditions. Plants were then stored under glasshouse conditions (as above) until fruit maturation. Following the maturation of fruit, a sub sample of three seeds from up to three pollinator exposed fruit per plant (depending on the number of fruits produced) were sown and were raised under glasshouse conditions (as above). When at reproductive maturity, flowers from second generation plants were continually ‘tripped’ (agitated) to encourage self-fertilisation (given that hilum colour is maternally expressed). Upon maturation, one fruit from each second generation plant was then examined (given that all fruit produced by a maternal plant will produce seeds exhibiting the same hilum colour). For each radiating plant/array the number of the second generation plants comprising black hilum and the number comprising white hilum were then recorded. This system precludes discrimination of Wizard to Arthur movements as well as Wizard to Wizard or Arthur to Arthur movements (Bishop et al. 2016) and thus is only capable of detecting a proportion of pollen movement. Given this, pollen movement in this study represents detected between- and within-plot pollen movement.

5.3.3 Pollinator communities

The activity density of pollinators known to visit *V. faba* plants was quantified within each plot using pan traps (Westphal et al. 2008). Traps comprised three water-filled circular plastic bowls (80x200mm) painted with non-toxic fluorescent paint (one yellow, one blue and one white; UV Gear, UK). Pan traps have been deployed to describe pollinator species richness and activity density (Westphal et al. 2008) and, in some cases, to provide a surrogate measure of visitation (Ricketts et al. 2008). However, this survey method has been recognised to exhibit bias (Roulston et al. 2007) because the attractiveness of pan traps depends upon habitat and landscape context (Baum and Wallen 2011). Pollinators are less likely to encounter traps when floral resources are abundant and more likely to encounter traps when floral resources are scarce i.e. capture rates are proportional to visitation rates per unit flower area (Veddeler et al. 2006). I exploited this phenomenon to measure the attractiveness and pollinator activity density at the experimentally rare plant arrays located within different habitats.

Pan traps were placed in the centre of each plot immediately following the temporal replicate to avoid influencing plot attractiveness. Samples were collected at each plot (12 in experiment one and three in experiment two) on four occasions (or three in the case of experiment two) during 32-day study periods. Each survey was carried out in a randomised order between 0930 and 1700. After traps had been deployed for 24 hours, the catch was strained through fine muslin. All flower visitors were assigned to a broad taxonomic group (*Bombus* spp., *Apis mellifera*, solitary bee (small, medium and large), Syrphid (small, medium and large), Lepidoptera, Diptera and Coleoptera) and were subsequently counted. Of the total pollinators, the number of pollinator individuals from groups known to pollinate *V. faba* (Apidae: *Apis mellifera*, *Bombus* spp., solitary bee (small, medium and large) and Diptera: Syrphidae (small, medium and large)) was determined. Given the positive relationship between body mass and foraging distance in Apoidea (Greenleaf et al. 2007), the distance between the wing bases (Inter-tegular span, henceforth ‘IT’ span), which provides a proxy for dry body mass in Apoidea (Cane 1987), was determined. This relationship is less clear in Lepidoptera and Syrphidae, however inter-tegular span has previously been used as a measure of dry body mass in the latter given observed correlations (Folkö 2014). I therefore adopted this measure to maintain consistency, but it is important to note the potential inaccuracy in estimations due to differences between pollinator groups. The IT span was therefore measured for up to five insects from up to 13 species representing each pollinator group listed above using digital callipers. From this, an average IT span was calculated for each group of potential *V. faba* pollinators (listed above) and applied across the whole pollinator catch. A community weighted IT span was then calculated for pollinators within each plot.

5.3.4 Heterospecific pollen deposition

The deposition of heterospecific pollen on *V. faba* plants was measured in experiment two to assess the directness of within-plot movement through the occurrence of interspecific pollen transfer. Prior to the transference of plants into pre-marked locations, three buds (early stage) on each Arthur plant were covered with fine muslin to exclude pollinators and thus, serve as a control. After the seven-day study period, stigmas were removed from three pollinator-excluded and three pollinator-exposed flowers (using dissecting tweezers rinsed with ethanol) on each of the three Arthur plants within the central arrays. Each stigma was transferred directly to separate Eppendorf tubes containing 50µl of 70% ethanol.

Prior to measuring heterospecific pollen deposition, ethanol was left to evaporate from tubes. Following this, three drops of Calberla’s solution (5ml glycerine, 10ml 95% ethanol, 15ml distilled water and 2-5 drops of fuchsin mix (50:50 distilled water and fuchsin crystals); Dafni et al. 2005) was added to each Eppendorf in order to stain pollen and aid identification. Tubes were sonicated and the solution was mounted on a slide using a pipette and sealed with a coverslip. Slides were examined under a compound microscope at X20 magnification and the number of heterospecific and conspecific pollen grains were recorded.

5.3.5 Plant reproductive success

The number of viable seeds were counted for each of the fruits produced by Wizard plants in order to obtain a measure of seed set in plots varying in floral resources (described in section 5.2.1). Following this, the seeds from each fruit were placed in separate sealed paper envelopes and oven dried at 80°C for seven days to obtain a standardised desiccation state (Bishop et al. 2016). Upon drying, the total seed set of each fruit was weighed to obtain the biomass of reproductive output and then divided by the total number of seeds produced per fruit to obtain the mean seed weight from plants subjected to field conditions.

5.3.6 Statistical analysis

Generalised linear mixed effects models (GLMMs) were used to analyse pollinator communities, heterospecific pollen deposition and plant reproductive success. Where random effects did not account for variation in the data generalised linear models (GLM's) and linear models (LM's) were used instead. For pollinator community models, a GLMM with a Poisson error distribution was used both for the total activity density of pollinating insects (n = 48) and the activity density of potential *V. faba* pollinators (n = 48) in experiment one, whereas an LM with a Gaussian error distribution (n = 9) was used in both cases in experiment two. In contrast, the community weighted mean IT span of potential *V. faba* pollinators was analysed using an LM and a GLMM with a Log-normal error distribution (to account for non-integers) in experiments one (n = 30) and two (n = 9) respectively. Further, the deposition of heterospecific pollen was modelled using a GLMM with a Poisson error distribution (n = 81). When analysing plant reproductive success, seed set was modelled using a GLM in experiment one (n = 330) and a GLMM in experiment two (n = 125). This was analysed with a Binomial error distribution, where the number of seeds produced by a field-exposed fruit was modelled against the maximum number produced across the experiment (given that seed set was constrained at low numbers). The weight of seeds (both mean seed weight and reproductive biomass) was similarly modelled using a GLMM with a Log-normal error distribution in both experiments one (n= 330) and two (n= 125), with the exception of mean seed weight in experiment two which was modelled with a Gaussian error distribution.

In all models, fixed effects comprised the abundance and diversity of floral resources within experimental plots. In addition, the distance of separation between plants was included as a fixed effect in experiment two. To account for the structure of the experimental design, temporal replicate (nested within plot) was included as a random effect in all GLMMs using Poisson and Binomial error distributions. Alternatively, in GLMMs using Gaussian error distributions, only the temporal replicate was included as a random effect given the restrictions of the model. Stepwise selection using Laplace approximation (suitable for <3 random effects: (Bolker et al. 2009)) was then used to determine the minimal adequate model. Minimal models were subsequently compared to a null model with an identical random error structure, using an analysis of variance (ANOVA).

Given the sparsity of between- and within-plot pollen movements, statistical analysis of movement in relation to floral resources was precluded. The relationship between pollen movement and both pollinator activity density and pollinator community weighted mean IT span was however analysed using a GLM with a Poisson error distribution. All analyses were conducted in ‘R’ (version x64; R Core Team, 2013), using the lme4 package (Bates et al. 2015).

5.4 Results

5.4.1 Pollen movement

Outcrossing was observed in the majority of the central *V. faba* arrays examined, indicating an occurrence of intra-array pollen movement within this study (88.89% (n = 9) and 75% (n = 4) of arrays examined in experiments one and two respectively (only 13 arrays examined given the sparsity of fruit production). However, low levels of between-plot pollen movement (15m) was observed in experiment one (11.43% of examined fruit (n = 105)), which precluded formal statistical analysis. When plots were grouped into classes based on floral abundance using the Braun-Blanquet scale, overall, between-plot pollen movement was greatest over plots comprising intermediate floral abundance (Fig.5.VIII).

When measuring within-plot pollen movement at different distances from the central array in experiment two, fruit production was limited and, consequently, low levels of within-plot pollen movements were observed (8.3% of examined fruit (n = 24)), similarly precluding statistical analysis. These within-plot pollen

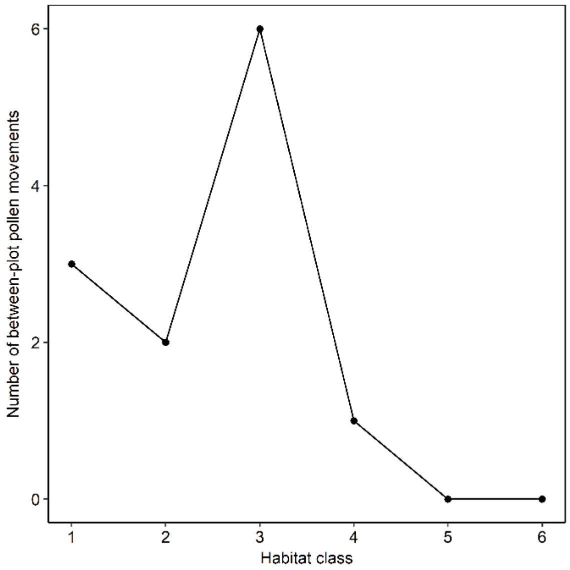

Figure 5.VII The total number of between-plot pollen movements when plots were split into classes based on floral abundance using the Braun-Blanquet scale (Floral units/0.5m² 1 = 0, 2 = 1-5, 3 = 5-25, 4 = 25-50, 5 = 50-75, 6 = >75).

movements were observed in plots three (n = 1) and nine (n = 1), where floral abundance was relatively low (1.4-11.4 flowers/0.5m²), between plants separated by a distance of 5m.

5.4.2 Pollinator communities

Overall the activity density of pollinators was low (total catch: experiment 1 = 1920 individuals; experiment 2 = 251 individuals). Of the potential *V. faba* pollinators (Apoidea and Syrphidae) only 72 individuals (experiment 1, dominated by solitary bees, 76%) and 32 individuals (experiment 2, dominated by syrphids, 34%) were recorded. In experiment one, the total number of pollinating insects was not significantly related to the diversity or abundance of floral resources within a plot (p = 0.504 and p = 0.705 respectively). In contrast, the activity density of pollinators known to pollinate *V. faba* (Apidae and Syrphidae) was negatively correlated with a plot's floral abundance (GLM z = -2.27, df = 44, p = 0.024, R²C = 0.37; Fig.5.IX) but not diversity (p = 0.164). Of these pollinators, no relationship was observed between the diversity or abundance of floral communities within a plot and the community weighted mean IT span of potential *V. faba* pollinators, though a weak positive relationship was measured in the latter (p = 0.692 and p = 0.097 respectively). In contrast, in experiment two, neither the activity density (the total number or the number of potential *V. faba* pollinators), nor the community weighted mean IT span of potential *V. faba* pollinators was affected by the abundance (p = 0.993, p = 0.673 and p = 0.984 respectively) or diversity (p = 0.864, p = 0.583 and p = 0.928 respectively) of floral resources within a plot.

Assessment of the relationship between pollinator communities and pollen movement was only analysed in experiment one given the particularly low incidence of pollen movement in experiment two. In doing this, between-plot pollen movement (15m) exhibited no relationship with either the activity density of pollinators or the community weighted mean IT span of potential *V. faba* pollinators (p = 0.376 and p = 0.360 respectively).

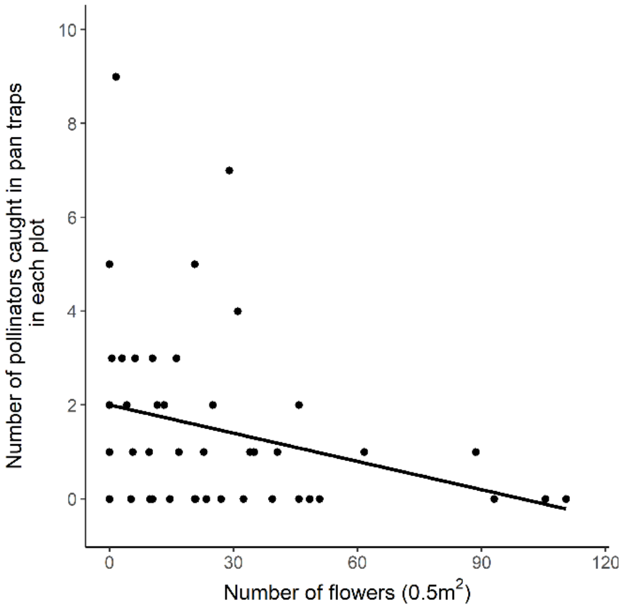

Figure 5.VIII The total number of potential V. faba pollinators (e.g. Apidae and Syrphidae) caught within pan traps in experiment one in relation to the abundance of floral resources within a plot.

5.4.3 Heterospecific pollen transfer

In experiment two, which looked at within-plot pollen movement, the number of heterospecific pollen grains deposited at a flower level was low (mean ± SE heterospecific: 0.81 ± 0.13; conspecific: 462.75 ± 64.17) and on flowers containing such pollen, this only represented 1-3 plant species, indicating a low incidence of interspecific pollen transfer within central *V. faba* arrays. Unsurprisingly therefore, the deposition of heterospecific pollen was not significantly related to the abundance or diversity of floral resources within a plot ($p = 0.207$ and $p = 0.235$ respectively). Although all heterospecific pollen was not identified to species level, pollen observations included plant species from within plots (e.g. *Plantago lanceolate,* a predominantly wind pollinated plant), plant species from within nearby plots (e.g. *Leontodon hispidus*) and plant species from the wider landscape (e.g. *Pinus* spp. (wind pollinated)).

5.4.4 Plant reproductive success

The number of seeds produced by *V. faba* plants was relatively consistent (mean ± SE Experiment 1 = 2.81 ± 0.15; Experiment 2 = 2.62 ± 0.23). Unsurprisingly therefore, no relationship was observed between seed set and the abundance and diversity of floral communities within a plot in either experiment one ($p = 0.643$ and $p = 0.833$ respectively) or experiment two ($p = 0.136$ and $p = 0.134$ respectively) (when analysed against the maximum seed set observed; eight and five respectively). Similarly, the biomass of the reproductive output (total weight of a fruit’s seed set) together with the mean weight of seeds was not significantly correlated with the abundance ($p = 0.785$ and $p = 0.950$ respectively) or diversity ($p = 0.789$ and $p = 0.558$ respectively) of floral communities within a plot in experiment one. Similarly, in experiment two, no relationship was observed between the reproductive output or the mean weight of seeds and the abundance ($p = 0.172$ and $p = 0.651$ respectively) or diversity ($p = 0.227$ and $p = 0.769$ respectively) of floral communities within a plot. When analysing the effect of the distance between central populations and radiating plants in experiment two, no effect was observed on seed set (p = 0.881), seed weight (p = 0.291) or reproductive biomass (p = 0.500).

5.5 Discussion

5.5.1 The effects of a habitat's floral composition on pollen movement between conspecific plant populations

This study indicates that the connectivity of conspecific plant populations is a function of the floral composition within the intervening habitat. This was illustrated in experiment one (looking at between-plot pollen movement) through an absence of pollen movement over plots comprising high floral availability. Although the sparsity of between-plot pollen movement in experiment one precludes concrete conclusions, these findings offer support to hypotheses. Moreover, these patterns reflect findings from chapter four, where inter-population pollen movement was reduced in habitats of high floral cover when compared to habitats of low floral cover (Evans et al. 2017). Findings here further this research and demonstrate a benefit of an intermediate cover of floral resources in enhancing pollen movement between conspecific plant populations. This is likely to be driven by the opposing effects of facilitation (recruitment of pollinators to habitat patches) and competition (between plants for higher visitation and constancy of visits between conspecifics). When habitats comprise low-intermediate levels of floral resources, co-flowering plants enhance the attractiveness of a habitat, facilitate visitation and increase pollen transfer between conspecifics. However, as floral resources increase, pollen transfer declines as pollinators are co-opted by competitive co-flowering plants (Ghazoul 2006, Jakobsson et al. 2009). The degree to which facilitation or competition occurs depends on the relative competitive advantage (e.g. large population size) offered by co-flowering plants in relation to conspecifics (Ghazoul 2006). A high degree of competition for pollinators is reflected in this study by a reduction in between-plot pollen movement.

In experiment two, the incidence and distance of within-plot pollen movement was similarly low. Where observed, pollen movement was between plants separated by close distances (5m) or between plants within central arrays (intra-population movements). Although conclusions can not be drawn here, this is suggestive of localised foraging. These findings support hypotheses which predict that pollinators base decisions on the trade-off between energy loss and energy gain. This is explained by optimal foraging theory and the benefit of shorter handling times when moving between plants separated by closer distances (Marden and Waddington 1981). The tendency of pollinators to forage locally, particularly in response to a habitat's floral cover, is illustrated by previous research on pollen movement in *V. faba* plants, where intra-population pollen movement increased in response to low floral availability (chapter three). The relationship between dispersal distance and floral availability has been observed by a wealth of research where a negative relationship has been demonstrated between foraging distance and the abundance and diversity of floral resources in the wider landscape (Jha and Kremen 2013, Danner et al. 2016, Redhead et al. 2016). As distances between plants increase, pollinators are faced with a trade-off between moving greater distances in search of preferred flowers or relaxing their constancy and switching to another plant species (Charnov 1976). Results from experiment two however demonstrate low levels of heterospecific pollen transfer irrespective of floral resources within a plot. This suggests that pollinators did not relax their constancy in line with habitat context as hypothesised. These findings are inconsistent with previous research which has shown reductions in constancy in response to a scarcity of floral resources (Kunin and Iwasa 1996). Inconsistencies may be explained by low visitation to *V. faba* plants and thus, low levels of pollen movement in general. However, the response of pollinators to habitat context is expected to vary with pollinator taxon (Rader et al. 2016). For instance, foraging distance in generalist central place foragers, such as *Bombus spp*., has been shown to be driven more by the diversity than the abundance of a habitat's floral resources (Jha and Kremen 2013). This is arguably due to the importance of *Bombus spp.* maintaining a stable food supply across temporal and spatial scales in order to cater for their life cycle and foraging capability. In this study, no relationship was observed between floral diversity and pollen movement, probably due to the modest differences in species composition of floral communities between plots and the low incidence of between- and within-plot pollen movement. This study took advantage of the standardised size and distance of separation between study plots. In future experiments, it would be important to capture a greater variation in floral communities to reduce the unbalanced categorisation of habitats, while increasing sampling effort in order to better detect the low incidence of between- and within-plot pollen movement.

5.5.2 The influence of pollinator communities on pollen movement

In this study, the activity density of potential *V. faba* pollinators did not influence the incidence of between-plot pollen movement in experiment one. This contradicts previous research which demonstrated a positive relationship between pollinator activity density and inter-population pollen movement (chapter four). These inconsistencies may be attributable to the relationship between pollinator activity density and visitation rates and the biases attributed to using pan traps for measuring pollinator activity and richness (see 5.3.3). In contrast to chapter four, no direct observations were made between pollinator groups and visitation to *V. faba* plants and therefore relationships between pollinators and focal plants and biases of pan traps cannot be ascertained. Findings are however consistent with research looking at pollen movement in *V. faba* (chapter three). This indicates that implications of altered pollinator behaviour are plant specific and dependent upon interactions with pollinators. Findings here demonstrate reductions in pollinator activity density with increases in the abundance of floral resources. While this is not a direct measure of visitation, this is consistent with previous research demonstrating a negative relationship between *'per floral unit'* visitation and the abundance of floral resources within a habitat (Veddeler et al. 2006). This pattern supports hypotheses and is explained by the landscape-moderated dilution and concentration hypothesis (Tscharntke et al. 2012), which suggests that ‘per *floral unit’* visitation is reduced in response to a high availability of alternative resources (Veddeler et al. 2006, Sjodin 2007). Reductions in pollinator visitation are expected to correspond to reductions in pollen movement and therefore, the dilution of pollinators within florally rich habitats may explain the absence of between-habitat pollen movement in these habitats in this study.

Pollinator species differ considerably in their foraging behaviour. For instance, dispersal distance has been shown to vary between species (Rader et al. 2011, Danner et al. 2014) in relation to characteristics such as IT span (a proxy for body size (Greenleaf et al. 2007) and length (Gathmann and Tscharntke 2002). Here, however, no relationship was observed between floral resources and the community weighted mean IT span of potential *V. faba* pollinators within a plot. This may be due to the small number of pollinators observed across surveys, combined with the dominance of communities by small sized pollinators (solitary bees and Syrphids in experiments one and two respectively). This community composition may explain the low levels of pollen movement observed across the two experiments given that both are recognised to be poor pollinators and infrequent visitors of *V. faba* in the field (Garratt et al. 2014). Moreover, this may offer an explanation for the absence of a relationship between pollinator activity density pollen movement. Syrphids, in particular, have been shown to carry smaller pollen loads, comprising less viable pollen than bees (Rader et al. 2011). In this study, as I had focussed on pollen movement rather than pollinator movement *per se*, I did not discriminate the effects of different pollinator species, and this could be a productive avenue for future studies.

5.5.3 The implications of altered pollinator communities and pollen movement on plant populations

In both experiments one and two, the seed set of *V. faba* plants was not affected by the abundance or the diversity of floral resources within a plot. Seed set is positively related to the deposition of compatible pollen, which is a function of pollinator visitation (Engel and Irwin 2003, Mayer et al. 2012). Pollinator activity density in experiment one were reduced within plots comprising high floral abundance, suggesting reduced visitation. Given that seed set was not reduced in parallel, this indicates that the supply of outcrossed pollen was not a limiting factor for *V. faba* reproduction in this experiment. Although pollen limitation, illustrated in chapter four, has been observed frequently in self-incompatible plants (Harder and Aizen 2010), this either suggests that limited pollen is required for seed set (Garratt et al. 2014), or alternatively, that selfing provided reproductive assurance when pollinator availability was low (Eckert et al. 2010). The quantification of self-fertilisation was precluded in this study given limitations of the plant system and high abortion rates of pollinator excluded flowers. In experiment two however, given that radiating plants comprised of an individual plant and that low levels of within-plot movement were observed, a high degree of self-fertilisation is expected. While self-fertilisation can have negative implications for plant seed set, previous research demonstrated comparable seed set in selfed and outcrossed *V. faba* plants (Garratt et al. 2014). This suggests that *V. faba* plant reproduction is not negatively affected by the source of pollen (i.e. whether it was outcrossed or selfed).

When measuring the fitness of progeny, findings from both experiment one and two indicate that *V. faba* seed weight, similarly, was unaffected by habitat context in this study. This is suggestive that self-fertilisation does not affect the fitness of *V. faba* plants. However, conclusions on the comparative fitness of plants were precluded as self-fertilisation rates could not be measured due to a sparsity of within-habitat pollen movement data. *Vicia faba* plants have however previously been shown to exhibit comparable fitness following reproduction by either outcrossing or self-fertilisation (Garratt et al. 2014). This contradicts hypotheses which predicted negative effects of self-fertilisation, but instead reflects the effectiveness of the self-compatibility system in *V. faba.* This may be driven by the capacity of *V. faba* plants to purge deleterious effects or, alternatively, because such plants are shown to carry a lower genetic load (fewer deleterious recessive alleles then obligate outcrossers which have higher genetic loads) as they are regularly exposed to selection. Given this, fitness effects in selfers may not be observed in studies observing immediate, short term effects (Lopez-Cortegano et al. 2016). The implications of self-fertilisation however will vary depending on a plant's life stage (Husband and Schemske 1996) and may only be detected in specific fitness traits (Thiele et al. 2010). This was illustrated by a study which demonstrated that while selfed progeny were shown to be 50% less fit than outcrossed progeny when analysing seed set and pericarp mass, no effect was observed when examining seed weight or germination (Dudash 1990). This emphasises the need to measure a greater variety of traits when examining the fitness effects of selfing on a plant's progeny.

Plant fitness is further influenced by the genetic distance of outcrossed pollen, where progeny fitness is greatest when the distance between the pollen source and the maternal plant is optimal (Dudash 1990). This results from spatial genetic structure (SGS) in plant populations, where relatedness declines with distance between plants (Loveless and Hamrick 1984). In experiment one, radiating arrays comprised three plants to mimic rare and isolated plant populations. Given a low incidence of between-plot pollen movement and an occurrence of outcrossing within central arrays, a high degree of intra-population movement is expected. As plants were artificially placed in the field, SGS was not expected to impact the arrays in this study. However, under natural conditions, where populations may exhibit SGS, a higher incidence of inbreeding and reduced genetic variation is expected where pollination occurs in localised patches (Ellstrand and Elam 1993). Given the observed absence of between-habitat pollen movement over habitats comprising high floral abundance, the potential increase in SGS-driven inbreeding may be a long-term concern for plants in these conditions.

5.5.4 Connectivity in fragmented landscapes

Plant populations in nature are becoming increasingly isolated from conspecifics, separated by large distances and a heterogeneity of habitats. Increasing the distance of separation between plant populations and altering the composition of intervening habitats has been shown to disrupt pollen movement between plant populations (Murphy and Lovett-Doust 2004, Leimu et al. 2006). Findings here support this research, illustrating an effect of floral resources on between-plot pollen movement. At a local scale, between-plot pollen movement can be enhanced by low levels of floral resources. Where these resources increase, intra- and inter-population pollen movement is reduced (illustrated in chapters three and four respectively). Contrary to this, high levels of floral resources are demonstrated to increase the permeability of habitats to pollinator movement (Rosa et al. 2015). This indicates that while pollinators may be attracted to florally rich habitats, they become distracted by an abundance of floral resources reducing inter-population pollen movement (Lander et al. 2013).

This, along with previous research (chapter four), indicates that connectivity is a function of the floral communities within the intervening habitat together with the distance between plant populations (Aavik et al. 2014). Given this, inter-population pollen movement may be enhanced by reducing distances between plant populations (e.g. through restoration plantings) together with managing intervening habitats to provide an intermediate floral cover. To enhance interactions between populations however, a greater understanding is needed of the combined effects of a habitat's structural and functional attributes, scaled up to a level which represents a typical foraging bout of a pollinator species. Indeed, while landscape composition was demonstrated not to effect intra-population pollen movement (chapter three), little is known of the effects of floral resources at a landscape scale on population connectivity. Inter-population pollen movement has been demonstrated to positively affect the genetic diversity of a plant population (Aavik et al. 2014). Faced with reductions in pollen movement, the genetic diversity of a plant population is expected to decline through a combination of mating between close relatives and genetic drift (Frankham 2010). Such losses reduce a plant population's evolutionary potential and long-term survival potential (Etterson 2004). This understanding is thus important to inform management interventions to ensure pollen movement between conspecific plant populations and thus, gene flow across fragmented landscapes (Rosa et al. 2015).

5.5.5 Summary

Findings from this study suggest an effect of floral resources on the incidence of inter-population pollen movement, where between-plot movement was maximised over habitats comprising an intermediate abundance of floral communities. This suggests that pollen movement is facilitated by low levels of floral resources surrounding focal plant populations. Between-plot pollen movement was however not related to pollinator activity density, which was reduced in response to high floral abundance in experiment one. Changes to pollinator activity density and pollen movement in experiment one however had no measurable effect on plant reproductive success. This was mirrored by an absence of an effect of floral resources on plant reproductive success in experiment two, reflecting comparable levels of heterospecific pollen transfer across plots in this experiment. The implications for plant reproductive success however are expected to vary depending upon a plant's mating system; the degree of genetic structure within a plant population, and the fitness traits explored.

In modified landscapes, plant populations are often separated by a matrix of heterogeneous habitats and by larger distances than explored in this study. It is therefore fundamental that future research focuses on the effect of a habitat's structural and functional attributes at greater scales to determine the leading drivers of inter-population pollen movement within modified landscapes.

CHAPTER 6

Discussion

Landscape modification alters the spatial and temporal availability of floral resources. By altering the attractiveness of habitats to pollinators, this is expected to influence pollen movement in terms of incidence, distance and directness, which may in turn impact the long-term survival of plant populations. However, knowledge of the effects of habitat and landscape context (e.g. the availability of floral and nesting resources), particularly in relation to the importance of spatial scale, on pollinator foraging behaviour and thus, pollen movement is limited. Moreover, the implications of variations in pollinator foraging behaviour for plants which are rare in the landscape needs further investigation in order to prescribe targeted management interventions. With this in mind, this thesis explored how variations in the availability of floral resources at different spatial scales affected plant-pollinator interactions and the impacts of this on the fitness and longer-term survival of plant populations. Overall, I focused on two overarching questions:

1. How do variations in the availability of floral resources at different spatial scales alter pollinator foraging behaviour and what are the implications of this for pollen movement?
2. How do changes to pollinator behaviour at different spatial scales affect plant outcrossing, reproduction and fitness?

These questions were addressed through a number of large-scale and plot level field experiments. Chapter two specifically focused on the importance of floral resources at a local and landscape scale on plant-pollinator interactions. Here, findings demonstrated no relationship between floral resources and pollination behaviour (visitation rates and pollinator constancy). Furthermore, no effect was observed with the reproduction or fitness of plant species exhibiting different mating systems. The amount of pollen removed from study plants was however affected by the availability of floral resources at a landscape level, where pollen removal increased with the availability of floral resources. On this basis, chapter three explored the effects of floral availability using a larger range of habitats at a wider range of spatial scales, to account for differences in pollinator floral preferences and foraging capabilities. In this chapter I assessed the fate of removed pollen through measures of intra-population pollen movement. This illustrated changes in pollinator foraging behaviour through reductions in pollinator activity density and richness where the availability of floral resources was high at a local scale. This was paralleled by changes to intra-population pollen movement. No effects however were observed when floral resources were measured at spatial scales greater than 50m. Chapter four therefore focused on the availability of floral resources at a local scale, this time exploring the effects on inter-population pollen movement and plant outcrossing rates within a habitat. Additional findings here demonstrated changes in pollinator function through reductions in inter-population pollen movement and outcrossing rates when plants were located within florally rich habitats. The implications of changing pollinator function on plants present at low frequency was illustrated through a higher incidence of pollen limitation coupled with reductions in reproduction and fitness. With this in mind, chapter five explored how movement of pollen and the effects on plant fitness varied with a gradient of floral communities at a local scale. Here additional findings demonstrated changes in pollinator function through a higher incidence of inter-population pollen movement over habitats comprising intermediate floral resources, though given the paucity of data, statistical analysis in this case was precluded.

In this chapter, I evaluate how findings from each of these chapters have contributed to answering the overarching questions and explore the wider implications for the management and conservation of threatened plants.

6.1 How do variations in the availability of floral resources at different spatial scales alter pollinator foraging behaviour and what are the implications of this for pollen movement?

6.1.1 Pollinator behaviour

In this thesis, variations in pollinator foraging behaviour was quantified by the activity density, richness and inter-tegular span (‘IT’ span) of pollinators. I show that the abundance of floral resources at a local scale had a negative effect on the activity density and richness of pollinator species (per unit area), while positively affecting pollinator IT span (a proxy for body size). This does not reflect the overall density and diversity of pollinators within a habitat, but instead illustrates the effect on the potential for pollinator visitors at a plant level. Reductions in pollinator activity density and richness at a plant level have similarly been observed in previous studies and are argued to be a result of a dilution effect; as pollinators are unable to utilise all available floral resources, ‘per floral unit’ visitation rates are reduced (Veddeler et al. 2006, Jha and Vandermeer 2009, Holzschuh et al. 2016). Under these conditions, plants are faced with competition for a limited number of shared pollinators (Schuett and Vamosi 2010, Grab et al. 2017). Indeed, in this thesis, communities within florally rich habitats were shown to comprise larger pollinators, reflecting a greater number of *Bombus* spp. Given the tendency for *Bombus* spp. to exhibit constancy to a flower species (Gegear and Laverty 2005), visitation to isolated plants within florally rich habitats may be expected to be further reduced. Throughout this thesis, focal plants were present at low frequency (designed to mimic rare plant populations) and thus did not exhibit the competitive advantage required to benefit from a facilitative effect and co-opt pollinators. When, however, plants increase their competitive advantage (illustrated in this thesis through greater population size and enhanced attractiveness associated with a self-incompatible mating system syndrome), they become better able to co-opt pollinators. Although the relationship between population size and visitation is inconsistent (Mustajarvi et al. 2001), findings here mirror patterns from a previous study which demonstrates a positive relationship between plant population size and pollinator visitation rates in populations comprising less than a few hundred plants (Bernhardt et al. 2008), as was the case in this study. This suggests that interventions aimed at increasing the attractiveness of plants occurring at low frequencies may negate the negative effect of high floral cover in the surrounding habitat.

Variations in pollinator foraging behaviour were observed in this thesis to be governed by floral resources at a local (<50m) rather than a landscape scale (50-1500m). While the effect of co-flowering heterospecific plants on pollinator visitation rates is consistent with previous research, the absence of an effect of floral resources in the wider landscape contradicts research which has demonstrated an importance of landscape context on pollinator visitation (Steffan-Dewenter et al. 2002, Woodcock et al. 2013, Pisanty et al. 2016). Contrasting patterns at different spatial scales have however been observed in previous research (Veddeler et al. 2006) and suggest that while landscapes comprising a high availability of floral resources may attract a greater number of pollinators (Ricketts et al. 2008) as illustrated in this thesis, it is floral communities at a local scale which determine whether plants benefit from the greater number of pollinators. It is noteworthy however that findings from this thesis were inconsistent across experiments, where no significant difference was observed between pollinator communities in response to the availability of floral resources at any spatial scale in chapters two and five (experiment two). This may have arguably been driven by the low number of recorded visitors in chapter two as a result of differences in survey method. While visitation observations provide direct measures of ‘per floral unit’ visitation rates, they yield low pollinator numbers and thus a paucity of data. In contrast, in chapter five, the absence of a relationship in experiment two may have been caused by floral communities being not as distinct as intended due to the management schedule at Parks grass (e.g. late cutting of plots). These inconsistencies between experiments highlight the context specific nature of plant-pollinator relationships and the importance of the composition of floral communities and the local pollinator pool in governing plant-pollinator interactions.

6.1.2 The implications for pollen movement

In this thesis, pollen movement was explored through the removal and deposition of pollen together with intra and inter-population pollen movement across a range of distances. Findings demonstrate a negative relationship between the abundance of floral resources at a local scale and pollen movement, indicating a change to pollinator foraging behaviour in response to floral availability. Typically, pollinator foraging is localised, displaying a distance decay distribution (Pasquet et al. 2008, Rader et al. 2011, Matter et al. 2013). This was reflected by patterns of pollen movement in this thesis, where the incidence of intra-population pollen movement was high and comparably, inter-population pollen movement (50-150m) was limited, particularly when the distance between populations exceeded 50m. This pollen movement was demonstrated to be affected by the surrounding floral communities, where both intra (1m) and inter (50-150m) population pollen movement was reduced as floral abundance increased at a local scale. This offers support to a previous study which showed inter-population pollen movement to be a function of the intervening habitat, where the presence of alternative resources led to a reduction in pollen transfer (Lander et al. 2011). In contrast, where floral resources were scarce, pollinators depleted resources (illustrated by increased intra-population pollen movement) and then moved longer distances in search of other resources. While pollen movement is not a direct measure of pollinator behaviour (Roulston et al. 2007), findings demonstrating a positive correlation between the activity density of pollinators and the incidence of inter-population pollen movement suggest an importance of pollinator visitors. The relationship between pollinator activity density and pollen movement was however not observed in chapter five. This inconsistency between experiments was likely due to a combination of a low incidence of between- and within-plot pollen movement caused by a low abundance of potential *Vicia faba* pollinator visitors.

Heterospecific pollen deposition and pollen removal however were unaffected by local floral communities, indicating comparable pollinator constancy and efficiency in habitats varying in floral resources. This contradicts previous studies which demonstrate changes in pollinator constancy and behaviour in response to a high availability of floral resources (Kunin and Iwasa 1996, Lander et al. 2011, Carvell et al. 2012). Given that deposition of conspecific pollen was not quantified, it is unclear as to whether the high degree of constancy observed in this thesis irrespective of surrounding floral communities reflects constancy to focal plants or to heterospecific plants, though given the low visitation rates, the latter is more likely. The removal of pollen however, while unaffected by floral resources at a local level, increased with floral resources at a landscape scale (1km^2). Given that visitation was not affected by floral resources at a landscape level, this suggests a difference in the efficiency of pollinators in relation to landscapes varying in floral availability (Boscolo et al. 2017). However, since intra-population pollen movement was not affected by floral resources at a landscape level, the fate of this removed pollen is unclear.

6.1.3 Summary

In this thesis, pollinators were shown to respond to the availability of floral resources at a local (<20-50m) and, to a lesser extent, a landscape scale (1km^2). This was illustrated through changes in pollinator foraging behaviour in terms of activity density, species richness and pollinator IT span (per unit area). This had direct implications for pollen movement, affecting pollen removal rates and the transference of pollen, illustrated through changes to both intra- and inter-population pollen movement. These findings therefore highlight the importance of local floral communities in governing the activity of potential pollinator visitors and affecting pollinator foraging behaviour.

6.2 How do changes to pollinator function at different spatial scales affect plant outcrossing, reproduction, and fitness?

The implications for plants of changes to pollinator function in response to the availability of floral resources was measured in this thesis through the incidence of pollen limitation, outcrossing rates, seed set and progeny fitness. Changes to pollinator function (including dispersal distance and pollen transfer efficiency) in response to the availability of floral resources was demonstrated to affect a plant's outcrossing rates and reproductive success. Outcrossing rates were reduced where the abundance of floral resources was high surrounding experimental arrays (illustrated in chapters three (through intra-population movement) and chapter four). This is consistent with previous research and reflects the low activity density and richness of pollinators within such habitats, driven in part by competition from other floral resources (Bell et al. 2005). Under these conditions, plants in chapter four were shown to receive an insufficient pollen supply, offering support to previous studies which have similarly demonstrated a high incidence of pollen limitation in plants when surrounded by rich floral communities (Jakobsson et al. 2009). Self-compatible plants, which can offer reproductive assurance, were observed in chapters three and five to be unaffected by changes to pollen movement. In contrast, plants which could not readily utilise self-pollen, were shown to suffer reduced seed production in chapter four. This pattern however was inconsistent and no such relationship was observed between floral resources and the seed set of plants exhibiting a low propensity to reproduce by self-fertilisation in chapter two. This variation between experiments reflected parallel inconsistencies with measures of pollinator activity density in this chapter and thus is unsurprising.

Findings from this thesis further demonstrate an effect of changes to pollinator behaviour on plant fitness. This was illustrated in chapter four through reductions in the germination of seeds produced by plants exhibiting a low propensity to self when located within florally rich habitats. Given that this reflected parallel reductions in outcrossing rates, these findings imply a negative effect of self-fertilisation. This was supported by findings from chapter two, where seed weight was demonstrated to be lower in plants potentially reproducing by self-fertilisation. Effects on plant fitness were however inconsistent across this thesis, with no relationship observed between seed germination and floral resources in either plant species in chapter two. Moreover, the negative implications of self-fertilisation on plant fitness were only observed in early stage fitness traits and not later stage fitness traits as measured in chapter four (e.g. the number of flowers at reproductive maturity etc.). This illustrates a plant's ability to negate the negative effects of self-fertilisation (Husband and Schemske 1996). This supports hypothesises suggesting a plant's ability to purge the deleterious effects of self-fertilisation (Smith and Maynard-Smith 1978). The immediate effects of changes to pollinator function in response to variations in the availability of floral resources on plants are therefore expected to be minimal. In the longer-term however, self-fertilisation has been demonstrated to lead to more pronounced inbreeding and deleterious effects on plant survival (Lynch et al. 1995, Koelling et al. 2011, Sicard and Lenhard 2011). For instance, over four years, seeds from selfed plants exhibiting a mixed mating system were shown to suffer reductions in leaf area and height when compared to seeds from outcrossed plants (Mooney and McGraw 2007). More work is therefore needed to assess longer term fitness implications, using population models to incorporate other limiters to population survival (e.g. herbivory), in order to decipher the long-term implications for plants.

6.2.1 Summary

Changes to pollinator function (e.g. foraging behaviour) in response to the availability of floral resources can be seen here to reduce the quantity and quality of pollen transfer. This was illustrated through a higher incidence of pollen limitation, coupled with reductions in plant outcrossing rates. Although this led to negative implications for plant reproduction and fitness, findings recognise differences between plant species. In the short-term, plants here have demonstrated the capacity to recover from the negative effects on fitness.

However, in the long-term, the negative effects on plant fitness will be determined by the degree of inbreeding within the next generation of plant progeny.

6.3 Limitations of approach

Associated with each experiment were a number limitations involving both the experimental system and the methods used which, in some instances, constrained the experiment and led to restrictions on the inferences being made.

6.3.1 Experimental system

When exploring the effects of habitat composition on plant-pollinator interactions, I utilised agri-environment scheme options; wildflower and pollen and nectar mixes to represent florally rich habitats and thus ensure that the availability of floral resources was standardised across spatial replicates. These sown habitats comprise a limited array of plant species, which have been shown in some cases to be particularly attractive to a small suite of pollinators (e.g. *Bombus* spp.; Wood et al. 2015). Exploring whether pollinators associated with these habitats included species known to visit the focal plant species was beyond the scope of these experiments. It is recognised however that findings may have varied if plants were instead introduced into habitats where a greater diversity of bees known to pollinate the focal plant species were expected. This is particularly the case for *Silene gallica* which is pollinated predominantly by syrphids which, given differences in morphology (e.g. to *Bombus* spp.), vary in their floral preferences. Moreover, when quantifying floral availability at a landscape scale, it was a combination of these habitats, along with woodland and grassland, that were classified as having a high floral richness. Although this categorisation was similarly used in previous experiments to mitigate the constraints of quantifying floral resources at a landscape scale (Steffan-Dewenter et al. 2002), it is recognised that habitats can vary considerably in their floral availability. Future studies may therefore benefit from separating habitats into categories based on prior knowledge of the availability of floral resources. Further benefit may also be gained from maintaining greater consistency between sites or study systems to allow for cross comparison with fewer confounding variables. While this was the intention and was in some cases achieved (e.g. the use of Hillesden and *E. California* in multiple chapters), it was not always practical to answer the questions being asked. For example, Hillesden was too small for sufficient replicates at a landscape scale and variation in floral resources at these scales across a site is often limited. These experiments however, offer preliminary data and when scaling these experiments up, it is advised that the study system (e.g. plant species) should remain consistent but that populations are introduced into habitats which are more reflective of natural populations.

6.3.2 Methods selection

The experimental methods were chosen in order to maximise data return in light of time and funding constraints. Indeed, when measuring pollinator visitation, pan traps were often used to make inferences on visitation. This was following a paucity of low resolution data (i.e. recognisable taxonomic units) from visitation observation surveys. Pan traps alternatively provided a species inventory over a much longer time period (Ricketts et al. 2008). While, in some cases, visitation observations were conducted alongside pan traps to ensure consistency in catches between methods, pan traps do not reflect visitation rates and further, are shown to exhibit biases in catch (e.g. more generalists are often caught) (Roulston et al. 2007). In future, this could be remedied by increasing the sampling effort of visitation observation surveys or by utilising digital recording devices, enabling direct associations with plant visitation. It is important however that recording devices are used in combination with either pan traps or visitor observations given that they are shown to be limited by the depth and field of view (Gilpin et al. 2017). Similarly, when measuring pollen movement, time and funding constraints restricted the number of molecular markers used. This led to a large number of progeny samples being disregarded based on uncertainty in paternity assignment. Further, given that inter-population pollen movement was low across all experiments, this indicated a need for greater sampling effort; a factor which should be borne in mind for future studies within this field.

6.4 The implications for the management and conservation of threatened plants

6.4.1 Habitat management

The floral communities surrounding plant populations can compete for pollinator visitors or alternatively, depending upon flowering stage, they can instead facilitate pollination services (Grab et al. 2017). In this thesis, plants which were present at low frequency were outcompeted for ‘diluted’ pollinators when surrounding floral communities were abundant and diverse. While this may reflect the artificial nature of habitats introduced as part of agri-environment schemes, which provide provisions for a limited pollinator community (Wood et al. 2015), it highlights the importance of considering the surrounding floral community when managing threatened or isolated plants. Management of threatened or isolated plants should therefore focus on maximising facilitation of pollinator visitation whilst also enabling isolated plants to better compete and co-opt pollinators (Grab et al. 2017). This could be done through limited intervention and instead, by allowing nature to take its course. This approach should encourage an intermediate cover of flowers with different phenotypes and flowering periods and thus limit competition between plant species. Alternatively, interventions can be used to facilitate visitation and pollen transfer, including increasing the attractiveness of floral displays through the introduction of magnet species (Molina-Montenegro et al. 2008) as well as by increasing the duration of floral availability through introducing plants with a range of flowering periods (Moeller 2004, Ghazoul 2006). This will enhance the activity density of shared pollinators within a habitat, or within non-target, neighbouring habitats through a spill-over effect (Brudvig et al. 2009). Management interventions designed to help isolated plants better co-opt pollinators should focus on directly maintaining and increasing their population size (and indeed this is illustrated here by greater visitation when plant populations were large). The co-opting of pollinators can be further enhanced by targeted restoration plantings of heterospecific species, selecting for plant species which flower sequentially to isolated plants (Menz et al. 2011, Grab et al. 2017) and exhibit complementary phenotypes (e.g. different colour forms; Ghazoul 2006).

6.4.2 Managing the intervening habitat

The wider habitat matrix is shown here to be important for determining the ability and likelihood of pollinator movement between optimal habitat patches and thus, gene flow between plant populations. Indeed, through this thesis I demonstrate gene flow between conspecific plant populations and thus, connectivity, to be a function of the attributes of the intervening habitat. For instance, pollen movement was shown to be reduced between conspecific plant populations when separated by florally rich habitats. Example management interventions designed to enhance connectivity have included the use of corridors and stepping stone habitats (Bruckmann et al. 2010, Menz et al. 2011). While these create a directional response and provide a means of facilitating the movement of organisms between patches (Cranmer et al. 2011, Dick et al. 2003), findings from this thesis indicate that corridors can impede pollen movement between isolated plants by distracting pollinators which move to more rewarding, abundant flowers. For conservation and restoration efforts, the intervening habitat therefore needs to be taken into consideration in order to create self-sustaining plant populations and thus, lessen a plant’s vulnerability to extinction; a key objective of management interventions (Reiter et al. 2016). Therefore, where re-introduction or translocation programs of threatened plants are planned, care should be taken to ensure a network of low floral resources between plant populations. In natural populations, this can instead be achieved by reducing the isolation between conspecific plants by establishing additional populations where connectivity may be low (Hodgson et al. 2009, Auffret et al. 2015). It is noteworthy, however, that while gene flow between populations can provide genetic rescue to inbred populations (e.g. heterosis) (Pickup et al. 2013), pollen from distant populations can result in outbreeding depression and thus, negatively affect plants (Ruane et al. 2015). Identifying an appropriate genetic source is therefore necessary prior to such interventions.

6.4.3 The scale of intervention

Floral resources at the landscape scale have been highlighted as driving pollinator attraction and thus governing pollinator communities within an area (Montero-Castano and Vila 2012, Boscolo et al. 2017). This, through ensuring relationships with pollinators, is key to the success of conservation efforts for threatened plants (Reiter et al. 2016). However, findings from this project indicate that threatened or isolated plant species may instead be affected to a greater degree by floral resources at a habitat scale. Therefore, although pollinators may be enhanced at a landscape scale, surrounding floral communities determine the level of competition for increased shared pollinators and thus, the benefit for plants. This highlights the need for focused habitat-level management when introducing conservation or restoration interventions for plant populations. It is noteworthy however that competition for pollinator visitation has been argued to be mitigated by increases in the activity density and richness of pollinators, which is expected to be greater in landscapes with a higher proportion of semi-natural habitats (Ricketts et al. 2008, Diekotter et al. 2010, Holzschuh et al. 2016). While this was not shown in this thesis, this suggests that in certain circumstances landscape composition may still be important when designing management for plant populations. A greater understanding of the role of landscape composition in plant-pollinator relationships is therefore needed in order to decipher the indirect implications for plants.

6.5 Future directions

Following experiments conducted during this thesis, findings have uncovered gaps in our understanding of plant-pollinator interactions which warrant investigation. These are important to further our understanding of changes to pollinator behaviour in response to landscape modification and enable targeted restoration and conservation of threatened and isolated plant species. Recommendations for future work are listed below.

- *How do the functional and structural attributes of a habitat alter the connectivity between plant populations which reflect conditions observed in nature?* Given that the connectivity of plant populations has been shown to be a function of a habitat's structural and functional attributes, research would now benefit from exploring the synergy of these variables. Indeed, in nature, plant populations are often separated by large distances together with a heterogeneity of habitats and barriers obstructing pollinator movement. A greater understanding of connectivity under different conditions would thus allow for more focused management of threatened plant populations.

- *Does the availability of floral communities affect the fate of pollen and a plant's paternal success?* With increased pollen removal in response to a high availability of floral resources at a landscape scale observed in this thesis, research would now benefit from understanding where pollen is being moved to in relation to the associated pollinator species. Indeed, pollinator species differ considerably in their foraging behaviour and work has tended to focus on specific species (e.g. *Apis mellifera*). By discriminating pollinator behaviour and foraging paths at a species level, management interventions could be targeted to enhance effective pollen transfer by associated pollinators.

- *What is the balance between competition and facilitation in plant populations which are present at low frequency in nature?* Plant populations under natural conditions are likely to have established pollinator communities. Given this, it would be beneficial to explore the balance between competition and facilitation in threatened plant populations, with particular focus on how plant populations could mitigate the negative effects of competition and maximise facilitation. This is particularly important for determining how best to manage plant populations which are threatened or isolated due to anthropogenic modification.

6.6 Conclusion

Overall, findings from this thesis highlight the importance of floral resources at a local scale on pollinator foraging and pollination services to threatened or isolated plant populations. Co-flowering heterospecific plants, by offering alternative resources, compete for ‘diluted’ pollinators, resulting in reductions in potential pollinator visitors. The consequences of these changes are demonstrated through disruptions in the dispersal distance and incidence of pollen movement, with negative implications for plant outcrossing and intra- and inter-population gene flow. This was demonstrated to result in negative implications for plant reproduction and fitness in plants exhibiting a low propensity to self, driven in part by a higher incidence of pollen limitation in florally rich habitats. Although effects on reproduction and fitness were not observed in all plant species studied, due in part to differences in the ability to offer reproductive assurance provided by different mating systems, long-term fitness is expected to be affected by reductions in gene flow.

Given this, conservation and restorative efforts should focus on the surrounding and intervening habitat between threatened or isolated plant populations. Emphasis should be put on maximising the facilitative effect of neighbouring plants to encourage pollinator visitation and pollen transfer, while enabling plants to better withstand competition. In order to better prescribe management interventions however, further research is needed into changes to plant-pollinator interactions under more realistic conditions which are representative of plant populations in nature. This will enable the application of findings across a wider range of plants and landscape contexts.

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Appendices

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	Chapter 2	Chapter 3				Chapter 4	Chapter 5	
Plant species	Hillesden	Widmere	Waddesdon	Earth trust	Hillesden	Hillesden	Park Grass (May)	Park Grass (August)
Achillea millefolium L.	R							
Anthriscus sylvestris L. emend. Hoffm.	R						A	
Borago officinalis L.	R							
Capsella bursa-pastoris L. emend. Medik.						R		
Centaurea nigra L.	F	R	R			F		
Cerastium fontanum Baumg.	O	R			R	R	R	
Chenopodium album	O							
Cirsium vulgare Savi. emend. Ten.	R							
Conopodium majus Gouan. emend. Loret.							R	
Daucus carota L.	O					R		
Echium vulgare L.	O							
Epilobium montanum L.	O							
Filipendula ulmaria L. emend. Maxim.	R							
Galium aparine L.	A							

Galium verum L.	A					F		
Geranium dissectum L.	O	R	R		R	O		
Heracleum sphondylium L.	O					O		
Hypochaeris radicata L.						R	R	
Lapsana communis L.	F							
Leontodon hispidus L.								A
Leucanthemum vulgare Lam.	F	O	F	F		D		
Lotus corniculatus L.	D	R	R				F	F
Lychnis flos-cuculi L.	R				R			
Matricaria recutita L.	A					F		
Medicago lupulina L.		O		F				
Melilotus officinalis L. emend. Pall.	A							
Myosotis arvensis L. emend. Hill.	F							
Onobrychis viciifolia Scop.	F							
Phacelia tanacetifolia Benth.	O					O		
Plantago lanceolata L.		O	R		O	R	O	O
Polygonum persicaria S.F.Gray.	O							
Prunella vulgaris L.	O					O	R	
Ranunculus acris L.	O	F	O	O	D	A	D	
Ranunculus repens L.	R							

Rhinanthus minor L.	F	F		R	R	O		
Rosa canina L.	R							
Rubus fruticosus L. agg.	F							
Rumex acetosa L.	A						O	
Rumex obtusifolius L.	F							
Silene dioica L. emend. Clairv.	O					R		
Sonchus arvensis L.	R							
Sonchus asper L. emend. Hill.	O							
Taraxacum agg F.H.Wigg.							O	
Torilis japonica Houtt.								O
Trifolium hybridum L.	F							
Trifolium incarnatum L.	R							
Trifolium pratense L.	D	O	F		R	A	A	R
Trifolium repens L.		R				A		
Veronica chamaedrys L.							O	
Vicia cracca L.						F		
Vicia hirsuta L. emend. Gray.	R				O	A		
Vicia sativa L.		O			O	R		

Primer note: Cross-species transfer of microsatellite markers in the native wildflower, *Silene gallica* (Caryophyllaceae)

Abstract

1. Premise of study: Cross-species transfer of nuclear microsatellite markers was attempted in the rare arable wildflower, *Silene gallica* to provide a means of assessing the effects of local and landscape context on outcrossing in a self-compatible species.
2. Methods and Results: 29 microsatellite markers amplifying di- and trinucleotide repeats were transferred from three closely related species within the *Silene* genus to *S. gallica.* Reliable amplification was achieved for eight markers, though all showed little or no polymorphism (1-4 alleles per locus). Sequencing alleles at one locus indicated a substantial reduction in the length of the microsatellite repeat within *S. gallica*.
3. Conclusions: These results indicate limitations in cross-species transfer of microsatellite markers for *S. gallica* because of reduced allelic diversity. Further, this study indicates that even when transferred primers are successful, sequencing of amplified fragments should be undertaken to check their properties.

Introduction

Silene gallica (Caryophyllaceae) is a self-compatible wildflower that, following substantial declines within Britain during the 20th century (Preston et al. 2002), is now listed as a priority species under the UK biodiversity action plan (Gibson et al. 2006). Our understanding of the fitness of remaining *S. gallica* populations is limited. Analysis of neutral molecular markers allows quantification of outcrossing rates and levels of genetic diversity, which are positively correlated with the fitness and long-term viability of plant populations (Dostalek et al. 2010). By studying these factors in relation to local and landscape context, an understanding of the mechanisms determining outcrossing rates in this species, and the implications for plant survival, can be obtained. This is essential for the effective conservation of remaining populations.

Given the absence of molecular markers for *S. gallica,* I followed previous studies (Gode et al. 2014) and attempted the transference of primers developed for closely related species. The results allowed validation of the approach and should be of use to researchers looking to use cross-species transfer as a cost effective means of marker development in future.

Methods and Materials

Silene gallica plants were grown from seed (Herbiseed, Reading, UK) and DNA was extracted from 50mg of leaf material following a DNeasy protocol (Qiagen Ltd, Manchester, UK) (n = 8). Microsatellite markers were sourced from closely related species; *Silene ciliata*, *Silene latifolia* and *Silene nutans* (Rautenberg et al. 2012), some of which, had shown evidence of previous successful cross-species transfer (Gode et al. 2014). Initial screening was done using touchdown polymerase chain reaction (PCR) with annealing temperatures 68-30ºC, before conditions were optimised at 95°C for 5 minutes, 30 cycles of 94°C for 30 seconds, 56°C for 60 seconds, 72°C for 30 seconds, followed by a final elongation phase of 72°C for 10 minutes. Standard reaction conditions were as follows; 20ng DNA, 2µl PCR buffer, 0.2µl Primers (20µM), 0.16µl dNTPs (100µM), 0.2µl BSA and 0.1µl Taq DNA polymerase in a 20µl reaction. Fragment analysis was then performed on an ABI3730 under the following conditions: 0.5µl Liz 500 size standard, 9µl HiDi formamide and 0.5µl PCR product. Results were analysed using Genemarker V.1.95. To verify that markers were amplifying the target regions, and that variation in fragment sizes were primarily driven by microsatellite repeat length, I cloned (TOPO® TA cloning kit®; Invitrogen™, California, USA) and sequenced alleles at a locus that was particularly unreliable during amplification (SL-eSSR22).

Results and discussion

Out of 29 pairs of microsatellite primers tested (Teixeira and Bernasconi 2007, Moccia et al. 2009, Garcia-Fernandez et al. 2012), only eight successfully amplified in *S. gallica* and all showed low heterozygosity (Observed heterozygosity mean ± SE 0.27 ± 0.1) and limited allelic diversity (Alleles per locus mean ± SE 1.75 ± 0.62; Table 7.1). Furthermore, alleles at each locus were systematically smaller in *S. gallica* when compared to the size range in the original Silene species (71% of the alleles (n = 14) fell below the reported size range; Table 1). When sequencing alleles from SL-eSSR22, mutations were observed in the binding site, providing a reason for the unreliablity in amplification. Only one allele at SL-eSSR22 was successfully sequenced, though within this allele, a substantial reduction was observed in the mean microsatellite repeat length from (ACA)8 in the original species (*Silene latifolia*) to (ACA)3 in *S. gallica*. The reduced repeat length and size range of alleles compared to those found in the original species could provide an explanation for the reduced allelic diversity found across loci in this study. However, given, that a small sample of a largely selfing plant species was anlaysed in this study, greater numbers may be needed to decipher whether low variability was down to reduced repeat length or instead, ascertainment bias.

This study indicates that while markers can successfully be transferred between closely related species, their applicability depends on the allelic diversity within loci. This is affected in part by the propensity of the target species to self-fertilise (Barbara et al. 2007) as well as the repeat length, which has been positively correlated with mutation rate and hence, allelic diversity (Schug et al. 1997). This study therefore emphasises the need to sequence amplified fragments in order to determine the nature of the microsatellite repeat, and hence, the usefulness in genetic studies.

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Table 7.I. Characteristics of 8 microsatellite markers successfully transferred to Silene gallica. Shown for each marker are forward and reverse primer sequence, repeat type, size of the fragment (bp), annealing temperature (Ta), the number of alleles (A), the observed heterozygosity (Ho) and Genbank accession number. Values for S. gallica are shown in parentheses (n = 8).

Primer	Original species	Sequence	Repeat	Size	Ta	A	Ho	Genbank reference
Sci-1224	*Silene ciliata*	F: ACCTGATTAGAAGACACAGGAGGA R: TTTATGTTGCCGCATCCTTATC	(CT)18	140–196 (117-119)	56 (56)	26 (2)	(0)	JF979125
SL-eSSR17	*Silene latifolia*	F: CCCCTTTTCTTCTCCTCCAA R: CACCAGTTCCTGCACAAAAC	(ATT)13	238-280 (168-182)	- (56)	10 (4)	(0.83)	-
SL-eSSR22	*Silene latifolia*	F: CACCATTTCTTCACGGCTTC R: GCTGTTGTTAATGGCGGATT	(ACA)8	142-151 (138-144)	60 (56)	7 (2)	(0.66)	-
37H	*Silene latifolia*	F: TTCGTGGCGGTCTCTAATCT R: CCGACTGATGGAACACACAC	TRI (?)	211-253 (222-234)	60 (56)	12 (2)	(0.66)	-
Sci-0106	*Silene ciliata*	F: AAACAAACGAGCGATCATCTAA R: TTCCGATGCTTCTGGTACTTCT	(CT)7	111-135 (104)	56 (56)	12 (1)	(0)	JF979127
SL_eSSR16	*Silene latifolia*	F: AACCAACACCAGCAACCTTC R: TTCTTTGCCACTTCTTCACTCA	(ATC)5	185-200 (171)	- (56)	5 (1)	(0)	-
SIL03	*Silene nutans*	F: AAGCTTCATCAAATGAAATCGG R: GGTGGAGGAGAAGACCACAG	(AG)8	208-214 (200)	55 (56)	4 (1)	(0)	KJ671557
SL_eSSR08	*Silene latifolia*	F: GCATGAAATCATTTTTCAGAGG R: CGAAAAACACCACCAAAACC	(TAA)21	196-259 (215)	60 (56)	19 (1)	(0)	-

		Chapter 3				Chapter 4
Order	Pollinator species	Widmere	Waddesdon	Earth trust	Hillesden	Hillesden
Lepidoptera	*Agrotis exclamationis* (Linnaeus, 1758)	0	0	0	0	1
	Apamea monoglypha (Hufnagel, 1766)	0	0	0	0	1
	Autographa gamma (Linnaeus, 1758)	0	0	0	0	1
	Euclidia glyphica (Linnaeus, 1758)	1	0	0	0	0
	Lepidoptera spp.	0	2	1	1	3
	Maniola jurtina (Linnaeus, 1758)	0	0	1	0	0
	Ochlodes sylvanus (Esper, 1777)	1	3	0	0	0
	Polyommatus icarus (Rottemburg, 1775)	0	0	0	0	1
Hymenoptera	*Andrena bicolor* (Fabricius, 1775)	3	0	0	0	0
	Andrena chrysosceles (Kirby, 1802)	0	2	1	2	2
	Andrena cineraria (Linnaeus, 1758)	0	0	0	3	1
	Andrena flavipes (Panzer, 1799)	0	0	0	0	4
	Andrena fulvago (Christ, 1791)	0	0	2	0	0
	Andrena haemorrhoa (Fabricius, 1781)	1	0	0	0	0
	Andrena leaiana (Kirby, 1802)	0	0	0	1	0
	Andrena minutula (Kirby, 1802)	2	0	3	0	1

Andrena nigroaenea (Kirby, 1802)	1	0	0	0	9
Andrena nitida (Müller, 1776)	0	1	0	0	0
Andrena subopaca (Nylander, 1848)	0	0	2	0	0
Apis mellifera (Linnaeus, 1758)	1	12	2	17	28
Bombus barbutellus (Kirby, 1802)	1	0	0	1	0
Bombus hortorum (Linnaeus, 1761)	1	2	5	8	9
Bombus hypnorum (Linnaeus, 1758)	0	0	0	0	1
Bombus lapidarius (Linnaeus, 1758)	2	1	0	9	9
Bombus lucorum (Linnaeus, 1761)	2	0	0	1	0
Bombus pascuorum (Scopoli, 1763)	1	0	3	3	2
Bombus pratorum (Linnaeus, 1761)	0	2	0	0	2
Bombus ruderarius (Müller, 1776)	0	0	0	0	1
Bombus rupestris (Fabricius, 1793)	0	0	1	0	0
Bombus slyvestris (Le Peletier, 1832)	0	0	0	0	1
Bombus spp.	0	0	0	0	1
Bombus terrestris (Linnaeus, 1758)	8	2	1	4	8
Bombus vestalis (Geoffroy, 1785)	0	0	0	1	0
Halictus rubicundus (Chris, 1791)	1	0	0	0	0
Halictus tumulorum (Linnaeus, 1758)	1	1	1	0	1
Lasioglossum calceatum (Scopoli, 1763)	8	1	3	1	3

	Lasioglossum fulvicorne (Kirby, 1802)	7	1	0	1	0
	Lasioglossum malachurum (Kirby, 1802)	2	11	12	0	13
	Lasioglossum minutissimum (Kirby, 1802)	1	0	18	0	1
	Lasioglossum morio (Fabricius, 1793)	3	4	0	0	0
	Lasioglossum parvulum (Schenck, 1853)	7	0	0	0	0
	Lasioglossum pauxillum (Schenck,1853)	3	2	0	1	2
	Lasioglossum puncticolle (Morawitz, 1872)	0	0	1	0	1
	Lasioglossum quadrinotatum (Kirby, 1802)	0	1	0	1	0
	Lasioglossum villosulum (Kirby, 1802)	1	1	0	0	3
	Nomada fabiciana (Linnaeus, 1767)	0	0	0	0	1
	Sphecodes ephippius (Linnaeus, 1767)	1	0	0	0	0
Diptera	*Cheilosia albitarsis* (Meigen, 1822)	0	3	0	0	5
	Cheilosia antiqua (Meigen, 1822)	0	3	0	0	0
	Cheilosia vernalis (Fallén, 1817)	0	3	1	0	12
	Chrysogaster hirtella (Loew, 1843)	0	1	0	2	2
	Chrysotoxum elegans (Loew, 1841)	0	1	0	1	0
	Epistrophe euchroma (Kowarz, 1885)	0	0	0	1	0
	Episyrphus balteatus (De Geer, 1776)	1	3	32	28	18
	Eristalis arbustorum (Linnaeus, 1758)	0	34	3	25	9

Eristalis pertinax (Scopoli, 1763)	0	1	0	0	1
Eristalis tenax (Linnaeus, 1758)	2	8	4	3	7
Eupeodes corollae (Fabricius, 1794)	56	249	320	174	24
Helophilus pendulus (Linnaeus, 1758)	0	1	0	0	0
Melanostoma mellinum (Linnaeus, 1758)	0	0	1	0	1
Melanostoma scalare (Fabricius, 1794)	0	0	0	1	1
Merodon equestris (Fabricius, 1794)	3	1	1	0	4
Metasyrphus luniger (Meigen, 1822)	1	3	4	3	0
Neoascia podagrica (Fabricius, 1775)	0	0	1	0	0
Platychierus spp	1	0	0	0	0
Rhingia campestris (Meigen, 1822)	0	0	0	0	2
Rivula sericealis (Scopoli, 1763)	0	0	0	0	0
Sphaerophoria scripta (Linnaeus, 1758)	2	1	0	0	2
Syphidae spp	0	0	0	1	1
Syritta pipiens (Linnaeus, 1758)	0	1	1	0	0
Syrphus ribesii (Linnaeus, 1758)	0	0	0	4	3
Volucella bombylans (Linnaeus, 1758)	0	1	0	1	0
Total	126	363	425	299	203

			Floral resources							
Site	Habitat	Plant array	1m radius	20m radius	50m radius	100m radius	1500m radius	Outcrossed progeny	Progeny examined	Proportion of outcrossing
The Earth trust	Fallow	F1	0	0.11	0.05	0.15	0.29	1	6	0.17
		F2	0	0.14	0.46	0.52	0.29	2	4	0.5
		F3	0	0	0.24	0.33	0.29	6	9	0.67
	Wildflower mix	WF1	25.67	0.55	0.25	0.14	0.29	2	4	0.5
		WF2	7.33	0.62	0.28	0.26	0.29	3	7	0.43
		WF3	28.33	0.83	0.51	0.29	0.29	1	2	0.5
Widmere farm	Fallow	F1	0	0	0.15	0.25	0.55	0	4	0
		F2	0	0.01	0.35	0.20	0.55	4	11	0.36
		F3	0	0.01	0.35	0.20	0.55	0	0	NA
	Wildflower mix	WF1	24.33	0.80	0.54	0.39	0.55	1	4	0.25
		WF2	24.33	0.80	0.54	0.39	0.55	0	0	NA
		WF3	23.33	0.61	0.36	0.22	0.55	6	8	0.75
The Hillesden estate	Fallow	F1	0	0.01	0.23	0.17	0.22	0	1	0
		F2	0	0.09	0.10	0.16	0.22	2	3	0.67
		F3	0	0.08	0.26	0.31	0.22	1	2	0.5
	Wildflower mix	WF1	97.33	0.95	0.32	0.14	0.22	0	10	0
		WF2	43.33	0.98	0.72	0.53	0.22	1	2	0.5
		WF3	13.67	1	0.73	0.53	0.22	2	2	1
The Waddesdon estate	Fallow	F1	0	0	0.06	0.27	0.53	5	6	0.83
		F2	0	0	0.03	0.20	0.53	1	3	0.33
		F3	0	0	0	0	0.53	0	2	0
	Wildflower mix	WF1	42.33	0.82	0.52	0.45	0.53	1	4	0.25
		WF2	15	0.92	0.88	0.71	0.53	1	4	0.25
		WF3	12.33	0.95	0.65	0.43	0.53	3	7	0.43