Flowers and bees in the city:

The impact of urbanisation on native plants and their insect pollinators

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Declaration and Contribution

I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary educational institution. I declare that I am the primary author of all chapters.

The work contained in this thesis is my own, completed under the supervision of Dr R. Standish, Dr J. Fontaine, and Dr P. Ladd. Collection of flora and fauna occurred under the following licences: Scientific or Other Prescribed Purposes (SW017912, SW018769, SW019528), Regulation 4 (CE005328, CE005339), and Regulation 17 (SF010891, 08-000828-1, 08-000828-2). Dr T. Houston was paid to identify the insect specimens detailed in chapter three. The Department of Biodiversity, Conservation and Attractions (DBCA) and Murdoch University funded the genetics work in chapter five. Dr D. Coates facilitated the work in chapter five and S. McArthur assisted with lab work and analyses.

	Manuscript	Status	Contribution
Chapter three	Remnant bushlands and residential gardens provide complementary resources and habitat for insects in an urban landscape	To be submitted	E. Eakin-Busher - 80 % R. Standish - 7 % J. Fontaine - 5 % P. Ladd - 6 % T. Houston - 2 %
Chapter four	Mating strategies dictate the importance of insect visits to native plants in urban fragments	Published	E. Eakin-Busher - 80 % R. Standish - 8 % J. Fontaine - 4 % P. Ladd - 8 %
Chapter five	Gene flow and pollination of a rhizomatous herb in a fragmented urban landscape	To be submitted	E. Eakin-Busher - 80 % R. Standish - 5 % P. Ladd - 5 % D. Coates - 8 % S. McArthur - 2 %

In the absence of all authors' signatures, I hereby confirm that the above statement of contribution is a true representation of my work with validation from the Principal Supervisor of this thesis.

Emily L. Eakin-Busher
2020

Rachel J. Standish
2020

Abstract

Urbanisation is one of the most intensive and irreversible ecosystem changes facing the plants, animals, and people that live in the world's cities. Where native vegetation is cleared, habitat loss and fragmentation have major impacts on the viability of animal and plant populations. Removal of vegetation reduces plant population size, which impacts the resources available to flower visitors. In turn, this can reduce plant pollination and subsequent reproductive success. Understanding how landscape alteration affects ecosystem services such as pollination is fundamental to the conservation of native plants and their pollinating insects.

The aim of my research was to contribute knowledge of the interaction between native plants and pollinating insects in an urban environment. The study region included remnant native vegetation and native gardens in Perth, Western Australia that likely provide novel resources for some insects, while degrading resources for others. I aimed to establish the importance of insects to the reproductive success of native plants, to identify the site and landscape characteristics that influenced particular insect groups, and to determine pollen movement within a remnant bushland.

After hours (50) of careful observations of insects visiting flowers of five common native plant species, and pollinator exclusion studies, I found that four plants were reliant on insects for pollination to varying extents, but predominantly outcrossing species such as *Dianella revoluta* and *Jacksonia sericea* may be more vulnerable to urbanisation. I passively sampled insects and found that remnants and gardens were complementary in providing resources and introduced and native bees were observed, but their richness was generally low. Using detailed genetic data, I found that *Patersonia occidentalis* showed moderate outcrossing, which was maintained by insects moving pollen up to 116 m between plants within a small remnant. My studies point towards the importance of connectivity between small remnants, gardens and larger remnants for maintaining plant–insect interactions in the world's urban landscapes.

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1. General introduction

This thesis examines native plants and their insect pollinators in an urban environment—the Perth metropolitan region of Western Australia. The general introduction provides a brief overview of the ecological concepts and features of urbanisation which underpin my research questions, followed by an outline of the thesis structure.

1.1. Urbanisation background

Urbanisation describes the process of making a landscape suitable for large numbers of people to live and work. In some parts of the world, urbanisation has occurred over land that was previously cleared for agriculture or other land uses. In other parts of the world, urbanisation has started with the clearing of native vegetation. In such cases, loss of habitat and habitat fragmentation are key impacts of urbanisation (Grimm et al. 2008). Over half of the world's human population resides in urban areas, with the prediction that 68 % will be living in urban areas by 2050 (United Nations 2018). This shift towards urban living has resulted in rapid modification of the structure of landscapes.

Vegetation removal directly reduces the abundance and diversity of plants and animals in particular areas (Seto et al. 2012) while fragmentation results in a landscape where small, isolated remnants are all that remain of the formerly continuous native vegetation (Fahrig 2003; Hahs et al. 2009). These heavily modified landscapes present challenges for the organisms that live there, such as reduced population size, less vegetative cover, more isolation, disruption of plant–pollinator mutualisms (Harris and Johnson 2004), and reduced genetic fitness (Tarazi et al. 2013). Native plants and their insect pollinator species may, therefore, be increasingly at risk in urban landscapes (Memmott et al. 2007).

1.2. Insect pollination

Pollination is essential for the sexual reproduction of many plant species, and while some angiosperms receive pollen via abiotic sources (e.g., wind or water) or via autogamy (within-flower self-pollination), an estimated 87.5 % depend on animal vectors (Ollerton et al. 2011). Of the animal vectors, around 90 % are insects (Aizen and Feinsinger 2003), so insects will be my focus here. Pollinating insects include beetles, flies, wasps, moths, butterflies, and bees (Cane 2005; Ollerton 2017). Bees are well-adapted for pollination and are widely considered to be the most important pollinator group (Didham et al. 1996; Klein et al. 2007; Neff and Simpson 1993). Historically, the fragmentation and degradation of habitat has been considered to be the leading cause of pollinator declines (Bates et al. 2011).

1.3. Impacts of urbanisation on insect pollinators, plants, and their interactions

Given the dependence of plants on pollinating insects and vice versa, urbanisation can have significant ecological impacts on both plants and pollinators (Aguilar et al. 2006).

Pollinating insects

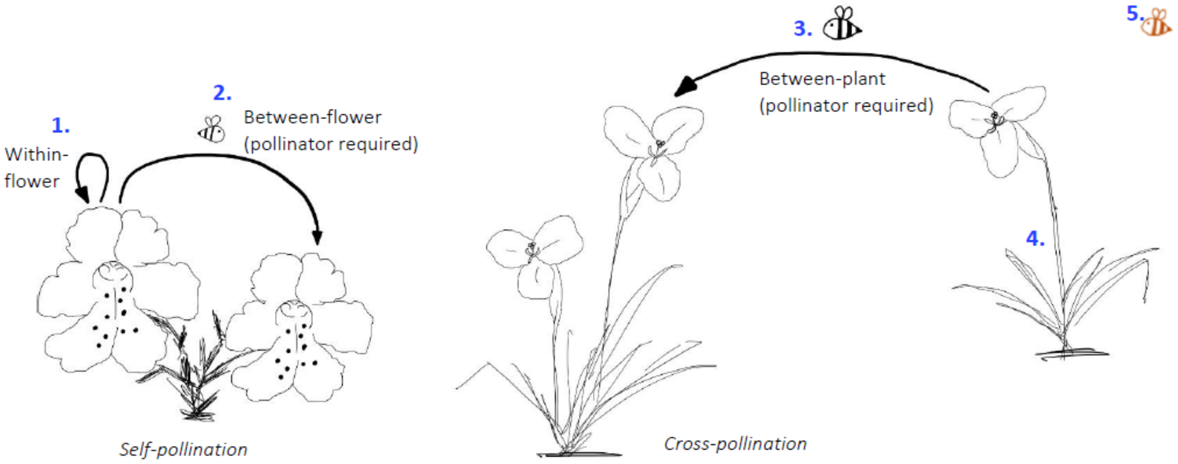
The impact of urbanisation on pollinating insects is varied, so although landscape structure and local habitat can influence pollinators (Kennedy et al. 2013), their responses are dependent on many interacting factors. For example, insect pollinators need to be able to access the habitat, food, and nesting material required for their survival (Cane et al. 2006; McIntyre and Hostetler 2001). The ability of insect pollinators to access these resources is dependent on species' traits, particularly those associated with resource acquisition (Bennett and Lovell 2019; Williams et al. 2010). In addition, insect pollinators' access to resources could be influenced by the extent of habitat loss, and the connectivity between these resources. Fragmentation may reduce pollinator species richness and abundance (Bates et al. 2011; Fortel et al. 2014; McIntyre and Hostetler 2001), and can result in altered pollinator behaviour and flight patterns (Didham et al. 1996).

Plants

Many plants require animal vectors to transfer pollen, and in most cases, the vector is an insect (Aizen and Feinsinger 2003). Plant reproduction can decrease as a result of fragmentation

(Aguilar et al. 2008; Tomimatsu and Ohara 2006); however, clear trends in urban environments are lacking. Overall, plants have different susceptibilities to impacts depending on their ecological traits (Aguilar et al. 2006). In particular, plant mating systems may determine the sensitivity of plant populations to urbanisation (Figure 1.1). For example, when the size of plant or pollinator populations has been reduced, self-compatible species may experience an increase in inbreeding (Coates et al. 2007). On the other hand, self-incompatible plant species are susceptible to changes in their pollinator assemblages because they require outcross pollen (Aizen et al. 2002; 2003).

The link between urbanisation and plant mating systems can influence pollen and gene flow. Given plants cannot move, connected habitats are important for their reproductive success. If remnants are more isolated than the distance insect pollinators can travel, pollen flow for plant species in these remnants (serviced by particular pollinators) may be reduced (Kearns and Inouye 1997). The rate of pollen flow in and between remnant vegetation may impact genetic and reproductive processes and population viability (Whelan et al. 2000; Young et al. 2010). Studies of optimal outcrossing distance have shown that pollen transfer between nearest neighbours can result in a reduced seed set (Rigney et al. 1993), but for particular plant species, the optimal outcrossing distance can still be within the first 10 metres (Ayre et al. 2019; Price and Waser 1979). The extent of outcross pollen transfer may, therefore, determine the genetic structure and subsequent long-term viability of a plant population following fragmentation.



Direct effects	Possible ecological consequences
1. Autogamy increases in capable self-compatible species	Reduced genetic diversity in progeny
2. Loss of plants/pollinators increases self-pollination	Pollen limitation Reduced genetic fitness in population Loss of reproduction in specialist plant species
3. Loss of insects reduces outcross pollen transfer	Reduced genetic diversity and genetic fitness Self-incompatible species unable to reproduce
4. Loss or isolation of plants reduces outcrossing opportunity	Reduced genetic diversity and genetic fitness
5. Local loss of particular pollinator	Reduced reproduction in specialist species

Figure 1.1: The potential influence of mating system on the susceptibility of plants to urbanisation.

Plant–pollinator interactions

Declines in the abundance or diversity of insect pollinators tend to reduce pollination (Potts et al. 2010; Verboven et al. 2014) and hence fertilisation and seed set. Determining the impacts on interactions is further complicated by the scarcity of intact landscapes available for comparison. Without comparisons, impacts could be deemed as minimal if there is redundancy among pollinators (e.g., if plants are visited by generalist pollinators, or if plants are autogamous) despite insect pollinator decline. However, plants with fewer pollinators are vulnerable in the event more pollinators are lost, and autogamous plants are vulnerable to loss of genetic diversity. Distinguishing among these possibilities is challenging, but large, connected plant populations are likely to provide adequate outcrossing opportunities as well as sufficient floral resources for pollinators (Potts et al. 2010; 2016).

1.4. Native gardens as habitat for pollinating insects

Gardens and patches of connected vegetation have the potential to support a diverse assemblage of pollinators, particularly if the urban landscape is accompanied by an increase in flowering plants (Bates et al. 2011). The richness and density of flowering species can have a positive impact on the richness of bee species in a variety of urban habitats (Bates et al. 2011; Hennig et al. 2012; Matteson et al. 2013). Gardens and remnant vegetation together may be able to provide habitat and floral resources on which pollinators can forage (Tommasi et al. 2004). The hypothesised habitat quality of potential combinations of sites is given in Figure 1.2.

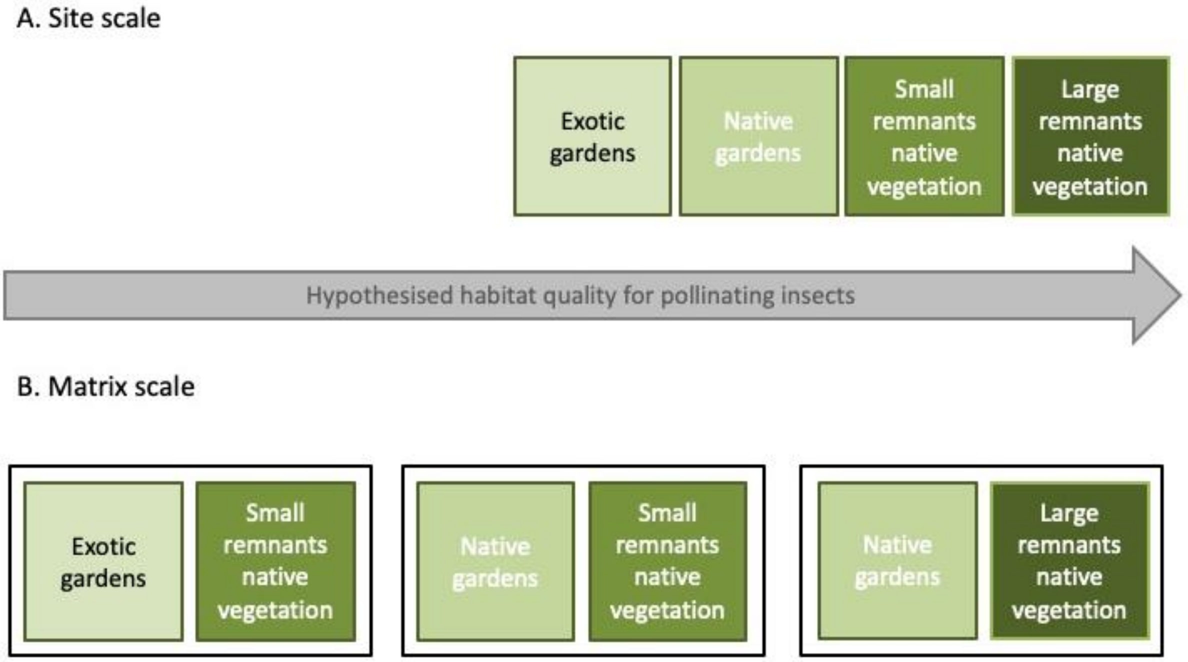


Figure 1.2. Hypothesised habitat quality for pollinating insects, where darker green represents improved quality. It is expected that a combination of native gardens and large remnants of native vegetation would be an ideal site arrangement for pollinating insects in an urban environment.

1.5. Research purpose

Pollination is one of the most important ecosystem services, and pollinating insects facilitate reproduction of many of the world’s flowering plant species (Ollerton et al. 2011). In recent years, the importance of insect pollinators has become increasingly recognised, in part due to concern about the possibility of pollinator loss in cities and farms (Potts et al. 2010; Sánchez-

Bayo and Wyckhuys 2019). However, there has been a bias in research effort towards some species and locations. European honey bees have received substantial media coverage as they are present in cities around the world and are subject to a "global bee decline" (Potts et al. 2010). There are more data on insect pollinators for Europe and the United Kingdom than for other locations such as Australia (De Palma et al. 2016; Harrison et al. 2015). More generally, bees account for one-third of all pollinators (Ollerton et al. 2011). Other insects (e.g., wasps) also contribute to pollination; however, their significance or vulnerability to decline (sensu global bee decline) is generally unknown (Sánchez-Bayo and Wyckhuys 2019). Regardless, the consequences of fragmentation are likely substantial for insect pollinators and sexually reproducing plant species. More research is needed to determine whether urbanisation has a negative impact on different insects in different locations (Hernandez et al. 2009; Winfree et al. 2011).

In order to develop conservation and management options for native plants and their pollinators in the future, it is necessary to determine which pollinating insect species are present in urban environments, which garden and remnant characteristics are important for their persistence, and what plant–pollinator interactions are taking place. Given ongoing and rapid environmental changes, we must anticipate further declines and prevent them where possible. Understanding the effects of urbanisation on pollinator assemblages is important for the intrinsic conservation value of urban pollinators, the ecosystem service of pollination, and for predicting the future effects of continuing urbanisation (Bates et al. 2011). This effort is especially important in biodiversity hotspots, such as the southwest of Western Australia.

1.6. The study system: southwest of Western Australia

In Australia, 86 % of the human population resides in urban areas (United Nations 2018). In the southwest of Western Australia, most vegetation has been profoundly altered following European settlement (Figure 1.3), hence many species here are of conservation concern

(Hopper and Gioia 2004). The region is one of only five Mediterranean type ecosystems classed as a global biodiversity hotspot, characterised by a high diversity of endemic plants (62 % of the >8,500 plant species; Fitzpatrick et al. 2008) with less than 30 % of the original vegetation remaining.

We can look to the example of the nearby Wheatbelt region of Western Australia, where extensive clearing for agriculture has resulted in a landscape of small patches of remnant vegetation surrounded by a relatively barren matrix, to understand impacts of fragmentation on native ecosystems (Saunders 1989). Native species that were previously widespread are now generally only found in remnants of the original communities (Williams 2011). Such communities are at risk of species decline, hence identifying factors which influence survival is critical for management decisions (Williams 2011), and this is true for urban landscapes too.

Western Australia is home to an estimated 800 bee species (Houston 2011), so, by understanding the plant and pollinator traits which promote resistance to urbanisation, and also which habitat attributes are important for pollinating insects will ultimately assist in their conservation, the conservation of insect-pollinated plant species, and plant–pollinator interactions. It is also beneficial to explore the value of managed systems within the urban matrix, such as gardens, for biodiversity conservation and maintenance of pollination services. In fragmented areas, gardens and remnants together may be able to provide habitat and potentially provide a continuous food source and variety of floral resources on which pollinators can forage (Tommasi et al. 2004).

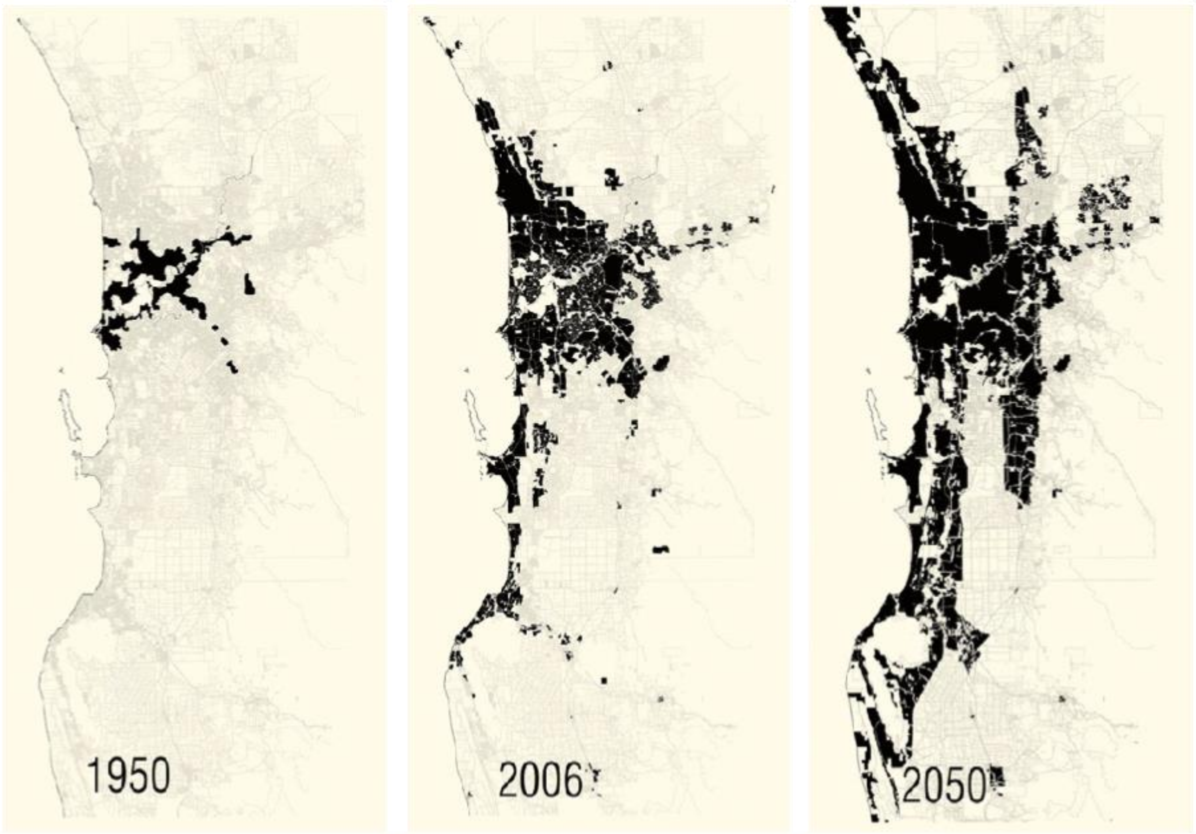


Figure 1.3. Urban growth from 1950 to 2006 in Perth, Western Australia, with a projection of 2050. Adapted from Weller (2009).

1.7. Research questions and thesis outline

The underlying theme of this research is the fragmentation of native vegetation in an urban setting, and whether particular attributes of the fragmented landscape impact native plants and their insect pollinators. I examined flower-visiting insects in the Perth metropolitan region and documented the features of a range of study sites to assess whether site characteristics influence insects, their visitation to flowers, and the resulting plant seed set. I also investigated the mating systems of five native plant species to investigate the susceptibility of common species to pollinator loss. Finally, I examined gene flow in a population of an outcrossing species to determine pollinator movement within an isolated urban remnant.

The relationship between plants, their environment, and their pollinators is fundamental knowledge for informing conservation practices and prioritising efforts. This is particularly important in urban environments where plant reproductive success may be compromised due to factors such as habitat loss and limited access to pollinators. The following research is presented as a literature review and three scientific papers, concluding with a general discussion. The relationship between these chapters is presented in Figure 1.4.

- **Chapter two** examines the literature on the impacts of urbanisation and fragmentation on native plants and insect pollinators. There has been considerable research on the effects of fragmentation on plant and animal populations in rural and urban areas. Here I focus on urban areas, plant communities and insect pollinators. I reviewed the evidence for the potential for remnant vegetation and gardens to mitigate adverse impacts of fragmentation.
- **Chapter three** is a data chapter explaining a two-season survey of insect taxa in gardens and remnants throughout the Perth metropolitan area. Insect data were examined in relation to various landscape measures, including remnant isolation, age of isolation, and site size. I aimed to determine the impact of site characteristics (e.g., size, vegetation type, vegetation cover), and landscape matrix characteristics (e.g., distance to nearby remnants, time since urbanisation, location) on the insect assemblages observed.
- **Chapter four** is a data chapter which describes a field experiment that determined the mating systems of native plant species that have a wide and common distribution in the Perth metropolitan area (*Dianella revoluta, Hemiandra pungens, Jacksonia sericea, Patersonia occidentalis, and Tricoryne elatior).* The importance of insect visitors to the reproductive success of these species was examined by observation and harvest of fruit at the end of the flowering season. Chapter four is published:

  Eakin-Busher EL., Ladd PG, Fontaine JB., and Standish RJ. 2020. Mating strategies dictate the importance of insect visits to native plants in urban fragments. *Australian Journal of Botany* **68**, 26–36.

- **Chapter five** describes the study of gene flow in *Patersonia occidentalis* in one urban remnant using microsatellite markers to establish paternity of seeds produced over one flowering season. These data infer pollinator movement in a population of the species and can be used to estimate gene flow, and specifically if pollinators are moving pollen within the remnant or if pollen has been sourced from individuals living in populations external to the remnant.
- **Chapter six** is a general discussion which summarises the main findings of each data chapter and explains their overall significance.

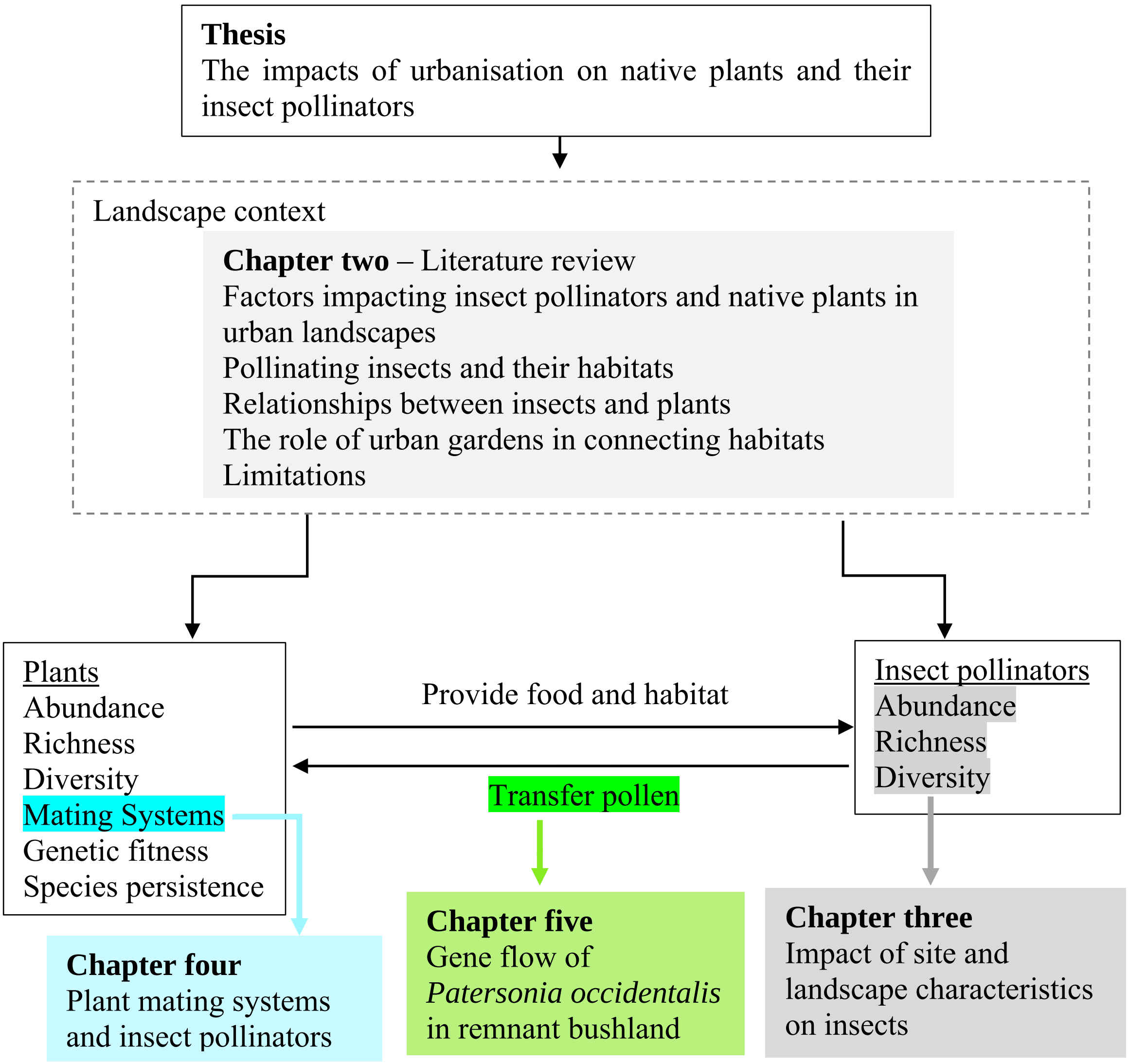


Figure 1.4. Urbanisation impacts plants, pollinators, and their interactions. The literature review contains a broad overview, chapter three focuses on the factors influencing insects, chapter four on plant mating systems of insect-pollinated native plants, while chapter five investigates gene flow in an urban plant population.

2. Factors impacting insect pollinators and native plants in urban environments

2.1. The impacts of urbanisation

Urbanisation is the process of increasing and concentrating human habitation, often characterised by an increase in energy consumption and extensive modification of natural habitats (Mcdonnell and Pickett 1990). Anthropogenic impacts in urban areas take on many forms, including an increase in pollution and disturbance, and significant changes to landscape composition (Mcdonnell and Pickett 1990). As a result of urbanisation, terrestrial environments are increasingly subjected to habitat loss and changes in landscape composition, such that formerly continuous vegetation is transformed into isolated remnant patches (Fortel et al. 2014; Tomimatsu and Ohara 2006). Such changes pose a significant threat to biodiversity (Seto et al. 2012).

While vegetation loss and the associated increase in habitat fragmentation are commonly reported impacts of urbanisation (Fahrig 2003; Grimm et al. 2008), these have a range of underlying effects on ecological functions and processes. Potential impacts include an increase in alien invasive plants (Albrecht et al. 2016), reduced connectivity, altered microclimates (Li et al. 2018), altered gene flow (Jha and Kremen 2013) and mating systems (Eckert et al. 2009), disrupted plant–pollinator mutualisms (Harris and Johnson 2004), increased competition with introduced pollinators (Thomson 2016), and threats to insect biodiversity (Sánchez-Bayo and Wyckhuys 2019). Given the extent of these pressures, understanding their impacts is crucial in maintaining functional ecosystems for the future (Fitch 2017).

The objective of this literature review is to outline the ecological impacts of urbanisation on native plants and insect pollinators, to summarise research that has already been undertaken in this field, and to determine important directions for future research. The literature was chosen based on keyword searches in Web of Science. Search terms were "plant pollinator" or "pollinator"; plant pollination; fragmentation; urbani?ation; urban or town or city or suburb. This chapter will provide an overview of the present literature regarding urbanisation and its influence on insect pollinators, particularly bees. I will first outline the importance of insect pollination, followed by the published negative impacts of urbanisation. I will then examine the complexities of research in urban ecology by commenting on the ability of urban environments to mitigate urbanisation impacts. Finally, I will discuss inconsistent findings and finish the literature review with a conclusion.

2.2. The crucial role of animals for pollination

Pollination is one of the most important ecosystem processes as it facilitates the reproduction of the majority of flowering plants. Over 85 % of known flowering plant species depend on animal pollinators (Ollerton et al. 2011), most commonly insects (Aizen and Feinsinger 2003; Kremen et al. 2007). Pollinating insects include particular beetles, flies, wasps, moths and butterflies, and bees (Cane 2005). There are a number of specialised beetle-pollinated plants (Bernhardt 2000), and some wasps (particularly Vespidae, Scoliidae and Pompilidae families) commonly visit flowers. Lepidoptera, especially the moths, are a highly diverse group with more than 140,000 flower-visiting species expected (Ollerton 2017). Although Diptera is a diverse group overall, the main flower visitors are only within Syrphidae, Bombyliidae, and Tachinidae families.

Despite the diversity of pollinating insects, bees are widely considered to be the most important pollinator group and are adapted for pollination in many ways (Didham et al. 1996; Klein et al. 2007; Neff and Simpson 1993). There are over 20,000 species of bees globally, displaying a range of behaviours and traits (Cariveau and Winfree 2015; Zanette et al. 2005). Australia is home to approximately 1,600 native bee species (Schwarz and Hogendoorn 1999), with around 80 of these occurring in Perth (T. Houston, pers. comm. July 2019). Diverse bee communities persist in urban areas in many parts of the world (Matteson et al. 2008;

McFrederick and LeBuhn 2006; Threlfall et al. 2015; Tommasi et al. 2004). However, despite bees being the most heavily studied pollinator guild (Potts et al. 2010), the effect of urbanisation on bee assemblages is not well understood (Zanette et al. 2005). Although this literature search included any urban insect pollinators, publications were heavily skewed towards bees, perhaps owing to their importance as pollinators. Bee pollination is critical not only to plants for reproduction but also to humans who are heavily reliant on crop pollinators for many food plants (Garibaldi et al. 2013; Klein et al. 2007). Understanding and maintaining populations of bee species and other insect pollinators should, therefore, be of great importance to ecologists and to land managers.

2.3. Pollinating insects in urban environments

Insect pollinators are crucial components of urban ecosystems as they maintain ecosystem functions in areas which are ecologically fragile (Bellamy et al. 2017). However, habitat loss and land use change (i.e., to intensive agriculture or urban area) drive pollinator declines (Mallinger et al. 2016; Sánchez-Bayo and Wyckhuys 2019), with bee species richness declining by 45 % in one study (Cardoso and Gonçalves 2018). In addition, disturbances can alter pollinator behaviour and flight patterns (Didham et al. 1996), and reduce successful foraging. Despite this, urban environments have the potential to support diverse communities of wild bees (Normandin et al. 2017), including solitary and eusocial bee species, and especially generalist and cavity-nesting species (Cariveau and Winfree 2015; Hernandez et al. 2009).

Pollinator declines are occurring across the globe (Potts et al. 2010) but it is difficult to determine exactly how pollinators are affected by humans, and how they will respond to future impacts, especially in different locations (Comba et al. 1999). Studies in Europe and North America highlight a decline in pollinator diversity (Potts et al. 2010), and there is a well-documented decline of honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.) and some wild bees (Sánchez-Bayo and Wyckhuys 2019). Despite the publicity of such declines, their extent for many bee species is unknown.

One of the most important anthropogenic drivers of bee decline is land-use change (Cariveau and Winfree 2015). This is primarily through loss, degradation and fragmentation of habitat (Bartomeus et al. 2013; Potts et al. 2010; Vanbergen et al. 2017; Winfree et al. 2009), which can reduce nesting sites and crucial floral (nutrition) resources (Gill et al. 2016). In Western Australia, sites further from the capital city were large and less isolated, with better vegetation than sites closer to the city, and this favoured many butterflies and moths (Williams 2011). Similarly, in Mexico, fragmented landscapes negatively affected the pollinator abundance for an understorey palm (Aguirre and Dirzo 2008). Habitat area, quality and isolation can also be important characteristics in determining the survival of insect populations (Samways 2007). In addition, other drivers of bee declines include pesticide use (Rortais et al. 2005), pathogens (McMenamin and Genersch 2015), introduced invasive species (Stout and Morales 2009), and climate change (Kerr et al. 2015; Potts et al. 2010).

Many studies report a decrease in species richness and abundance of pollinating insects in urban areas compared to rural and natural habitats (Bates et al. 2011; Fortel et al. 2014; McIntyre and Hostetler 2001). In Chicago, for example, green roofs supported native bees, but more bee species were observed in the nearby prairies and parks (Tonietto et al. 2011). Again within the city of Chicago, only 68 species were found, while there were 138 species in the overall region (Lowenstein et al. 2015). A review by Hernandez et al. (2009) also identified a negative correlation between bee species richness and urban development, suggesting that urbanisation does have the tendency to reduce species richness. Such observations reinforce the idea that urbanisation and fragmentation have the potential for detrimental impacts on insect pollinators. A summary of these impacts is provided in Table 2.1.

Table 2.1. Summary of published negative impacts of urbanisation on plants and pollinators

Response variable	Taxa	Key explanatory variable	Location	Key result
Pollination services	Bees	Hardscape	Illinois, USA	Fruit set decreased with increasing hardscape (Bennett and Lovell 2019)
	Bees	Urbanisation	Xining, China	Decline in pollinator visits, *Gentiana dahurica* seed set declined and pollen limitation increased (Hou et al. 2019)
	Bees and hoverflies	Land use	Flanders, Belgium	Negative influence on seed set (Verboven et al. 2014)
	Pollinators	Habitat fragmentation	Literature review	Reduced pollination (Aguilar et al. 2006)
	Trillium camschatcense	Habitat fragmentation	Hokkaido, Japan	Reduced plant reproduction (Tomimatsu and Ohara 2006)
	A. brachybotrya *E. glabra* Flower visitors	Habitat fragmentation	New South Wales, Australia	Plants faced a decline in pollination when growing in fragmented sites (Cunningham 2000)
Pollinator reproductive fitness	*Tetragonula carbonaria* (Australian social stingless bee)	Floral diversity		Floral diversity drives bee fitness (Kaluza et al. 2018)
Pollinator abundance/richness	Bees	Increased canopy cover and impervious surface	Toledo, Ohio USA	Decline in bee abundance (Burdine and McCluney 2019).
	Bees and hoverflies	Habitat loss	Germany	Decline in taxa and decrease in specialisation (Jauker et al. 2019)
	Bees	Urbanisation	Southern Brazil	Since sampling in the 1980s, bee species richness declined by 45% (Cardoso and Gonçalves 2018)
	Bees	Conversion of habitat to row crops	United States	Modelled bee abundance declined (Koh et al. 2016)
	Bees and hoverflies	Land use	Birmingham, UK	Lowest pollinator abundance and diversity with more urban gardens (Bates et al. 2011)
	Bumble bees	Change in land use	Stockholm	Nesting and foraging sites decreased (Ahrné et al. 2009)
	Bumble bees	Urbanisation	San Francisco	Bumble bee abundance lowered with lower resource availability. Species richness was negatively associated with a competitor

				(McFrederick and LeBuhn 2006)
Pollinator species composition	Bees	Change in land use	Southern Costa Rica	Shift from mostly native to mostly non-native bees (Brosi et al. 2007)
	Insect pollinators	Habitat loss	Southern Ontario, Canada	Shift from specialist to generalist insect pollinators (Taki and Kevan 2007)
	Bees	Urbanisation	Michigan, USA	Increase in male bees and a decrease in ground-nesting females (Fitch et al. 2019)

Lentini et al. (2012) suggest remnants are important for bees in agricultural landscapes of New South Wales. The suitability of urban areas for bee habitation (i.e., habitat quality) is influenced by site and landscape characteristics, including floral abundance, garden size, and plant species richness (Frankie et al. 2005; Ahrné et al. 2009). Hall et al. (2019) identified key features, such as wooded vegetation, required to support diverse bee communities in fragmented agricultural landscapes of Victoria. Diverse landscapes therefore have the potential to support insects by meeting the requirements of numerous species, as well as providing flowers throughout the entire foraging period (Mallinger et al. 2016). Understanding the pollinator traits which promote resilience, and also which habitat attributes are important for pollinating insects will ultimately assist in their conservation.

Insect traits

The highly variable nature of urban environments causes variation in their suitability for insect habitation (see New 2018). There are over 20,000 species of bees globally, and they display different behaviours and traits (Cariveau and Winfree 2015). Thus, the impact of urbanisation is also likely to be different for different pollinator species. For example, the abundance of large-bodied bees (*Bombus* and *Apis* spp.) increased with increasing impervious cover, while small soil-nesting *Halictus* increased with increasing flower cover (Bennett and Lovell 2019). In another study, small, eusocial ground-nesters had the strongest responses to urban warming

(Li et al. 2018). However, generalisable trait-based predictions regarding species responses are not always evident (Bartomeus et al. 2018).

For particular bee species that happen to do well in disturbed or highly modified environments, an increase in abundance and/or diversity may be expected (see Matheson et al. 1996). For example, in homogenous plant cover (e.g. monocultures), a small group of specialised bees may thrive, decreasing bee diversity, but potentially increasing the density of some groups (McIntyre and Hostetler 2001). Urbanisation may decrease the abundance of floral specialists (insects that collect pollen from a limited number plant species), meaning there may be relatively more generalised pollinators with increased urbanisation (Deguines et al. 2016; Taki and Kevan 2007). However, urban bees may visit a lower proportion of the available flowering plants (higher specialisation) (Theodorou et al. 2016).

Native versus introduced bees

Global biodiversity may be threatened by alien invasive species in many environments (Goulson 2003). Invasions often result from anthropogenic activities and threaten ecological functions such as pollination, reproductive success and population dynamics of co-flowering native species (Albrecht et al. 2016; Memmott et al. 2007). Introduced pollinators may prey on native species or compete with them for resources (Goulson 2003). A well-known example is the introduced honey bee (*Apis mellifera*), which shares many flowers with native bees, and can outcompete them (Johanson et al. 2019; Thomson 2016).

Urbanisation can have a positive influence on exotic bee abundance, and exotic bees may negatively affect native bee populations (Carman and Jenkins 2016; Fitch et al. 2019). Competition is variable, with review results spanning from negative effects on wild bees (53 %), and no effect or mixed effect (28 % and 19 % respectively; Mallinger et al. 2017). In one study, honey bees increased with flower abundance and Hung et al. (2019) suggested that native insects may therefore forage other plants to avoid competition from the honey bees.

Similarly, a bumble bee species—*Bombus sitkensis*—was negatively associated with the presence of *Bombus vosnesenskii,* a dominant competitor (McFrederick and LeBuhn 2006). This was attributed to the reduced number of nesting sites available for *Bombus sitkensis*. In this case, urbanisation was not necessarily the cause of the decline in species richness, which shows the importance of considering other factors when determining the cause of pollinator declines. However, findings remain speculative because, although less urbanisation (i.e., potentially more nest sites) could allow an increase in bee species richness, the relationship remains unverified.

Honey bees (*Apis mellifera*) are the most frequent floral visitors in natural habitats worldwide (Hung et al. 2018), but many flowering plant taxa may be dependent on non-honey bee visitors for pollination (Hung et al. 2018). Honey bees may be less effective pollinators than other bees (Garibaldi et al. 2013; Hung et al. 2018), which in turn influences plant reproductive success (Hausmann et al. 2016; Ne'eman et al. 2010).

2.4. The benefit of insect-pollinator interactions for plants

The major advantage of insect-pollinator interactions is the transfer of outcross pollen, which allows genetic variability of progeny (Faegri and Van Der Pijl 1979; Kearns et al. 1998) and increased reproductive output (Eakin-Busher et al. 2016; e.g., Greenleaf and Kremen 2006). Plant–pollinator interactions are, therefore, integral to ecosystem functionality and the maintenance of biodiversity worldwide.

Generally, there is thought to be a negative relationship between fragmentation and pollination (Aguilar et al. 2006, Aguilar et al. 2008). A reduction in the number or diversity of flowering plants can lead to a decrease of pollinators (McIntyre and Hostetler 2001), which then alters biotic interactions (Hahs et al. 2009; Harrison and Winfree 2015), and reduces the reproductive output in plants (Tomimatsu and Ohara 2006). Indeed, the best-documented impact of fragmentation in animal-pollinated plants is reduced plant reproduction (Tomimatsu and Ohara 2006). Insect pollinator may risk being lost from habitat fragments (Tscharntke et al. 2002), especially those with low ability to disperse (Thomas 2000). Therefore plants with fewer pollinator interactions (i.e., more specialised interactions) would be at greater risk than widespread plant species (Norfolk et al. 2018). As a result of pollinator declines, many native plant species have gone locally extinct in cities worldwide (Hahs et al. 2009).

2.5. Mating systems and gene flow

The ecological traits of a plant species have some predictive capacity over a plant species' susceptibility to impact (Aguilar et al. 2006). Mating systems, the method by which plants reproduce, are one such example. The mating systems of sexually reproducing plants range from autogamous (within-flower) self-pollination, through to obligate outcrossing (Lloyd 1979). Outcrossing depends on plant–pollinator interactions and is generally thought to be advantageous given the genetic fitness of outcrossed compared with inbred progeny (Stephenson et al. 2000). Mating systems influence gene flow and genetic drift and are therefore highly influential in generating genetic differentiation among plant populations (Duminil et al. 2009).

Habitat fragmentation can be detrimental to plant populations, as it may result in reduced reproduction. For example, if fragmentation reduces the pollinators in an area, plants may experience less outcrossing, and populations may suffer reductions in genetic fitness and population viability (e.g., Young et al. 1996; Donaldson et al. 2002; Harris and Johnson 2004; Pauw 2007). The loss of plants has a similar impact, as fewer potential sires lead to lower genetic diversity. However, some plant populations show resilience to fragmentation, for example, Thavornkanlapachai et al. (2019) found that reproduction in naturally fragmented populations of mammal-pollinated *Banksia nivea* subsp. *uliginosa* was not negatively impacted by the size of the plant population. Generally though, reduced pollen dispersal, along with inbreeding, can result in reduced fitness of progeny (Gibson et al. 2012; Krauss et al. 2007;

Llorens et al. 2013; Yates et al. 2007b) and reduced reproductive success in successive generations (Fenster and Dudash 1994). It is therefore useful to understand which pollinators contribute to plant reproduction, particularly for plant species which cannot self-pollinate, and rely completely on pollinator assemblages for their reproduction (Aizen and Feinsinger 2003; Aizen et al. 2002). Plant species which are pollinated by birds tend to have higher paternal diversity and increased pollen dispersal compared to plants which are pollinated by insects, as birds can travel greater distances (see Krauss et al. 2017). However, wild bees can still be important pollinators of outcrossing plant species in urban areas (Verboven et al. 2014).

The absence of pollinators may increase self-pollination in self-compatible plant species, potentially leading to reduced genetic fitness of offspring (Kearns and Inouye 1997). Population size and habitat disturbance may impact pollinator availability and behaviour, inbreeding (self-compatible species), the ability to find mates (outcrossing species), and the size of the pollen pool (Coates et al. 2007). Through their influence on plant fitness and genetic variability, mating systems can also influence adaptation (Levin 2012; Munoz et al. 2016). For example, where low pollinator activity causes pollen limitation (e.g., Knight et al. 2005; Newman et al. 2013), this may lead to selection for self-fertilisation (Lloyd 1992).

Gene flow (i.e., pollen and seed dispersal) is a critical component of plant mating systems and influences population fitness. Gene flow can reduce the effects of genetic drift and prevent loss of genetic diversity (Sork and Smouse 2006), but if remnants are more isolated than the distance pollinators can travel, pollen flow may be reduced (Kearns and Inouye 1997). The rate of pollen dispersal in and between remnants may therefore impact genetic and reproductive processes and population viability (Whelan et al. 2000; Young et al. 2010).

2.6. Can urban gardens mitigate the impacts of urbanisation?

In the literature regarding bee pollinators in urban parks and gardens, negative impacts are well documented. For example, gardens often include a high proportion of horticulturally modified plants and exotic species which provide poor quality nutrition for many pollinators (Bates et al. 2011). Herbicides, insecticides and other chemicals are also used in garden management, and gardens often correlate with the presence of domestic cats, a major predator of vertebrate pollinators (Goddard et al. 2010). In addition, the loss of stable forage and the abundance of homogenous plants in urban areas could limit the yield of plant fruit and seed (Lowenstein et al. 2015), and the abundance of floral resources around urban gardens may not facilitate pollination of all plants equally (Moeller 2004). In Birmingham (United Kingdom), the poorest pollinator abundance and diversity was associated with the largest percentage of suburban gardens (Bates et al. 2011). In some plant species, pollination may decrease due to an increase in competition for pollinators (Lowenstein et al. 2015).

Contrary to expectations, urbanisation does not always have a net negative impact on pollinators, most likely due to the availability of novel food and nesting resources (Martins et al. 2017; Quistberg et al. 2016; Tommasi et al. 2004). Urban plant populations are becoming increasingly important, and high-quality urban habitats are capable of supporting pollinators (Baldock et al. 2019; Dylewski et al. 2019). In comparison to concrete-dominated landscapes, residential gardens may provide high-quality bee habitat where pollination of particular flowering plant species is enhanced (Cussans et al. 2010; Samnegård et al. 2011).

Some studies show a positive effect of urbanisation on certain bee taxa and functional groups such as bumble bees and cavity-nesters (Cane et al. 2006), while others found that bee species richness, abundance and functional diversity increased with increasing urbanisation (Martins et al. 2017). Studies have also found similar bee assemblages between urban parks and wild parks (McFrederick and LeBuhn 2006) while others found pollinator numbers were highest in suburban gardens, and not the nearby forests (e.g., Kaluza et al. 2016; Winfree et al. 2007) or agricultural areas (Theodorou et al. 2016). Similarly, a study in Vancouver (Canada) showed that although parks only constituted 2.5 % of the total land use, they were productive and diverse ecosystems and maintained high species diversity (Tommasi et al. 2004). Moderate anthropogenic land use may, therefore, be compatible with the conservation of some bee species (Winfree et al. 2007).

Although pollinator diversity and abundance may be greater in wild areas, urban areas also have the potential to be important pollinator reservoirs, especially if heterogeneity is maintained through sustainable urban planning (Tommasi et al. 2004). Small habitat patches, such as public urban parks have been studied for their contribution to biodiversity maintenance in otherwise hostile urban areas (see Bates et al. 2011; Tommasi et al. 2004; Zanette et al. 2005) and parks and gardens form interconnected networks and are therefore important providers of habitat for pollinators (Goddard et al. 2010).

Parks and remnants are often the focus of urban nature conservation; however, private gardens potentially offer an extensive and undervalued resource for enhancing urban biodiversity (Goddard et al. 2010). Domestic gardens can support high biodiversity and even a high density or productivity of species that have experienced severe local or regional declines (Gaston et al. 2005; Goddard et al. 2010). Because bee diversity and species richness can be driven by floral abundance and habitat, garden managers could promote bee conservation by managing for floral connectivity and abundance within urban habitats (Plascencia and Philpott 2017; Samnegård et al. 2011). However, because domestic gardens are privately owned, they typically lie outside of the management requirements and immediate control of administrative authorities (Gaston et al. 2005).

Floral resources

Gardens and corridors have the potential to support a diverse assemblage of pollinators, particularly if the urban landscape is accompanied by an increase in flowering plants (Bates et al. 2011). Because pollinators are dependent upon plants for nesting and floral resources, the abundance and activity of insect pollinators are often associated with the abundance and diversity of flowering plants (Frankie et al. 2005; Kaluza et al. 2018; McFrederick and LeBuhn

2006; Potts et al. 2010; Quistberg et al. 2016). Similarly, plant richness and density can also have a positive impact on the richness of bee species in urban habitats (Bates et al. 2011; Hennig et al. 2012; Matteson et al. 2013). However, there is a lack of basic information concerning bee biology and its association with flowering plant species (Gill et al. 2016).

Garden flowers are often considered valuable if they produce pollen or nectar (Comba et al. 1999). Highly efficient pollinators such as bumble bees can also respond positively to urban land use, as they may visit flowers frequently (Verboven et al. 2014). Bumble bees are also abundant in urban community gardens (Matteson and Langellotto 2010), where they can make use of both artificial and natural nesting sites (Osborne et al. 2008), which in turn benefits pollination of urban plants. Even when urban plants are located next to resources that may compete for pollinators, research by Lowenstein et al. (2015) found that co-occurring resources in gardens did not cause competition for pollination and may, in fact, lead to complementary visitation to adjacent plant species. However, these findings vary, thus pollination services are less generally reliable in highly disturbed environments (Ahrné et al. 2009).

Increasing the floral resources in urban environments has the potential to neutralise negative effects from characteristics such as impervious surface (Burdine and McCluney 2019). Flower richness has the potential to be greater in urban areas than agricultural areas for example, and in such cases, visiting insects tend to forage from a greater number of plant species (Theodorou et al. 2016). However, the structure of plants is likely important, with increased tree cover decreasing bee abundance and richness (Burdine and McCluney 2019; Hall et al. 2019).

Maintaining good quality floral resources is important for bee conservation (Plascencia and Philpott 2017). Pollinators may use a combination of different habitats to meet their needs (Cole et al. 2017), with good quality urban habitats even able to act as a source of pollinators for surrounding agricultural areas (Bates et al. 2011). Characteristics such as increased floral abundance, taller vegetation, and more woody plant cover can correlate with an increase in bee species richness and abundance (Pardee and Philpott 2014). Additional considerations include the presence of specific plant species, as certain plants may be more important than overall plant species diversity for supporting insect communities (Warzecha et al. 2018). Landscape heterogeneity and the spatial arrangement of flowers, has also been shown to predict bee abundance and richness (Plascencia and Philpott 2017; Senapathi et al. 2017).

Native plants

In urban gardens, pollinator activity has been shown to increase with an increased proportion of native plant cover (Fukase and Simons 2016). Where alien and native species co-flower and share pollinators, consequences for native plant pollination, reproductive success, and evolution may occur. These consequences range from negative (competition) to positive (facilitation), but the ecological drivers which determine the severity of such interactions are poorly understood (Albrecht et al. 2016).

Alien invasive plant species can influence co-flowering native plants through their shared pollinators (Albrecht et al. 2016). For example, generalist pollinators may preferentially visit invasive plants with large, attractive floral displays (Morales and Traveset 2009). Invasive plants can, therefore, alter the reproductive success of native plants through reducing pollinator visits to native plants (Albrecht et al. 2016). The removal of exotic plants can have a positive effect on pollinator richness (Rafferty 2017). However, some studies have found no difference in insect attraction between native and non-native plant species (Martins et al. 2017; Rollings and Goulson 2019).

Connectivity

Connectivity among patches of vegetation may reduce the negative impacts of urbanisation. According to the theory of island biogeography, vegetation remnants, urban parks and gardens can act as islands of good quality, useable habitat, isolated within a hostile matrix (Macarthur and Wilson 1967). Within an urban matrix, gardens have the potential to provide a connection (corridor) between natural areas (Tommasi et al. 2004). Gardens and remnants together may therefore be able to provide habitat and a continuous food source on which pollinators can forage (Tommasi et al. 2004).

Although patches of habitat may exist, many factors influence whether particular insects will be able to use it. For example, for insects such as bees to reach other patches, the distance between them needs to be short enough to allow migration (Hanski 1999). If a lack of resources and poor connectivity between suitable habitats does reduce the capacity of pollinating insects to disperse and forage, there may be a reduction in the presence of pollinators, and their service to plants (Bennett and Lovell 2019; Charlesworth 2006; Donaldson et al. 2002; Townsend and Levey 2005; Ward and Johnson 2005). Similarly, isolated patches of suitable habitat may not be discoverable by bee colonists, and if the habitat is too distantly isolated, then local population extinction can occur (Cane 2005).

Research on pollinator movement, however, (e.g., along a gradient of urbanisation) may not always accurately represent pollinator responses in a wider habitat. For example, Bates et al. (2011) suggested there may have conceivably been a greater abundance and diversity of pollinators in urban areas (than rural) if most species of pollinator along this gradient use gardens as habitat. Therefore, to determine the response of pollinators to urbanisation, several habitat types should be studied considering site and matrix scales and associated characteristics.

Surrounding surface cover

Surrounding surfaces, such as soils or impervious surfaces can influence insect presence, usually with decreasing insect pollinators with increasing impervious cover (e.g., Bennett and Lovell 2019, Burdine and McCluney 2019). Soils rather than impervious surfaces are necessary for different ground-nesting bees (Geslin et al. 2016; Tonietto et al. 2011), while species that tunnel into wood or plant stems likely have preference for these. Sites containing bare ground without mulch has also been shown to increase abundance in urban gardens (Quistberg et al. 2016).

Impervious surfaces often influence the bee assemblages found, with negative correlations often reported (Geslin et al. 2016; Sivakoff et al. 2018). This extends to the area surrounding gardens, where the abundance of bees and of honey bees (*Apis mellifera*) can decrease at sites with more surrounding urban land cover (Plascencia and Philpott 2017) and paved areas (Cardoso and Gonçalves 2018). A combination of substrates therefore favours bee diversity (Cane 2005), and maintaining habitat within developed landscapes is likely beneficial, as is minimizing impervious cover at large spatial scales (Bennett and Lovell 2019).

2.7. Evidence of urbanisation having no impact

Results that counter the prediction that urbanisation has a detrimental impact are not uncommon in the literature. Such results may reflect the complexity of urban environments and the different study scales and response variables used in research. For example, in a review of 265 published studies on pollinator responses to anthropogenic land use, bee responses to change in land use were negative in 40 % of cases, but 13 % were positive, and 47 % were neutral (Winfree et al. 2011). The idea that urbanisation has a negative impact on plants and pollinators, and the idea that gardens provide ‘green infrastructure’ are simplified views. In reality, findings are often not as expected, or there are neutral or “no impact” results (Table 2.2).

Table 2.2. Summary of urbanisation and fragmentation impacts on insect pollinators

Winners	No overall impact	Losers
Cavity nesting bees (Cane et al. 2006)	Bees (Kearns and Oliveras 2009)	Bumble bees (McFrederick and LeBuhn 2006, Ahrné, Bengtsson and Elmqvist 2009)
Honey bees (*Apis mellifera*) (Aizen and Feinsinger 1994)	Eusocial bees (Zanette et al. 2005)	Bees (Fortel et al. 2014)
Non-native species (Costa Rica) (Brosi et al. 2007)	Bees (Fischer et al. 2016)	Insect pollinators (Hostetler and McIntyre 2001)
Exotic bees (Michigan, USA) (Fitch et al. 2019)	Bees (Hinners et al. 2017)	Bees and hoverflies (Bates et al. 2011)
		Ground nesting bees (Cardoso and Gonçalves 2018; Geslin et al. 2016)
		Native bees (Costa Rica) (Brosi et al. 2007) Native bees (Michigan, USA) (Fitch et al. 2019)
		Specialist pollinators (Taki and Kevan 2007)

In some cases, changes in land use due to urbanisation may not cause significant decreases in species diversity or abundance. Kearns and Oliveras (2009) predicted that increased development would decrease species richness of flower-visiting bees. However, they found 108 species out of the 116 that were present in 1907 and concluded there was no significant loss in diversity despite the urban development which had occurred. Neither the number of species nor their abundance differed significantly among the plot types, although the trend did indicate increasing diversity with increasing distance from urbanisation (Kearns and Oliveras 2009). Similarly, in Minas Gerais (Brazil), generalist bee species did not appear to decrease in abundance when urbanisation caused habitat change, and no relationship was found between the solitary and the primitive eusocial wasps and bees and habitat changes (Zanette et al. 2005). Matteson et al. (2013) also found no direct influence of urban development intensity on insect pollinator communities.

Bee species richness and abundance have the potential to be greater in anthropogenic landscapes than surrounding areas (e.g., Winfree et al. 2007). Overall, it seems that abundance and diversity may not be predicted by the landscape composition itself, but rather whether adequate resources are present for particular insect species. For example, 62 bee species studied by Cane et al. (2006) had diverse responses to fragmentation. In smaller vegetation patches, there was no difference in the absence of small-bodied and large-bodied bee species. Similarly, the area of parks, distance to the nearest source population, and perimeter to area ratio did not predict bumble bee community structure in San Francisco's (USA) urban parks (McFrederick and LeBuhn 2006). Overall, the responses of bees, at least in the case of Cane et al. (2006) appeared to be predicted by nesting and dietary requirements.

More recently, a study by Fischer et al. (2016) suggested that detected bee species were not affected by environmental variables such as site type, flower coverage, and plant species. Additionally, urban characteristics, such as area and isolation did not have an overall negative impact, but affected different species in different ways (e.g., some thrived, while others declined, Fischer et al. 2016). In Colorado (USA), Hinners et al. (2017) also reported no overall effect of urbanisation on bee abundance, with highly variable bee abundance and species density within study sites. The variability of results for different species and different locations highlights the complexity of insect research in urban environments.

2.8. Topic importance and knowledge limitations

Global urban populations are predicted to reach five billion by 2030 (United Nations 2018). Little is known about the future impacts of urban expansion (Seto et al. 2012), thus developing an understanding of ecosystem responses to urbanisation is crucial. In addition, there have been fewer publications on urban bee ecology than bees in agricultural and wild environments (Hernandez et al. 2009). Bees can be sensitive to changes, but publications continue to report varied responses of bees to the impacts of urbanisation (Cariveau and Winfree 2015; McFrederick and LeBuhn 2006).

The idea that habitat loss threatens biodiversity is widely documented (e.g., Taki and Kevan 2007). However, the results of published studies do not always support this idea, and some authors suggest the contrary: that urban environments have the potential to provide a rich mosaic of habitats (Sadler et al. 2006) and may, therefore, be a haven for biodiversity. The variability of findings is also a clear reason to improve our ecological understanding, especially for future management of biodiversity in urban areas.

A frequent limitation to studies in urban ecology is the complex nature of urban environments. Researchers commonly test assumptions along a gradient of urbanisation (Sadler et al. 2006), where human pressure is greatest in the city centre (McDonnell and Hahs 2008). Studies using gradients (e.g., Ahrné et al. 2009; Bates et al. 2011; Choate et al. 2018; Fortel et al. 2014; Geslin et al. 2013; Theodorou et al. 2017) show that there can be changes in species composition and richness with variation in urbanisation. Sampling along gradients is a useful, simple method as samples do not need to be taken from across an entire landscape. However, urban ecology would benefit from a systematic, standard method for experimental designs, as McDonnell and Hahs (2008) found that of 300 papers published between 1990 and 2007, only five addressed how the urban gradient was actually quantified. Similarly, many surveys have classified habitats as urban without defining what was meant by the term. Quantitative descriptors, ideally of ecological significance, are therefore necessary to define urban habitats (McIntyre et al. 2000). If there is a continued absence of quantitative definitions, research findings may be compared in cases where the data are not necessarily comparable.

Urbanisation may be expected to have different effects on pollinators in different urban areas. Pollinator responses in one area may not necessarily be true for other urban areas (Bates et al. 2011), especially when comparing pollinator responses at an international scale. The current literature is biased towards cities in Europe and North America (De Palma et al. 2016;

Harrison et al. 2015), and urban bee research is lacking in Africa, Asia, most of South America (Hernandez et al. 2009) and Australia. Archer et al. (2014) found that there was also a geographic bias in the literature investigating how pollinators respond to land use changes, which is a concern because response differences and sensitivity of pollinators to change can vary greatly in different locations. This geographic skew also promotes bias towards the species found in particular regions (Winfree et al. 2011). Collecting accurate information on pollinators is necessary to identify any vulnerable species, set conservation goals and determine whether the current literature reflects what is happening globally (Archer et al. 2014).

Overall, little is known about how bee communities are affected by urban development (Kearns et al. 1998; Price and Waser 1998). Few surveys of bees in urban areas exist, and the species present in published studies cannot usually be compared with historical records as these records are often incomplete, or do not exist. This issue is significant because even if the number of extant species is high, it may only be a portion of the historical species pool (McFrederick and LeBuhn 2006). As bees facilitate the reproduction of most flowering plant species (Didham et al. 1996; Klein et al. 2007; Neff and Simpson 1993), their service is of critical importance to plants.

One approach which could serve to standardise bee research could be to implement collaboration across countries. For example, an international project (Globenet) exists to determine whether anthropogenic landscape change has the same effect on carabid beetles in different regions (Niemelä et al. 2000). Globenet uses common field methodology (pitfall trapping) along urban-rural gradients (i.e., visually similar land mosaics). Carabids are used because of their taxonomic and ecological variation, and sensitivity to environment type. Despite species differences, the general patterns of community response to anthropogenic disturbance were similar. International efforts such as this are more likely to reliably reveal the extent to which general patterns are applicable in various parts of the world. Thus future work on the response of pollinators to urbanisation would benefit from a standardised approach

(Bates et al. 2011). Collaboration between ecologists, urban planners and the community could also be used to extend landscape ecology to include the study and management of gardens, as patches of interconnected habitat in the ecosystem (Goddard et al. 2010).

Future directions for studying urban bee ecology include incorporation of landscape-scale assessments and conducting manipulative experiments (Hernandez et al. 2009). Hernandez et al. (2009) reviewed 68 studies and found remnant habitats and managed gardens were studied more than home gardens. Additional shortfalls in the literature include the effects of urban land use on bees and the pollination services they provide (Verboven et al. 2014), the influence of habitat condition and remnant characteristics on pollination success (Harrison and Winfree 2015), and ecological connectivity within urban areas (LaPoint et al. 2015). Urban development continues; thus, urban parks and gardens will become even more important as a sanctuary for organisms. To maximise the efficacy of urban parks, we need to know which characteristics influence biodiversity (McFrederick and LeBuhn 2006).

Knowledge gaps in the pollination field were recognised in 2002, and the initiative for the Conservation and Sustainable Use of Pollinators was established, aiming to promote the conservation of pollinator diversity. This thesis aims to contribute to such global efforts by determining the impacts of urbanisation on native plants and insect pollinators in Perth, Western Australia. Little is known about the ecology of insect visitors and subsequent pollination for native plants here. This research will provide records for researchers in order to know which bee species exist in certain habitats in the Perth area and may also lead to better decisions for environmental management. For example, if larger remnants contain more pollinators than small remnants, then perhaps it will be important to preserve habitats of suitable size in the future. I also used genetic research to help determine the source and scale of movement of pollen in urban environments.

2.9. Concluding remarks

Urbanisation continues at a rapid rate, with a projected increase in the proportion of the world population living in urban environments, which will stimulate further clearing and disturbance. Despite the role of gardens and remnants as habitat in urban environments, this review suggests that different vegetation types (i.e., gardens and remnants) are not likely to be beneficial to every insect species, as an individual's requirement for particular patches of vegetation depends on its needs at the time. There is a lack of generality among published findings in many cases, and thorough research in a number of geographical locations has not been carried out. To date, the available evidence suggests the negative impacts of urbanisation outweigh the biodiversity maintenance afforded by small patches of habitat. Despite the suggestion that if key species can persist, pollination services can continue, we cannot underestimate the value of pollinator diversity and redundancy, especially for recovery from disturbances (e.g., drought, fire). Patches of habitat and the surrounding urban matrix are complex environments and therefore require further research in an effort to understand their processes. With reliable findings, urban planners and developers may be informed on how to establish and maintain connected environments with high biodiversity in urban areas.

3. Remnant vegetation and residential gardens provide complementary resources and habitat for insects in an urban landscape

3.1. Abstract

The fragmentation of native vegetation is a global issue resulting from increasing alienation of land due to urban development, mining, and agriculture. In south-western Australia, there has been a profound change to the landscape through extensive removal of native vegetation. Plants and animals which remain in remnants may experience threats to their persistence, particularly from reduced access to resources and increased isolation. In cities, the mosaic of retained vegetation and urban gardens provide habitat to support native plants and animals. While urban gardens may not provide enough area to support vertebrate native animals, invertebrates may gain benefits if the plants in the gardens provide resources similar to those in the native vegetation. However, the relative value of remnants versus gardens to invertebrate fauna has been little studied. There is growing concern regarding threats to conservation of invertebrates, in particular insect pollinators. Honey bee declines have attracted global attention due to the importance of honey bees as pollinators of food crops; however, more data are needed for other insects to determine if declines are common responses to habitat loss and fragmentation.

In Perth, the largest city on the west coast of Australia, a number of remnants and urban gardens planted predominantly with native plants in the same general area were selected to examine invertebrate fauna, to see if they were complementary or different in their composition. I predicted that garden insect fauna would be different from that of remnants in recently alienated areas but would become more similar with time since development and would be influenced by proximity to remnants. I used blue and yellow pan traps to investigate insects, in particular, whether their abundance was were related to the site and landscape matrix characteristics of urban remnants and residential gardens. I used generalised linear mixed

effects models to examine the effects of remnant age and area, and their interaction, on the relative abundance and functional diversity.

Insect composition and abundance differed between garden and remnants with a greater abundance of insects in gardens, particularly older ones. The two most abundant groups were Coleoptera, prominent in remnants, and Diptera most abundant in gardens. In general, insects were more abundant in older gardens, but this was driven mainly by Diptera abundance. Pollinating insects were much less abundant than other groups and were lower when gardens were further away from remnant vegetation ($z = -2.48$, $p < 0.05$), pointing towards the importance of connectivity. Overall, my data suggest that remnant vegetation and native gardens may both provide resources and habitat for insects living in this urban landscape. As connectivity seems to be important for pollinating insects, a denser distribution of small remnants may be more useful than sparse large remnants in the urban matrix.

3.2. Introduction

Fragmentation of natural habitat is considered to be one of the greatest threats to species biodiversity and ecosystem function worldwide (Fahrig 2003; Foley et al. 2005; Rathcke and Jules 1993). Habitat fragmentation involves two main processes: a reduction in habitat extent and an increase in the spatial isolation of habitat patches (Fahrig 2003). Fragmentation therefore results in a landscape where native vegetation is divided into a series of smaller patches, occurring in isolation, surrounded by a matrix of changed land (Saunders et al. 1991). The loss of habitat in particular has large, consistently negative effects (Fahrig 2003) such as a reduction in the abundance and diversity of plants and animals (Seto et al. 2012). In addition, habitat loss and fragmentation can lead to negative consequences for plant–pollinator interactions (Burkle et al. 2013; Ferreira et al. 2013).

The value of pollinators is well known, with insects sustaining food crop supply and facilitating the reproduction of wild plants (Aizen and Feinsinger 2003; Ollerton et al. 2011; Potts et al. 2016). There is growing concern regarding the decline of insect pollinators (Bartomeus et al. 2013; Cameron et al. 2011; Koh et al. 2016; Potts et al. 2010), again with land use and habitat change implicated as major drivers (Brown and Paxton 2009; Sánchez-Bayo and Wyckhuys 2019). When vegetation is divided into smaller, more isolated patches, the organisms living in these areas face threats to their persistence, such as reduced population size which can alter species composition (Yates et al. 2007a), gene flow (Coates et al. 2007) and edge effects (Didham et al. 1996; Harrison and Bruna 1999). In general, a decrease in habitat size and floristic diversity tends to decrease the diversity and richness of insect assemblages, as well as the pollination they provide (see Burdine and McCluney 2019; Hall et al. 2019). Impacts on insects other than pollinators are less studied.

Given the immense diversity of insects (Zhang 2011), their individual responses to impacts are likely to vary widely. Insects require food sources and nesting material to complete their life cycles (Williams et al. 2010), so traits such as their body size, flight distance, and flexibility in their choice of food and nesting resources may determine their response to fragmentation (Gathmann and Tscharntke 2002; Saunders et al. 1991). For example, small-bodied bee species typically forage within a few hundred meters of their nests, so they may be more susceptible to local disturbances than larger bee species capable of travelling several kilometres (Greenleaf et al. 2007; Zurbuchen et al. 2010). Similar relationships exist for other pollinating insects such as butterflies, which, if exhibiting traits such as low mobility, low reproduction, and narrow feeding niches, were most strongly affected by habitat loss (Öckinger et al. 2010). On the other hand, solitary bees and below-ground nesters, which can make use of the surfaces in built-up areas, may be more suited to isolation from remnant vegetation than social bees and above-ground nesters (Williams et al. 2010). Overall, large, connected plant populations are more likely to provide sufficient floral resources (i.e., pollen and nectar) for insect pollinators than small, isolated plant populations (Potts et al. 2016).

Urban environments can provide insect pollinators with some food and nesting resources (Cane et al. 2006; Hall et al. 2017), yet insects are likely to also be dependent on native habitat features. For example, plant species richness and the presence of flowering plants may determine whether the habitat is suitable for a particular insect (Frankie et al. 2005; Matteson and Langellotto 2010). Pollinating butterflies and hoverflies are generally nectivorous as adults (Winfree et al. 2011) and therefore require nectar-producing flowers. However, taxa with specialist relationships may require particular prey species or particular plants for laying their eggs (Winfree et al. 2011). Meanwhile, bees use both wooded and open parts of the landscape while foraging (Winfree et al. 2015), but small, isolated remnants are expected to support fewer pollinators than continuous habitats (e.g., Donaldson et al. 2002). In some locations, the increased cover of insect-pollinated plants allows for increased bee density (Banaszak-Cibicka et al. 2016). The inverse is also possible as a greater cover of shrubs and undergrowth may have a negative impact on ground-nesting bees, due to the reduction in soil nesting sites (Banaszak-Cibicka et al. 2016; Potts et al. 2006). These associations can influence other pollinating insects in turn, for example, beeflies may increase where landscape characteristics favour ground-nesting taxa, as beeflies are parasitic on ground-nesters (Boesi et al. 2009). In addition, landscape features such as the time since isolation, distance between remnants, and the degree of connectivity are also likely predictors of insect persistence (Kennedy et al. 2013; Saunders et al. 1991).

The southwest of Western Australia is well-known for its diverse and endemic native vegetation, yet as little as two percent of the original vegetation is left in some areas (Hobbs 2001). The characteristic vegetation of the region, woodland containing *Eucalyptus* and *Banksia* species, is adapted to the dry climate and well-drained, nutrient poor soils (Beard 1989). However, extensive historical clearing has left behind fragments of this species-rich bushland, now situated within an urban matrix that includes developed areas, vegetated road verges and residential gardens. Although gardens may be near remnant vegetation, they represent a highly modified environment, not equivalent to the original vegetation.

Flower-visiting insects documented in the southwest region include bees, wasps, flies and beetles (e.g., Yates et al. 2005), but a consensus on which habitat characteristics are most important for pollinating insect survival is lacking. In wider Australia, Hall et al. (2019) identified key features, such as wooded vegetation, required to support diverse bee communities in fragmented agricultural landscapes of Victoria, and Lentini et al. (2012) suggested the importance of fragments for bees in agricultural landscapes of New South Wales. Western Australia is home to an estimated 800 species of native bee (Houston 2011), with about 80 species found in the Perth metropolitan area (Houston, pers. comm. July 2019). A decline in bumble bee and European honey bee populations has been reported in other parts of the world (Potts et al. 2016). However, it is not clear whether such declines of native or introduced bee species have also occurred in Western Australia.

Understanding how habitat fragmentation affects insect species will inform conservation of local native insect pollinators and insect-pollinated native plants. Similarly, collecting data on other insects can provide insight into the ecological processes taking place in the chosen study sites. I sampled insects in banksia woodland remnants and residential gardens within the urban landscape of the city of Perth. I quantified insect abundance, morphospecies richness, and diversity, and tested the following predictions:

1. Garden insects will be different from remnant insects, and these differences will be predicted by their responses to local site characteristics such as overall site size, percentage native vegetation, canopy cover, and, in the case of gardens, time since garden establishment.
2. Insect responses to urbanisation will be apparent at the landscape scale. Insects will respond positively (e.g., increased abundance, richness and diversity) where there has been more time since urbanisation (i.e., time since a major disturbance), and where remnants and gardens are connected to nearby remnants. Connectivity was measured by distance to nearby remnant vegetation, percentage cover of roads and remnant vegetation within a 2 km radius of each site.
3. Insect responses to urbanisation will change when multiple landscape and site characteristics are considered together. For example, time since urbanisation may have a positive or negative effect on insects in gardens depending on other characteristics of the landscape and garden. A garden with a high percentage of native vegetation, close to remnant vegetation, may attract more insects than a garden with comparatively less native vegetation that is distant from remnant vegetation.
4. Insects from different functional groups will differ in their responses to urbanisation. I was particularly interested in determining responses of insect pollinators given the importance of this group to pollination.

3.3. Methods

Study location

My study was carried out within the metropolitan region of Perth, Western Australia (-31.9505, 115.8605). Perth is a relatively young, rapidly expanding metropolitan area of ~2 million people, centred around a river estuary and extending north and south along the coast (ABS 2017). While the greater Perth region contains 79 % of the state's population (ABS 2017), the density of people remains comparatively low by world standards, with only 315 people per km^2 for the Greater Perth area (ABS 2017) decreasing with increasing distance from the city centre.

Perth is located within the Southwest Australian Floristic Region, a globally recognised hotspot with rich and threatened biodiversity (Hopper and Gioia 2004). Perth also sits within the Swan Coastal Plain IBRA bioregion (Interim Biogeographic Regionalisation for Australia; Thackway and Cresswell 1995). The Perth subregion is approximately 30 km wide and extends along the coast covering 1,142,334 ha. Soils are old and infertile and include the Bassendean Dune System (aeolian sandplain with mostly quartz sand) and the Spearwood Dune System (slightly calcareous aeolian sand) (McArthur and Bettenay 1974). Prior to European arrival, vegetation across the Swan Coastal Plain was largely woodland, with more *Eucalyptus* and *Allocasuarina* spp. dominant on the Spearwood Dune System, and banksia woodland characteristic of the Bassendean (Beard 1989). In the case of banksia woodland, the vegetation type is now designated as a threatened ecological community (Department of the Environment and Energy 2016). Banksia woodlands of the Swan Coastal Plain are now highly fragmented with a reduction from approximately 132 patches (median 146 ha) to over 12,000 patches with an estimated median size of 1.6 ha (Department of the Environment and Energy 2016). The banksia woodland overstorey is a relatively open canopy (up to ~30 % cover) with trees predominantly comprising *Banksia*, *Allocasuarina*, and *Eucalyptus* spp. The understorey consists of woody shrubs, including species from the Proteaceae, Myrtaceae, and Fabaceae families, over perennial herbs (Dodd and Griffin 1989). *Eucalyptus*-dominated woodlands in the surrounding region are similar but contain an overstorey dominated by *Eucalyptus marginata* and *Corymbia callophylla* rather than *Banksia*. The climatic pattern conforms to the summer drought and winter rainfall characteristic of Mediterranean-climate regions, with Perth experiencing annual precipitation of 735 mm, and annual mean temperature of 24.8 °C (1994–2019; Bureau of Meteorology 2019).

Site selection

I selected nine woodland remnants and 32 residential gardens for study (n = 41 study sites; Figure 3.1). To minimise variation in the vegetative features and local climate which may influence insect presence, I deliberately selected sites on the Bassendean and Spearwood sands of the Swan Coastal Plain. I advertised for native gardens through local community groups focused on native plants (Friends of Kings Park; https://www.friendsofkingspark.com.au/). Each garden was planted mostly with local native species with varying amounts of retained lawn. The most commonly observed garden plant genera were *Grevillea*, *Conostylis*, *Callistemon*, *Anigozanthos*, *Banksia*, and *Eremophila*, but composition of the 'understorey' layer in gardens was highly variable. Distance from the city centre provides an index of time since isolation and population density. The total bushland area of remnant sites ranged from 1.92 ha–267 ha, and sites ranged from <1 km–14 km from the city centre. The total area of gardens ranged from ~10 m^{2}–1650 m^{2,} and these were approximately 2–15 km from the city centre. Gardens ranged from 0.3–10 km away from remnant sites, though most were within 4 km of a remnant. Neighbouring gardens abutting the selected site were not measured as part of this study.

I used remnant native vegetation, lakes and waterways, roads, and urban tree cover land-cover data (DPIRD Geographic Information Services 2019; Main Roads WA 2019;

Principal Coordinator Wetlands 2019; Spatial Data 2018) to determine the land-cover surrounding each study site. I categorised these data into roads and buildings, any vegetative cover, and remnant native vegetation cover. The percentage of each cover type within 200 m, 500 m, 1.5 km and 2 km radii of each sampling site was calculated using ArcMap (ESRI 2011). Using multiple scales could detect insect sensitivity to different distances. I chose a 2 km maximum because although larger social bees may travel further to forage (e.g., honey bees travelled 5.5 km on average from a hive in Sheffield, UK; Beekman and Ratnieks 2000), others, such as solitary bees, are not expected to have foraging distances beyond 1.5 km from their nests (Zurbuchen et al. 2010). Even bees which can cover large distances have been shown to prefer to forage within 500 m from their nests (Osborne et al. 2008). Zurbuchen et al. (2010) therefore recommended nesting and foraging resources should be in close proximity (within a few hundred metres) to maintain populations of certain solitary bees.

Native vegetation was largely cleared during the construction of the Perth suburbs where I sampled. To determine the approximate year each remnant I surveyed was left within developed area, I examined historical aerial imagery and council records. The native gardens were generally established on bare sand, which enabled me to use aerial photography to determine the year that each garden was planted. I asked residents to provide details too. As 2007 was the earliest year of high-resolution photography accessible, gardens which existed in 2007 were given an establishment year of <2007. Thus, gardens were established from 4 to more than 11 years ago.

Field data collection

At each remnant plot (26 total) and garden (32 total), I gathered data on environmental attributes which may influence insect presence. I collected vegetation data from September–December in 2016 and 2017. In remnants, I established square 5 m × 5 m plots to measure remnant woody vegetation and a 1 m × 1 m subplot to record small, annual plants. I identified all understorey plant species inside the plot and recorded all overstorey plants within ten metres of the plot edge. In most remnants, multiple plots were established to detect variation across the overall site. In gardens, I sampled sections which conformed with the 25 m^2 plots established in remnants as closely as possible. I used the list of identified plant species to calculate the percentage of native plant species present. I estimated percentage of canopy cover and percentage of vegetation cover in percentile categories.

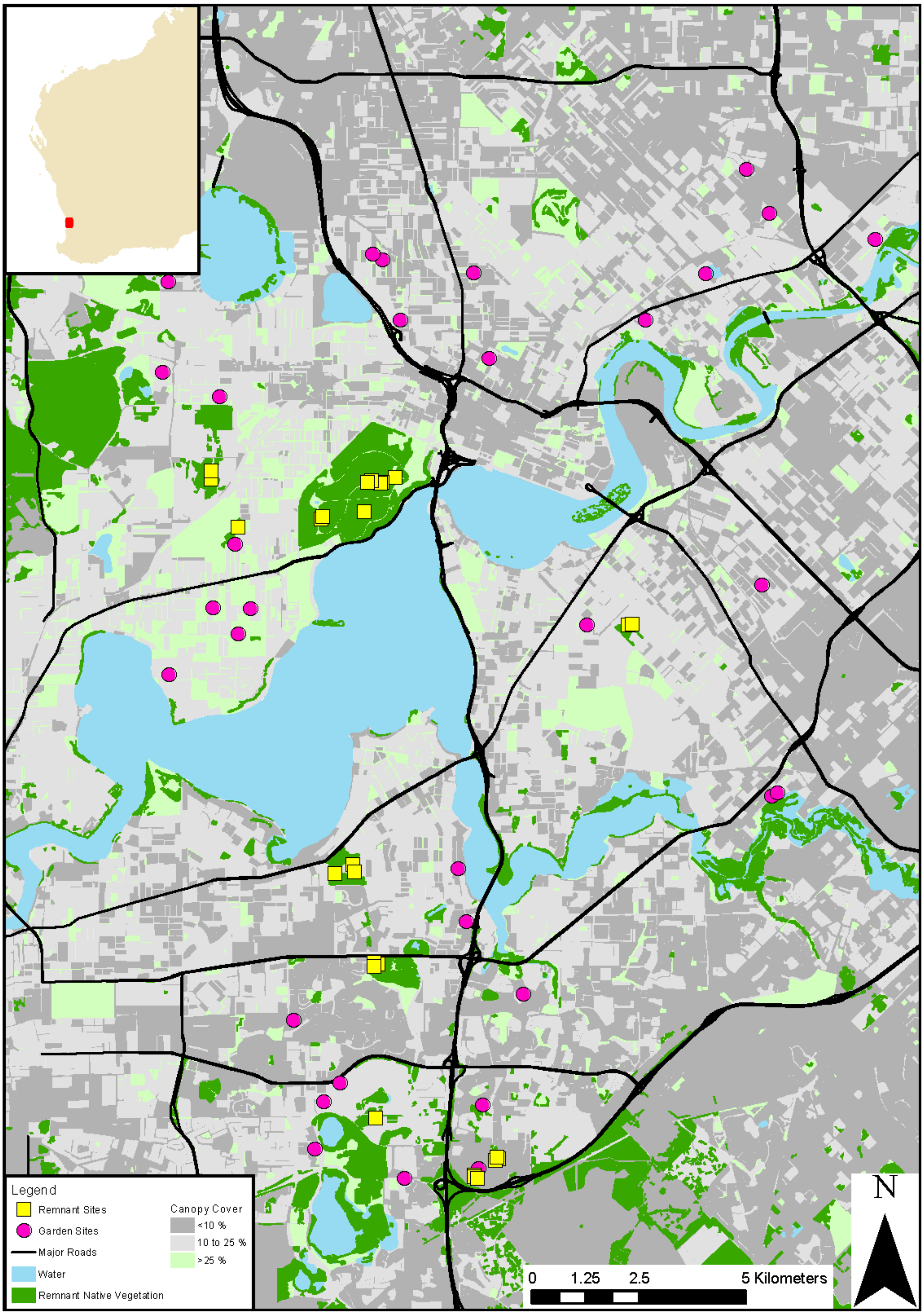


Figure 3.1. Study sites in remnants and gardens across metropolitan Perth. N= 9 (26 plots) and 32 respectively.

Insect sampling

Yellow and blue pan traps were used to capture insects between September and December in 2016 and 2017. Pan traps are a simple, cost-effective method of collecting a variety of insects (Vrdoljak and Samways 2012). This method was chosen because it allowed all sites to be surveyed within the same selected season, effort was identical for each site, and it eliminated sampling bias associated with multiple observers (Roulston et al. 2007). Blue and yellow traps were chosen as these are common flower colours in the region. Blue traps tend to attract certain Hymenoptera (Aguiar and Sharkov 1997), while yellow traps catch many Diptera (Disney 1982). Traps were open at each plot for a total of six days over the two seasons (three days each year), totalling 42 h per plot. I trapped at 41 sites (58 plots altogether), totalling 2,436 trap hours. I also recorded a log scale estimate of the number of open flowers present per trapping day (0, 10, 100 etc.) and recorded dominant flower species and colours. For each plot or garden, two pairs of traps were set, one blue and one yellow, both 18 cm in diameter. The traps were fixed to the ground using Velcro dots on weed mat pegs. Paired traps were spaced 20 cm apart, and the next pair was at least 2 m away. Each bowl was filled with 150 ml of a water and surfactant mixture (~2 ml of unscented hand wash in 2 L of water). The traps were set and collected in the same order to ensure all traps were active for the same length of time. Insect samples were identified to species where possible, but otherwise to morphospecies (i.e., groups with morphological differences). I also categorised the insects into ecological groups: all insects, pollinating insects, Hymenoptera, pollinating Hymenoptera, Coleoptera, Diptera, pollinating Diptera, wasps, native bees, and ants.

Weather

Pan traps were set on warm, sunny days from approx. 08:00 until 15:00 hrs. On trapping days, precipitation, air temperature, wind speed and air pressure were obtained from the Perth Bureau of Meteorology weather radar for the time the traps were open. None of the trapping days included meaningful precipitation (>0.1 mm) between 08:00 and 15:00 hrs. The minimum air temperature during sampling was 7.9 °C in 2016 and 8.0 °C in 2017. The maximum air temperature was 34.4 °C in 2016 and 35.1 °C in 2017, while the mean air temperature was 21.2 °C in 2016 and 21.7 °C in 2017. The mean wind speed (13.3 km/h in 2016 and 12.7 km/h in 2017) and mean air pressure (1016.3 hPa in 2016 and 1016.4 hPa in 2017) were also similar. I explored the effects of climate variables on insect response variables but did not find any effects, probably due to the consistency of temperature, rainfall, wind speed and air pressure between years. Therefore, these data were not included in the statistical models.

Insect observations

To gain an indication of insect behaviour and further investigate the composition of insect assemblages, pan trapping was supplemented by observations of insects visiting flowers of selected plant species. Observations were recorded on warm, sunny days between 09:30 and 15:00 hrs in 2017. Insects were recorded while watching focal plants (*Dianella revoluta*, *Hemiandra pungens*, *Jacksonia sericea*, *Patersonia occidentalis*, and *Tricoryne elatior*) in 10-minute intervals, usually for at least a total of 30 minutes per session. Time, date, weather conditions, and insect visits were recorded. Insect visitors were recorded as pollinators when they interacted with the stigma and male floral parts, or in the case of buzz-pollinated *D. revoluta*, when sonication was audible. When insects landed on the flower but did not contact the anthers or stigma, they were recorded as visitors.

Pollinator likelihood

For the purpose of identifying likely insect pollinators, a pollinator likelihood classification system was established. The insects sampled were ranked on a scale of 1 (unlikely pollinator) to 5 (likely pollinator). This was loosely based on a pollination efficiency estimation system for Apiaceae L. (Lindsey 1984). Field observations and literature were used to determine insect traits and behaviours such as their size, whether they landed on flowers, interacted with reproductive parts of flowers, moved between flowers, and whether they collected and/or transferred pollen. Visiting insects ranked 1–2 had rare–occasional contact with flowers (respectively), did not contact the flower's reproductive parts and had no adaptations for carrying pollen. Visitors ranked 3 had more consistent flower visits but were still unlikely to transfer pollen frequently. Pollinators (4–5) were usually bees, with specific requirements and adaptations for collecting pollen, as well as frequently visiting flowers and interacting with anthers and stigmas. This index allowed me to quantitatively analyse the floral interactions observed. Despite having the capacity to transfer pollen, Coleoptera were not included in the pollinator group as they had regularly consumed the reproductive floral parts during my observations.

Data analysis

My overall objective was to quantify how landscape and site-scale attributes influenced insects. To achieve this, I first conducted community analysis (NMDS) and then performed regression on community-level attributes such as total abundance, richness, and diversity (Figure S1). Finally, I modelled insect abundance as a response to the characteristics of the study sites and the wider landscape.

Statistical analyses were conducted in R, version 3.4.1 (R Core Team 2017). To visualise differences in the species composition of remnants and gardens, I used insect abundance counts to calculate dissimilarity values using the Bray-Curtis dissimilarity index in the vegan package (Oksanen et al. 2013). I then performed ordination of sites using non-metric multidimensional scaling (NMDS) and the metaMDS function in vegan. I performed Permanova on Bray-Curtis dissimilarity values between remnant and garden sites using adonis in vegan (Anderson and Walsh 2013).

To investigate the influence of site characteristics on insects, the abundance, richness, and Shannon diversity of each insect group was calculated. I implemented generalised linear mixed models (GLMM, package glmmTMB, Brooks et al. 2017) for the response variable (insect abundance) against predictor variables. Note that a model was created for each insect group (i.e., all insects, pollinating insects, etc.) and remnant and garden sites were modelled separately given their different histories. Rarefied species richness (i.e., adjusting for differences in the number of individuals and the number of samples collected) was not used as all calculations returned zeros. Insect richness and Shannon diversity both correlated with insect abundance and were therefore not modelled. Predictor variables consisted of continuous fixed effects (distance to remnant, site size, plant diversity, nativeness, impervious surfaces, remnant vegetation), categorical fixed effects (bare ground, flower number, time since urbanisation, year) and random effects (site, trapping day; Table 3.1). To allow for comparison of parameter estimates, continuous predictor variables were scaled by subtracting the mean and dividing by the standard deviation. Collinearity was assessed among all predictors with a threshold of $p = 0.05$. Two pairs of predictors (site size–distance to remnant; plant diversity–nativeness) showed collinearity, so only distance to remnant and plant diversity were retained in models with multiple predictors. To verify models met underlying assumptions, residuals were visually assessed. Where counts were adequate (groups: all insects and Diptera in both site types, and Coleoptera in remnants), abundance models were fitted using the negative binomial (Poisson) family. Where counts were inadequate for models to run (i.e., fewer than five records per morphospecies per site, or sampled inconsistently), I fitted binomial models to presence/absence data. The model responses and predictors are given in Table 3.1.

Table 3.1. The response and explanatory variables for investigating the impact of site and landscape characteristics on insects. Units are indicated in brackets. The response variable is insect abundance, calculated for the following ecological groups: all insects, all Diptera, all remnant Coleoptera (Poisson), pollinating Hymenoptera, wasps, garden Coleoptera (binomial).

Variable	Description
Responses	
Insect abundance	Number of insects sampled per trap day, per site
Insect richness*	Number of distinct, identifiable insect taxa sampled per trap day, per site
Insect diversity*	Shannon diversity (H), where $H = -\sum_{i=l}^{s} p_i ln\, p_i$ Pi = proportion of population made up of species i S=number of species in sample
Predictors	
Time since urbanisation	Approximate number of years since the area within 5 km of the site became urbanised (as indicated by road construction)
Remnant vegetation (%)	Percentage of remnant vegetation within a 500 m radius
Hard surface (%)	Percentage of hard surface within 500 m
Site size (ha)*	Calculated using aerial imagery
Distance to nearest remnant (km)	Calculated using aerial imagery
Garden establishment year	Approximate year garden was established
Plant abundance*	Number of plants per garden or remnant plot
Plant richness*	Number of different plant taxa per garden or remnant plot
Plant diversity	Shannon diversity (see above) per garden or remnant plot
Canopy cover (%)	Percentage of garden or quadrat covered by tree canopy estimated in 0–25 %, 25–50 %, 50–75 % and 75–100 % categories (measured from midpoint of quadrat or garden)
Bare ground (%)	Percentage of garden or quadrat that is soil, mulch, or gravel without plants
WA native plants (%)*	Percentage of plant species native to Western Australia
Flowering	Estimate of flowers within 10 m (ordinal scores: 0, 10, 100, 1000)

*Not modelled in multiple predictor models due to correlation with another variable

3.4. Results

More insects were sampled in gardens (3,807) than remnants (1,987), however this was largely driven by large numbers of Diptera in gardens (Figure 3.2). There were slightly more Coleoptera sampled in remnants (934) than gardens (586), but Hymenoptera were found in similar numbers (381 in remnants and 405 in gardens, Figure 3.2). Overall, orders Hemiptera, Lepidoptera and Odonata had fewer than 20 individuals sampled, so these groups were excluded from further analyses. Although these results reflect the taxa present in traps, not necessarily all taxa within the study site, they provide a preliminary insight into the insect assemblages at the chosen sites. Yellow and blue pan traps generally sampled different groups, with more Coleoptera in blue traps, more Diptera in yellow traps, and mixed results for Hymenoptera (Figure 3.2). Hymenoptera were the most diverse family sampled as most specimens were identified to at least genus level, however it is noted that Diptera diversity may be greater than shown due to a number of taxa grouped as 'other' (i.e., different to the Diptera families presented below).

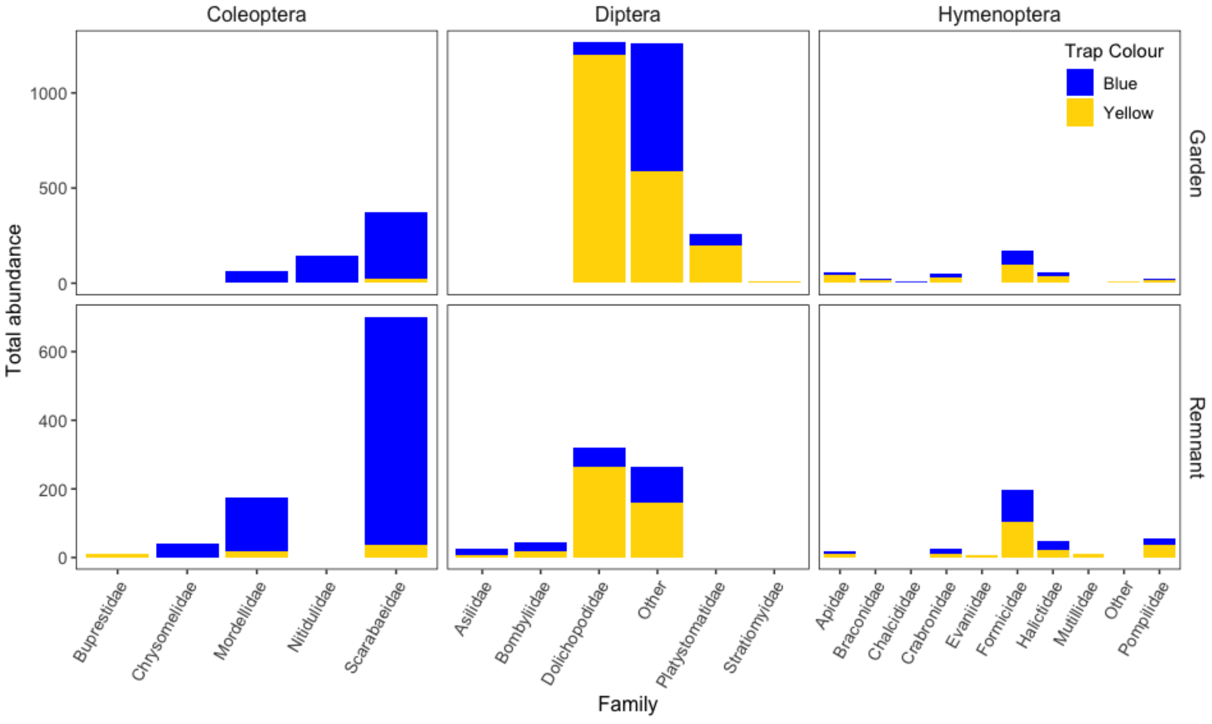


Figure 3.2. Total abundance of insects in remnants and gardens, displayed by family, site type, and trap colour

Prediction 1: Garden and remnant insects will be different, depending on site characteristics

Overall, the total abundance of insects was greater in gardens (n=32) than in remnants (n=26 plots), with Diptera more abundant in gardens than in remnants (Table 3.2). There were also slightly more Coleoptera in remnants (mean=35.92 ±6.57) than in gardens (mean=18.31 ±6.02). In remnants, I collected a total of 1,987 insects from orders Coleoptera (934), Diptera (660), Hymenoptera (381, 196 ants), Lepidoptera (6), Odonata (5), and Hemiptera (1; Table 3.2). In gardens, I collected a total of 3,807 insects from the orders Coleoptera (586), Diptera (2,797), Hymenoptera (405, 172 ants), Lepidoptera (12), Hemiptera (4), and Odonata (3). In remnants, there were six different Coleoptera families, 11 Hymenoptera families and at least six Diptera families sampled. In gardens, there were six Coleoptera families, 14 Hymenoptera families and at least eight Diptera families. Of the 348 sampling occasions, there were only six instances where traps did not contain insects: four in gardens (1.1 %) and two in remnants (0.6 %).

Table 3.2. Summary of most abundant insects sampled in gardens and remnants by total number and percentage of sites each group was observed in

Order	Insect morphospecies ID	Garden	Remnant	Frequency Gardens (%)	Frequency Remnant (%)	Pollinator likelihood ranking
Coleoptera	Mordellidae	64	175	66	77	1
	Nitidulidae	146	5	63	12	1
	Scarabaeidae	371	702	22	96	1–2
	Other Coleoptera	5	52	9	19	1–3
	Total	586	934			
Diptera	Asilidae	2	27	6	46	1
	Bombyliidae	1	44	3	46	4
	Dolichopodidae	1266	320	100	100	1
	Platystomatidae	258	0	63	0	1
	Syrphidae	2	3	6	12	4
	Other Diptera	1268	266	100	96	1–3
	Total	2,797	660			
Hymenoptera	Ampulicidae Dolichurus	0	1	0	4	1
	Apidae *Amegilla chlorocyanea*	2	8	6	27	5
	Apidae *Apis mellifera*	13	10	28	31	5
	Apidae *Exoneura*	43	0	6	0	5
	Other native bee	8	6	13	12	4
	Braconidae	21	3	16	4	1
	Chalcididae	7	0	19	0	1
	Crabronidae	49	24	41	23	3
	Evaniidae	6	11	13	31	1
	Formicidae	172	196	81	88	1
	Halictidae	54	49	46	65	5
	Ichneumonidae	4	0	9	0	1

Order	Insect morphospecies ID	Garden	Remnant	Frequency Gardens (%)	Frequency Remnant (%)	Pollinator likelihood ranking
	Mutillidae	1	13	3	19	3
	Pompilidae	22	57	16	54	3
	Sphecidae	0	2	0	8	3
	Tiphiidae *Diamma bicolor*	1	1	3	4	3
	Vespidae *Polistes*	2	0	6	0	3
	Total	405	381			
Hemiptera	Hemiptera	4	1	3	4	-
	Total	4	1			
Lepidoptera	Hesperiidae	3	2	9	12	-
	Lycaenidae	0	2	0	4	-
	Pieridae *Pieris rapae*	9	2	25	8	-
	Total	12	6			
Odonata	Odonata	3	5	9	15	-
	Total	3	5			
Overall Total		**3,807**	**1,987**			

Non-metric multidimensional scaling (NMDS, plot stress=0.26) produced weak groupings (Figure 3.3). Permanova showed that the community composition of insects in remnants and gardens was significantly different (F.model=11.53, R^2=0.03, P<0.001).

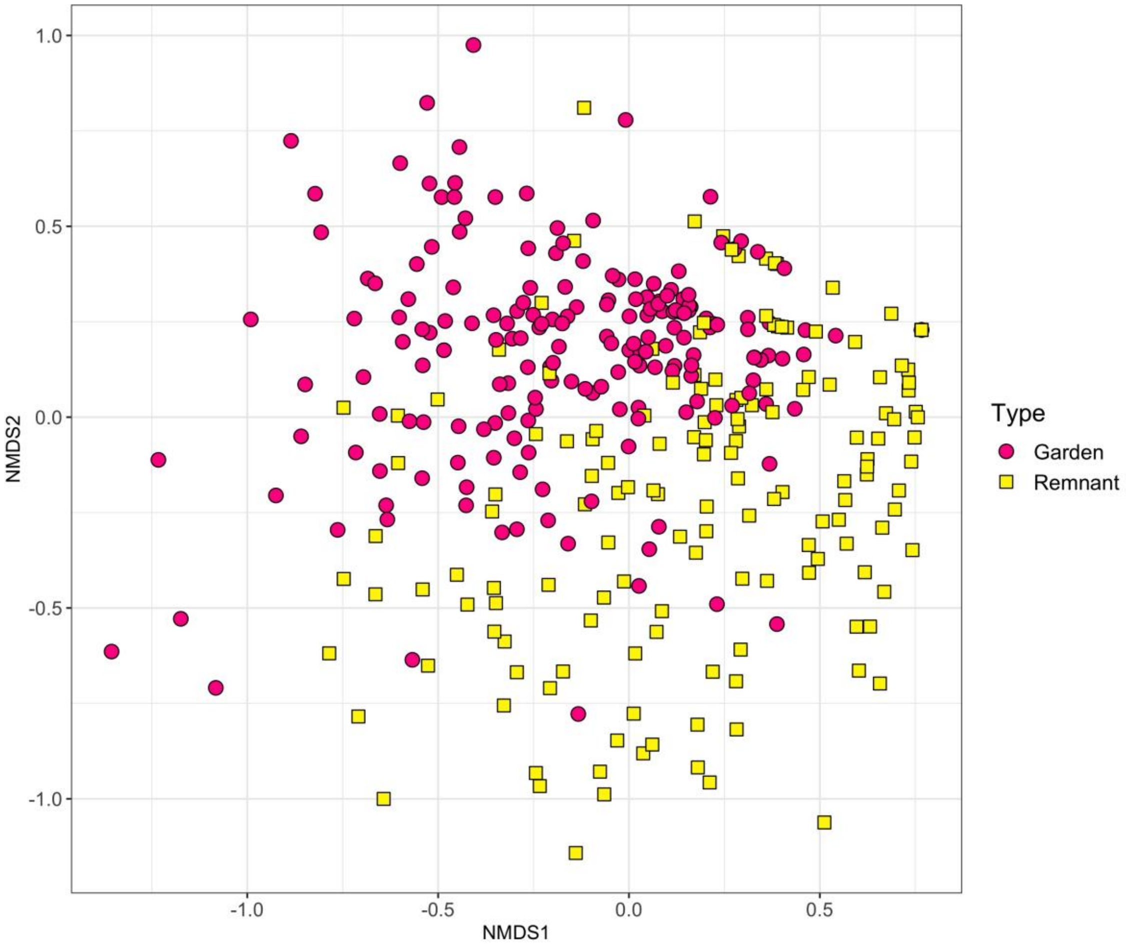


Figure 3.3. Non-metric multidimensional scaling plot using Bray-Curtis dissimilarity values to show dissimilarity between the insect communities found in remnants and gardens. Environmental predictors (distance to remnant, remnant vegetation within 500 m, and plant diversity) had little effect. Plot stress: 0.26.

The characteristics of garden sites and remnant sites were similar in some ways and different in others (Figure 3.4). Gardens had greater plant diversity and impervious surroundings, as well as some sites with canopy cover above 50 % (Table 3.3). Remnants (mean=95.01 ±23.09 ha) were much larger than gardens (mean=0.04 ±0.01 ha), but the proportion of native plants was similar for both site types (Table 3.3). Gardens and remnants were located similar distances from the nearest remnant, and bare ground, flowering, and time since urbanisation seemed equally variable for both site types (Table 3.3). There was no relationship between garden age and size.

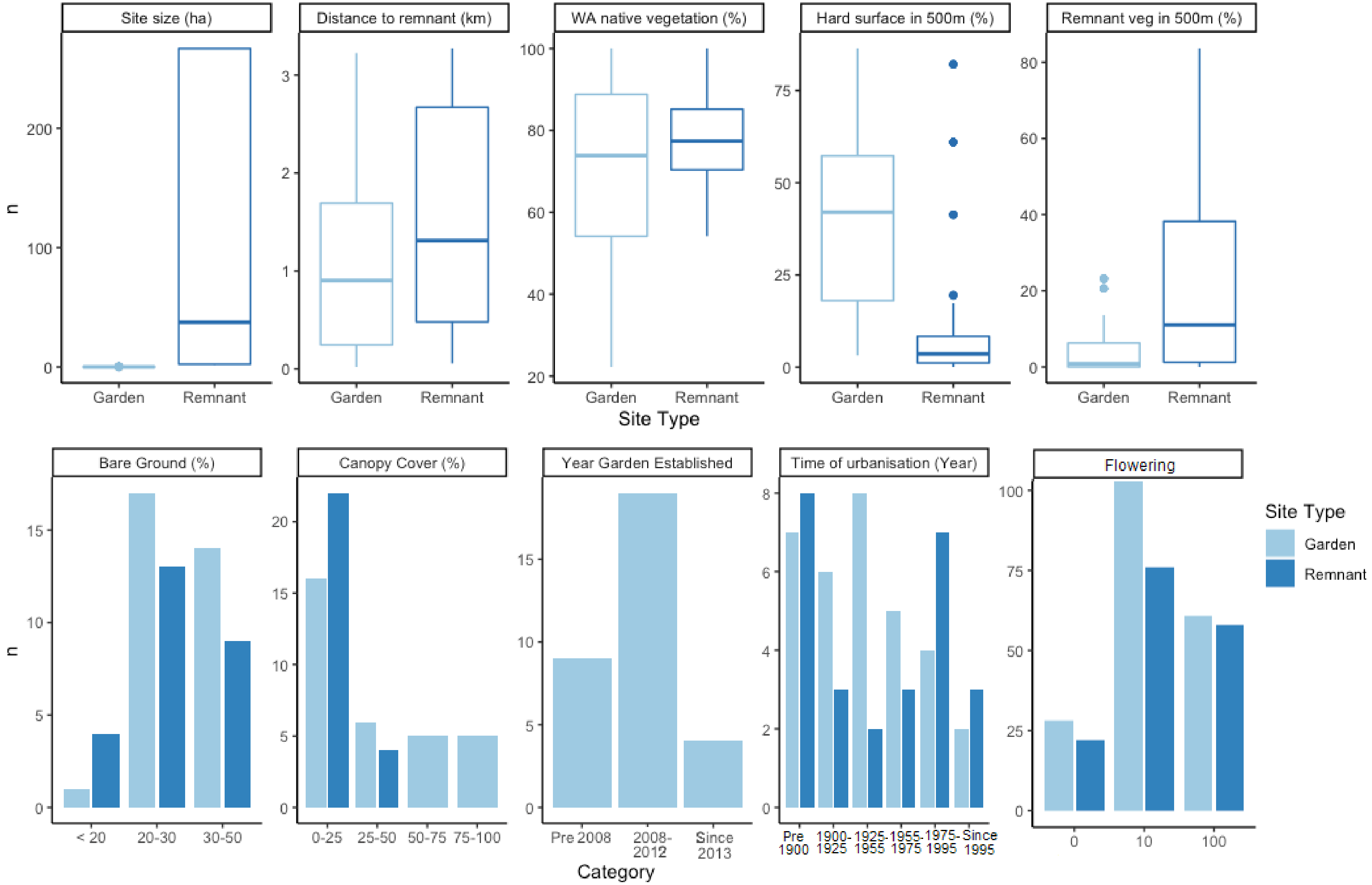


Figure 3.4. Similarities and differences between native gardens and remnant vegetation sites. Native gardens were smaller than remnants and had canopy cover greater than 50 % in some cases. Garden sites were surrounded by more buildings and roads, while remnant vegetation plots were generally surrounded by remnant vegetation.

Table 3.3. Range, mean and standard error for continuous variables; site size (ha), distance to nearest remnant (m), proportion native to WA, plant abundance, plant richness, plant diversity (within ~25m^2), impervious surfaces within 500 m (%) and remnant vegetation within 500 m (%).

	Garden		Remnant	
Variable	Range	Mean (SE)	Range	Mean (SE)
Site similarities				
Distance to remnant	20.25–3223	1129.09 (170.66)	55.4–3270	1488.24 (202.06)
Native to WA	0.22–1	0.71 (0.04)	0.54–1	0.77 (0.02)
Plant abundance	11–145	59.81 (6.10)	27–127	68.88 (5.64)
Plant richness	7–60	27.31 (2.33)	14–28	22.96 (0.81)
Site differences				
Size (ha)	0.003–0.26	0.04 (0.01)	**1.92–266.67**	**95.01 (23.09)**
Plant diversity	**1.39–4.01**	**2.59 (0.13)**	1.67–2.73	2.30 (0.04)
Impervious surfaces (%)	**3.19–86.38**	**38.78 (4.24)**	0.05–82.07	11.30 (3.92)
Remnant vegetation (%)	0–23.2	3.97 (1.09)	**0–83.63**	**21.38 (4.85)**

Bold groups are significantly greater ($p < 0.05$)

In general, few trends were observed from the models of individual gardens and remnant site characteristics. The only consistent effect for gardens and remnants was a slight decrease in the abundance of Diptera with an increased proportion of native vegetation (Table 3.4). In gardens, wasp presence declined with the increase in the proportion of native vegetation, and the presence of pollinating Hymenoptera increased with increased plant abundance (Table 3.4). In remnants, insect abundance declined at sites with more canopy cover. The remaining local site variables I measured (i.e., plant diversity, bare ground, and site size) did not predict the abundance or presence any of the insect groups, regardless of site type. However, site size may have had an indirect effect on insect abundance, as site size was related to distance to remnant vegetation (Table 3.1, Table 3.4). The effects of time since urbanisation, distance to remnant, and surrounding surface cover are addressed in the following section.

Table 3.4. Main effects of site characteristics on insect abundance for gardens and remnants

	Gardens	Remnants
Site similarities		
Time since urbanisation	Fewer Diptera in most recently urbanised sites (z = −2.41, p<0.05)	
Distance to remnant vegetation	Fewer pollinating Hymenoptera with increasing distance to remnant (z=−2.46, p<0.05)	More Diptera with increasing distance to remnant (z=2.07, p<0.05)
Native vegetation (%)	Decrease in Diptera and wasps with increased native vegetation (z=−2.02, p<0.05, z=−2.00, p<0.05)	Decrease in Diptera with increased native vegetation (z=−2.07, p<0.05)
Plant abundance	Increase in pollinating Hymenoptera with increased plant abundance (z=2.25, p<0.05)	
Site differences		
Impervious surfaces (%)	Diptera and wasps decreased with increased proportion of impervious surfaces (z=−2.08, p<0.05, z=−2.02, p<0.05)	
Remnant vegetation (%)		Slight increase Diptera with increasing proportion of remnant vegetation (z=2.19, p<0.05)
Canopy cover (%)		Fewer insects at sites with more canopy cover (z=2.29, p<0.05)

Prediction 2: Insect responses to urbanisation at the landscape scale

Insect responses to landscape characteristics were examined using the previous models (Table S1). Diptera were the most abundant group overall, and their abundance in gardens was greatest in sites which became urbanised before 1900 in comparison to those urbanised after 1995 (z = −2.41, p<0.05. Overall insect abundance therefore followed a similar pattern (Figure S1). Diptera abundance was greater in the oldest gardens (established prior to 2007) than in more recently established gardens (2008–2012: z = −3.44, p<0.01, since 2013: z = −2.05, p<0.05). Diptera in remnant sites were more abundant with increasing distance to the nearest remnant, while in gardens, pollinating Hymenoptera declined with increased distance to the nearest remnant (Table 3.4). At garden sites, an increase in the proportion of impervious surface was related to a decrease in Diptera abundance and a decrease in the presence of wasps (Table 3.4). At remnant sites, Diptera abundance increased with increased proportion of remnant vegetation.

Prediction 3: Effects of multiple site and landscape characteristics on insects

At garden sites, the overall insect abundance was greatest in the oldest gardens (established before 2007) than those established from 2008–2012 or after 2012 (Table S2). This model also indicated that overall insect abundance was lower in gardens urbanised from 1900–1925, 1975–1995, and since 1995, than those urbanised before 1900 ($z = -2.66$, $p < 0.01$, $z = -2.85$, $p < 0.01$, and $z = -3.46$, $p < 0.001$ respectively) (Figure 3.5). The abundance of Diptera in gardens matched the pattern for total insect abundance, with fewer Diptera observed in the gardens established from 2008–2012 than those established prior to 2007 ($z = -2.47$, $p < 0.01$). For Coleoptera in gardens, abundance increased at sites that had more plants flowering ($z = 1.99$, $p < 0.05$).

In remnants, the total insect abundance was greater at sites with 0–25 % canopy cover than sites with 25–50 % canopy cover ($z = -2.29$, $p < 0.05$). Diptera abundance increased with increasing vegetation within 500 m ($z = 1.57$, $p < 0.01$). The remaining predictor variables did not have significant effects (Table 3.5).

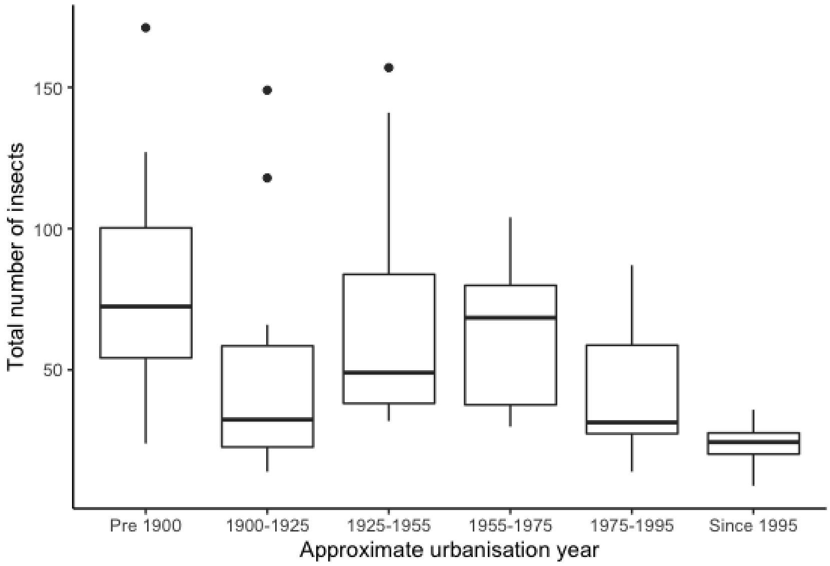


Figure 3.5. Boxplot of urbanisation year and overall insect abundance of garden sites. Boxplots display the median (thick bar), interquartile range, minimum and maximum values and outliers.

Table 3.5. Predictor variables which performed best in generalised linear mixed effects models

	Group	Predictor variables of best model (* if variable significant)	Effect of increasing predictor on insects
Remnants	All insects	Any vegetation within 500 m	
Poisson		Canopy cover (%)*	↓
	Coleoptera	Any vegetation within 500 m	
		Canopy cover (%)	
	Diptera	Any vegetation within 500 m*	↑
		Distance to remnant	
Binomial	Likely pollinators	Distance to remnant	
	Hymenoptera	Plant diversity	
	Pollinating Hymenoptera	Distance to remnant	
	Wasp	Flower number	
	Native Bee	Distance to remnant	
	Ant	Remnant vegetation within 500 m	
	Pollinating Diptera	Bare ground (%)	
		Canopy cover (%)	
		Plant diversity	
Gardens			
Poisson			
	All insects	Impervious surface within 500 m	
		Time since urbanisation*	↓
		Time since garden established*	↓
	Diptera	Impervious surface within 500 m	
		Time since garden established*	↓
		Distance to remnant	
Binomial	Coleoptera	Vegetation within 200 m	
		Flower number*	↑
	Likely pollinators	Distance to remnant*	↓
	Hymenoptera	Distance to remnant	
	Pollinating Hymenoptera	Distance to remnant*	↓
	Wasp	Any vegetation within 500 m	
		Distance to remnant	
		Plant diversity	
	Native Bee	Canopy cover (%)	
		Distance to remnant *	↓
	Ant	Flower number	
		Bare ground (%)	
		Distance to remnant	
	Pollinating Diptera	Impervious surface within 500 m	

Prediction 4: Insect-pollinator responses

From the total insects sampled, 240 individuals (~4 %) were classed as likely flower pollinators, 191 Hymenoptera (79.6 %), and 49 Diptera (20.4 %). In gardens, there were 121 likely pollinators, 118 Hymenoptera (97.5 %) and three Diptera (2.5 %). In remnants, there were 119 likely pollinators, 73 Hymenoptera (61.3 %) and 46 Diptera (38.7 %) (Figure 3.6).

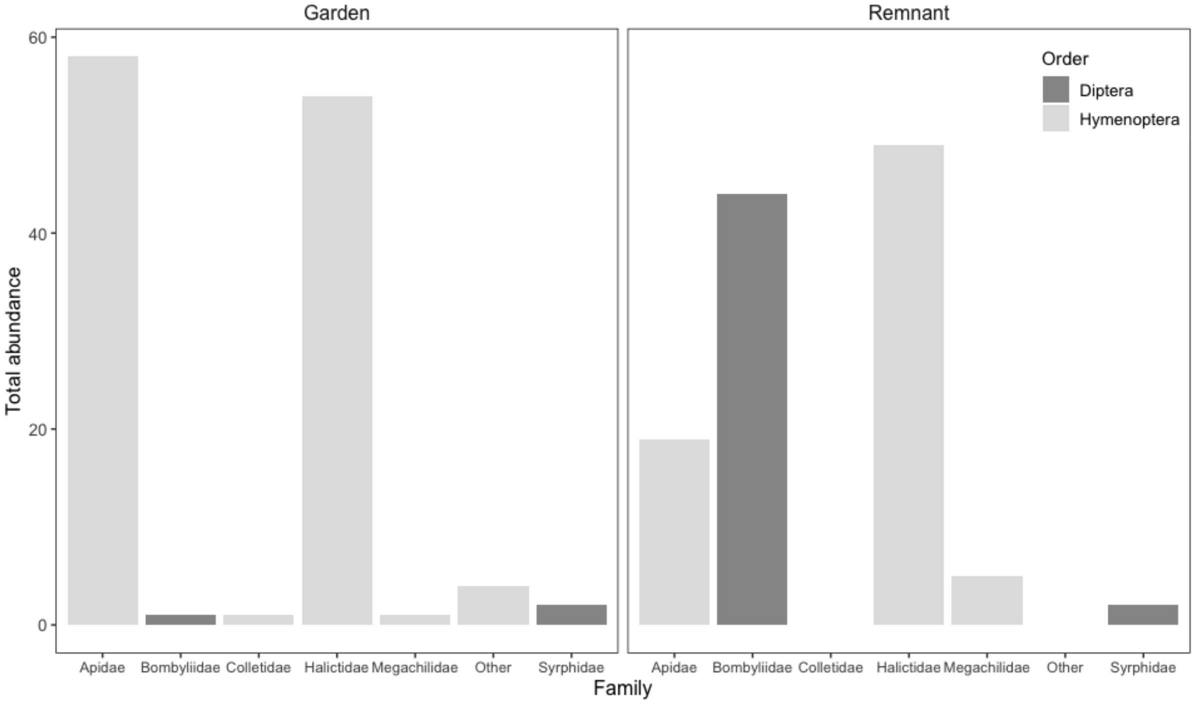


Figure 3.6. Total abundance of pollinating insects sampled (Orders Diptera and Hymenoptera).

The abundance of pollinating insects varied among sites (Figure 3.7). Pollinating Diptera were more frequently observed in remnants. On the other hand, the number of different pollinating Hymenoptera taxa observed in gardens (10 morphospecies) and remnants (8 morphospecies) was similar. Overall, the abundance of pollinating insects was much lower than the other insect groups. The only binomial model with a significant result was for the pollinating Hymenoptera at garden sites. Pollinating Hymenoptera in gardens declined with increasing distance to the nearest remnant vegetation ($z = -2.64$, $p < 0.01$).

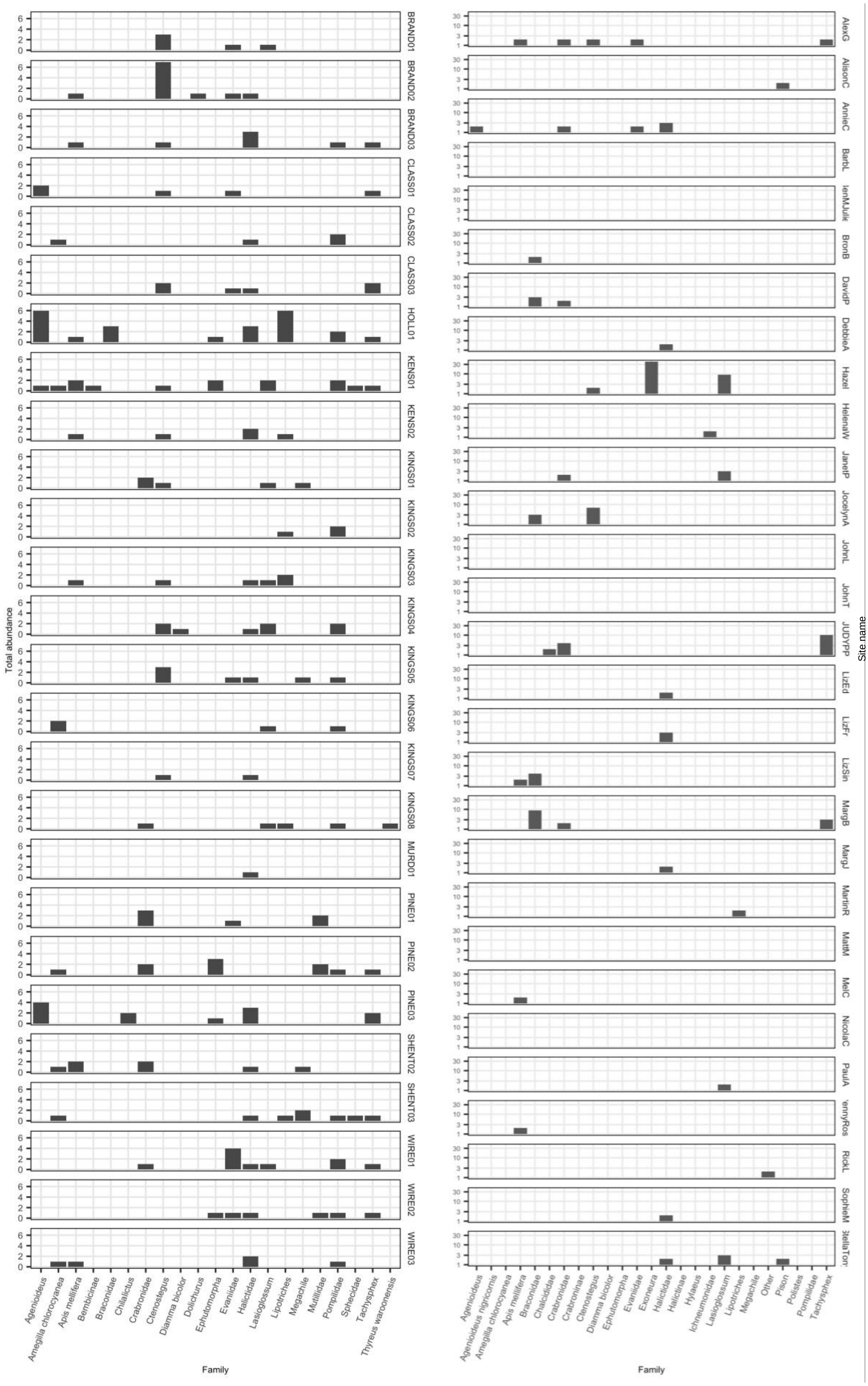


Figure 3.7. Total abundance of pollinating insects per remnant (left) and garden site (right).

3.5. Discussion

My data show that the influence of site and landscape characteristics on insect abundance was mixed. Some morphospecies were observed at a similar proportion of garden and remnant sites (e.g., long-legged flies, other flies, ants, and honey bee—*Apis mellifera*). However, some morphospecies were only observed at one site type (e.g., Platystomatidae flies only sampled in gardens), or were observed more often at one site type than the other (e.g., Bombyliidae more often at remnants than gardens). Site and landscape characteristics were generally only weakly predictive of insect abundance, richness and diversity, if at all, and their effects varied depending on whether they were considered individually (prediction one) or collectively (prediction three). It is recognised that insect abundance recorded by passive sampling, though providing a robust comparison among sites, may be complemented by other methods (e.g., observational records and targeted sweep netting; Prendergast et al. 2020). I acknowledge that some taxa may be underrepresented here. For example, there were fewer pollinating insects than other insect groups overall. Nonetheless, passive sampling reduces temporal variation compared with observational records and targeted sweep netting, and overall provides evidence for both similarities, between garden and remnant sites and the insects sampled within them, and also differences, within the insect assemblages. These variations are likely due to the diversity of insect responses, with these reflecting differences in resource use and perhaps some plasticity in resource use to enable their persistence in urban environments (Fenster et al. 2009). This finding highlights the importance of site characteristics which are examined next.

Prediction 1: Garden and remnant insects will be different, depending on site characteristics

The number of different morphospecies was similar across site types, perhaps due to the similarities between gardens and remnants (i.e., the similar proportion of native vegetation, connectedness to the nearest remnant vegetation, time since urbanisation, bare ground, and flowering, Figure 3.8). The insects I sampled are likely those which can persist in modified environments (e.g., Matheson et al. 1996), however, the composition of the insect assemblages at each site type differed slightly (Table 3.2, Figure 3.3). Insects that were sampled at both gardens and remnants may have been quite generalist in nature, meaning they have a broad range of food sources, wide distributions, and rapid development (New 2015). Generalist insects are therefore less selective with their resource use and would be able to utilise a range of habitats.

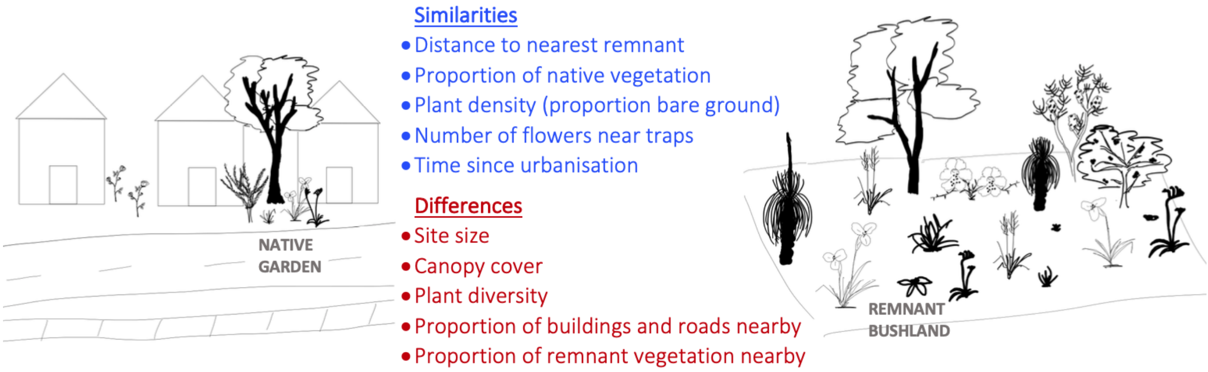


Figure 3.8. Summary of similarities and differences between native gardens and remnant vegetation sites.

It is expected that the local features of remnants, such as their large size and availability of resources could moderate negative effects of urbanisation to some degree. Old, large native gardens may have similarities to remnant sites in terms of their proportion of native plants and plant density, yet their differences likely give rise to differences in their insect assemblages, unless the resources available are similar. The native gardens I selected to study tended to be large and floristically diverse, in comparison to other gardens in the landscape such as predominantly-lawn gardens or rose gardens with low plant species diversity. The native gardens sampled therefore likely provided food and shelter resources to a number of insects, which may account for the lack of effect of some site variables.

I found more Diptera individuals in gardens than in remnants, and this is likely due to the traits of the sampled Diptera. Most were long-legged flies (Diptera: Dolichopodidae), which feed on aphids and insect eggs and are often found on leaves and moist soil (Gardiner

2014), which were plentiful in gardens. There is also a tendency of Dolichopodidae to be abundant in pan traps (CSIRO 2020). It is probable that both factors have contributed to high numbers of Diptera in my study.

My models showed that the abundance of Diptera decreased with increasing proportion of native vegetation. The Diptera sampled were diverse and are generalist in nature, and thus may have benefitted from the increased cover of exotic species associated with decreasing plant nativeness. Additionally, another study in the Perth region showed that plant species origin was a strong predictor of nectarivore use (Kennedy et al. 2018). Thus, it could be possible that if native plants attract other bird or insect species, the abundance of flies decreases. However, Diptera were recorded more frequently than any other insect group, which may suggest they are better suited to urban environments than other insect groups, and are particularly attracted to the pan traps I used. Diptera are common in other urban environments, e.g., the City of Melbourne, Australia, particularly in lawn habitats (Mata et al. 2015), which are also common in the landscape sampled here and together with flowering gardens, may have provided suitable habitats for Diptera.

Different species are likely to be influenced and respond in different ways (e.g., Fischer et al. 2016), and therefore, considering insect assemblages at a broad taxonomic level may have concealed species-specific responses in my study. High-resolution studies of specific groups, such as pollinating flies or native bees, are needed to explore this possibility.

Prediction 2: Insect responses to urbanisation at the landscape scale

The effects of individual landscape characteristics (i.e., time since urbanisation, distance to nearest remnant, and proportion of surface cover in the surrounding area) were mixed depending on the insect groups considered. The prediction that insect abundance would increase with more time since urbanisation was only supported by Diptera at garden sites. However, Diptera also showed a positive response at remnant sites that were more urbanised, and at remnant sites with a greater proportion of remnant vegetation nearby. Diptera abundance at various sites may be due to their ability to exploit multiple resources for nutrition (e.g., Burkle et al. 2013). Alternatively, because Diptera were numerous, they were analysed using count data (rather than presence/absence) and may therefore be the most likely group to have significant responses. It is not surprising to see inconsistent effects of urbanisation on a large group of insects, as responses are likely related to particular traits of the individual species (Wenzel et al. 2020). Even studies specifically studying insect traits have found that traits are not necessarily strong response predictors and that responses are not consistent (Bartomeus et al. 2018).

In gardens, Diptera abundance decreased with an increase in the proportion of hard surfaces in the surrounding area, and the wasp group showed a similar response. For wasps, this could be due to a lack of nest sites. Although not modelled, the diversity of wasps tended to be greatest at some of the larger gardens, possibly due to a wider range of habitats in large compared with smaller gardens. Hymenoptera: Crabronidae and Pompilidae (solitary predators) were the most commonly sampled wasps and would be reliant on the presence of prey species. Overall, these data point towards gardens allowing some insects to access the resources they need for survival (i.e., foraging and nesting resources; McIntyre and Hostetler 2001), but that connectivity between sites is ideal.

Prediction 3: Effects of multiple site and landscape characteristics on insects

When considered together, the overall insect abundance was greater in the oldest gardens than in more recently established gardens, and at gardens in neighbourhoods with longer time since urbanisation but this pattern was driven by the high Diptera numbers. This finding suggests that colonisation of gardens may increase with time, probably as a result of increased availability of flowers and habitat as the gardens grow. Some insects may move between garden and neighbouring remnant sites while others may preferentially live at one or other.

Overall, Coleoptera were more abundant in remnants than in gardens, and samples were dominated by Mordellidae and Scarabaeidae families. Mordellidae can be found on dead or partly dead trees (Liu et al. 2018) and Scarabaeidae feed on vegetation and clusters of flowers (Gleeson 2016). In gardens, sampled Coleoptera consisted mostly of Scarabaeidae and Nitidulidae (sap beetles, which feed on sap and decaying vegetative matter). Coleoptera in gardens were the only group with a positive response to flowering despite flowers generally known to be attractive to bees (Pardee and Philpott 2014; Threlfall et al. 2015).

At remnant sites, I found that overall insect abundance was greater at sites with reduced canopy cover, a result consistent with findings from North American studies where percent tree cover and canopy cover were negatively related to bee species abundance (Burdine and McCluney 2019; Miljanic et al. 2019). It is likely that multiple site characteristics contribute to my result; for example, while less canopy cover could decrease foraging on canopy flowers, it may increase the likelihood of insects visiting understorey flowering plants. Beyond these few patterns and despite sampling a number of variables expected to influence insect abundance and diversity, there are still many more factors beyond the scope of this study (e.g., competition, alien plants, warming) which may have influenced the insects found in both gardens and remnants.

Prediction 4: Insect-pollinator responses

I expected that insect communities would vary according to the availability of suitable foraging and nesting resources, but insect pollinators were one of the least sampled groups in this study, which may account for their lack of response to many of the site characteristics. The paucity of pollinators in remnants is emphasised by observations of five plant species that were studied in detail. The introduced *A. mellifera* was the only visitor to two plant species, and two others were only visited by two native bee species and visits were infrequent (Eakin-Busher et al. 2020). More sampling is necessary in order to gain a better idea of the full suite of insects present at the chosen study sites, for example, active sampling has been shown to capture more bees in the region (see Prendergast et al. 2020).

The bees I sampled were ground-nesters (Halictidae and female *Amegilla chlorocyanea*), stem nesters (Exoneura), and socially nesting species (*Apis mellifera*). Ground-nesting bees have been found to have a significantly higher probability of occurrence in older green space with less impervious surface (Geslin et al. 2016; Threlfall et al. 2015). However, the presence of these species at a site could also indicate that the required resources for the species are in proximity, rather than within the site itself.

Since urban gardens are designed to encourage abundant flowering, they should be beneficial to pollinators (Verboven et al. 2014). The presence of pollinating Hymenoptera increased with increased plant abundance here, which could point towards plant abundance improving habitat, such as hollow stems for Exoneura and other pollinators to nest in. The plant abundance variable was related to plant diversity, so increased plant abundance could indicate diverse plant species, which may provide constancy of floral resources if different species are flowering at different times throughout the year. This accords with the idea that gardens can provide abundant floral resources compared with other habitats in urban landscapes (Kaluza et al. 2016; Salisbury et al. 2015).

When considered alongside other variables, the main influence on the abundance of pollinating Hymenoptera was the distance to the nearest remnant. So even if these insects use gardens, the remnants nearby likely provide critical resources such as nesting sites and or particular floral resources (see Hinners 2008). Detailed studies of floral resources (Johanson et al. 2019; Theodorou et al. 2016) found that bee richness and visitation increased as a response to local flower richness and coverage, respectively. Most of the observed pollinating Diptera were observed in remnants, which may suggest remnants are better for this particular insect group. Overall though, the number of different pollinator taxa observed in gardens and remnants was similar. Maintaining remnants in close proximity (~500 m) to native gardens is additional insurance for insect-pollinator communities in the likely event that some insects are using the resources in both habitats.

In native gardens, the ecosystems and flowers present may appeal to particular (e.g., native) pollinators (Pardee and Philpott 2014; Threlfall et al. 2015). Indeed, the greatest number of native bees I observed was in a garden which contained a mass-flowering eucalypt tree. Yet despite some gardens being able to support native bee communities (Makinson et al. 2017), fragmentation can drive pollinator declines (Harrison and Winfree 2015; Potts et al. 2010), and can also lead to insect communities being dominated by the introduced European honey bee *Apis mellifera* (Aizen and Feinsinger 1994). This may have already happened in Western Australia, as *Apis mellifera* is a generalist which visits many plant species, while native bees (of which ~80 occur in the Perth region; T. Houston, pers. comm. July 2019) have not been recorded on as many plant species, and are less frequently observed (e.g., Eakin-Busher et al. 2020; Yates et al. 2005).

3.6. Concluding remarks

I encourage further complementary studies using additional sampling methods, such as visual observations and sweep netting. Such an approach may result in a larger dataset with more insects sampled more frequently, which could provide for more robust modelling (i.e., count-based over presence/absence data). Pan traps may over, or under-sample certain taxa, so the overall pollinator fauna may be underrepresented (Cane et al. 2000; Roulston et al. 2007) despite providing a means to collect comparative samples in remnants and gardens. There is also merit in more detailed observations of insects across fewer sites and so increasing opportunity to collect additional data per site. For example, although I characterised each native garden in detail, sampling at the neighbouring houses may have provided additional insight into the preferences of insects. Lastly, sampling across the complete spectrum of urban environments, from lawn-only to native-only gardens, may improve the capacity to predict insect responses.

The diversity of insect responses suggests that maintaining heterogenous habitats and configurational complexity (e.g., patches of open ground without mulch in gardens to provide nest habitat) could assist in maintaining insect assemblages within the modified urban landscape (Miljanic et al. 2019). My data highlight the potential for gardens with floral compositional diversity to support a rich insect fauna. The response of Diptera and wasps to the increasing cover of exotic species suggests that deliberately planting exotic species could benefit some urban insects. For example, exotic flowering plants can extend the availability of food resources for pollinators and thus increase their diversity in gardens (Salisbury et al. 2015). Building knowledge on the relationships between these insects and their specific plant and habitat requirements will inform conservation efforts in the broader landscape. This study reaffirms the complexity of results from studies in urban ecology and warrants further research of targeted pollinators. Overall, remnant vegetation and gardens are likely complementary in providing resources and habitat for pollinating insects in urban environments and maintaining diverse native and exotic gardens could assist in maintaining viable and biologically diverse plant and insect assemblages.

4. Mating strategies dictate the importance of insect visits to native plants in urban fragments

4.1. Abstract

Plant species conservation relies on their reproductive success and likelihood of population persistence. Plant mating systems, and particularly the relationship between plants and their pollinators, is fundamental knowledge to inform conservation efforts. This knowledge could be critical for prioritising efforts in human-dominated fragmented landscapes, such as the world's biodiversity hotspots, where reproductive success may be compromised due to habitat loss, limited access to pollinators or other factors. Yet, fundamental data on plant mating systems are lacking for many Australian plants. Here, we determined the mating systems of native plant species growing in native woodland fragments within Perth's urban landscape in south-western Australia. We manipulated insect access to flowers and pollen transfer on five locally common native species, then observed floral visitors and examined reproductive success. *Hemiandra pungens* and *Patersonia occidentalis* had mixed mating systems with some ability to self-pollinate, while *Dianella revoluta* and *Jacksonia sericea* were reliant on insects for outcross pollination. The fruits and seeds produced by *Tricoryne elatior* were too low to draw conclusions about its mating system. The introduced honey bee *Apis mellifera* was the sole visitor to the mixed mating species, while native bees visited *D. revoluta* and *J. sericea* (one bee species each). Overall, our data suggest that *D. revoluta* and *J. sericea* are more vulnerable to fragmentation than *H. pungens* and *P. occidentalis*. While insects significantly contributed to the reproductive output of the two former plant species, our observations suggested low frequency and richness of insect visitors to these urban fragments. More research is required to determine the generality of our findings. A comparative study in larger native woodland fragments would help estimate the impact of fragmentation on insect pollinators and consequences for the insect-reliant plant species.

4.2. Introduction

Plant species conservation requires an understanding of plant mating systems. Most plant species are hermaphroditic, and many species are self-compatible (Eckert et al. 2009). Therefore, many plants produce seeds through self-fertilisation (i.e., ‘selfing’). At the opposite extreme, plants produce seeds through cross-fertilisation with unrelated conspecific plants (i.e., ‘outcrossing’). In these cases, plants are reliant on vectors to transfer pollen, and in most cases, the vector is an insect (Aizen and Feinsinger 2003). Outcrossing rate depends on plant-pollinator interactions and can vary with the abundance and foraging behaviour of pollinating insects, which in turn is affected by the density and dispersion of conspecific plants (Holsinger 1996). Consequently, plasticity is inherent in plant mating systems, and has presumably evolved in response to selection pressure to reproduce in the absence of pollinators but not at the expense of a reduction in fitness of inbred compared with outcrossed progeny (Stephenson et al. 2000). Understanding the extent to which human disturbance, especially habitat fragmentation, might drive the expression of this plasticity has been a key focus of plant species conservation efforts (e.g., Aguilar et al. 2006).

There has been considerable research on plant mating systems and plant-pollinator interactions in fragmented landscapes of Europe (e.g., Aguirre-Gutiérrez et al. 2015; Andrieu et al. 2009; Grass et al. 2018; Jauker et al. 2019) and the Americas (Bennett and Lovell 2019; e.g., O’Connell et al. 2006). There are comparatively fewer studies of plant mating systems in Australia and especially for plants with insect pollinators (Johanson et al. 2019). Research has tended to focus on unmodified landscapes (Ladd et al. 2019; Loy et al. 2015; Popic 2013), although there are some exceptions. In eastern Australia, native *Dianella revoluta* showed evidence of self-incompatibility and low fruit set in native forest fragments (Duncan et al. 2004). Lentini et al. (2012) suggest the importance of fragments for bees in agricultural landscapes of New South Wales. Similarly, Hall et al. (2019) identified key features, such as wooded vegetation, required to support diverse bee communities in fragmented agricultural landscapes of Victoria. These studies used vane traps to sample bees, many of which were pollinators and did not link bee responses to plant reproductive success. In Western Australia, Newman et al. (2013) measured the reproductive success of four insect-pollinated self-incompatible orchid species in 11 urban fragments near Perth and attributed pollen limitation to low pollinator activity. Most studies of fragmented landscapes are limited by lack of knowledge of the plant-insect communities and interactions that existed prior to habitat clearing. Despite this shortcoming, studies in Australia and elsewhere suggest the possibility of simplified insect pollinator communities in fragmented landscapes. Detailed studies of plants and their pollinators are needed to determine the generality of findings and the broader consequences for plant species persistence in fragmented landscapes.

In this study, we explore the insect visitation, pollination, fruit and seed set of five insect-pollinated plant species in urban fragments in Perth, Western Australia. We determine the mating systems of these plant species and the importance of insect pollinators to fruit and seed set by manipulating insect access to flowers. We couple this study with detailed observations of insect visitors on plant species in the same urban landscape. Specifically, we aim to answer the following questions:

Are plant species capable of selfing?

Does outcrossing improve fruit production and seed set?

Which insects are potential pollinators of the target native plants?

4.3. Methods

Study species

We studied the mating systems of five native Western Australian plant species: *Dianella revoluta* R.Br., *Hemiandra pungens* R.Br., *Jacksonia sericea* Benth, *Patersonia occidentalis* R.Br., and *Tricoryne elatior* R.Br. The floral morphology of these species suggests a role for insect pollinators (Table 4.1). There are records of native anthophorine bees visiting *D. revoluta* growing in banksia woodland near Perth (Houston 2000). We selected these particular native plant species because they are common across south-western Australia (Western Australian Herbarium 1998), and have quantifiable fruit and seed sets. In addition, the study species had a range of flower colours and flowering phenologies (Table 4.1). We anticipated that these and other differences (e.g., habit, presence of nectar; Table 4.1) would attract a range of insect visitors and pollinators, and therefore, a representative sample of insects in the urban landscape.

Study sites

We selected three fragments of native banksia woodland vegetation near Perth, Western Australia: Classon Park Leeming (32°04'56.69" S, 115°51'34.22"E), Nicholson Road Reserve (32°03'02.64"S, 115°55'57.19"E), and Wireless Hill (32°1'46.99"S, 115°49'45.99"E). All three sites experience a Mediterranean-type climate with cool winters and hot summers (mean maximum annual temperature, 24.8 °C; mean minimum annual temperature, 12.8 °C), and rainfall is c. 732 mm per year (Bureau of Meteorology 2019). Nicholson Road is bordered by residential areas and a highway, with an industrial area, sporting oval and golf course nearby. Classon Park is in a residential area, surrounded by streets and houses. Wireless Hill is located next to a highway, also surrounded by streets and houses with a shopping complex to the southeast. *Hemiandra pungens* was studied at Nicholson Road, *P. occidentalis* at Classon Park, and the remaining three species at Wireless Hill. Pollination experiments were carried out on individuals approximately 40 m from reserve edges.

Table 4.1. Characteristics of focal plant species and sites at which they were studied in September–December 2017

	Dianella revoluta	*Hemiandra pungens*	*Jacksonia sericea*	*Patersonia occidentalis*	*Tricoryne elatior*
Image (not to scale)					
Approx. flower size	2.1 cm	1.7 cm	2.0 cm	3.0 cm	1.8 cm
Flowering start	Early–mid November	Mid September	Mid–late November	Mid September	Mid October
Flowering end	Late December	Early November	Mid-January	Mid November	Mid November
Flower symmetry	Actinomorphic	Zygomorphic	Zygomorphic	Actinomorphic	Actinomorphic
Anther display	Exposed	Hidden	Hidden	Exposed	Exposed
Stigma display	Well exposed	Initially hidden, later exposed	Hidden	Well exposed	Well exposed
Colour	Purple/blue	White/purple	Orange	Purple	Yellow
Nectar	No	Yes	Yes	No	Yes
Duration of flower display	< 1 day	Several days	Several days	< 1 day	< 1 day
Family	Asphodelaceae	Lamiaceae	Fabaceae	Iridaceae	Asphodelaceae
Habit	Rhizomatous herb	Prostrate shrub	Low spreading shrub	Rhizomatous herb	Rhizomatous herb
Life history	Perennial	Perennial	Perennial	Perennial	Perennial
Site	Wireless Hill	Nicholson Rd	Wireless Hill	Classon Park	Wireless Hill
Size of fragment	37.5 ha	11.2 ha	37.5 ha	1.9 ha	37.5 ha
Distance to nearest fragment	1800 m	650 m	1800 m	400 m	1800 m

Experimental design and data collection

Having selected five plant species, we opted to focus our efforts on one population of each species. While modest, this design was necessary given the lack of available data on mating systems to inform a more complex design. Additionally, from a logistical perspective, the design had to be executed with the limited flowering season of each plant species. We selected six plants of each species in September 2017 and manipulated insect access and pollen transfer based on techniques outlined by Kearns and Inouye (1993); multiple inflorescences or individual flowers on six plants per species were randomly assigned pollination treatments. We tagged flowers corresponding to their specified pollination treatment, either open, autogamy, self, outcross or supplementary pollination (Table 4.2). Given the window for hand pollination was limited by flowering time, supplementary pollination was undertaken at Wireless Hill, where the majority of pollination experiments occurred (supplementary pollinations completed for *D. revoluta*, *J. sericea* and *T. elatior*).

Table 4.2. Pollination types used to determine the role of insects in fruit and seed set of five native plant species.

Pollination treatment	Description	Bagged	Insect interaction
Open	Flowers unmanipulated (control) with free access to visitors	No	Allowed
Autogamous	To determine whether within-flower pollination could occur without insect visitors, flowers were not hand pollinated	Yes	Excluded
Self	Pollen from one flower was applied to stigmas of another on the same plant to test for self-compatibility (i.e., geitonogamy)	Yes	Simulated by hand
Outcross	The recipient flower was emasculated and pollen from a plant at least 5 m away was applied to stigmas (i.e., xenogamy) to test whether outcross pollen produced more fruit and seed than self-pollen	Yes	Simulated by hand
Supplementary	Outcross pollen was applied to stigmas of uncovered flowers of *T. elatior*, *J. sericea* and *D. revoluta* to determine whether additional outcross pollination increased open fruit and seed set. The first two experiments (*P. occidentalis* and *H. pungens*) did not include supplementary pollination.	No	Simulated by hand and allowed

Self, outcross and supplementary pollinations were achieved by hand. To facilitate pollination, we used sharp tweezers to scrape pollen directly from the anther of a donor flower and wipe it onto the stigma of the recipient. As *D. revoluta* has poricidal anthers, donor anthers were pierced and sliced with the tweezers to access pollen, which was then distributed onto the specified recipient. Given the close proximity of stigma and anthers in *H. pungens*, hand pollinations had to be undertaken on mature buds before autogamy could occur. The peroxide test of stigmas produced bubbles, which suggested that stigmas from mature buds were likely to be receptive to pollen (King 1960). Between 40 and 179 flowers of each native plant species were treated from 18 September to 22 December 2017 (Table 4.3). We treated as many flowers as possible within the constraints of flowering times and the intricate nature of hand pollination on small flowers. Autogamy, self and outcross pollination treatments were covered with organza bags to exclude insect visitors (Table 4.2). We collected fruits close to maturation in late 2017 and recorded the number of fruits and seeds. Sites were visited daily, and the first flowering dates were recorded when five or more flowers of the species were open. The end of flowering was recorded when flowers of the focal plant species remained at five or fewer.

Floral visitor observations

Flower visitor observations were recorded on warm, sunny days between 0930–1530 hours. We varied the timing of observations to span the duration of the flowering season, as well as morning and afternoon recordings for each plant species. These staggered observation times gave us a better chance of detecting insects which may have been active at different times throughout the day. We also recorded weather conditions, which we periodically reviewed to ensure a similar range of weather conditions while observing insects on each plant species. Observations for each plant species were undertaken during their flowering period (Table 4.1) with one plant watched by an observer (EEB) sitting about one metre away. The observer sat still for one minute before recording any observations to limit observer effects (Wade et al.

2005). The selected plants usually had around ten flowers, but the minimum was two flowers, and the maximum was ~100 flowers on a large *T. elatior*. To document nearby flowering, we estimated the total number of flowers (of any plant species) within a 10 m radius. Observation effort for each species varied due to the different number of flowers produced and overlaps in their flowering times (Table 4.1). The total observation time was greatest for *P. occidentalis,* but there was a long delay before any bees started visiting flowers.

Insects were recorded in 10-minute intervals while watching focal plants, with overall observations on each occasion lasting at least 30 minutes (Table S3). In the case of 'buzz'-pollinated *D. revoluta*, we recorded pollination when bees grasped a flower from underneath, and vibration was audible. Buzz pollinators, usually bees, remove pollen from poricidal anthers using vibration (De Luca and Vallejo-Marín 2013). For the other plant species, insect visitors were listed as potential pollinators when they interacted with the anthers and stigmas of flowers. Where insects landed on the flower but did not contact the anthers or stigma, they were recorded as visitors and as these were not crucial to our study, have not been included.

Data analysis

All statistical analyses were completed in R version 3.4.1 (R Core Team 2017). We present means and 95 % confidence intervals for fruit and seed sets produced by different pollination treatments. To determine the effect of pollination treatment on fruit and seed sets, we used generalised linear mixed models (lme4 package; Bates et al. 2015) following suggestions of Zuur et al. (2009) to check model assumptions were met (i.e., normal residuals, homogeneity of variance, and overdispersion). Individual models were applied to data for each species, and open pollination was the baseline against which each pollination treatment was compared. Fruit set data (response variable: fruit presence/absence) were fitted using a binomial distribution with logit-link, as the per-flower outcome was binomial (i.e., 1 for fruit, 0 for no fruit). We anticipated that number of seeds per fruit would vary across the plant species (P. Ladd, pers.

obs., Spring 2016). In most cases, seed set data (i.e., response variable: number of seeds produced per fruit) were fitted using a Poisson distribution, as recommended for discrete counts (Bolker et al. 2009). However, as pollinated *J. sericea* produce either one or two seeds per fruit, a binomial distribution was fitted for the *J. sericea* models. All models included pollination treatment as the explanatory fixed-effect, and because there were multiple observations per plant, individual plants were included as a random effect. In some cases (*D. revoluta* and *J. sericea*), treatments resulted in zero fruits across all flowers and plants, leading to a lack of model convergence. In these cases, we do not report statistical effects. Residuals were visually assessed, and models were fitted using the Laplace approximation, as recommended by Bolker et al. (2009) for models with binomial and Poisson distributions that include less than three random effects. Comparisons between other groups were investigated using 95 % confidence intervals of treatment means. Where supplementary treatments were undertaken, we calculated pollen limitation (Larson and Barrett 2000): L= 1 – (Po/Ps), where Po is the percent fruit set from open-pollination and Ps is the percent fruit set by plants that received supplementary pollen. L= 0 indicates no pollen limitation in the study population. Fruit set of *T. elatior* was not statistically analysed because there were too few data.

4.4. Results

Mating systems

In three of five focal plant species, outcross pollination improved fruit production (Figure 4.1a, Figure S2a). Autogamy provided a minor contribution to *P. occidentalis* fruit and seed set and a major contribution to *H. pungens* fruit and seed set (Table 4.3). Conversely, *J. sericea* and *D. revoluta* required insects to produce fruit and seed. The fruit and seed set data for *T. elatior* were too sparse to draw conclusions regarding treatment effects.

The *D. revoluta* plants we studied were predominantly self-incompatible, as flowers did not produce fruit from autogamous pollinations and produced just one fruit from self-pollination (Figure 4.1a). The insect contribution to *D. revoluta* pollination was therefore major, with almost all fruit produced by treatments which simulated or allowed insect pollination. The mean proportion of *D. revoluta* flowers setting fruit was greatest for supplementary pollination (47 %, $z = 2.65$, $p<0.01$, Table S4). For each treatment type, the mean number of seeds per fruit was not significantly different from the seeds produced in the open (control) fruits (all $p>0.05$, Figure 4.1b. Figure S2b).

Table 4.3. Experimental units and reproductive output for five native plant species.

	Dianella revoluta	*Hemiandra pungens*	*Jacksonia sericea*	*Patersonia occidentalis*	*Tricoryne elatior*
Image (not to scale)					
Number of flowers in each treatment					
Open	60	49	65	179	40
Autogamy	60	77	64	142	98
Self	60	81	62	160	107
Outcross	60	69	65	140	167
Supplementary	60	NA	60	NA	52
Total	**300**	**276**	**316**	**626**	**464**
Number of fruits produced					
Open	14	42	15	55	1
Autogamy	0	37	0	9	0
Self	1	52	0	22	0
Outcross	20	43	18	67	2
Supplementary	28	NA	21	NA	0
Total	**63**	**174**	**54**	**153**	**3**
Proportion setting fruit (%)					
Open	23 (±4.22)	86 (±7.37)	23 (±4.62)	31 (±6.09)	3 (±5.00)
Autogamy	0	48 (±13.24)	0	6 % (±1.79)	0
Self	2 (±1.52)	64 (±15.57)	0	14 % (±6.52)	0
Outcross	33 (±6.03)	62 (±9.07)	28 (±8.92)	48 % (±3.54)	1 (±0.80)
Supplementary	47 (±10.22)	NA	35 (±4.28)	NA	0
Mean proportion setting fruit (%)	21 (±4.07)	65 (±6.13)	17 (±3.37)	25 (±4.01)	1* (±0.66)
Percentage of fruit set produced by:					
Open	22	24	28	36	33
Autogamy	0	21	0	6	0
Insect-simulated	78	55	72	58	67*
Pollen Limitation	0.51	Not available	0.34	Not available	Not available
Possible seeds per individual fruit	1–6	1–4	1–2	1–40 or more	Up to 3 1-seeded mericarps
Mean number of seeds per fruit	1.5 (±0.14)	3.2 (±0.07)	1.2 (±0.06)	5.5 (±0.70)	1
Range of seeds produced					
Open	1–3	1–4	1–2	1–19	1
Autogamy	0	1–4	0	1–11	0
Self	1–1	1–4	0	1–8	0
Outcross	1–6	1–4	1–2	1–41	1
Supplementary	1–4	NA	1–2	NA	0
Mean number of seeds produced					
Open	1.5 (±0.17)	3.2 (±0.15)	1.2 (±0.11)	5.5 (±0.63)	1 (±5.00)

Autogamy	0	3.2 (±0.18	0	2.7 (±1.05)	0
Self	1 (±0.00)	3.4 (±0.13)	0	3.2 (±0.50)	0
Outcross	2.2 (±0.33)	2.9 (±0.14)	1.3 (±0.11)	15.2 (±1.12)	1 (±0.00)
Supplementary	2.1 (±0.19)	NA	1.2 (±0.10)	NA	0

*Small proportion of treatments setting fruit prevented *T. elatior* results from inclusion in statistical analysis.
NA is where treatment was not applied and numbers in brackets are standard error.

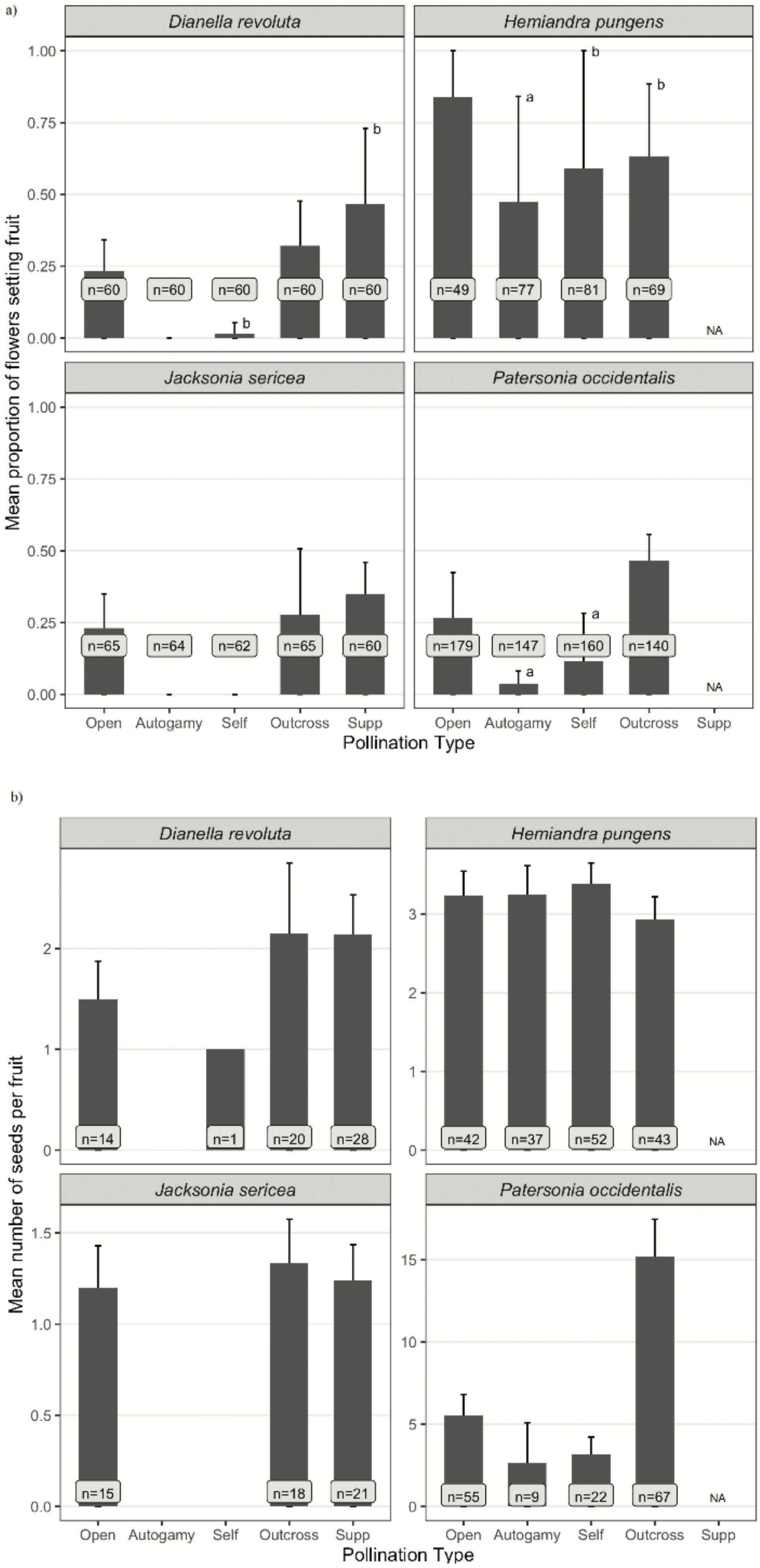


Figure 4.1 a) Mean proportion of flowers setting fruit and b) mean number of seeds per fruit from each pollination treatment on each of the four study species which set fruit. Bars indicate +95% CI and numbers are sample sizes. Note the different Y scales for 1 b). Open = flowers not bagged, Autogamy = flower bagged to test for within-flower pollination, Self = hand pollinated with pollen from the same plant then bagged, Outcross = hand pollinated with pollen from a different plant then bagged, Supp = hand pollinated with outcross pollen, not bagged. NA indicates supplementary pollinations were not carried out. [a] result is significant (compared to open) at $p<0.001$, [b] result is significant at $p<0.01$.

Fruit set in *H. pungens* was more consistent across pollination treatments than for our other focal species (Figure 4.1a), though supplementary pollination was not tested. All treatments produced a lower proportion of flowers setting fruit (48–64 %) than the open group (86 %, z = 3.23, p<0.01, Table 4.3), yet seed set was comparable for all *H. pungens* pollination treatments (p>0.05, Figure 4.1b). Of our study species, *H. pungens* appeared to be the least reliant on insects, as 48 % of the autogamous pollinations produced fruit. However, in this treatment, the results varied markedly, from 8 to 87 % fruit set for individual plants.

For *J. sericea*, the mean proportion of flowers developing into fruit was less than half for all samples (Figure 4.1a). In this population, *Jacksonia sericea* was self-incompatible, as autogamy and self-pollination produced no fruit or seeds (Figure 4.1b). For treatments that produced fruit, there was no significant difference in seed set between the open group compared with outcross and supplementary pollinations (p>0.05, Table S4). The total failure of fruit set in the autogamy and self-pollination treatments and <50 % fruit set with pollen supplementation led us to conclude that the species is reliant on insect pollinators. The species is reliant on insect pollinators, but even with supplementary pollen, the mean fruit set was less than 50 %.

In *P. occidentalis*, fruits were produced by pollination treatments simulating or open to insects (i.e., non-autogamous pollinations) in 94 % of cases. Outcross pollination in *P. occidentalis* produced the greatest proportion of flowers setting fruit (48 %) and the greatest mean number of seeds, while autogamy produced the least (6 %, Figure 4.1), though supplementary pollination was not tested so we do not know if the species was pollen limited.

Insect observations

Insects were the only flower visitors observed for all focal species. In general, insect visits were infrequent, ranging from 1–8 insects/hour (Table S3). Introduced honey bees (*Apis mellifera*), which cannot vibrate anthers, were the most frequently observed flower visitors on three focal plant species (*P. occidentalis*, *H. pungens*, and *T. elatior*). At the start of flowering, *P. occidentalis* was not visited. However, after about one month, *A. mellifera* began to visit *P. occidentalis* flowers. Some *H. pungens* plants we observed were individuals planted at reserve edges, where honey bees were the only observed insect visitors (Table S3). *Apis mellifera* did not visit *D. revoluta,* which requires vibration to remove pollen. The only insect observed on buzz-pollinated *D. revoluta* were female native blue-banded bees (*Amegilla chlorocyanea*). The only visitor observed on *J. sericea* was a species of native bee from the Megachilidae family. The greatest number of different insect species (3 species) was observed on *T. elatior*, yet no fruit was produced by the plants on which we observed these insects at the study site. In general, insects were not abundant at the study sites (mean 0–1), and there was no statistical relationship between insect abundance and approximate flowering within a 10 m radius of focal plants.

4.5. Discussion

Outcross pollination was beneficial to the plant species studied here to varying extents. *Dianella revoluta* and *Jacksonia sericea* were generally self-incompatible and so required insects for outcross pollen. On the other hand, *Hemiandra pungens* and *Patersonia occidentalis* had mixed mating systems with some ability to self-pollinate, but *P. occidentalis* produced significantly more seeds per fruit from outcrossing than from selfing. *Dianella revoluta* and *J. sericea* may be more vulnerable to fragmentation than autogamous plant species, as they are reliant on insect vectors to transfer pollen.

The bee fauna observed visiting the target native plant species at our study sites was depauperate, with *D. revoluta*, *J. sericea,* and *P. occidentalis* apparently only visited by one bee species each. Native bee taxa have been recorded on some of these plant species previously (a total of two bee taxa on *H. pungens*, six on *J. sericea*, and three on *D. revoluta*, Houston 2000). A limited pollinator community may, therefore, be unusual, particularly since only *D. revoluta* has a somewhat specialised (buzz) pollination. Around 80 species of native bee inhabit the Perth region (T. Houston, pers. comm.), but the only bee species we observed on *D. revoluta* was a native blue-banded bee (*Amegilla chlorocyanea*), an important pollinator of many buzz-pollinated plant species (e.g., Eakin-Busher, Fontaine and Ladd 2016). Other bee species capable of buzz-pollination do occur in Perth urban reserves (PGL and EEB, pers. obs.), so it is surprising they were not recorded on *D. revoluta*. In comparison, *D. revoluta* flowers in non-urban areas in south-eastern Australia were also visited by *A. chlorocyanea* and other native bee species (Duncan 2003). However, in a study of buzz-pollinated *Conostephium,* only *Leioproctus* and *Lasioglossum* bees were observed to visit the flowers, despite *A. chlorocyanea* inhabiting the study site (Houston and Ladd 2002), so perhaps it is not unusual for particular bee taxa to visit particular flowers. Given the correlation between pollinator species diversity and increased fruit set (Albrecht et al. 2012), if the low frequency and richness of insects is a general characteristic of these native plant populations, they could be susceptible to reproductive risk if they were to lose insect pollinators or become isolated from potential pollen donors in the landscape (Aguilar et al. 2006).

The Australian bee fauna is considered to be very diverse (Houston 2018), yet studies of bee visitors to native flowers in fragmented and intact landscapes show varied results. Our study can be contrasted against another from the same fragmented landscape, which surveyed insect visitors to jarrah (*Eucalyptus marginata*) trees. While we recorded just three bee species, Yates et al. (2005) reported 83 species, of which introduced *A. mellifera* was the most frequently recorded. Our plant species provide a comparatively minor food resource compared with a mass flowering tree, but nonetheless, it is unusual not to have seen more insect visitors. Other comparative studies, but for intact landscapes, include semiarid eucalypt woodland in Western Australia, where only three native bee species and introduced *A. mellifera* visited selected native plants (mostly species in the Asteraceae; Loy et al. 2015), and research in the arid zone of New South Wales, where a quarter of the plant species studied had only one bee visitor (Popic et al. 2013). Taken together, these studies suggest *Apis mellifera* is a common generalist insect visitor to Australian native flowers compared with native bees, which appear to be more specialist in their visiting habits, but more data are needed to confirm this assertion.

The *J. sericea* population was obligately xenogamous and required insects for fruit set. We know of no other studies on the mating systems of *Jacksonia* R.Br. ex Sm., an endemic Australian genus of Fabaceae comprising ~55 species of which the greatest diversity is in the south-west of Western Australia (Chappill et al. 2007). However, for three species of *Dillwynia,* which is related to *Jacksonia* (tribe: Mirbelieae; Toon et al. 2014), and for *Pultenaea densifolia* (Fabaceae) from eastern Australia, mating systems were also obligately xenogamous (Gross 1990; 2001). Only one native bee species was observed visiting *J. sericea* flowers despite the fact that similar pea flowers are visited by native and introduced bees in New South Wales (Gross 2001; Lomov et al. 2010). So, it seems that *J. sericea* is vulnerable to the loss of its sole pollinator, but more data are needed to determine whether other pollinators visit, to properly assess the risk of coextinction (i.e., where species loss can cause further extinctions; Plein et al. 2017).

We can only speculate as to the pollination efficiency of the insects we observed. Despite the small number of bee species visiting native plants, the open fruit set for *J. sericea, P. occidentalis* and *H. pungens* was similar to outcross or supplementary pollination, which indicates sufficient pollination. In a fragmented landscape in eastern Australia, visitation rates to *Dillwynia juniperina* varied from 1.5 to 6.4 bees/hr for native bees and from 10.1–16.7 bees/hr for introduced bees (Gross 2001). For a buzz-pollinated plant in arid Western Australia, visits by *Lasioglossum* species ranged from 0.4–29.6 bees/hour (Ladd et al. 2019). This result is within the range of our observations of native bees, but higher than our results for introduced European honey bees. In our study, the three focal species that have one-day flowers (*D. revoluta, P. occidentalis, T. elatior*) all had at least one visitor per hour. *Dianella revoluta* showed some pollen limitation (PL = 0.51), which could indicate inadequate visitation rates, or that visitors were not transferring outcross pollen to the flowers. The greater seed set from outcross versus facilitated self-pollination in *D. revoluta* is consistent with evidence from southeastern Australia, where self-pollen caused ovule abortion (Duncan 2003; Duncan et al. 2004).

Despite the careful and consistent observation of *P. occidentalis* flowers at the beginning of the flowering season for over a month, no visitors were recorded until one day *Apis mellifera* began visiting and continued to do so for the remainder of the observation period. We cannot discount possibility of an observer effect, though instances where native bees attempted to visit *D. revoluta* and *J. sericea* flowers while they were being held for hand pollination suggests they were unperturbed by the observer. For *P. occidentalis*, 25 % of the open pollinated flowers converted to fruits, so visits by *A. mellifera* provides some insurance against seed set failure. However, the resultant seed set from open pollinated *P. occidentalis* flowers was less than the seed set by manually outcrossed flowers. This is perhaps unsurprising, as the generalist *Apis mellifera* was first introduced into Australia in 1826 (Doull 1973), and would not have been the original pollinator for *P. occidentalis*. We did not observe any native bees on *P. occidentalis*, and can find no records of them from sources such as Houston (2000), but observed that insect pollination did not produce the maximum possible fruit set. Of the other species visited by *A. mellifera, H. pungens* was strongly autogamous, *T. elatior* produced only one fruit from open pollination, and *P. occidentalis* may not have had optimal fruit set. However, as *A. mellifera* was the only or main visitor to three plant species, it appears to be the sole provider of essential pollination service to these species.

Given its self-compatibility, *Hemiandra pungens* appeared well buffered against poor pollinator attention. The fruit set variability among *H. pungens* plants for the autogamy treatment (large CI in Figure 1a) indicates a possible mechanism whereby if pollinators were absent, autogamous plants could produce more seeds and hence more offspring than plants with poor self-pollination ability. Lack of pollinators has been identified as a factor influencing the evolution of selfing (Charlesworth 2005), so if pollinators disappeared, a *H. pungens* population could theoretically become entirely autogamous. In this way, *H. pungens* would be conserved in the landscape if there were no negative genetic consequences of autogamy and no other factors limiting its survival.

Indeed, local plant population attributes may also influence fruiting success. *Tricoryne elatior* was the exception in our study with almost no fruit formation despite abundant attention from potential pollinators. As fruits formed at other sites (E. Eakin-Busher, pers. obs. 2018), there may be a genetic condition or lack of genetic diversity among the plants that were studied, which may have prevented fertilisation, particularly if *T. elatior* is obligately outcrossing. Many plant species have variable mating systems depending on where they grow and may have variation in their self-incompatibility in different parts of their range (Roda and Hopkins 2019), so the mating systems identified here may not apply universally for each species. However, the similar results for *D. revoluta* with those from New South Wales (Duncan et al. 2004) do point towards consistency for at least one species.

Further research to compare plant reproduction in small fragments and intact landscapes in the Perth area would be valuable. However, finding large areas of intact natural vegetation similar to Perth remnants is increasingly difficult due to urban expansion. It may be necessary to instead examine changes over time at particular sites, so our study provides a baseline against which future studies can be compared. Future studies could also compare mating systems among plant populations to delineate the impact of fragment size and isolation on mating systems. Available evidence suggests that plant mating systems respond to selective pressures (e.g., Teixido and Aizen 2019) and this topic is worthy of further research in the context of urbanisation and other impacts of human activity. Given the prevalence of these impacts, conservation relies on the capacity for native plant and animal populations to be self-sustaining. Global concerns about declines of insect pollinators and plant species loss (Humphreys et al. 2019; Potts et al. 2010) highlight the growing importance of plant-pollinator studies to understand and manage synergistic effects.

5. Gene flow and pollination of a rhizomatous herb in a fragmented urban landscape

5.1. Abstract

The reproductive success and population persistence of native plants living in fragmented habitats can be influenced by factors including their mating system, pollen dispersal, and genetic fitness. I used six microsatellite genetic markers to investigate the mating system and patterns of pollen flow in native *Patersonia occidentalis* in remnant native vegetation in the southwest of Western Australia. *Patersonia occidentalis* is a self-compatible herb with insect pollinators. Microsatellite markers indicated that adult *P. occidentalis* in this population had 3–17 alleles per locus (H_e = 0.34–0.82), with the potential for null alleles (0.01–0.31). These genetic data can be used to estimate the extent of genetic connectivity among remnant populations reliant on pollinators and ultimately, the long-term viability of the species.

I collected seed from 22 different mothers and assigned paternity to 106 seedlings at 80 % confidence or greater using the potential sire with the greatest probability of being the true sire. There were 50 unique sires assigned, with 27 contributing pollen to more than one progeny. Approximately 70 % of paternity assignments resulted from outcrossing within the remnant, and nearest neighbours were rarely the assigned sire. On average, the distance between the mother and assigned sire was 33.99 m, including cases where self-pollination occurred. The assigned paternity described here suggests that this *P. occidentalis* population is largely outcrossing. Introduced European honey bees (*Apis mellifera*) were the only observed pollinator of *P. occidentalis*, so the frequent outcrossing is likely maintained by insect-facilitated pollen transfer.

Failure to assign sires in some cases could be due to null alleles, or pollen transfer from other remnants within pollinator flight distance. Information is lacking on whether native pollinators contribute to this system, but the remnant vegetation here supported enough

introduced pollinators to maintain pollen movement and therefore genetic diversity in this plant population.

5.2. Introduction

Understanding the environmental impacts of human activity in urban locations, particularly where biodiversity is already threatened, is of utmost importance for developing conservation strategies. However, the consequences of urbanisation on plants and pollinators, particularly in the long-term, are not well understood (Seto et al. 2012). In urban areas, major environmental impacts associated with human activities include habitat loss and fragmentation (Fahrig 2003), which can, in turn, impact plant reproduction (Aguilar et al. 2006). However, the exact impacts and plant responses can be species-specific or depend on complex ecological interactions, which are likely different for different locations (see Hobbs and Yates 2003). Because plants may have different responses to impact, fragmentation does not always adversely impact plant reproductive success (e.g., Thavornkanlapachai et al. 2019).

Following fragmentation, plant species generally persist in small remnants situated within a hostile matrix. The ecological and genetic consequences for these plants include invasion of exotic species, reduced population size, reduced pollen pool (Coates et al. 2007), isolation, and disruption to pollinators and seed dispersers (Cunningham-Minnick and Crist 2020). Because angiosperms depend on pollen flow for successful reproduction, pollination provides one of the strongest cases for managing natural habitats. Many preferentially outcrossing plants are reliant on animal vectors to transfer pollen (Aizen and Feinsinger 2003), and limited pollen diversity and dispersal may, in the presence of inbreeding, result in reduced fitness of progeny (Gibson et al. 2012; Krauss et al. 2007; Llorens et al. 2013; Yates et al. 2007) and reduced reproductive success in successive generations (Fenster and Dudash 1994). These are compelling reasons to understand fragmentation impacts.

The impact of fragmentation on plant mating systems, the patterns in which gametes combine in plant populations, can be substantial. For example, in animal-pollinated species, habitat fragmentation can lead to reduced pollen flow, increased inbreeding in normally outcrossing species, reduced genetic fitness, and local extinction of populations (see Young et al. 1996; Young and Clarke 2000). Thus generally, a negative relationship between habitat fragmentation and pollination services is assumed (e.g., Aguilar et al. 2006). Pollen dispersal and the factors which influence it are therefore important for genetic and reproductive processes and understanding the patterns is important for managing plant populations in fragmented urban landscapes.

Perth in the southwest of Western Australia is a sprawling city in which there has been habitat fragmentation and subsequent isolation of native vegetation. Remnant native vegetation therefore provides an ideal location to examine the effects of urban fragmentation on pollen dispersal and the mating system in animal pollinated species. In this study, I investigate pollen flow in a population of *Patersonia occidentalis* R.Br. in a representative urban remnant in Perth, Western Australia. *Patersonia occidentalis* is insect-pollinated and has a mixed mating system, with one study demonstrating the species produces a significantly greater seed set following outcross pollination than self-pollination (Eakin-Busher et al. 2020). The presence of this native, insect-pollinated plant species within a small remnant provided the opportunity to explore the outcomes of insect-pollination. Specifically, I aim to determine what percentage of progeny are produced by self-pollination and to what extent progeny are sired by plants within the same remnant. I expect that most sires will be situated within the remnant, near the maternal plant and that more outcrossing than selfing will occur in the population.

5.3. Methods

Study species

Patersonia occidentalis R.Br. (Iridaceae) is an endemic native iris, commonly known as purple flag. The rhizomatous, perennial herb produces inflorescences of purple flowers from August to December, with larger plants being capable of abundant flowering. Flowers are open for less than one day, during daylight (E. Eakin-Busher, pers. obs. Spring 2016) and supply visitors only with pollen. Multiple fruits are produced within each infructescence, and at maturity, seeds are oval shape, approximately 5 mm long by 1 mm wide. In a previous study, *P. occidentalis* was visited by *A. mellifera*, with low overall visitation, and there was no evidence of other pollinators (Eakin-Busher et al. 2020). *Patersonia occidentalis* flowers were observed for pollinators during site visits between 0900 and 1500.

Study site

The study was undertaken in Brandwood Reserve in Leeming (-32.0851, 115.8555), 17 kilometres south of Perth, Western Australia. Brandwood Reserve covers 3.21 hectares and is comprised of lawn, playgrounds, and two hectares of remnant vegetation. The remnant vegetation is banksia woodland, dominated by *Banksia* trees with scattered *Allocasuarina* spp. The understorey is dominated by native shrubs and herbs, with weedy, non-native grasses dominant at the unsealed track, sealed path, and remnant edges. Unsealed tracks are widespread and are commonly used by children on bikes and residents walking dogs. The site is managed by the City of Cockburn and is bordered by roads and houses. There is evidence that management of the reserve consists of weed spraying within the remnant vegetation, and lawn mowing and waste collection. The climate is Mediterranean-type with cool, wet winters and hot, dry summers (Bureau of Meteorology 2019). Approximately 350 m along the road, there is another reserve of similar size, Classon Park, which also contains *P. occidentalis*.

Study design

Brandwood Reserve was searched for flower-producing *P. occidentalis* individuals during September 2017. Each individual was mapped using a Global Navigation Satellite System (GNSS). A 10 cm leaf sample was collected from each flower-producing adult within the community (i.e., possible sires). All leaf samples were freeze-dried. Following flowering, seeds were collected from maternal individuals that had produced at least one swollen infructescence (Figure 5.1). Seeds were collected from 1–8 infructescences and amalgamated into a bulk sample, per mother, which totalled 29–226 (mean 70) seeds each. To determine the most effective method for germinating *P. occidentalis*, trials were undertaken on additional seeds (Table S5). To prevent deterioration while germination trials took place, seeds were stored at −15 °C with ~15 % relative humidity for approximately five months. These conditions are consistent with conventional seed banking, and suitable for other Western Australian flora with orthodox seeds (Crawford et al. 2007). On July 1st, 2017, seeds were treated with aerosol smoke produced by burning native plants and were left in the sealed container for 24 hours. Seeds were then placed onto coarse quartz sand in a covered container at 18 °C and watered every few days. The first seeds germinated after three weeks. Germination was scored when the radical reached 2 mm. Every germinant was planted into a 50:50 mix of coarse quartz sand and native potting mix. Seedlings were kept in a glasshouse and watered twice per day until they were around ~4 cm tall when they were harvested for DNA extraction and genotyping. Seeds produced 3–181 (mean 41) germinants, a germination success of 10–87 % (mean 54 %). I harvested up to 16 seedlings from the maternal plants, i.e., all germinants if there were 16 or fewer. For plants with more than 16 germinants, harvest selection was haphazard for individuals at 4 cm. A total of 280 seedlings from 22 different mothers were harvested for genotyping.

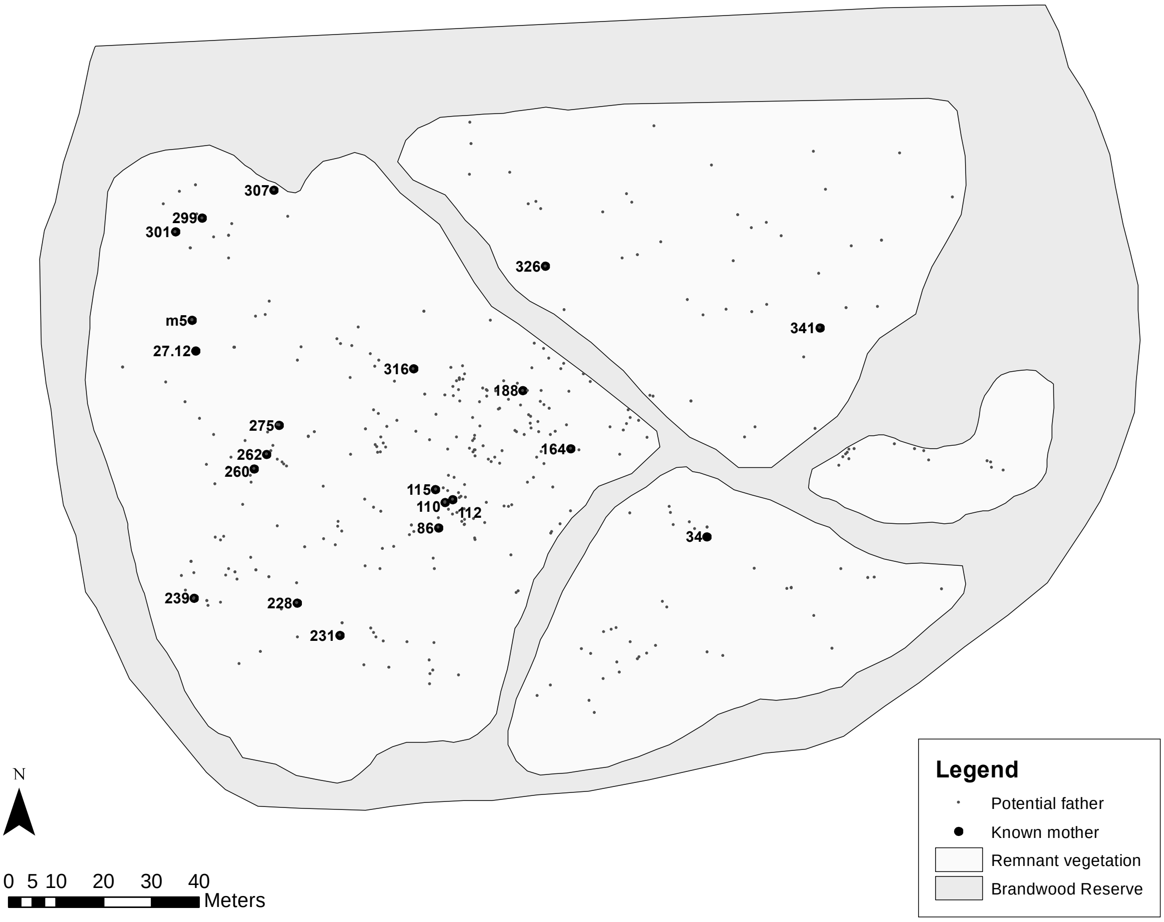


Figure 5.1. Location of all flower-producing *Patersonia occidentalis* plants in the remnant area of Brandwood Reserve, Leeming Western Australia. Labelled individuals are maternal plants from which seeds were collected.

DNA extraction and genotyping

I used a modified version of the Doyle and Doyle method (Doyle and Doyle 1987) to extract *P. occidentalis* DNA from freeze-dried leaf material. The Doyle and Doyle buffer solution had sodium sulfite added to minimise DNA degradation (Byrne et al. 2001). Extracted DNA was sequenced on a MiSeq (Illumina Inc., San Diego, USA) at Monash University, Malaysia. DNA was quantified and sheared (500 bp) on a Covaris ultrasonicator (Covaris, Woburn, USA). A NEBNext Ultra DNA preparation kit for Illumina (New England Biolabs, Ipswich, Massachusetts) was used for library preparation after sequencing., 23 primers were selected for further trials on six *P. occidentalis* individuals. Of the 23 primers, 13 were monomorphic, six polymorphic, and four failed to amplify. Six primer pairs produced clear variable loci (Po2, Po6, Po10, Po11, Po18, and Po23, Table S6).

The G5 label set was used to label forward primers with a fluorescent colour (either VIC, PET, NED or FAM). Microsatellite regions were then amplified by polymerase chain reaction (PCR) in multiplexes of two sets using a Qiagen Multiplex kit (Qiagen, Hilden, Germany). Each multiplex reaction contained 1µl of DNA, 3.75µl of Qiagen mix, 0.75µl primer mix and 2µl water (from Qiagen kit). All primer mixes underwent PCR using the Multiplex60 program. PCR cycling was programmed as follows: an initial activation step at 95°C for 15 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s, and extension at 72 °C for 60 s, with a final extension at 60 °C for 30 min and a further 1 min at 25 °C. I diluted the DNA with sterile H_2O (1:5), and 1.0 µL of the dilution was added to a mix of 12.0 µL Hi-Di Formamide (Applied Biosystems) and 0.1 µL GeneScan LIZ®500 Size Standard (Applied Biosystems). PCR products were separated by capillary electrophoresis on a 3730xl DNA Analyzer (Applied Biosystems) at the WA State Agricultural and Biotechnology Centre (SABC). Genotypes were scored in GeneMapper v3.7 (Applied Biosystems), and allele bins were manually assigned and adjusted where necessary.

Individual loci of adult plants were tested in Genepop v4.0 (Raymond and Rousset 1995) for departure from Hardy-Weinberg equilibrium using a Markov chain algorithm to estimate p-values of exact tests. Genepop was also used to test for linkage disequilibrium (LD). Micro-Checker v2.2.3 (Van Oosterhout et al. 2004) was used to screen all adult individuals for null alleles using the Brookfield 1 Estimator (Brookfield 1996).

Genetic diversity

I used GenAlEx v6.5 (Peakall and Smouse 2006) to calculate genetic diversity parameters for six microsatellite loci in mature individuals and progeny of *P. occidentalis*. Parameters comprised the number of individuals genotyped (N) averaged over all loci, the mean number of alleles per locus (Na), the number of effective alleles (N_e), expected (H_e) and observed (H_o)

heterozygosity, and the Fixation index (*F*). Between-group ANOVA was conducted in Excel to assess significant differences ($\alpha = 0.05$) between estimates for adults and progeny.

Mating system

Mating system parameters for maternal *P. occidentalis* were estimated using MLTR version 3.4 (Ritland 2002). Estimates of the means and standard deviations were calculated for the multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), estimated biparental inbreeding ($t_m - t_s$), correlation of selfing or outcrossing among maternal plants (r_s) and multi-locus correlation of paternal plants (r_{pm}) using the expectation-maximization (EM) method. Standard deviations were based on 100 bootstraps and an effective number of pollen donors (N_e) was determined as $1/r_{pm}$.

Pollen dispersal and spatial structure

Likelihood-based paternity analysis was conducted using Cervus (Marshall et al. 1998) and included all potential pollen donors within the population, regardless of their flowering time. The simulation parameters were: 10,000 cycles, 269 candidate parents, 0.98 proportion sampled, and the proportion of loci mistyped was 0.01. Confidence in paternity assignment was measured as the difference in the critical logarithm of odds (LOD) scores between the candidate sire with the highest score and the candidate sire with the second-highest score under strict (95 %) and relaxed (80 %) confidence levels.

A total of 280 progeny were genotyped for paternity assignment and estimating pollen immigration. Genotypes for progeny from known mothers were assessed for paternity with genotypes of all flowering plants in the population. As *P. occidentalis* is capable of self-pollination (Eakin-Busher et al. 2020), known mothers were also included as potential male parents. The percentage of selfed progeny (i.e., progeny assigned their female parent as their most likely male parent) and the number of different sires that were pollen donors for at least one progeny were also calculated. The most likely sire assigned at ≥80 % confidence was considered to be the true most likely sire. Failure to assign paternity to 174 seedlings (62 %) was due to either insufficient power of the molecular markers to assign sires, null alleles, or the pollen donors falling outside of the sampled population.

Paternity exclusion was used to measure pollen immigration into the population using the Pollen Flow program (Slavov et al. 2005). Pollen immigration (m) and standard error were estimated for the offspring from each maternal plant and averaged over all families for an estimate of the mean pollen immigration for the population (Slavov et al. 2005). I used the diploid sampling option and tested for immigration using a minimum of three progeny/parent mismatches for exclusion. The paternal contributors to the seeds of each maternal parent were sampled using TwoGener in GenAlEx. TwoGener estimates the effective number of pollen donors per average maternal parent (N_{ep}) and the average distance of pollen dispersal.

Effective pollen dispersal distances were calculated from paternal assignments made under relaxed and strict analysis conditions (>80 % confidence) using the GNSS locations of the maternal plant for a particular seed and the most likely pollen donor. The frequency distribution of the geographic distance between parent pairs was graphed, and locations of parent pairs were mapped using ArcGIS (v10.7.1, ESRI, Redlands, CA). Spatial autocorrelation analysis (SAA) was carried out in GenAlEx 6.5 (Peakall and Smouse 2006). Spatial genetic structure was assessed using a Mantel test of correlation between genetic and spatial distances. I used 10 m distance classes in order to provide an approximately even number of samples in each class.

5.4. Results

Genetic diversity

Overall, the density of *P. occidentalis* plants within the remnant vegetation was 0.02/m^2, and 0.01/m^2 for flowering individuals (i.e., potential sires). A significant deficit of heterozygotes (departure from Hardy-Weinberg equilibrium) was observed for loci Po2, Po11, and Po18, but only one of 15 pairs was significant for linkage disequilibrium (Po2 vs Po11). The possible presence of null alleles was detected, with potential frequencies generally 0.21 or less, except in locus Po2 (Table 5.1).

Table 5.1. Allelic diversity values for microsatellite markers in *Patersonia occidentalis* at Brandwood Reserve. Na, number of alleles per locus; Ho, observed heterozygosity; He, expected heterozygosity; Excl, non-exclusion probability where one parent is known; Potential null allele frequencies (Brookfield Estimator 1 frequencies are reported).

	Locus	N_a	H_o	H_e	Excl	Null (B1)
Adults (n=269)	Po2	10	0.27	0.82	0.53	0.31
	Po6	5	0.32	0.49	0.87	0.12
	Po10	5	0.48	0.51	0.87	0.01
	Po11	17	0.37	0.73	0.63	0.21
	Po18	9	0.50	0.71	0.71	0.12
	Po23	3	0.19	0.34	0.94	0.11
	Mean	8.17	0.36	0.60	0.76	0.15
Progeny (n=280)	Po2	9	0.15	0.82	0.53	0.32
	Po6	5	0.33	0.51	0.87	0.16
	Po10	3	0.39	0.44	0.91	0.03
	Po11	12	0.41	0.75	0.62	0.02
	Po18	7	0.46	0.66	0.77	0.12
	Po23	3	0.19	0.34	0.94	0.12
	Mean	6.5	0.32	0.59	0.77	0.13

Observed heterozygosity ranged from 0.19 (Po23) to 0.50 (Po18) in adults and 0.15 (Po2) to 0.46 (Po18) in progeny. Observed heterozygosity was lower than the expected heterozygosity in all cases (Table 5.1). A total of 50 alleles were detected in 549 genotyped individuals. Adults (49 alleles) had a mean of 8.17 alleles per locus, while progeny (40 alleles) had a mean of 6.50 alleles per locus (Table 5.2). No significant difference was observed between adults and progeny for any of the genetic diversity parameters (Table 5.2).

Table 5.2. The number of mature individuals and progeny genotyped averaged over all loci (N) and genetic diversity parameters for six microsatellite loci in *Patersonia occidentalis*. Values are the mean number of alleles per locus (N_a), the number of effective alleles (N_e), observed (H_o) and expected (H_e) heterozygosity, and the Fixation index (F_{is}). Standard errors are in parentheses. Statistical comparison between adults and progeny comprises degrees of freedom (df), F-value and P-value.

	N	N_a	N_e	H_o	H_e	F_{is}
Adults	268.33 (0.21)	8.17 (2.07)	3.03 (0.62)	0.36 (0.05)	0.60 (0.08)	0.38 (0.09)
Progeny	274.08 (0.17)	6.50 (1.46)	2.97 (0.65)	0.32 (0.05)	0.59 (0.08)	0.42 (0.10)
df	-	1	1	1	1	1
F-value	-	0.43	0.00	0.27	0.02	0.08
P-value	-	0.53	0.95	0.61	0.90	0.78

Mating system

Patersonia occidentalis had a mixed mating system with the estimates of outcrossing (t_m and t_s) significantly lower than one (Table 5.3). Apparent selfing due to biparental inbreeding among close relatives was significantly greater than zero. The correlation of selfing (r_s) was not significantly greater than zero, but r_p was, and MLTR estimated an effective number of pollen donors of 2.22 (N_e), while TwoGener estimated N_{ep} of 2.38.

Table 5.3. Mating system parameter estimates for six microsatellite loci in adult *Patersonia occidentalis* plants with standard deviation.

Parameter	Value
MLTR	
Multilocus outcrossing rate (t_m)	0.71 (0.06)
Singlelocus outcrossing rate (t_s)	0.52 (0.05)
Bi-parental inbreeding rate ($t_m - t_s$)	0.19 (0.05)
Correlation of selfing or correlation of outcrossing among plants (r_s)	0.09 (0.09)
Multilocus correlated paternity (r_{pm})	0.45 (0.09)
Effective number of pollen donors (N_e)	2.22
TwoGener	
Effective number of pollen donors (N_{ep})	2.38
Average estimated pollen dispersal distance	7.76 m

Pollen dispersal

Paternity assignment identified one pollen source for 106 (38 %) of the 280 seedlings at 80 % confidence or greater (Table 5.5). The combined probability that the loci will exclude an unrelated candidate male parent from paternity of progeny when the maternal genotype is known was 0.83.

Table 5.4. Details of paternity assignments obtained using Cervus as percentages of progeny, for six microsatellite loci in progeny of *Patersonia occidentalis*. Progeny were assigned to most likely sires at confidence intervals of 80 % or greater.

Parameter	Value
Number of progeny genotyped	280
Number (%) of progeny assigned a male parent at ≥80 % confidence	106 (38 %)
Number (%) of progeny assigned a male parent at ≥95 % confidence	37 (13 %)
Number (%) of progeny not assigned a male parent	174 (62 %)
Number of different sires	50
Percentage of assigned progeny with a unique sire	47 %
Percentage of assigned progeny that were selfed	19 %
Percentage of progeny assigned a nearest neighbour as the sire	1.9 %
Mean pairwise distance among maternal and potential paternal plants (potential pollen dispersal distance, m)	55.41 m (±0.32)
Mean pairwise distance among mothers and assigned sires (pollen dispersal distance, m)	33.99 m (±3.06)
Mean pairwise distance among mothers and assigned outcrossed sires only (m)	39.59 m (±2.96)
Maximum pairwise distance among maternal and potential paternal plants (m)	158.83 m
Maximum pollen dispersal distance (m)	116.46 m

Paternity was assigned to 50 unique sires, 27 of which contributed pollen for more than one progeny. In 20 cases, two or more progeny shared the same mother and father, including four sets of selfed siblings. Six different maternal plants were considered the most likely sire (total of 20 cases, 19 %). Four of these six which produced seeds via selfing also produced seeds through outcrossing. The mean number of assigned sires per mother was 2.12, and the maximum number of progeny sired by one father was seven. Based on the paternity assignment results, 219 of the 269 possible parents did not contribute pollen to the genotyped progeny, despite producing at least one infructescence each. Introduced European honey bees (*A. mellifera*) were the only observed flower visitor during the study (Table 5.5).

Table 5.5. Number of *Apis mellifera* observed on *Patersonia occidentalis* at Brandwood Reserve

Date	Start Time	End Time	Minutes	Total visits observed
19/10/17	13:30	14:00	30	1
20/10/17	12:30	13:00	30	4
21/10/17	11:20	11:50	30	4
25/10/17	13:10	13:30	20	2
30/10/17	11:20	11:40	20	2
03/11/17	11:20	11:50	30	1
06/11/17	10:10	10:30	20	1
Total			**180**	**15**

Of the seedlings assigned paternity, pollen dispersal distances ranged from 0–116.46 m with the average pollen dispersal distance overall being 33.99 m (±3.06 m) (Figure 5.2). The average pollen dispersal distance for paternity assigned at 95 % confidence was 27.19 m (±3.97 m), and for paternity assigned at 80 %, the average pollen dispersal distance was 37.64 m (±4.14 m) (Figure S3). A south-westerly (218 degrees) directional mean was calculated for pollen movement overall, but this was not significant (T = 5.68, p = 0.59).

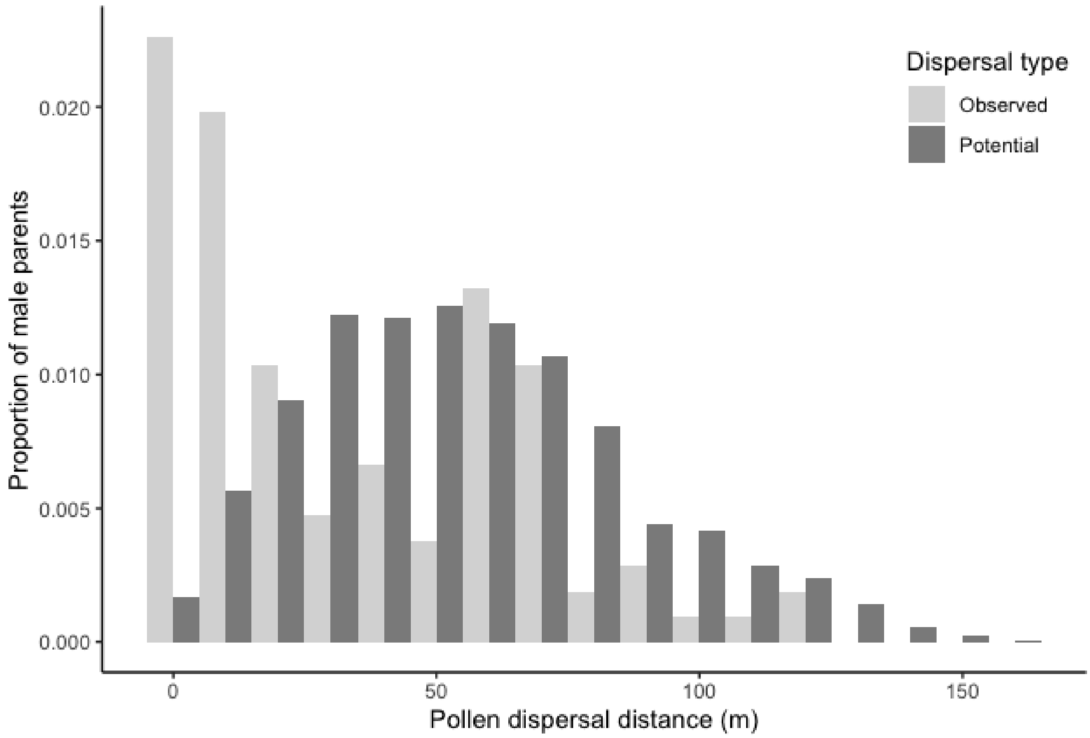


Figure 5.2. Frequency histogram of observed and potential pollen dispersal in the surveyed *P. occidentalis* population. Observed dispersal distance is the mean distance from each maternal plant to the assigned fathers (assigned by Cervus at 80 % or greater) and potential dispersal distance is the mean distance from each maternal plant to all potential fathers. Distance is given in groups of 10 m.

Spatial genetic structure

A Mantel test showed a significant relationship between genetic and spatial distance within this *P. occidentalis* population ($P < 0.05$). Spatial autocorrelation analysis detected significant spatial genetic structure in the smallest distance classes of 0–10 m and 10–20 m ($r = 0.06$, $P < 0.01$ and $r = 0.02$, $P < 0.01$ respectively). After 20 m, there was no significant autocorrelation between pairwise multilocus genotypes (Figure 5.3).

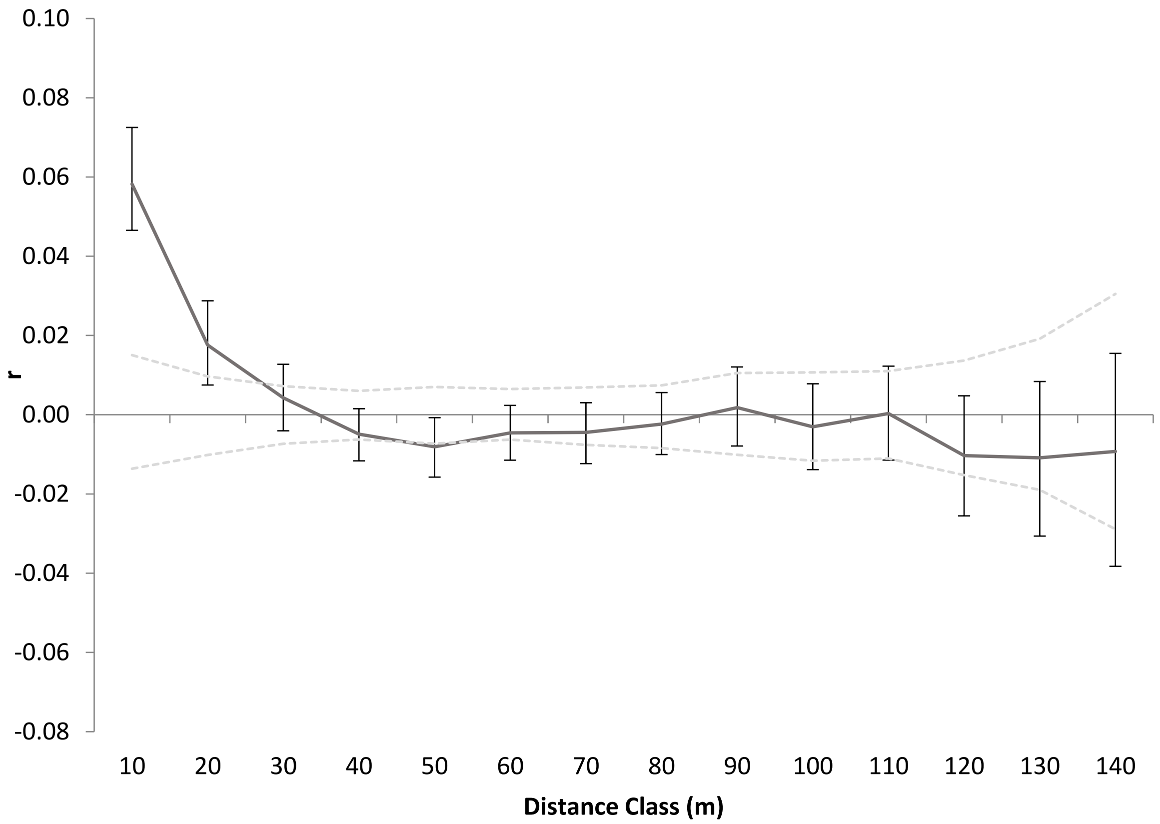


Figure 5.3. Correlogram showing the genetic correlation coefficient, r, as a function of geographic distance for *P. occidentalis*. Dashed lines indicate the 95 % confidence interval around the null hypothesis of a random distribution of genotypes. Error bars indicate the 95 % confidence of r as determined by bootstrapping.

5.5. Discussion

The *P. occidentalis* population studied here had a mixed mating system with more outcrossing than selfing occurring. I found limited nearest neighbour crossing probably associated with the relatively low density of open *P. occidentalis* flowers on any one day. Pollen was dispersed across the remnant despite only one species of pollinator being observed, which suggests pollen movement is sufficient to maintain moderate levels of outcrossing in this *P. occidentalis* population.

Genetic diversity and mating systems

Estimates of genetic diversity provide some insight into the mating system of this *P. occidentalis* population. Firstly, a moderate to high outcrossing rate was documented, suggesting that insects are maintaining outcross pollen transfer here. Of the few individuals which produced seed via selfing, most also produced seed via outcrossing. *Patersonia occidentalis* flowers only last one day, so outcrossing increases their likelihood of pollination, especially as flowers on small individual plants may not be produced simultaneously (E. Eakin-Busher, pers. obs. 2017).

In outcrossing species, it has commonly been reported that levels of observed heterozygosity are lower and the fixation index higher in progeny than in adult cohorts (e.g., Millar et al. 2020; Thavornkanlapachai et al. 2018). In self-compatible species, lower heterozygosity is generally attributed to post-zygotic seed abortion mechanisms, with the cumulative effects of high levels of inbreeding depression and strong post-germination selection against inbred progeny increasing throughout the life-cycle (Duminil et al. 2009; James 1992; Petit and Hampe 2006). Thavornkanlapachai et al. (2018) recorded a higher fixation index for progeny than adults in the mainly mammal-pollinated shrub *Banksia nivea*, and the same pattern was reported for bird and mammal-pollinated *Banksia media* (Millar et al. 2020). In contrast, I found the heterozygosity and fixation index values for adults and progeny were not significantly different (Table 5.2). This could indicate that some selfed individuals have survived to adulthood here, without selective mechanisms acting against them. However, given the presence of null alleles in multiple loci, this suggestion remains speculative. In addition, the rhizomatous habit of *P. occidentalis* makes it difficult to determine the origin of individuals, thus closely related or clonal plants may have had unsuccessful selfing occurring, but this remains unknown as selective mechanisms were not examined as part of this study.

Neighbours are typically more closely related than distant plants (Ellstrand 2014), and this was the case in the population studied here. *Patersonia occidentalis* individuals within 20 m of each other were more likely to be closely related than by chance, with no significant likelihood of close relatedness beyond 20 m. Relatedness of spatially close individuals could be a result of their rhizomatous growth, or from pollen dispersal occurring over a short distance. In addition, relatedness among nearby individuals may be due to short-range seed dispersal whereby most seeds fall near the mother, as seeds do not have visible appendages to facilitate long-distance wind or animal-mediated dispersal (Plate 5.1).

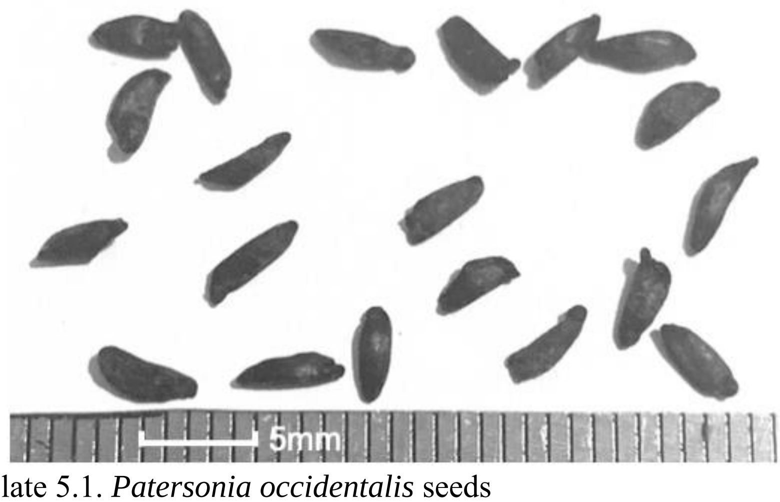


Plate 5.1. *Patersonia occidentalis* seeds

Although relatively low, significant levels of biparental inbreeding were observed in this *P. occidentalis* population, indicating at least some progeny may be the result of mating among relatives. This result is likely related to the density of plants as even for mammal pollinated

Banksia nivea the denser population had the lower biparental mating value and the shorter observed pollen dispersal distance (Thavornkanlapachai et al. 2018). *Patersonia occidentalis* is a rhizomatous herb which may already have quite high levels of relatedness among nearby plants and some biparental inbreeding would be expected. Although indicative only due to the presence of null alleles, my analysis suggests very little crossing between nearest neighbours, suggesting that the biparental inbreeding is likely due to crosses between related plants that are more spatially distant. However, it is also possible that crosses between neighbours occurred, but resulted in less seed due to self-incompatibility or post-zygotic rejection. While most seeds likely dispersed short distances, infrequent longer distance seed dispersal could explain spatial distance among related plants (i.e., leptokurtic dispersal; Levin and Kerster 1969).

In a small population such as this, with few potential sires, genetic theory predicts high values of correlated paternity; a greater proportion of seeds fathered by one individual within maternal progeny arrays, more self-pollination (e.g., Aguilar et al. 2008; Eckert et al. 2010) and potentially a loss of genetic diversity. Indeed, the estimates of the effective number of pollen donors suggested that a few fathers generally contributed to seed production on each maternal plant. Studies on a number of other south-west Australian plants report ‘very low' correlated paternity as 0.035 (Krauss et al. 2009), and ‘low’ as 0.06–0.16 (Ritchie et al. 2019). This *Patersonia occidentalis* population had a higher correlated paternity of 0.45. Higher correlated paternity may be expected in a small population of insect-pollinated plants, as near-neighbour mating is typical. Despite this, the realised pollen dispersal in *P. occidentalis* suggested outcrossing occurred beyond the nearest neighbour, which may account for the low to moderate level of genetic diversity observed within the population. The combined probability that the loci had the potential to exclude an unrelated candidate male parent from paternity of progeny when the maternal genotype was known was 0.83, so although it is likely that the inability to assign a sire for all progeny is due to the presence of null alleles, there could be the potential of pollen being imported from outside of the population.

Pollen dispersal

The relatively low density of open *P. occidentalis* flowers on any one day (i.e., much less than the density of flower-producing plants which was 0.01/m^2) may have reduced the likelihood of near neighbour visits. Pollinator movement between plants is more likely to span longer distances in sparse populations (Levin and Kerster 1974; Thavornkanlapachai et al. 2018). However, low plant density can also increase inbreeding if animal vectors do not travel between distant individuals. Here, honey bees were not concentrated in any part of the remnant and displayed quite unsystematic flight patterns, pointing towards their opportunistic movement among available flowers on a given day. However, I do not know which *P. occidentalis* flowers were open on the same day, or whether other plants were flowering and being visited in addition to the tagged *P. occidentalis* plants.

The number of *P. occidentalis* individuals and maternal plants was greatest in the southwestern section of the remnant, and pollinators may have been attracted to the higher number of flowers in this area. The paternal plants that sired multiple progeny were generally located in this section too, yet it is unlikely that these flowering plants produced any more pollen than flowering plants located in other parts of the remnant. Although larger maternal plants produced more seed, this did not correlate with successful germination nor the number of germinants assigned their mother as their sire (E. Eakin-Busher unpublished data). It is worth noting that seed collection may have also inadvertently selected more outcrossed individuals, as outcrossing produces more seed than selfing (Eakin-Busher et al. 2020), and seed was generally collected from plants which had one or more infructescences. Therefore, the outcrossing identified through parentage assignment of these seeds could have been overestimated.

The seed-producing plants which were also considered most likely sire of their own seed (20 cases total), did not have any noticeable difference in their proximity to other plants, nor were they plants of any particular size. In cases where maternal plants had multiple seeds sired by one father, pollinators may have spent longer foraging on multiple flowers on one individual plant (e.g., Ishii 2006). This is possible as multiple seeds and fruits were collected from each mother, but the number of open flowers on each plant on any given day is unknown. Pollinators are generally more likely to deposit pollen on near neighbours, and the probability of siring is assumed to decrease with the distance between mates (Levin and Kerster 1974). According to the genotypes scored here, the pollen dispersal distance here ranged from 0–116.46 m, but the most frequent was from 10–20 m (or 0–10 m including selfing). Insect pollinator movement, therefore, occurred between plants which were in close proximity to each other, yet rarely between nearest neighbours.

In this study, paternity assignment identified a pollen source for 38 % of the progeny assessed. Overall, this reflects that not every potential parent contributed pollen, and indicates that failure to assign paternity was a limitation of the markers used in this study. Alternatively, *A. mellifera* may be transporting *P. occidentalis* pollen from external sources such as the remnant 350 m along the road, but I did not have genotypes for plants from external populations to test this. *Apis mellifera* have been recorded transferring pollen up to 1 km between native trees in another study in Western Australia (Byrne et al. 2008), and are capable of travelling several kilometres (Beekman and Ratnieks 2000), so they could theoretically travel between nearby populations. However, the grooming behaviour of honey bees may reduce the likelihood of pollen transfer over long distances (i.e., pollen carryover) (see Krauss et al. 2017). There is limited information on pollen carryover after grooming and more generally, how far *A. mellifera* travel and transport pollen in their introduced range.

Studies of other Western Australian native plants (e.g., primarily bird-pollinated *Calothamnus quadrifidus* subsp. *teretifolius, Banksia menziesii*, and *Eucalyptus incrassata*), show that pollinators can still sufficiently transport pollen over degraded and fragmented habitat (Ritchie et al. 2019; Sampson et al. 2014). Although *P. occidentalis* is insect-pollinated, the presence of honey bees may counteract some negative impacts of fragmentation (e.g., reduced dispersal; Byrne et al. 2008). This is an important point as there have been many genetic studies of vertebrate pollen dispersal in Western Australia but few involving insects. Despite birds theoretically being able to fly between distant populations, *Calothamnus quadrifidus* subsp. *teretifolius* still only had few pollen parents per seed parent (Sampson et al. 2014) and this was generally the case here. Conversion to fruits was limited for *P. occidentalis* visited by *A. mellifera* (Eakin-Busher et al. 2020) so despite significant levels of outcrossing, the species is still likely pollen limited due to low pollinator attention.

Pollinators

Introduced European honey bees were the only observed visitors to *Patersonia occidentalis* during this particular study, and likely facilitated pollination. However, a native pollinator surely existed prior to the introduction of the European honey bee in 1826. Previous records of visitors to *Patersonia* flowers are lacking. Despite *P. occidentalis* flowering for several weeks, *A. mellifera* only started visiting flowers in a nearby reserve partway through the flowering season (Eakin-Busher et al. 2020). Similarly to my observations for this *Patersonia* population, South African Irids related to *Patersonia* often rely on a single species for pollination (Goldblatt and Manning 2006).

Insects other than *A. mellifera* were recorded opportunistically on *P. occidentalis* in Perth during the spring of 2016 and 2017 (E. Eakin-Busher, pers. obs.). *Liparetrus* beetles (Coleoptera: Scarabaeidae) were observed multiple times and could theoretically facilitate pollen transfer due to their interaction with anthers and stigmas. Most South African Iridaceae are pollinated by Hymenoptera (bees), but some are pollinated by Coleoptera (beetles) among other insects and birds (Goldblatt and Manning 2006). Actinomorphy (exhibited by *P. occidentalis*) is associated with pollination by hopliine beetles or pollen collecting bees in the Iridaceae in South Africa (Goldblatt et al., 1998). Beetles may therefore be a native pollinator for *P. occidentalis*. However, in this study the presence of beetles on a flower was generally as a herbivore, as these beetles tended to eat the reproductive structures. Other visitors were only observed once each and evidence of any pollen transfer was lacking. It therefore seems likely that introduced *A. mellifera* facilitated the pollination events occurring here.

Patersonia occidentalis may have some resilience to fragmentation due to being able to be pollinated by introduced bees, as these are thought to fly further than native bees. Native bees are more likely to nest locally (e.g., in soil or hollows) and remain close to their nest, whereas the introduced honey bees which have nests and can range widely (e.g., Beekman and Ratnieks 2000; Visscher and Seeley 1982). Therefore, introduced bees may be more likely to reach isolated remnants and facilitate pollen transfer between more geographically distant plants, while native bees may facilitate pollination between individuals in close proximity to each other.

5.6. Concluding remarks

Knowledge of pollinator movement in urban plant populations can inform the conservation of genetically diverse plant populations. The feral pollinator here seems to provide adequate pollen movement and levels of outcrossing to ensure plant population persistence and maintenance of genetic diversity. While it was not possible to confirm linkage between nearby remnants in terms of pollen movement, my study supports the idea that retention of urban native vegetation remnants can conserve small native plant populations such as this population of *P. occidentalis*. More detailed studies on the genetic structure of insect-pollinated species are needed as a comparison with those available for vertebrate pollinated taxa.

6. General discussion

The global diversity of pollinating insects leads to complexity in their responses to impacts of human activity, and this was the case for my study on the impacts of urbanisation on insects in the southwest of Western Australia. Few studies have investigated detailed interactions between native plants and insect pollinators here. Evidence from Europe and North America suggests that fragmentation impacts will differ with plant mating systems in terms of outcrossing and selfing (Eckert et al. 2009), which, in turn, will affect genetic consequences for insect-pollinated native plants.

The aim of my research was to contribute knowledge of the interaction between native plants and pollinating insects in an urban landscape. My study comprised remnant native vegetation and native gardens in Perth, Western Australia. I identified site and landscape characteristics that influenced insect groups, to establish the importance of insects to the reproductive success of native plants, and to determine pollen movement within a remnant vegetation fragment. I combined hand pollination trials, insect sampling and observations, and genetic analysis to provide a baseline impact assessment for this urban landscape.

The introductory chapters established the background information and current extent of global research on urbanisation and its impact on native plants and pollinators, while the data chapters outlined the major research aims and findings. I used a range of approaches to investigate my study questions. First, I passively sampled insects in remnant and garden sites and gathered information on the local and landscape characteristics of each site (Chapter three). Then, I observed insect visits to flowers and conducted pollinator exclusion studies (Chapter four). Finally, I used genetic data to determine pollen movement in a population of *P. occidentalis* within a remnant in the urban matrix (Chapter five). In this closing chapter, I summarise the main outcomes of the thesis, the management implications for urban environments, and directions for future research.

6.1. Overview of major findings

Global concerns about declines of insect pollinators and plant species loss (Humphreys et al. 2019; Potts et al. 2010) highlight the growing importance of plant–pollinator studies and provided strong impetus for my research. My sampling of insect assemblages in remnant vegetation and native gardens in urban Perth found fewer pollinators than other insects (Chapter three). While I was not able to compare my overall findings to an intact landscape, they do suggest that both the native and modified (garden) environments provided resources for insect assemblages. These data provide important baseline sampling for future studies using complementary sampling methods (e.g., sweep netting). Additionally, I showed that four native plant species, with a range of mating systems, were reliant on insect pollinators to varying extents (Chapter four). Genetic data could be used to validate my findings. My data suggest that two self-incompatible species —*Dianella revoluta* and *Jacksonia sericea*—may be more vulnerable to declines in reproductive output as a consequence of urbanisation (Chapter four). I also found that *P. occidentalis* showed moderate outcrossing, which was maintained by insects moving pollen within a remnant and possibly beyond it (~350 m, Chapter five). Understanding pollination requirements and pollinator visitation activity of these common native plants provides insight into how their disjunct populations may be maintained, and ultimately, conservation of the species in the urban landscape. Genetic data on additional populations of the species, and indeed, on additional native plant species, would provide further insight into the conservation requirements of native plants and insects in this biodiversity hotspot.

6.2. Discussion of overall results

Results for the influence of site and landscape characteristics on insect abundance were mixed. In itself, this reflects two major opposing ideas in this field. Firstly, that urban expansion has a negative impact on biodiversity (McKinney 2002, Rivkin et al. 2020), and secondly that insects can be abundant and diverse in urban environments (Hall et al. 2017; Rivkin et al. 2020). The inconsistency of effect of site and landscape characteristics accords with other studies in other locations (Bartomeus et al. 2018; Buchholz and Egerer 2020; Makinson et al. 2017; Rivkin et al. 2020) and points towards a need to refine studies of the attributes that are critical to understanding controls on insect assemblages.

The Perth region has been heavily modified through the anthropogenic influences of urbanisation. On the one hand, habitat fragmentation and isolation from intact plant communities can impact pollinator re-establishment (in a restoration context; Winfree et al. 2011). On the other hand, gardens can provide some resources for insects (Chapter three). Remnant vegetation exists in small patches across the study area, while the gardens have been subject to complete vegetation clearing and subsequent replanting. The re-established gardens can therefore play an important role in maintaining landscape connectivity and plant-pollinator interactions, and connectivity was likely important to pollinating insects here (Chapter three).

Urban gardens of flowering plants should support pollinating insects (e.g., Kaluza et al. 2016; Rudd et al. 2002; Wenzel et al. 2020) and even small patches of green space can provide habitat for pollinator groups (Daniels et al. 2020). However, despite the bee fauna of Perth being diverse (~80 species of native bee; T. Houston, pers. comm.), observations were limited during this research. From over 50 hours of observations on five native plant species, I observed two species of native bee. Reduced pollinator abundance could be attributed to urban cover (e.g., Levé et al. 2019; Plascencia and Philpott 2017), through the loss of structural diversity and degradation of habitat. Pollinator abundance also decreased when garden sites were isolated from remnant vegetation. Many of the observed bee taxa were small, solitary bees that have low mobility (Greenleaf et al. 2007; Zurbuchen et al. 2010). Therefore, gardens were likely suitable for these pollinators because of floral and nesting resources (e.g., Kaluza et al. 2016; Samnegård et al. 2011), but also because they were close to other gardens and remnants (Chapter three and see also Levé et al. 2019; McFrederick and LeBuhn 2006). My data suggested some insects were attracted to exotic plants in gardens, and this aspect may be worthy of further research given people's tendency to cultivate both native and exotic plants in urban gardens.

The *P. occidentalis* population studied displayed more outcrossing than selfing, thus pollen flow occurred despite only introduced honey bees visiting flowers (Chapter five). There appears to be sufficient insect pollinator movement to facilitate outcrossing of the species despite urbanisation. This study opens questions of the original *P. occidentalis* pollinator: what was it, and does it still visit *P. occidentalis*? Alternatively, what was it, and has the *A. mellifera* reduced its foraging or outcompeted it? Considering these questions, it was interesting to document the visitation of *A. mellifera* to *P. occidentalis* at multiple sites in the landscape. Plant and pollinator were both present at the sites, but no visitation of any sort occurred for a number of weeks, until suddenly *P. occidentalis* became of interest to the introduced bees.

Native bees were observed visiting other plant species at the same site, yet never observed visiting *P. occidentalis* flowers despite over 20 hours of observations. Due to the presence of native bees at the same site, perhaps the presence of *A. mellifera* impacts native bee foraging on *P. occidentalis*. Native bees appear to be more specialist in their visiting habits in terms of flowers visited, while *A. mellifera* are frequently recorded visiting a variety of flowers in Western Australia (e.g., Yates et al. 2005). Indeed, *A. mellifera* has the potential to dominate disturbed landscapes (Aizen and Feinsinger 1994; Carman and Jenkins 2016; Fitch et al. 2019), and this can negatively impact native bee populations through reducing floral resources (Gross 2001), or competition (Johanson et al. 2019; Thomson 2016).

The dominance of *A. mellifera* has consequences not only for native bees, but also for plants. *Apis mellifera* can decrease native plant pollination and reproduction (e.g., Gross and Mackay 1998) by depositing less pollen than native bees, or by actively removing pollen from stigmas (e.g., *Melastoma affine* in Queensland, Australia; Gross and Mackay 1998). For *P. occidentalis* studied here, *A. mellifera* did not facilitate the maximum possible fruit set.

However, *A. mellifera* was the main visitor to three of the studied native plant species, so it provided some insurance against seed set failure.

6.3. Overall themes and implications

The major themes I explored within this thesis are plant–insect interactions, physical environment–insect assemblage interactions, different plant mating systems, and pollen dispersal and gene flow. Each of these components is influenced by many interacting and complex processes, thus my results contribute knowledge but also prompt more questions. My results contribute knowledge on the insect assemblages and plant species reproduction in a fragmented landscape. My studies reinforced the idea that insect assemblages in urban environments are controlled by complex interactions. I have provided baseline data on insects and show that some, such as introduced *A. mellifera*, play an important role in maintaining the reproductive success of particular native plants. This baseline data will enable comparison of insect assemblages over time, which is especially important in the face of a global bee decline (Potts et al. 2010).

Little was known about the mating systems and pollinating insects for the native plants and sites studied here. Thus, my results contribute data which could underpin maintenance of the reproductive potential of these plant species as urbanisation continues. In the populations I studied, I showed that *Hemiandra pungens* and *P. occidentalis* were self-compatible, while *Jacksonia sericea* and *Dianella revoluta* were not and are therefore dependent on insect pollinators. In addition, my study shows that an introduced pollinator is important to the pollination of a native plant population.

6.4. Management implications

Understanding the insect assemblages present, and the underlying plant mating systems and pollen transfer, is fundamental to being able to conserve native plants and their insect pollinators moving forward. There are implications for management at landscape and site levels. My findings suggest connectivity among habitats is important for insects and that both remnants and gardens provide habitat for insects, including pollinators. The effectiveness of these habitats is improved by connectivity to remnant vegetation (Chapter three). Thus, for biodiversity conservation, I would advise city planners to conserve existing remnant vegetation and to leave uncleared patches of native vegetation as new suburbs are developed, along with removing invasive plant species. Additionally, it would be useful for management to support initiatives for people to plant native gardens, and to support research into the role of lawns and other green spaces for supporting insects, and the role of exotic plants in gardens (e.g., Yang et al. 2019).

To conserve native plant species into the future, consideration should be given to providing for plant species that depend on insects for outcrossing, as their reproduction is more vulnerable to pollinator losses. Despite this, management of pollination for the intrinsic conservation value of plant species is uncommon, even for threatened plant species. However, the management of pollination is well developed for food production (e.g., increasing yield with increasing bee abundance, Motzke et al. 2016). Management of pollination can therefore be possible if there are clear aims (i.e., to increase crop yield). If an insect-pollinated plant did face extinction, there might be the potential to increase pollination through managing bees using artificial nests or hives. However, pollination of the plant species reliant on native bees would be difficult to manage, as most native bees are solitary nesters. Although artificial nests (e.g., ‘bee hotels’) can provide additional habitat for solitary nesters, the high density of nests can also facilitate parasite invasion (MacIvor and Packer 2015). In addition, native bee species that nest in hives (e.g., *Tetragonula carbonaria*), are not found in Perth. Therefore, to safeguard native bee visits to plants here, preserving remnant vegetation and establishing gardens is perhaps the most viable option for maintaining native bee populations.

The management of urban landscapes for pollinator conservation is also challenging, as it requires the public to value urban spaces as potential insect habitat, rather than developing spaces for aesthetic and practical purposes (e.g., paved courtyards with no plants). This idea of conserving pollinators is gaining traction through 'save the bees' campaigns, but in general, there seems to be a focus on introduced honey bees, rather than native bee species.

Pollinating insects can be supported through increased flowering (Verboven et al. 2014) and plant diversity (Kaluza et al. 2018), but selecting favourable plant species is also important. For example, rather than insects being reliant on plant diversity, Warzecha et al. (2018) found that ~15 % of the available plant species were crucial to the entire insect assemblage observed. Similarly, native plants may appeal to native bees more than exotic gardens (Pardee and Philpott 2014). My findings suggested a possible role of exotic plants for supporting some insect groups and studies in other countries have also suggested that adding flowers, regardless of their origin, can support beneficial insects (Matteson and Langellotto 2011; Wenzel et al. 2020). Thus, identifying and planting the species preferred by native pollinators could be equally as important as planting diverse flowering plants. However, simply planting flowers does not guarantee the establishment of specialised pollinators (Warzecha et al. 2018). Beyond flowers, habitat structure and nesting resources need to be considered, such that suitable substrates and hollow-stemmed vegetation are available for solitary nesting native bees in urban environments.

At the site level, there are additional actions individual gardeners can do to encourage insects. Community gardens in Aachen, Germany, have shown that pollinators can quickly colonise a site, and become representative of the communities in rural reference sites (Daniels et al. 2020). These gardens considered ecological principles, such as avoiding intensive maintenance (manicuring and mowing), avoiding the use of pesticides and insecticides, and growing plants that are typical of the regional meadows (Daniels et al. 2020). Similar principles could be adopted more widely to benefit insects and plants in urban landscapes here. However, further research would help to ensure that management allows functional ecosystems to be maintained, including the survival of, and interactions between, plants and pollinators.

6.5. Suggestions for future research

Pollinating insects were generally under-sampled in this study, thus in many cases, species-specific responses were not able to be determined. Complementary studies would therefore benefit from high-resolution studies within specific groups, and including additional sampling methods, such as visual observations and sweep netting. This could allow for more robust (count-based) statistical modelling instead of presence/absence. Overall, the pollinating insects in this environment warrant further investigation, for a number of other reasons. For example, determining the extent to which introduced *A. mellifera* impacts native pollinators in this environment would allow for the future conservation of natives. Similarly, it would be beneficial to determine whether a particular bee group facilitates a better seed set for native plants, and whether certain bee groups can provide long-distance pollen movement among remnants in this urban landscape. A pollinator interaction network would also be a useful baseline to visualise which plants are visited by which insects, but this would have been ideal prior to the introduction of *A. mellifera* so that their impact on native pollinators could have been determined. Still, this information could help provide information to prevent future losses.

There is merit in more detailed observations across fewer sites and controlling for as many variables as possible so that only a few variables are tested. Focusing on a restricted number of insect taxa, in a wider variety of habitat types, could also help to narrow down the requirements of specific insect groups. A trait-based approach may help too. This could have a greater capacity to predict which insects would persist in particular locations.

Since I only studied native gardens here, further exploration around this theme would be ideal. For example, my observations pointed towards canopy cover being influential on insect abundance, similar to Miljanic et al. (2019) and Burdine and McCluney (2019). Instead of a purely ‘native garden’ criterion, it would therefore be interesting to consider sites based on strata. For example, lawn-only sites compared to those interspersed with herbs and grasses, a shrub layer, tall shrubs and overstorey trees.

The impact of selective pressures such as fragment size and isolation could warrant further investigation in urban landscapes. While the historical reference state has become increasingly difficult to locate for many urban landscapes, comparison to other benchmark states may be feasible and even more relevant to future biodiversity conservation (McNellie et al. 2020). Both these factors could be included in the sampling design of future research projects.

A focus on a smaller number of plant taxa, but from a wider range of locations or habitats would provide insight into the consistency of plant responses to urbanisation and so the potential for local adaptation. For example, the mating systems of a plant species can vary in different parts of their range (Roda and Hopkins 2019), depending on local environment and interactions. Equally, replicating my study of plant mating systems (chapter four) for other native plant species would improve our ability to generalise urbanisation impacts for different mating systems. Thus, studies of mating systems of and visitors to one plant species in multiple locations would be useful to determine the degree to which my results can be generalised. Finally, I have shown the benefit of pairing insect observations with genetic data for a more complete understanding of plant mating systems. This same approach could be adopted for other plant species, pollinators and landscape contexts.

6.6. Concluding remarks

Ultimately, understanding the complex interactions between native plants and pollinating insects is critical in order to better manage natural areas for the preservation of functional ecosystems and populations of native plants and insects. My studies point towards the importance of connectivity between small remnants, gardens and larger remnants for maintaining plant–insect interactions. Overall, this study has given insight into insect assemblages in the Perth region, particularly the pollinators of the selected native plant species.

It has provided information on how these plant species reproduce, which could be critical to their future management if pollinator species decline.

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8. Appendix

Chapter three

Table S1. Significant outputs from individual site characteristic models. Input format was: Insect group ~ Site characteristic + (1|Site) + (1|Year)

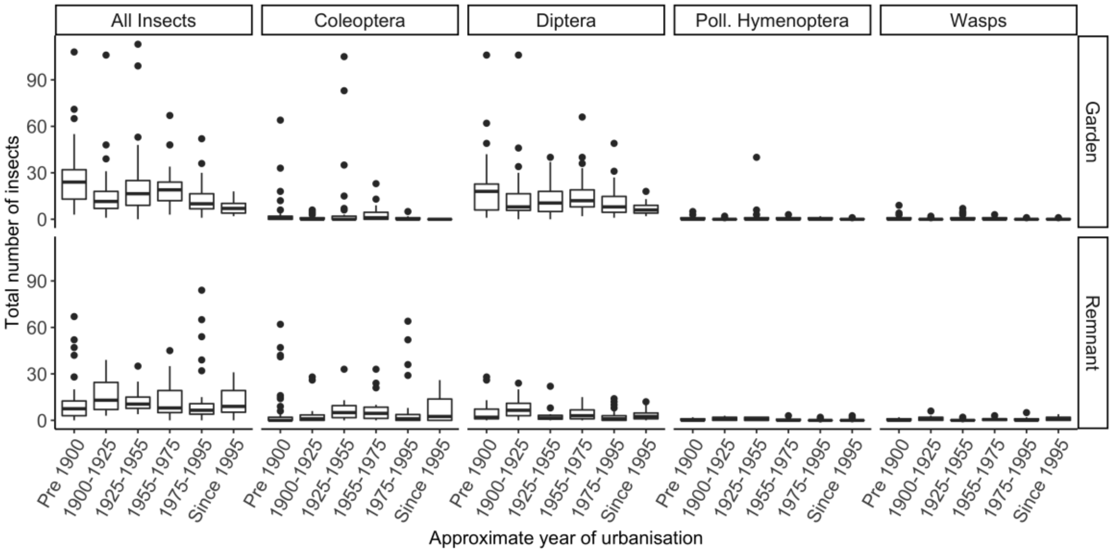
Site Type	Variable	Insect Group	Model outputs	Estimate	Standard Error	z value	Pr(>|z|)
Remnant	Distance to nearest remnant	Diptera	Intercept	2.44	0.12	19.72	<2e-16***
			Distance to remnant	0.25	0.12	2.07	<0.05*
	Remnant vegetation within 500m	Diptera	Intercept	2.20	0.16	13.53	<2e-16
			Remnant vegetation within 500m	0.00	0.00	2.19	0.03*
	Canopy cover	All insects	Intercept	3.68	0.09	39.18	<2e-16***
			Canopy cover 25-50%	-0.58	0.25	-2.29	<0.05*
	WA native plants (%)	Diptera	Intercept	2.43	0.13	19.38	<2e-16***
			WA native plants	-0.25	0.12	-2.07	<0.05 *
Garden	Time since urbanisation	All insects	Intercept	4.36	0.16	27.59	<2e-16
			Urbanisation year 1900-1925	-0.53	0.24	-2.18	<0.05*
			Urbanisation year 1925-1955	-0.20	0.22	-0.93	0.36
			Urbanisation year 1955-1975	-0.20	0.25	-0.81	0.42
			Urbanisation year 1975-1995	-0.63	0.26	-2.39	<0.05*
			Urbanisation year Since 1995	-1.21	0.35	-3.48	<0.001***
		Diptera	Intercept	4.03	0.19	21.72	<2e-16
			Urbanisation year 1900-1925	-0.37	0.28	-1.35	0.18
			Urbanisation year 1925-1955	-0.40	0.25	-1.59	0.11
			Urbanisation year 1955-1975	-0.20	0.29	-0.71	0.48
			Urbanisation year 1975-1995	-0.45	0.31	-1.47	0.14
			Urbanisation year Since 1995	-0.96	0.40	-2.41	<0.05*
	Distance to nearest remnant	Pollinating insects	Intercept	0.68	0.38	1.79	0.07
			Distance to remnant	-1.09	0.43	-2.53	0.01
		Pollinating Hymenoptera	Intercept	0.55	0.34	1.62	0.11
			Distance to remnant	-0.93	0.38	-2.46	0.01*
		Native bees	Intercept	0.27	0.29	0.93	0.35
			Distance to remnant	-1.02	0.36	-2.87	0.00*
	Hard surface within 500m	Diptera	Intercept	4.01	0.17	23.40	<2e-16***
			Hard surface within 500m	0.00	0.00	-2.08	0.04*
		Wasps	Intercept	1.74	0.71	2.43	0.01
			Hard surface within 500m	-0.01	0.00	-2.02	0.04*
	Garden establishment year	All insects	Intercept	4.40	0.15	29.33	<2e-16***
			Garden established 2008-2012	-0.49	0.18	-2.66	0.01
			Garden established Since 2013	-0.67	0.28	-2.42	<0.05*
		Diptera	Intercept	4.16	0.15	27.56	<2e-16
			Garden established 2008-2012	-0.63	0.18	-3.44	0.05*
			Garden established Since 2013	-0.57	0.28	-2.05	<0.05*
	Plant abundance	Pollinating insects	Intercept	0.82	0.43	1.90	0.06
			Plant abundance	1.31	0.56	2.32	<0.05*
		Pollinating Hymenoptera	Intercept	0.63	0.37	1.72	0.09
			Plant abundance	1.03	0.46	2.25	<0.05*
	Canopy cover (%)	Native bees	Intercept	0.71	0.44	1.61	0.11
			Canopy cover 25-50%	-0.71	0.75	-0.94	0.35
			Canopy cover 50-75%	0.22	0.88	0.25	0.80
			Canopy cover 75-100%	-2.80	1.30	-2.16	<0.05*
	WA native plants (%)	Diptera	Intercept	4.35	0.33	13.20	<2e-16***
			WA native plants	-0.90	0.44	-2.02	0.05*
		Wasps	Intercept	3.15	1.39	2.27	0.02*
			WA native plants	-3.54	1.77	-2.00	<0.05*

Table S2. Significant outputs from multiple site characteristic models. Input format was: Insect group ~ Site characteristics + (1|Site) + (1|Year).

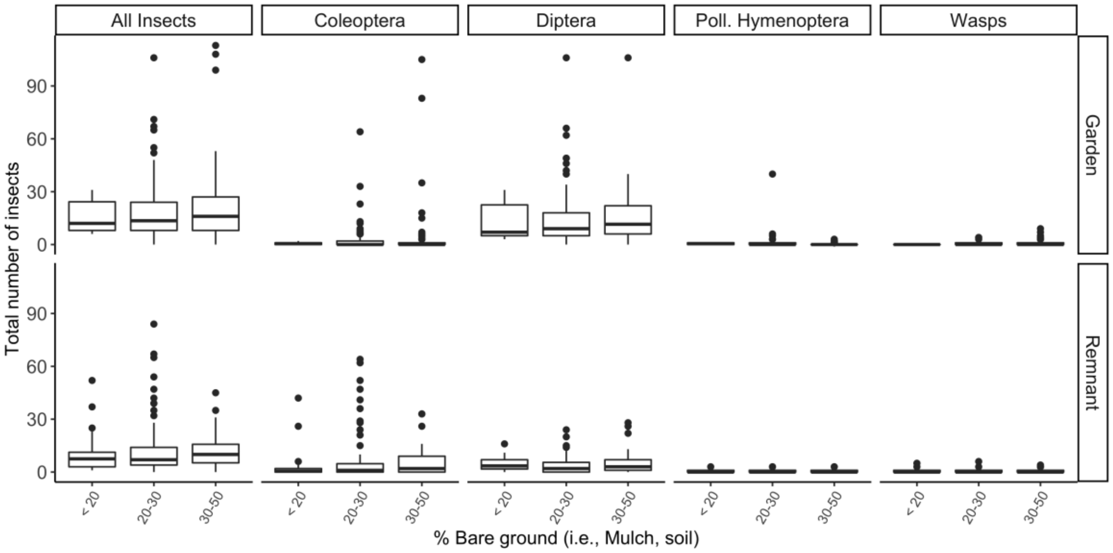
Site Type	Insect Group	Model outputs	Estimate	Standard Error	z value	Pr(>|z|)
Remnant	All insects	Intercept	3.52	0.17	21.01	<2e-16***
		Any vegetation within 500m	0.00	0.00	1.62	0.11
		Canopy cover 25-50%	-0.59	0.26	-2.29	0.02*
	Coleoptera	Intercept	3.52	0.17	21.01	<2e-16***
		Any vegetation within 500m	0.00	0.00	1.62	0.11
		Canopy cover 25-50%	-0.59	0.26	-2.29	0.02*
	Diptera	Intercept	2.44	0.13	18.42	<2e-16***
		Any vegetation within 500m	0.30	0.10	2.86	0.00**
		Distance to remnant	0.17	0.11	1.57	0.12
Garden	All insects	Intercept	4.63	0.22	21.33	<2e-16***
		Hard surface within 500m	0.03	0.08	0.44	0.66
		Urbanisation year 1900-1925	-0.51	0.22	-2.33	0.02*
		Urbanisation year 1925-1955	-0.14	0.20	-0.71	0.48
		Urbanisation year 1955-1975	0.10	0.22	0.45	0.65
		Urbanisation year 1975-1995	-0.61	0.25	-2.43	0.02*
		Urbanisation year Since 1995	-0.99	0.30	-3.27	0.00**
		Garden established 2008-2012	-0.49	0.16	-3.02	0.00**
		Garden established Since 2013	-0.67	0.23	-2.90	0.00**
	Diptera	Intercept	4.05	0.19	21.10	<2e-16***
		Hard surface within 500m	-0.13	0.08	-1.61	0.11
		Garden established 2008-2012	-0.51	0.19	-2.74	0.01**
		Garden established Since2013	-0.51	0.27	-1.89	0.06
		Distance to remnant	0.05	0.08	0.64	0.52
	Pollinating insects	Intercept	0.67	0.37	1.80	0.07.
		Distance to remnant	-1.13	0.43	-2.63	0.01**
		Plant diversity	0.44	0.37	1.19	0.24
	Coleoptera	Intercept	0.39	0.60	0.65	0.52
		Any vegetation within 200m	0.47	0.46	1.01	0.31
		Flowering	0.03	0.02	1.99	0.05*
		WA native plants	-0.46	0.44	-1.04	0.30
	Hymenoptera	Intercept	7.97	3.04	2.62	<0.01**
		Distance to remnant	-1.09	1.41	-0.78	0.44
	Pollinating Hymenoptera	Intercept	0.55	0.34	1.62	0.11
		Distance to remnant	-0.93	0.38	-2.46	0.01*
	Native bees	Intercept	0.17	0.44	0.39	0.70
		Canopy cover 25-50%	0.30	0.78	0.39	0.70
		Canopy cover 50-75%	1.60	1.08	1.49	0.14
		Canopy cover 75-100%	-2.11	1.23	-1.71	0.09
		Distance to remnant	-1.20	0.40	-3.04	<0.00**

Figure S1. Graphs showing the general pattern of insects vs. site characteristics

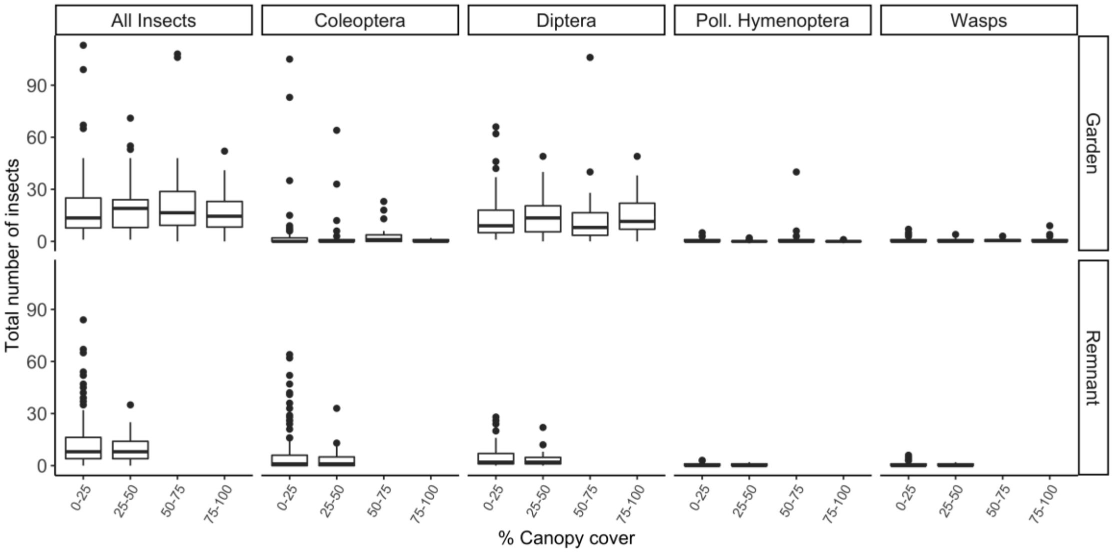
Insect Abundance ~ Urbanisation Year



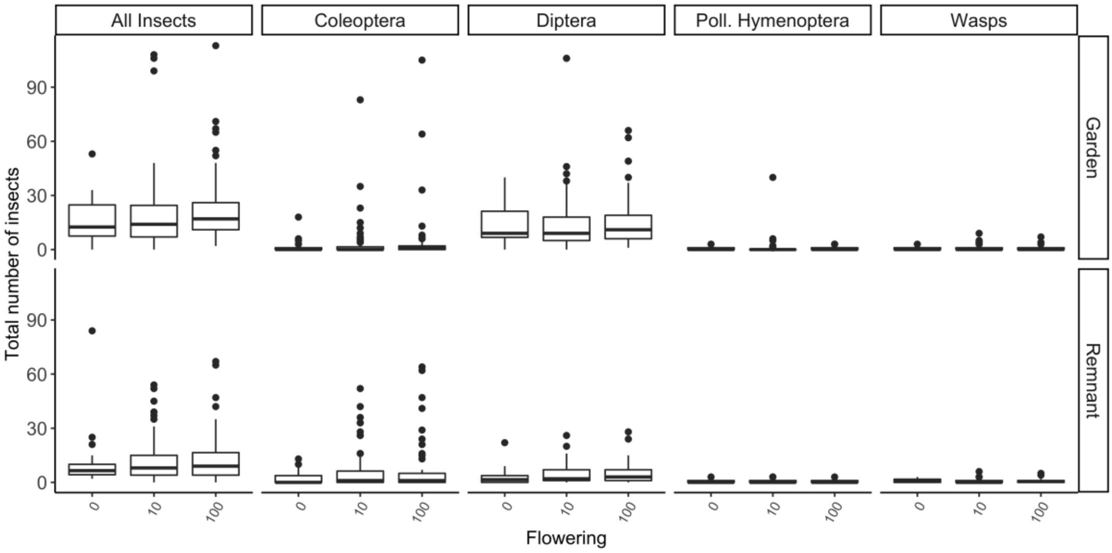
Insect Abundance ~ Bare Ground



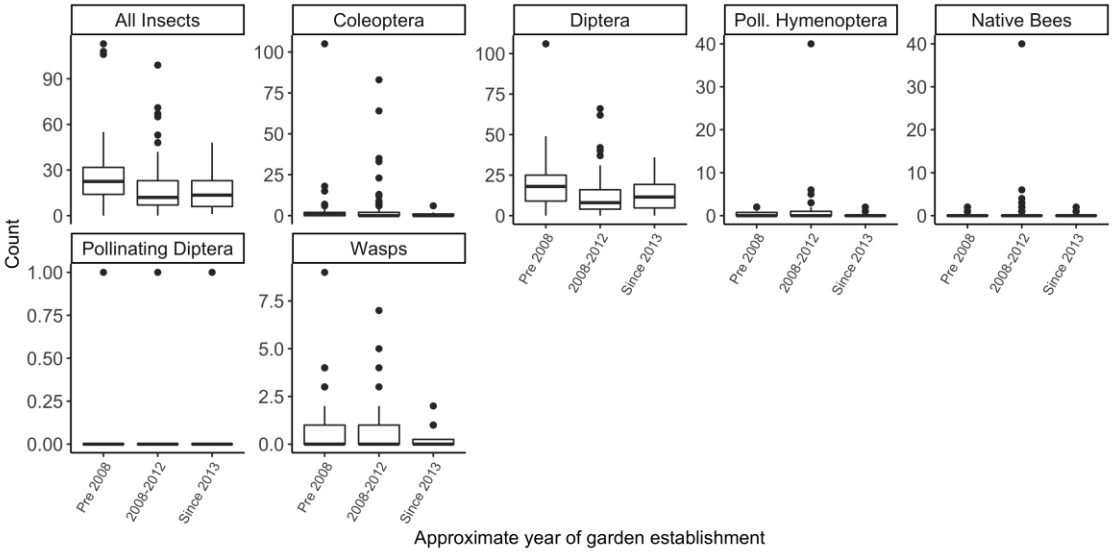
Insect Abundance ~ Canopy Cover



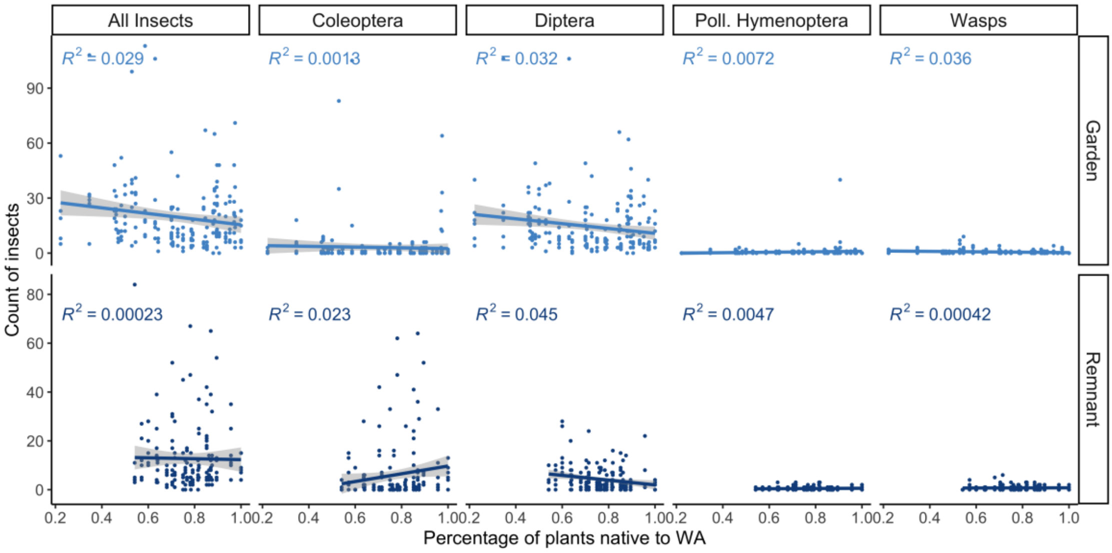
Insect Abundance ~ Flowering



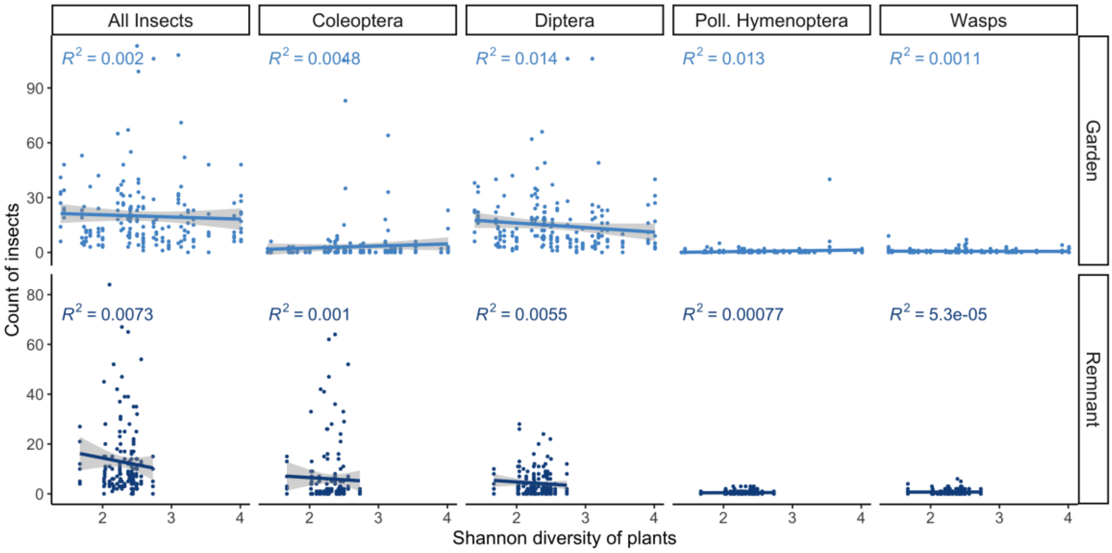
Insect Abundance ~ Garden establishment year



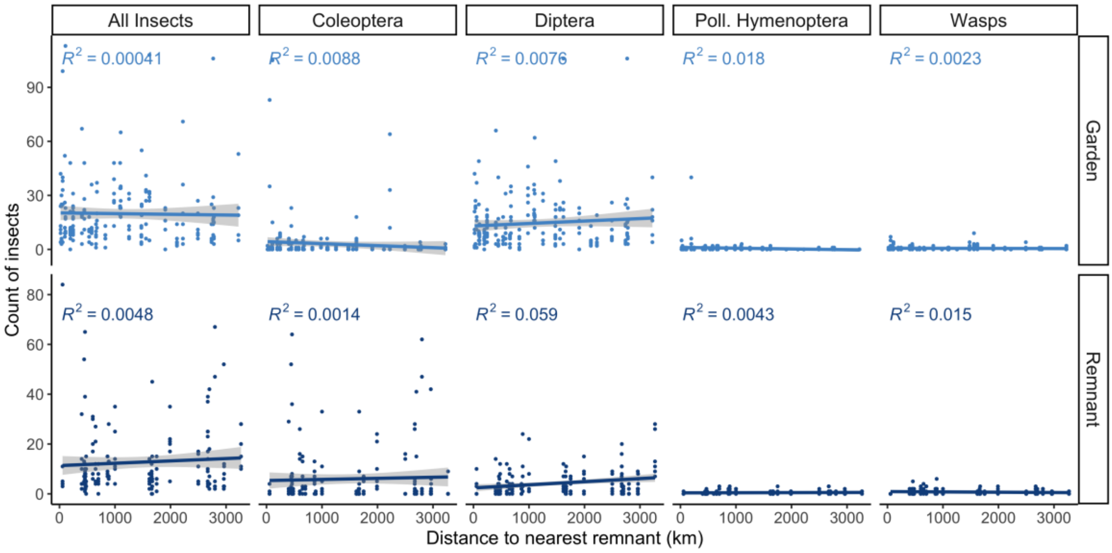
Insect abundance ~ Plant nativeness



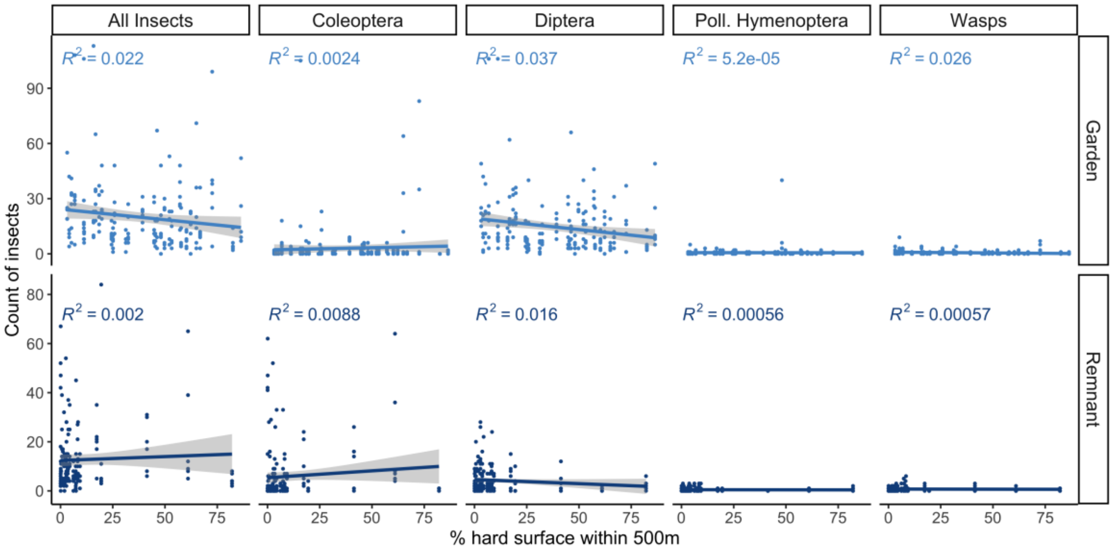
Insect abundance ~ Plant diversity



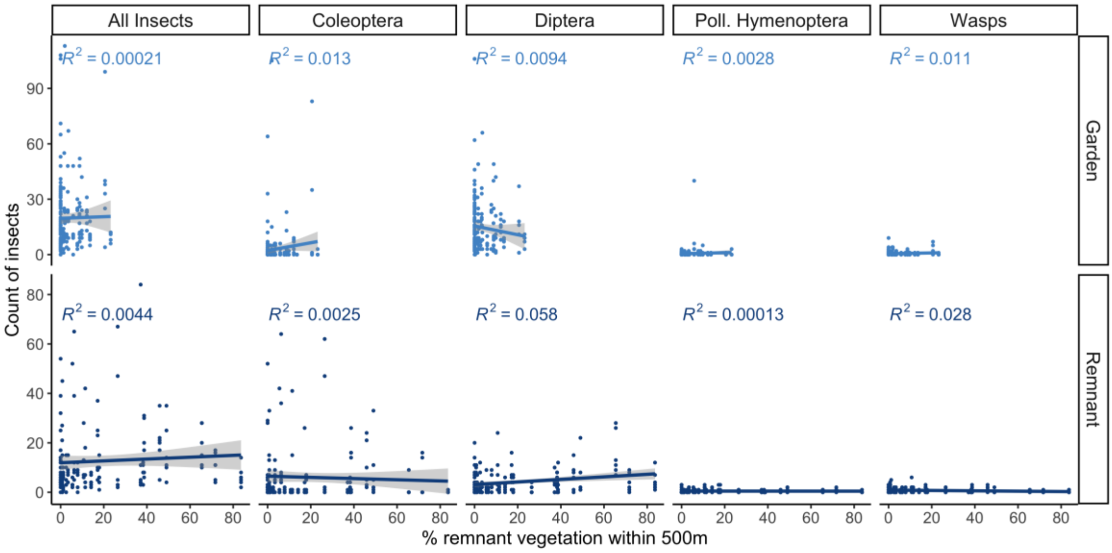
Insect abundance ~ Distance to nearest remnant



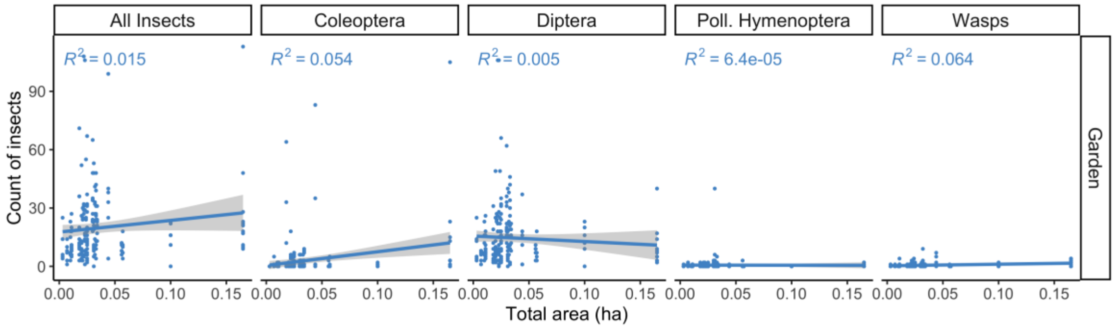
Insect abundance ~ Hard surface



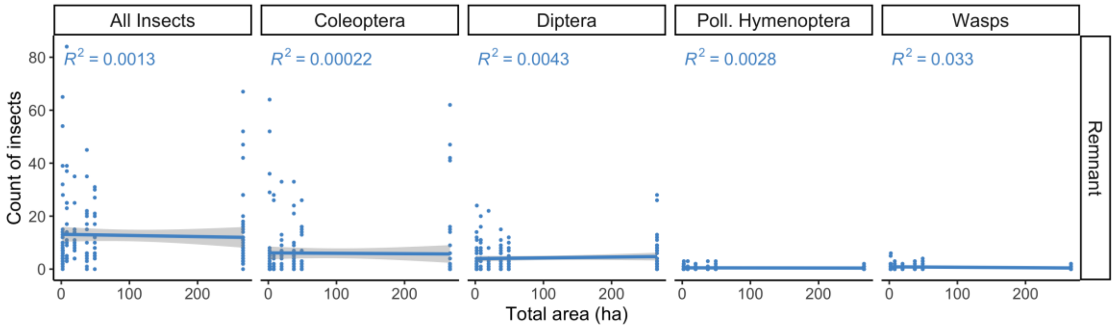
Insect abundance ~ Remnant native vegetation



Insect abundance ~ Garden site size



Insect abundance ~ Remnant site size



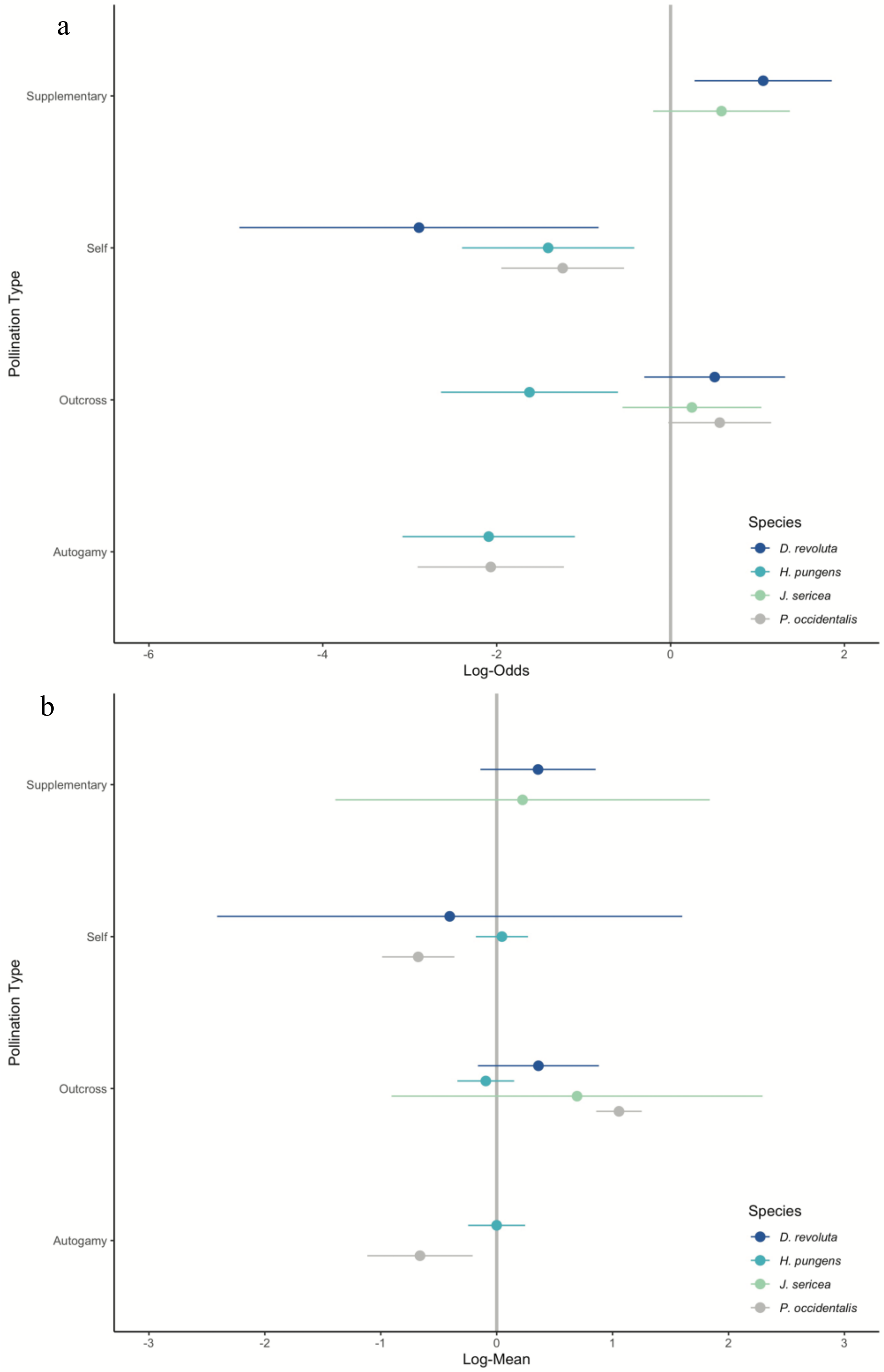


Figure S2 a) Coefficient plot showing log odds of flowers setting fruit and b) log mean number of seeds per fruit, compared to Open pollination from each pollination treatment on each of the four study species which set fruit. Autogamy = flower bagged to test for within-flower pollination, Self = hand pollinated with pollen from the same plant then bagged, Outcross = hand pollinated with pollen from a different plant then bagged, Supp = hand pollinated with outcross pollen, not bagged.

Table S3. Summary of potential pollinator observations for each plant species and approximate flowering totals within a 10 m radius. Observations were carried out in 10-minute intervals.

	Dianella revoluta	*Hemiandra pungens*	*Jacksonia sericea*	*Patersonia occidentalis*	*Tricoryne elatior*
Total observation time (minutes)	650	320	320	1410	390
Number of different days	21	7	10	13	11
Total flowers of focal species observed	659	298	441	772	896
Total number of flowers within 10m radius during observations	2900	860	410	7110	1980
Total visits observed	48	42*	30	33	32
Number of different insects recorded	1	1	1	1	3
Insect species	*Amegilla chlorocyanea* Blue-banded bee ♀	*Apis mellifera* Honey bee	Native bee (Megachilidae family)	*Apis mellifera* Honey bee	*Apis mellifera* Honey bee Hoverfly *Amegilla chlorocyanea* Blue-banded bee ♀

* No insects were observed on the pollination treatment population of *H. pungens* at Nicholson Road.

Table S4. Generalised Linear Mixed Model (GLMM) output for the fruit and seed set for each pollination treatment, compared with open pollination.

	Species	Pollination treatment	Estimate	Std. Error	z value	Pr(>|z|)
Fruit set	*Dianella revoluta*	(Intercept)	-1.20	0.32	-3.77	<0.001***
		Autogamy	NA	NA	NA	NA
		Self	-2.89	1.05	-2.75	<0.01**
		Outcross	0.51	0.41	1.23	0.22
		Supplementary	1.07	0.40	2.65	<0.01**
	Hemiandra pungens	(Intercept)	1.93	0.60	3.23	<0.01**
		Autogamy	-2.09	0.51	-4.14	<0.001***
		Self	-1.41	0.51	-2.79	<0.01**
		Outcross	-1.62	0.52	-3.13	<0.01**
		Supplementary	NA	NA	NA	NA
	Jacksonia sericea	(Intercept)	-1.21	0.30	-4.08	<0.001***
		Autogamy	NA	NA	NA	NA
		Self	NA	NA	NA	NA
		Outcross	0.25	0.40	0.61	0.55
		Supplementary	0.59	0.40	1.46	0.14
	Patersonia occidentalis	(Intercept)	-0.74	0.24	-3.11	<0.01**
		Autogamy	-2.06	0.43	-4.82	<0.001***
		Self	-1.24	0.36	-3.45	<0.001***
		Outcross	0.57	0.30	1.87	0.06
		Supplementary	NA	NA	NA	NA
Seed set	*Dianella revoluta*	(Intercept)	0.41	0.22	1.86	0.06
		Autogamy	NA	NA	NA	NA
		Self	-0.41	1.02	-0.40	0.69
		Outcross	0.36	0.27	1.35	0.18
		Supplementary	0.36	0.25	1.41	0.16
	Hemiandra pungens	(Intercept)	1.17	0.09	12.89	<0.001 ***
		Autogamy	-0.00	0.13	-0.00	1.00
		Self	0.05	0.11	0.39	0.69
		Outcross	-0.09	0.12	-0.76	0.45
		Supplementary	NA	NA	NA	NA
	Jacksonia sericea	(Intercept)	-1.39	0.65	-2.15	0.03 *
		Autogamy	NA	NA	NA	NA
		Self	NA	NA	NA	NA
		Outcross	0.69	0.82	0.85	0.40
		Supplementary	0.22	0.82	0.27	0.79
	Patersonia occidentalis	(Intercept)	1.67	0.10	16.42	<0.001***
		Autogamy	-0.66	0.23	-2.85	<0.01 **
		Self	-0.68	0.16	-4.26	<0.001***
		Outcross	1.05	0.10	10.54	<0.001 ***
		Supplementary	NA	NA	NA	NA

* result is significant at $p<0.05$.
** result is significant at $p<0.01$.
*** result is significant at $p<0.001$.

Table S5. Germination trials for *Patersonia occidentalis* seeds carried out from February to July 2018. C=seeds soaked in DI water for 24 hours, S=seeds soaked in 10 % Regen smoke water for 24 hours, GA=agar contained gibberellic acid, N=seed coat nicked, Str=25°C for the first 8 weeks, S2=aerosol sawdust smoke, S3=aerosol native plant smoke. Substrates were agar in petri dishes or a soil mixture or sand in foil trays. Seeds were incubated at the given temperatures. Grey indicates treatments which produced a germinant.

Substrate and temperature	ID	Treatment	Number of seeds	Proportion germinated	Genetic analysis
Agar 15°C	1	C	12	0.00	No
	2	S	12	0.00	
	3	GA	12	0.00	
	4	GA+S	12	0.00	
	5	C+N	12	0.00	
	6	S+N	11	0.00	
	7	GA+N	12	0.08	
	8	GA+S+N	11	0.00	
	9	C+StrA	12	0.00	
	10	S+StrA	12	0.00	
	11	GA+StrA	12	0.00	
	12	GA+S+StrA	12	0.00	
	13	C+StrGA	12	0.00	
	14	S+StrGA	12	0.08	
	15	GA+StrGA	12	0.25	
	16	GA+S+StrGA	12	0.00	
	17	C+N+StrA	12	0.00	
	18	S+N+StrA	12	0.08	
	19	GA+N+StrA	12	0.17	
	20	GA+S+N+StrA	12	0.08	
	21	C+N+StrGA	12	0.08	
	22	S+N+StrGA	11	0.00	
	23	GA+N+StrGA	12	0.08	
	24	GA+S+N+StrGA	11	0.00	
Agar 15°C	Br1	GA+S2	12	0.00	No
	Br2	S2	12	0.08	
	Br3	GA+S2	12	0.00	
	Br4	S2	12	0.00	
	Cl1	S2	12	0.08	
	Cl2	GA+ S2	12	0.00	
	Cl3	S2	12	0.17	
	Cl4	GA+ S2	12	0.08	
Agar 18°C	Br5	S3	15	0.13	No
	Br6	GA + S3	12	0.08	
	Br7	GA + S3	15	0.07	
	Br9	GA + S3	30	0.00	
	Cl5	GA + S3	15	0.07	
	Cl6	GA + S3	15	0.20	
	Cl7	GA + S3	12	0.25	
	Br10	S3	10	0.20	
Soil+sand mix 18°C	Br8	S3	20	0.00	
	Cl9	S3	19	0.11	
	Cl8	S3	10	0.10	
Sand only 18°C	ClS	S3	100	0.44	

Substrate and temperature	ID	Treatment	Number of seeds	Proportion germinated	Genetic analysis
Sand only 18°C	p34	S3	50	0.54	Yes
	beside34	S3	40	0.35	
	p86	S3	60	0.52	
	P110	S3	112	0.34	
	P115x	S3	42	0.83	
	p169	S3	154	0.66	
	p164	S3	226	0.80	
	p188	S3	46	0.76	
	p228	S3	122	0.87	
	p231	S3	48	0.65	
	p239	S3	24	0.79	
	p260	S3	77	0.75	
	p262	S3	42	0.64	
	p275	S3	119	0.41	
	np275	S3	66	0.64	
	p299	S3	54	0.56	
	p301	S3	91	0.67	
	301E	S3	49	0.39	
	p307	S3	40	0.10	
	p316	S3	97	0.27	
	p326	S3	26	0.50	
	p341	S3	29	0.10	
	M5	S3	40	0.55	
	27.12	S3	26	0.27	

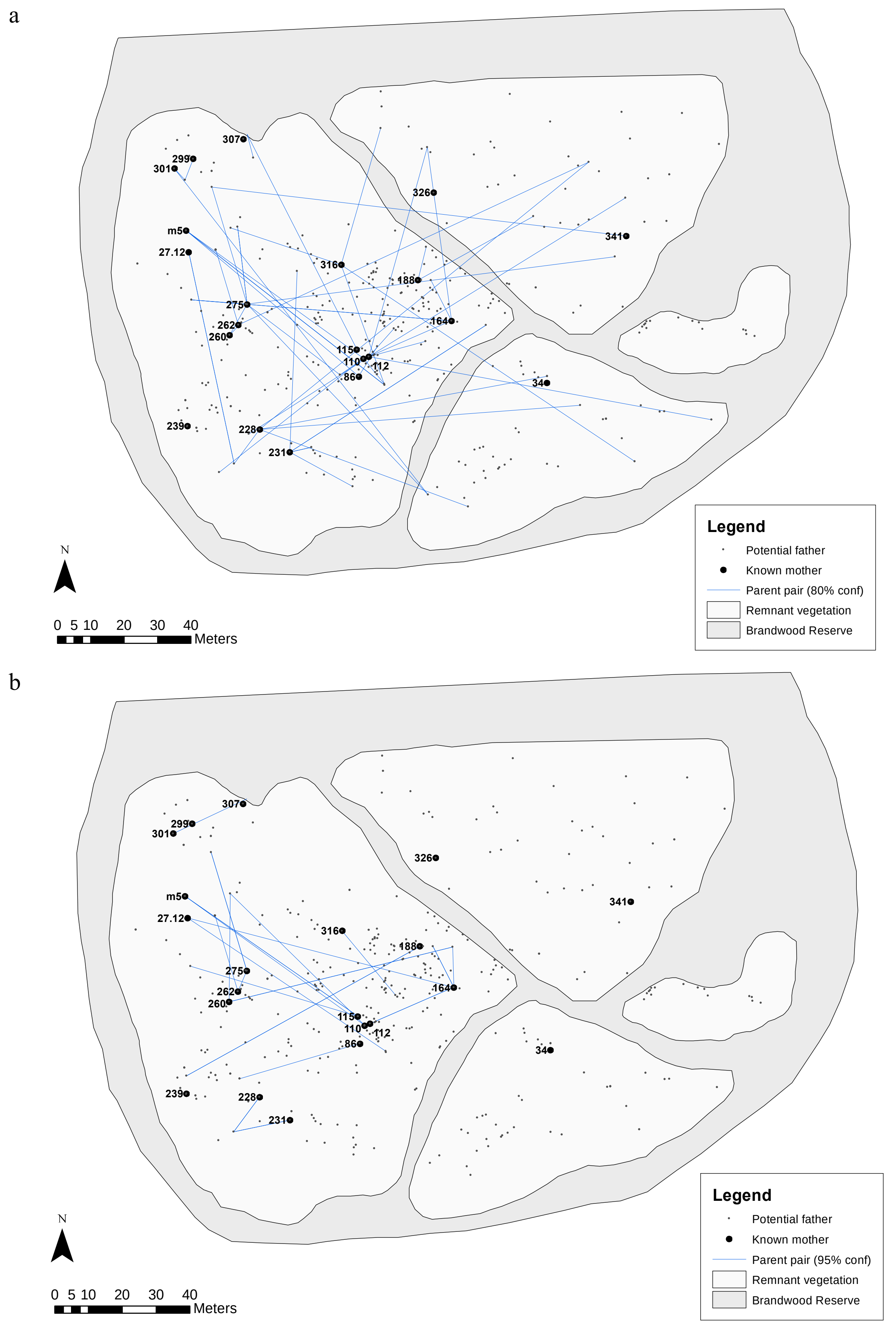


Figure S3. Inferred pollen dispersal events (based on paternity assignment) between known maternal adult and most likely sire at 80 % (a) and 95 % (b) confidence.

Table S6. Characterisation of six microsatellite loci in a population of *Patersonia occidentalis*. Details are provided for locus name, primer sequence, repeat motif and allele size for adult individuals.

Locus	Primer sequence	Primer sequence (R)	Repeat motif	Allele size range
Po02	F: CTTCACGCCCAGCAACAAA	R: GCAGATTTCACTTCCGCCAC	$(AG)_{13}$	102–124
Po06	F: TCCGGTCGGACTCTTCCTAG	R: GCCACTGCCTTCTCCATGG	$(TC)_6$	117–125
Po10	F: CCCAACAGTACCATGATTCTGC	R: AAACGACGGTCTTGCTTCCT	$(AAAT)_6$	152–168
Po11	F: TTGCTGCTAGCCAATTGTGA	R: TTGTCGCTACATGGTATTCGT	$(TC)_{16}$	164–200
Po18	F: TGGATGCACTGAGACTGAAGG	R: TAGTCCCACATTGGAAGCCC	$(GT)_5$	324–380
Po23	F: CAACTGCAGGAGACTCAGCT	R: TGAGAACACGGATGGCACG	$(AGA)_5$	286–292