Flinders University

Spatio-Temporal Variability in Plant Reproduction Across a Fragmented Landscape of Southern Australia

By

Alex Blackall

Bachelor of Science (Honours)

Thesis Submitted to Flinders University

for the degree of Doctor of Philosophy

Doctor of Philosophy

College of Science and Engineering

10/01/2023

Photos on Title page: **Left**, *Pultenaea daphnoides* J.C.Wendl. **Middle**, *Hibbertia exutiacies* N.A.Wakef. **Right**, *Stackhousia aspericocca* Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker

TABLE OF CONTENTS

THESIS STRUCTURE

Following the general introduction (Chapter One), which defines the overarching research aim and hypotheses, the empirical research in this thesis is presented as a series of chapters written in a manuscript format (Chapter Two, Three, Four and Five). Supplementary material is provided at the end of each of these chapters. The general discussion (Chapter Six) connects the findings of the previous four empirical research chapters to the overarching research aim and hypotheses defined in Chapter One, and discusses the implications of this research for conservation more broadly.

All chapters within this thesis were written by me. However, because their significant advice and input into this research my supervisors, Duncan Mackay and Molly Whalen, are co-authors of the four empirical chapters (Chapter Two, Three, Four and Five).

Chapter Two *- Reproductive Output of a Common Sclerophyllous Shrub, Pultenaea daphnoides J.C.Wendl. (Fabaceae) across a range of reserve sizes.*

I came up with the original research design (80 %), conducted the fieldwork, lab work, statistical analyses (90 %), and wrote the initial manuscript (80 %).

Chapter Three *- Spatial and Temporal Variability in Pollen-Limitation and Pre-Dispersal Seed Predation of a Common Sclerophyllous Shrub within a Fragmented Landscape.*

I came up with the original research design (80 %), conducted the fieldwork, lab work, statistical analyses (90 %), and wrote the initial manuscript (80 %).

Chapter Four *- Soil Moisture Limits Fruit-Set of Hibbertia exutiacies N.A.Wakef. (Dilleniaceae), a Pollen-Limited Sclerophyllous Shrub that Suffers Extensive Pre-Dispersal Seed Predation.*

I came up with the original research design (80 %), conducted the fieldwork, lab work, statistical analyses (90 %), and wrote the initial manuscript (80 %).

Chapter Five *- Impact of Reserve Area on Reproduction of a Moth-Pollinated Stackhousia Sm. (Celastraceae) Species in a Fragmented Landscape.*

I came up with the original research design (80 %), conducted the fieldwork, lab work, statistical analyses (90 %), and wrote the initial manuscript (80 %).

ABSTRACT

The research in this thesis explores spatio-temporal variability in pollination and reproduction of three plant species with contrasting pollination systems across a range of reserve areas protecting plant populations within a fragmented landscape of southern Australia. In particular, plants were chosen with ecological traits that have not usually been displayed by plants included in previous studies of landscape disturbance. Thus, this research examined three common species rather than the rare and threatened species which are more typically studied. This research also included the study of a plant species with high levels of insect pre-dispersal seed predation, which is an understudied biotic interaction in fragmented landscapes, and finally this research included a nocturnally moth-pollinated species whereas most studies have focused on plants with diurnal rather than nocturnal pollinators.

There was no evidence of pollen-limitation of reproduction of the papilionaceous legume *Pultenaea daphnoides* J.C.Wendl. (**Chapter Two**) in the two years studied. This may have been due to the relative diversity of bee visitors to flowers of *P. daphnoides*, including the introduced honeybee, *Apis mellifera*. However, viable seed production was mostly limited to the smallest reserves assessed in 2018, with almost complete abortion of viable seeds in larger reserves. This striking result occurred in a year of historically low early spring rainfall and highlights the possibility that smaller reserves may maintain at least some viable seed production across fragmented landscapes in particular years. However, plants within both small and large reserves were both successful at producing viable seeds in the previous year of 2017, and thus reserve area was not a consistent predictor of reproductive success.

In contrast to *P. daphnoides*, fruit production of the relatively more specialized buzz-pollinated *Hibbertia exutiacies* N.A.Wakef. (**Chapter Three**) was significantly pollen-limited in 2017, perhaps suggesting the more specialized pollination system of *H. exutiacies* increases the risk of reproductive failure. Viable seed production in 2017 was also positively related to the amount of native vegetation in the landscape surrounding sample sites, which was not necessarily related to reserve area, and this positive relationship appeared to be largely due to spatial variation in this species' high level of insect pre-dispersal seed predation. However, similar to *P. daphnoides*, *H. exutiacies* displayed almost complete abortion of seed development in the following year of 2018. An experimental field study of two populations in 2019 demonstrated that water stress may limit reproduction in particular years for some populations (**Chapter Four**).

In comparison to *H. exutiacies* and *P. daphnoides*, reproduction of the nocturnally moth-pollinated *Stackhousia aspericocca* Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker (**Chapter Five**) was comparable between smaller and larger reserves in 2018 and 2019 and there was no significant difference in reproduction between years.

Regarding the conservation of common plants within fragmented landscapes, the findings of this thesis do not suggest the studied plant species are at greater risk of reproductive failure within smaller reserves versus larger reserves. Nevertheless, the idiosyncratic responses of the three species studied emphasises the importance of continued empirical studies of pollination and plant reproduction within fragmented landscapes.

DECLARATION

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Alex Blackall
Signed..

10/01/2023
Date..

ACKNOWLEDGEMENT

First, I would like to thank my two longest standing supervisors in Duncan Mackay and Molly Whalen. In particular, I would like to thank Duncan for his statistical expertise and for introducing me to R, and Molly for her botanical knowledge and enthusiasm for plants, all of which greatly improved this research.

Second, I would like to thank my third supervisor in James Stangoulis. Your background and knowledge of plant physiology has given me a greater appreciation for the multiple complexities of plant reproduction. I would also like to thank Duncan, Molly, James, and my colleague in Kieren Beaumont for giving me casual demonstrating and marking in their various undergraduate topics over the last few years. I have greatly enjoyed and learned from this experience.

Third, I would like to thank Katja Hogendoorn and Mike Schwarz for reviewing chapters and listening to a number of presentations throughout my candidature. In particular, I would like to thank Katja for helping with the identification of native bees (**Chapter Two** and **Three**). I also would like to thank both Bill Barker and Hellmut Toelken of the State Herbarium of South Australia for their discussions regarding the plant genus of *Stackhousia* (**Chapter Five**) and *Hibbertia* (**Chapter Three** and **Four**), respectively.

Fourth, I would like to thank both the Department for Environment and Water and the National Trust of South Australia for giving me permission to conduct research within their conservation reserves.

Fifth, I would like to thank those students who volunteered their time in the field. A special mention to Immi Marshall and Mielle Kastelanac who volunteered on multiple occasions.

Sixth, I would like to thank my two former housemates and good friends in Jade Puletama and Jesse Farrand-Harbutt. Our regular knock off drinks, although perhaps sometimes overindulgent, definitely helped to keep me sane, particularly during the initial turmoil of COVID-19.

Lastly, I would like to thank all my friends and family for supporting me throughout this academic venture. This includes my family dogs in Woody, who is currently asleep on my bed, and Cooper, who very sadly passed away during the final stages of writing this thesis, he will be forever missed. However, the largest thanks must be made to my parents in Greg and Susan Blackall, both of whom have encouraged me from beginning to end. My dad, a man more in tune with the world of construction and mechanics, helped me in the field on a number of occasion and even captured the moth pictured in Fig. 5-3d in Chapter Five. My mum has always (from the moment of birth to this very second) supported and encouraged me, even while I finished the seemingly never-ending final edits of this work, and it is to her that I dedicate this thesis.

This research was supported by the **Australian Government Research Training Program Scholarship**.

CHAPTER ONE

1. GENERAL INTRODUCTION

Landscape disturbance (here used to describe the combined effects of habitat loss and fragmentation and other interrelated processes (e.g., edge effects)) is considered one of the major drivers of global declines of insect abundance and diversity (Sánchez-Bayo and Wyckhuys 2019; but see Simmons et al. 2019). Thus, within many fragmented regions of the world, the abundance and diversity of insect pollinators have declined (e.g., see Biesmeijer et al. 2006; Pauw 2007; Goulson et al. 2008; Potts et al. 2010; Ollerton 2017). Similarly, although a smaller number of vertebrates act as pollinators (Ollerton 2017), vertebrate pollinators have declined globally (Regan et al. 2015). Given the majority of the world's flowering plants are pollinated by animals (Ollerton et al. 2011), pollinator declines, potentially in conjunction with the plants they pollinate, are of serious conservation and economic concern. Indeed, a review of the contribution of animal pollinators to plant reproduction estimates an absence of pollinators would result in a third of plant species producing no seeds, and reproduction of half of all plants globally would decline by 80.00 % or more (Rodger et al. 2021). Worryingly, biotic pollination also appears to have a greater susceptibly to anthropogenic disturbance than other plant-animal interactions, such as seed predation and herbivory (Neuschulz et al. 2016). Thus, perhaps unsurprisingly, biotic pollination is often detrimentally affected by habitat loss and fragmentation (e.g., reviewed by Aizen et al. 2002; Hobbs and Yates 2003; Harris and Johnson 2004; Aguilar et al. 2006; Hadley and Betts 2012).

However, although pollination may be negatively impacted, declines in pollination (i.e., floral visitation rate, pollen tube numbers, etc.) do not necessarily translate into declines in plant reproduction (e.g., see the multi-species study by Aizen and Feinsinger 1994a). Similarly, an observed decline in plant reproduction does not necessarily result from an equivalent decline in pollination (e.g., reduced seed output can occur due to increased levels of pre-dispersal seed predation; Matesanz et al. 2015). Moreover, landscape disturbance does not inevitably result in a negative impact on the pollination and reproduction of all plant species in a landscape (Yates and Ladd 2005), and some plant species may perform relatively better within fragmented landscapes (Cunningham 2000a). Thus, the impact(s) of landscape disturbance on pollination and plant reproduction can still be somewhat idiosyncratic, and empirical studies remain important in understanding the reproductive changes that occur to plants within fragmented landscapes (Hobbs and Yates 2003; Broadhurst and Young 2007).

In the following general introduction to this thesis, I first define habitat loss and fragmentation before briefly reviewing causes as to why pollination and plant reproduction are negatively impacted by landscape disturbance, and highlight remaining gaps and biases in our understanding of these impacts. Subsequently, I describe the study region, review past studies relevant to pollination and plant reproduction in this locality, and describe the study species. Lastly, I summarise why this study will help to address gaps and biases in the literature and define the overarching aim of this research.

1.1 Habitat Loss and Fragmentation

The clearance of native vegetation typically, but not necessarily (e.g., see Fahrig 2019), results in the subdivision of remaining habitat into increasingly smaller and more isolated patches (Saunders et al. 1991; McIntyre and Hobbs 1999; Fischer and Lindenmayer 2007). Thus, habitat loss (a process) results in a landscape in which once contiguous vegetation is now fragmented (a pattern). Such fragmented landscapes are recognized by their strong contrast between remaining fragments of vegetation and the surrounding matrix, the matrix being defined as the dominant patch type in the surrounding landscape (e.g., urbanised areas, farmland, etc. Saunders et al. 1991; McIntyre and Hobbs 1999; Fischer and Lindenmayer 2007).

Although typically confounded, the ecological consequences of habitat loss and fragmentation per se, defined as the subdivision of habitat into smaller patches independent of habitat loss, may be considerably different (see review by Fahrig 2017). However, within the literature on the effects of habitat disturbance on plants and their pollinators, few studies have separately partitioned the effects of habitat loss and fragmentation (e.g., only six studies out of the 303 reviewed by Hadley and Betts 2012). Regardless, these studies still demonstrate that landscape disturbance (i.e., the combination of habitat loss and fragmentation) has an overall negative influence on both the pollinators and the pollination dynamics of plants (Aizen et al. 2002; Hobbs and Yates 2003; Harris and Johnson 2004; Aguilar et al. 2006; Hadley and Betts 2012). The few studies that have separated the effects of habitat loss and fragmentation also suggest negative consequences to plants and their pollinators via fragmentation alone (e.g., see Farwig et al. 2009 and additional studies in Hadley and Betts 2012). Here, to avoid further confusion, I have consistently used the terms habitat loss and fragmentation in combination or alternatively supplemented both terms with landscape disturbance. Likewise, here the terms fragmented habitat or landscape simply refer to a habitat or landscape under the influence of landscape disturbance.

1.2 Why Does Pollination and Plant Reproduction Decline?

1.2.1 Pollinators

Pollinator abundance and/or diversity may decline in smaller and/or more isolated vegetation fragments, leading to reduced visitation by pollinators to flowers and/or less efficient pollination by ineffective pollinators. This could be due to a decline and/or loss of pollinator populations within the fragments themselves (Aizen and Feinsinger 1994b), particularly when fragment-specific resources used by pollinators are lower in fragments of smaller area (Steffan-Dewenter 2003), or pollinators may avoid smaller and/or more isolated vegetation fragments versus larger and/or less isolated fragments (e.g., gap avoidance in some hummingbird species; Hadley et al. 2014). For example, an early study by Jennersten (1988) found that reproduction of the butterfly-pollinated herb, *Dianthus deltoides* (Caryophyllaceae), was pollen-limited (i.e., plant reproduction is constrained by the quantity and/or quality of pollen received) within two small habitat fragments of 0.15 ha and 0.32 ha (both embedded in a largely agricultural landscape) but not in a relatively larger fragment of 1.00 ha (surrounded by a mixture of meadows, cultivated and uncultivated fields, and forests) in southwestern Sweden. This difference was attributed, at least in part, to the lower visitation rate by pollinators to the flowers of *D. deltoides* in the smaller sized fragments. Indeed, even when pollinators can seemingly reach smaller fragments of vegetation and/or plant populations when foraging within the landscape, pollinators may still preferentially visit those larger fragments and/or plant populations which contain a greater availability of floral resources and this differential visitation may, in turn, result in lower levels of plant reproduction in those less visited smaller fragments and/or plant populations (Groom 1998; Ward and Johnson 2004). However, the relationship(s) between measures of fragment area and isolation, floral abundance, and pollinator visitation can be complex, and measures of pollinator visitation and plant reproductive output are not always significantly related to one another within fragmented landscapes (Lázaro et al. 2020). Thus, studies which examine the impact(s) of landscape disturbance on pollinator visitation alone are inherently limited in what they can conclude about the impact(s) on subsequent plant reproduction.

On a larger scale than that studied by Jennersten (1988), Hadley et al. (2014) observed a 40.00 % increase in seed production for the common, hummingbird pollinated herb *Heliconia tortuosa* (Heliconiaceae), across a gradient of areas of forest fragments ranging from 0.64 to 1300 ha in premontane tropical forest within Costa Rica. The availability of pollinating hummingbirds increased along this same gradient and was positively correlated with the seed production of *H. tortuosa*, suggesting seed production was limited in smaller forest fragments by lower access to the plant's pollinators (Hadley et al. 2014). In particular, the increased availability of pollinating hummingbirds in larger forest fragments was likely due to the gap avoiding behaviour of this plant species' pollinators, which avoid crossing gaps in forest cover as little as 30 m, highlighting the significant role that larger sized fragments of native vegetation may play for plant species with pollinators that otherwise tend to avoid dispersing through the surrounding matrix to either forage within and/or migrate to other vegetation fragments (Hadley et al. 2014). Pollinator size may also limit dispersal and/or foraging ability and plants in more isolated vegetation fragments and/or plant populations may be visited by larger pollinators on average (Steffan-Dewenter and Tscharntke 1999), although the impact(s) of such changes in the average size of visiting pollinators on pollination and in turn plant reproduction will be plant species-specific.

Altered communities of pollinators may also lower plant reproduction. For example, Delnevo et al. (2020) found that fruit-set increased with population size for the threatened shrub *Conospermum undulatum* (Proteaceae), within an urbanised area of southern Western Australia. This was due, at least in part, to smaller populations of *C. undulatum* having a lower diversity of weakly effective pollinators, including the total absence of the efficient specialized bee pollinator *Leioproctus conospermi* (Colletidae) (Delnevo et al. 2020).

In contrast, for those plant species pollinated by a relatively wider range of floral visitors (e.g., generalist-pollinated eucalypts; Ottewell et al. 2009), or plants pollinated by animals able to effectively utilise the modified matrix as habitat (e.g., highly mobile, dietary generalist, hawkmoth-pollinators; Skogen et al. 2016), pollination may be relatively unimpacted by landscape disturbance. Thus, for the generalist insect-pollinated endangered Australian shrub *Verticordia fimbrilepis* ssp. *fimbrilepis* (Myrtaceae), which is visited by a wide range of insect pollinators, smaller populations had similar or higher rates of floral visitation, pollination, and seed production relative to larger populations (Yates and Ladd 2005).

The degree of specialization by a plant on its pollinator, and vice versa, has thus been hypothesized as an important determinant of the impact of landscape disturbance on pollination and plant reproduction (Bond 1994). In support, Pauw (2007) demonstrated that the loss of a specialized oil-collecting bee *Rediviva peringueyi* (Melittidae) from small reserves within urban areas of the Cape Floral Region of South Africa resulted in zero seed-set for six orchids specialized on that pollinator. In comparison, seed-set was maintained for the co-flowering plant, *Hemimeris racemosa* (Scrophulariaceae), which is pollinated not only by *R. peringueyi* but also by three smaller species of *Rediviva* (Pauw 2007). Similarly, in the biodiversity hotspot of the Brazilian Atlantic forest, a region with only 11.00 % of original vegetation remaining, the magnitude of pollen-limitation of plant reproduction was greater for species increasingly specialized on fewer pollinators, suggesting a greater vulnerability to reproductive failure (Wolowski et al. 2014). However, both a qualitative review (Aizen et al. 2002) and a quantitative meta-analysis (Aguilar et al. 2006) found no effect of pollinator specialization on the vulnerability of pollination or plant reproduction to landscape disturbance. Thus, when assessed across a large collection of studies, specialization by a plant on its pollinator appeared to be an overall inconsistent predictor of how plant reproduction will respond to landscape disturbance, although both the review and meta-analysis necessarily required a coarse classification of levels of plant-pollinator specialization.

Similarly, although a recent comprehensive meta-analysis by Bennett et al. (2020) found pollen-limitation of plant reproduction was greater for those plant species specialized on a single pollinator versus two or more, there was no difference in the magnitude of this pollen-limitation between wild plants in natural versus managed habitats (i.e., landscapes of mostly agricultural crops, rangelands, and pastures). However, interestingly, pollen-limitation of plant reproduction was significantly greater for wild plants exclusively pollinated by bees in natural habitats versus similarly bee-pollinated plants in managed habitats, possibly as a result of supplementation of pollination by domesticated honeybees in managed habitats lowering the degree of pollen-limitation for these plants (Bennett et al. 2020).

1.2.2 Plant breeding systems

If there is a positive correlation between fragment area and plant population size (although this is not always true (e.g., see Donaldson et al. 2002)), then the genetic neighbourhood of plants growing in smaller fragments may have lower genetic diversity than that of plants growing within larger fragments, resulting in fewer compatible mates for self-incompatible plants, and otherwise possible greater levels of selfing, mating among close relatives (e.g., bi-parental inbreeding), and correlated paternity (see qualitative review by Eckert et al. 2010 and meta-analysis by Breed et al. 2015). For example, Glémin et al. (2008) found a small population of the rare, self-incompatible Mediterranean plant, *Brassica insularis* (Brassicaceae) was mate-limited due to the loss of genetic diversity controlling for self-incompatibly (i.e., low S-allele diversity in small populations reduces the average number of cross-compatible mates for an individual plant). Likewise, Young and Pickup (2010) found small populations of the endangered composite *Rutidosis leptorrhynchoides* a perennial of fragmented grasslands of eastern Australia, were mate-limited by the loss of genetic diversity controlling for self-incompatibly. Moreover, this mate-limitation reduced the seed output of plants even though the number of conspecific outcross pollen grains received by flowers were similar among populations of varying size (Young and Pickup 2010). Thus, landscape disturbance may still reduce plant reproduction even when pollinator services (i.e., flower visitation, outcross pollen transfer to stigmas, etc.) are not evidently impacted. As such, negative impacts on plant reproductive output should not be automatically attributed to negative impacts on their pollinators (e.g., plant reproduction in smaller vegetation fragments and/or populations may be pollen-limited due to the greater receival of genetically incompatible pollen rather than by lower pollinator visitation to plants in smaller fragments and/or populations).

Similarly, although self-compatible plants may not be mate-limited per se, reproductive output may decline if increased inbreeding results in higher seed abortion. Thus, Yates et al. (2007b) found small and fragmented populations of the mixed-mating bird-pollinated shrub *Calothamnus quadrifidus* (Myrtaceae) produced fewer seeds per fruit relative to larger populations in southern Western Australia. This occurred although the composition and visitation rate of bird pollinators to *C. quadrifidus* was unrelated to population size (Yates et al. 2007a). The highly mobile pollinators of *C. quadrifidus* also maintained outcrossing rates within smaller populations and allowed for long-distance pollen dispersal among fragments (Byrne et al. 2007). Indeed, pollinator mobility significantly determines the magnitude of selfing and correlated paternity of plants within fragmented populations (see meta-analysis by Breed et al. 2015). Nevertheless, the lower movement by pollinators between plants within smaller populations still resulted in the greater self-fertilization of ovules in *C. quadrifidus*, with lower seed-set resulting from greater post-zygotic abortion (Yates et al. 2007b). Thus, although the highly mobile pollinators of *C. quadrifidus* maintained outcrossing rates and genetically linked geographically separated populations, and the fitness of progeny within smaller populations was equal to those from larger populations (as measured by seed germinability, seedling mortality, and seedling growth), reproductive output was still reduced within smaller populations via inbreeding (Yates et al. 2007b).

While the results of Yates et al. (2007b) demonstrate that the reproduction of self-compatible plants may nevertheless decline via the negative effect(s) of greater selfing, the reproductive output of self-compatible plant species may be no different between self- versus cross-pollination (Patrick et al. 2018), and for those plants which regularly reproduce via self-fertilization, deleterious alleles which cause inbreeding depression may be otherwise purged from the gene pool (Vandepitte et al. 2010). Hence, even under increased inbreeding, self-compatibility may buffer plant reproduction from declining under landscape disturbance.

The breeding system of plants has thus been hypothesized as an important determinant of the impact of landscape disturbance of pollination and plant reproduction (Bond 1994). A meta-analysis by Aguilar et al. (2006) found that both pollination and reproduction of self-incompatible plants exhibited a greater decline in response to landscape disturbance than self-compatible plants. Moreover, reductions in offspring quality, as measured via offspring germination, growth, and survival, are also on average greater for self-incompatible plants versus self-compatible plants under landscape disturbance (see meta-analysis by Aguilar et al. 2019). Thus, overall, self-compatible plants appear to be less-susceptible to landscape disturbance than self-incompatible plants.

1.3 Gaps and Biases in Landscape Disturbance Studies

While a number of qualitative and quantitative reviews have found generally negative impacts of landscape disturbance on pollination and plant reproduction (e.g., Aizen et al. 2002; Hobbs and Yates 2003; Aguilar et al. 2006), these reviews along with particular studies (e.g., Pauw 2007) highlight gaps and biases that remain in our understanding of these impacts. In particular, the research contained within this thesis relates to the five broad gaps and biases listed below:

1.) Studies of rare and threatened plants, likely vulnerable to landscape disturbance to begin with, have been over-represented in the literature (as recognised by Hobbs and Yates 2003 and Aguilar et al. 2006; see similar viewpoint in Matesanz et al. 2015). However, common plant species, which are typically not the focus of conservation efforts, may be important for maintaining plant-animal interactions and other ecosystem functions throughout fragmented landscapes, which may in turn help support the conservation of threatened species (Gaston 2010; Knight et al. 2018; Cariveau et al. 2020). Thus, any negative impact(s) on common species will likely have more wide-ranging implications (Broadhurst and Young 2007). Conversely, it is possible that some common plant species, abundant across both small and large fragments, may be less impacted by current levels of landscape disturbance. If so, these species may be particularly valuable to current conservation and restoration efforts in fragmented landscapes. Thus, assessing which common plant species are impacted by landscape disturbance is an important topic of research in fragmented landscapes.

2.) Most studies are conducted over a single flowering season (Aguilar et al. 2006). Indeed, the lack of temporal replication of ecological field studies, particularly interannual replication, remains a significant problem for studies of plant-animal interactions (Medel et al. 2018;

CaraDonna et al. 2021), conservation ecology (Vaughn and Young 2010), and field ecology more generally (Filazzola and Cahill 2021). This is problematic given that temporal variability in pollination and plant reproduction are significant factors mediating the impact(s) of landscape disturbance (Hobbs and Yates 2003). For example, the detrimental effects of landscape disturbance may be masked in certain years, due to temporal variation in overriding factors, such as rainfall (e.g., see Morgan 1999).

3.) Many studies measure and find negative impacts on pollination and plant reproduction at the most severe scale(s) of disturbance, such as within small patches of remnant vegetation (Donaldson et al. 2002; Matesanz et al. 2015; Lázaro et al. 2020) or roadside verges (Lamont et al. 1993; Yates et al. 2007b; Llorens et al. 2018a). Thus, it is somewhat unclear how often the negative effects of landscape disturbance manifest themselves in larger conservation areas (e.g., see Cunningham 2000a and Pauw 2007).

4.) The literature has predominantly focused on plants with diurnal pollinators. For example, although moths represent the most diverse group of flower visiting insects globally (Ollerton 2017), and are common nocturnal flower visitors in many plant communities (see reviews by Macgregor et al. 2015; Hahn and Brühl 2016; Buxton et al. 2018), there remains a lack of understanding of the role of these nocturnal flower visitors for pollination (Van Zandt et al. 2020).

5.) Pollination is not the only biotic interaction which may be altered under landscape disturbance and impact plant reproduction (e.g., herbivory, seed predation, etc.). However, such processes, particularly antagonistic interactions, have been understudied in comparison to pollination even though they may directly impact the reproductive output of plants (Hobbs and Yates 2003; Chávez-Pesqueira et al. 2015; Teixido et al. 2022). For example, Cunningham (2000a) observed decreased fruit predation in linear strips of mallee woodland (e.g., roadside verges) versus larger woodland reserves for *Acacia brachybotrya* (Fabaceae) and *Eremophila glabra* (Scrophulariaceae) in New South Wales, Australia. Similarly, within the same study system, Duncan et al. (2004) observed a decline in flower damage by flower feeding beetles on plants of *Dianella revoluta* (Asphodelaceae) positioned at greater distances from a large nature reserve. More isolated populations of the legume *Colutea hispanica* suffered reduced predation of fruits by caterpillars in Central Spain (Rabasa et al. 2009). However, this relationship was evident in only one of three years studied, demonstrating significant spatial and temporal variability in the strength of the antagonistic interaction (Rabasa et al. 2009). Pre-dispersal seed predation by insect larvae was not correlated to population size for the endangered grassland herb *Gerbera aurantiaca* (Asteraceae) within southern Africa (Johnson et al. 2004). Significantly, however, although the seed-set of undamaged inflorescences of *G. aurantiaca* was positively related to population size, once pre-dispersal seed predation was accounted, no increase in final seed output with population size was evident (Johnson et al. 2004).

Ultimately, plant reproduction may be constrained simultaneously by a number of abiotic and biotic factors, all of which may vary spatially and temporally in their influence, and thus multi-year studies across a number of populations are essential to understanding the spatio-temporal dynamics of plant reproduction.

1.4 Study System: Southern Mount Lofty Ranges (SMLR)

The Southern Mount Lofty Ranges (SMLR) is an often-overlooked region of floristic diversity within Australia (Crisp et al. 2001). Located in the state of South Australia, Australia, on the Fleurieu Peninsula (Fig. 1-1a), the SMLR is composed of a series of hills rising to the south of the Gawler River (~32 km north of the city of Adelaide), following the peninsula to its eventual end (Armstrong et al. 2003; Fig. 1-1b). The highest point, Mount Lofty (720 m), lies to the east of the City of Adelaide (Fig. 1-1b), where the ranges rise steeply along a western escarpment before slowly descending as an elevated plateau to the east (Armstrong et al. 2003). The SMLR constitutes an area of approximately 6282 km^2, representing only 0.60 % of the land area of South Australia, and 0.08 % of Australia, respectively (Armstrong et al. 2003). Due to the increased rainfall associated with the ranges, the region as a whole is considered an island of temperate woodlands and forests, separated from similar regions in Australia by the sea and relatively drier environment(s) that surround it (Armstrong et al. 2003). As such, a number of plant and animal species common to the temperate woodlands and forests of Australia's eastern states, naturally exist as isolated populations within the SMLR (Armstrong et al. 2003).

1.4.1 Adelaide Hills

The impact(s) of landscape disturbance on pollination and plant reproduction were specifically examined in a peri-urbanised section of the SMLR known as the Adelaide Hills (Fig. 1-2). This region is representative of a typically fragmented landscape (a landscape where remaining native vegetation cover is between 10-60 %, as defined by McIntyre and Hobbs (1999)). Nevertheless, compared to other regions of the SMLR, the Adelaide Hills is still relatively well vegetated, reflective of the large remaining areas of native vegetation along the Adelaide Hills western escarpment (Armstrong et al. 2003; Fig. 1-1b, 1-2, 1-3). The predominant matrix surrounding reserves in the Adelaide Hills is a mixture of residential housing (Fig. 1-4a) and dryland agriculture and orchards (Fig. 1-4b, d-e). Larger reserves in particular can have a mixture of matrix types surrounding their boundaries. Additional native vegetation of varying degrees of quality also occurs outside reserve boundaries (Fig. 1-4c, f). The boundaries of many reserves have hard edges (e.g., Fig. 1-4d-e). However, in other instances, reserve boundaries are not as sharply defined (e.g., Fig. 1-4f). These so-called soft edges represent a modification gradient more typical of variegated landscapes (McIntyre and Hobbs 1999). Nevertheless, within the Adelaide Hills, distinct fragments of native vegetation can still be defined within an anthropogenically modified landscape (Fig. 1-2).

1.4.2 Vegetation clearance in the SMLR

Extensive clearance of native vegetation has occurred throughout the SMLR following European settlement (Lothian and Harris 2014). Thus, with only 13.00 % of the original native vegetation remaining, the present plant communities occur in fragments of various sizes, isolation, and levels of degradation (Armstrong et al. 2003; Fig. 1-2, 1-4a-b). Significantly, removal of native vegetation has not been random with respect to vegetation type, with the majority of remnant fragments associated with plant communities typical of nutrient poor soils (Armstrong et al. 2003). Thus, dry sclerophyll forest and woodlands (e.g., of messmate stringybark (*Eucalyptus obliqua* (Myrtaceae))) are over-represented in the present-day remnant vegetation (Armstrong et al. 2003). In contrast, savannah woodlands (e.g., of blue gum (*Eucalyptus leucoxylon*)) which were preferentially cleared for agriculture, are now under-represented (Armstrong et al. 2003). Present day native vegetation fragments are also commonly regrowth, and many have a long history of grazing by livestock (Armstrong et al. 2003). As Armstrong et al. (2003 p. 6) states “there is virtually no vegetation in the SMLR which not been altered since European settlement”.

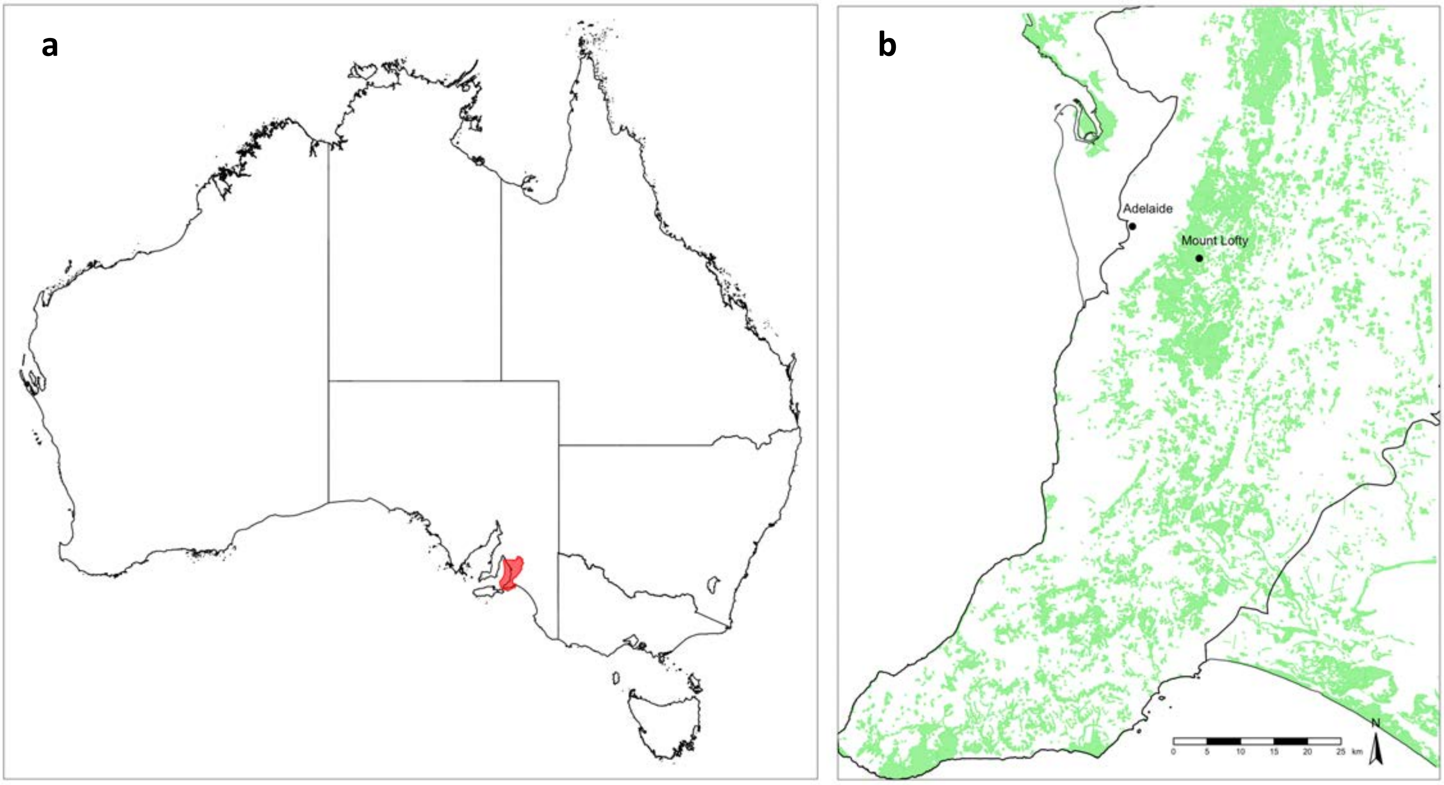


Fig. 1-1 (**a**) Map of Australia with borders of mainland states and territories. The natural resources management region for Adelaide and the Mount Lofty Ranges (more recently (01/07/2020) superseded by the separate landscape regions of Green Adelaide and the Hills and Fleurieu) has been highlighted in red. (**b**) Distribution of remaining native vegetation, highlighted in green, within the Southern Mount Lofty Ranges (SMLR), delimitated by the boundaries of the Fleurieu and Mount Lofty Ranges subregions defined by the Interim Biogeographic Regionalisation for Australia. The location of Adelaide, the capital city of South Australia, and Mount Lofty (720 m), the highest point within the SMLR, are given. The scale bar represents a total distance of 25 km. Native vegetation layer assembled by Department for Environment and Water (DEW) (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898). Maps constructed in the program R (R Core Team 2020) via the R package ‘tmap’ (Tennekes 2018).

Fig. 1-2 Native vegetation fragments remaining in the Adelaide Hills highlighted in green. This vegetation layer captures areas of remnant *Eucalyptus* forest and woodland, and other less prevalent plant communities present across public reserves and private land in the Adelaide Hills. However, note that not all native vegetation is necessarily captured by this dataset (e.g., native vegetation along roadsides, clumps of paddock trees, etc.). Data accessed via NatureMaps. Native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

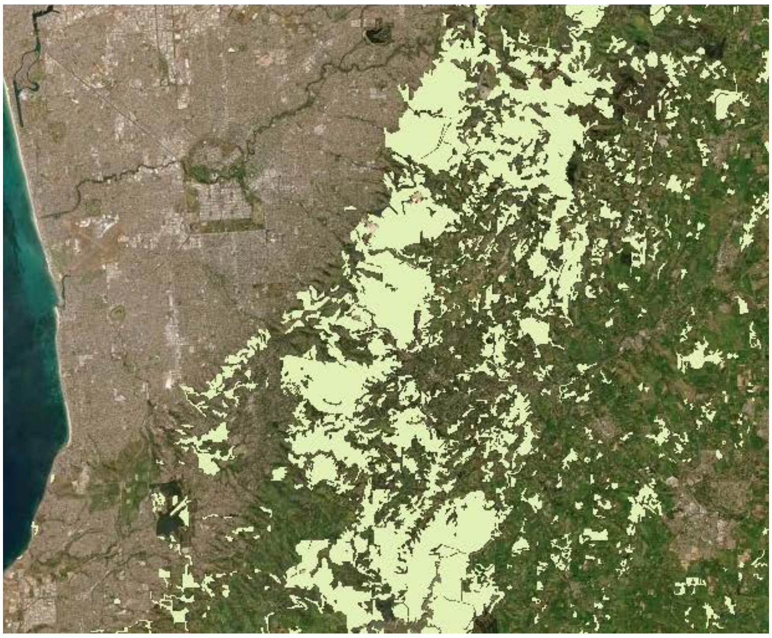


Fig. 1-3 A section of the western escarpment of the Adelaide Hills as viewed from Pakapakanthi, part of the Adelaide Park Lands which surrounds the Adelaide central business district. The summit of Mount Lofty (720 m), the highest point within the SMLR, is marked by an arrow.

Fig. 1-4 Surrounding matrix and edges of reserves in the Adelaide Hills. (**a**) Matrix surrounding The Knoll Conservation Park (CP). The matrix includes a number of residential homes intermixed with non-native and native vegetation and dryland agriculture. Data accessed via NatureMaps. (**b**) Matrix surrounding Kenneth Stirling CP, a reserve system made up of four independent reserves of native vegetation. Shown here are the reserves of Whites Scrub (smaller reserve) and Filsell Hill (larger reserve). The matrix includes dryland agriculture and orchards intermixed with non-native and native vegetation. Data accessed via NatureMaps. (**c**) Dirt road which marks the boundary of Nurrutti Reserve, located on the left-hand side. Note that some native vegetation is also present on the right-hand side, growing along roadside verges and the boundaries of private residential properties. (**d**) Part of the western boundary of Mark Oliphant CP. The private adjacent land is currently used for cattle grazing. Both (**e**) and (**f**) show a section of the southern boundary of Giles CP. (**e**) Private adjacent land currently used for dryland agriculture. (**f**) Private adjacent land with native vegetation. This area was burned in 2016 as part of a planned prescribed burn.

1.4.3 Landscape disturbance, pollination and plant reproduction in the SMLR

- ***Bird-pollination***

Most current evidence for an effect of landscape disturbance on pollination and plant reproduction in the SMLR comes from studies of bird-pollinated plants in southern Australia. The non-random clearance of native vegetation throughout the SMLR and southern Australia overall has had profound consequences for populations of nectarivorous birds (Paton 2000; Ford et al. 2001; Paton et al. 2004). Within southern Australia, bird-pollinated plants growing on poorer quality soils (e.g., dry sclerophyll forest and woodlands) typically flower throughout winter and spring, providing a food source for nectarivorous birds over this period (Paton 2000; Ford et al. 2001; Paton et al. 2004). In comparison, bird-pollinated plants growing on better quality soils (e.g., savannah-type woodlands) typically flower and provide nectar throughout summer and autumn (Paton 2000; Ford et al. 2001; Paton et al. 2004). However, due to the proportionally greater clearance of plant communities growing on better quality soils, nectar resources in these habitats are now scarce throughout summer and autumn causing nectarivorous bird populations to become food-limited and decline (Paton 2000; Ford et al. 2001; Paton et al. 2004).

Possibly as a result, the fruit-set of a number of common bird-pollinated plant species (e.g., *Astroloma conostephioides* (Ericaceae), *Epacris impressa* (Ericaceae), *Grevillea lavandulacea* (Proteaceae), *Xanthorrhoea semiplana* (Asphodelaceae)) is largely pollen-limited (i.e., fruit-set was significantly increased by manually supplementing flowers with additional pollen over that received naturally) within vegetation fragments of the SMLR (Paton 2000; Paton et al. 2004). However, pollen-limitation of plant reproduction alone does not imply a detrimental impact of landscape disturbance (i.e., pollen-limitation may result from causes unrelated to landscape disturbance).

There are no data on the reproduction of bird-pollinated plants in the Mount Lofty Ranges prior to vegetation clearance (as recognised by Paton 2000). Thus, the studied plant species may have always been naturally pollen-limited within these areas. This is a caveat from which most studies suffer due to an absence of historical data. However, the reproduction of at least one of these bird-pollinated plant species, *A. conostephioides*, while pollen-limited in vegetation fragments of the Mount Lofty Ranges, is not pollen-limited on Kangaroo Island, South Australia, where a number of habitat types remain protected within a large conservation reserve (i.e., Flinders Chase National Park (32 660 ha); Paton 2000). This supports the interpretation of negative impact(s) of vegetation removal and subsequent habitat loss of bird pollinators on the fruit-set of bird-pollinated plants in the SMLR. However, it is not clear whether the reproduction of any bird-pollinated plant species is more pollen-limited within smaller and/or more isolated vegetation fragments versus larger and/or less isolated vegetation fragments in the SMLR.

It should be noted that the fruit-set of plant species may also be limited by pollen quality, rather than, or in combination with, the quantity of pollen delivered. For example, floral visitation may be adequate to transport sufficient amounts of pollen to the stigmas of flowers to enable fertilisation. However, fruit-set may not be maximized due to the high levels of self-pollen and/or pollen of closely related individuals being delivered to stigmas. This could occur via the altered foraging behaviour of nectarivorous birds (e.g., greater within-plant foraging by pollinators; Yates et al. 2007a), and/or a smaller number of compatible mates (i.e., via mate-limitation; Young and Pickup 2010). Indeed, low fruit-set in small populations of the endangered, bird-pollinated *Grevillea repens* (Proteaceae) in Victoria, Australia, was at least partly attributed to mate-limitation (Holmes et al. 2008). Reproduction may also be limited by a combination of abiotic (e.g., available nutrients, soil moisture, etc.) and biotic factors (Campbell and Halama 1993).

Nevertheless, the evidence is strong that the reproduction of a number bird-pollinated plants is at least partly limited by efficient pollination (whether quantity and/or quality) in the SMLR. Likewise, it was found that the fruit-set and number of seeds per fruit of plants of the bird-pollinated eucalypt, *Eucalyptus leucoxylon*, within a revegetated woodland located in a semi-arid region to the east of the Adelaide Hills were also pollen-limited (McCallum et al. 2019a).

- ***Insect-pollination***

There have been limited studies on the reproduction of insect-pollinated plants in the SMLR in relation to landscape disturbance, and relatively few studies on insect pollination more generally.

Faast (2009) and colleagues (Faast and Facelli 2009; Faast et al. 2009; Faast et al. 2011), examined spatio-temporal variability of the generalist insect-pollinated, nectar producing orchid, *Caladenia rigida*, and the specialized sexual-deceptive congener, *C. tentaculata*, the flowers of which are pollinated by males of a single species of thynnine wasp within the SMLR. In support of the view that pollinator specialization results in a greater likelihood of reproductive failure (Bond 1994; Wolowski et al. 2014; Bennett et al. 2020), fruit-set of the specialist orchid *C. tentaculata* was consistently lower than that of the generalist *C. rigida* across the three consecutive years of the study (2005-2007). However, no statistically significant difference was found between populations, or across years, in the fruit-set of the specialist *C. tentaculata* (Faast 2009). In contrast, significant differences in fruit-set were evident between populations of the generalist *C. rigida* (Faast 2009). Nevertheless, variation in fruit-set between populations was not consistent across years, suggesting that characteristics of the populations per se (e.g., population size, area of vegetation fragments containing populations, etc.), were not a consistent explanatory factor across years (Faast 2009).

However, in the drought year of 2006 the two smallest populations of *C. rigida* failed to set any fruit, suggesting negative impacts of small population size (i.e., Allee effects) may only manifest themselves under stressful conditions (Faast 2009). Populations of *C. rigida* also showed significant spatio-temporal variability in the browsing of flowers by florivores (e.g., by the native bird, *Corcorax melanorhamphos* (white-winged chough)), with browsing of flowers typically being lower for populations nearer to the edges of roads and fire tracks (i.e., a positive edge effect; Faast and Facelli 2009), demonstrating the importance of considering biotic interactions other than animal pollination when examining factors determining plant reproduction.

It should be noted that the sizes of the orchid populations studied by Faast (2009) were not directly proportional to the areas of their vegetation fragments. For example, two of the smallest populations of *C. rigida* studied, which fluctuated between 15 to 60 flowering individuals depending on year, were both located in the same large vegetation fragment (~350 ha), versus, for example, a much larger population of between 800 to > 1500 flowering plants within a smaller vegetation fragment of only 4.5 ha (Faast 2009). Indeed, population size can be a better predictor of reproductive output than fragment area for some plant species (Ward and Johnson 2004). However, for those plant species with a generally extensive distribution across their vegetation fragment area, population size and fragment area will be positively correlated to a greater degree. Nevertheless, for rare or patchily distributed plants, impacts of fragment area and population size may not be equivalent (e.g., also see Donaldson et al. 2002). Indeed, Paton (2000) suggested insect-plant pollination systems may typically operate at spatial scales smaller than individual fragments of vegetation within southern Australia.

Another relevant study in the SMLR is that of Ottewell et al. (2009) who examined the effect of distance to the nearest conspecific on seed production of scattered paddock trees of *Eucalyptus camaldulensis*, a generalist insect-pollinated eucalypt, and *E. leucoxylon*, which is predominantly pollinated by nectarivorous birds. It was predicted that an increased isolation of individual trees from their nearest conspecific would negatively correlate with seed production, due to reduced outcross pollen received and greater within-plant movement of pollen by pollinators in more isolated individuals. Moreover, this effect was expected to be greater for plants of the insect-pollinated *E. camaldulensis*, given the more mobile bird pollinators of *E. leucoxylon* should in theory be better able to maintain outcross pollination between ever more distant trees (Ottewell et al. 2009). Indeed, as already mentioned, pollinator mobility significantly determines the magnitude of selfing and correlated paternity of plants within fragmented populations (see meta-analysis by Breed et al. 2015). Nevertheless, neither species of eucalypt showed the predicted relationship between seed production and distance to their nearest conspecific (Ottewell et al. 2009).

The results of Ottewell et al. (2009) contrast with a similar study by McCallum et al. (2019b), who found that the number of seeds per fruit of six species of eucalypt, including *E. leucoxylon* and a mixture of other insect- and bird-pollinated species, was higher for individuals when a reproductive conspecific was within 20 m of the sampled plant within a revegetated woodland located in a semi-arid region to the east of the Adelaide Hills. Thus, significant relationships between plant isolation and levels of plant reproduction may be quite contextual (i.e., vary with location, year, etc.).

While the results of Ottewell et al. (2009) and McCallum et al. (2019b) are informative, the reproduction of scattered paddock trees may not be equivalent to comparisons of populations of trees within smaller and/or more isolated fragments of vegetation, versus larger and/or less isolated fragments of vegetation. For example, Krauss et al. (2007) found seed production of *Eucalyptus salubris*, the flowers of which are visited by nectarivorous birds, significantly declined in smaller, fragmented populations (i.e., $<$ 10 individuals) versus larger, unfragmented populations (e.g., $>$ 1000 individuals) in southern Western Australia. However, in contrast, seed production of another studied eucalypt, *E. salmonophloia*, again visited by nectarivorous birds, was unrelated to population size in the same study region (Krauss et al. 2007). Thus, seed production of *Eucalyptus* species, whether measured for individual paddock trees or small fragmented populations, seems inconsistently impacted under landscape disturbance.

A mediating factor which may need to be considered in studies of pollination and plant reproduction in the SMLR is the prevalence of the introduced honeybee, *Apis mellifera*. Ottewell et al. (2009) hypothesized that the introduced honeybee, a now prevalent visitor to the insect-pollinated flowers of *E. camaldulensis*, facilitated longer distance pollen dispersal versus native insects (i.e., native bees, wasps, flies, etc.). This is because honeybees are able to forage over several kilometres from their nests (Visscher and Seeley 1982), versus a flight range of a few hundred meters for most native bees in Australia (Schwarz and Hurst 1997). Thus, high rates of floral visitation by *A. mellifera* to this generalist insect-pollinated eucalypt may have increased pollen dispersal distances and helped to buffer seed production against the negative impacts of increasing isolation (Ottewell et al. 2009). However, it should be noted that the efficiency of *A. mellifera* as a pollinator varies considerably among plant species (Paton 1993, 1997; Gross and Mackay 1998; Gross 2001; Celebrezze and Paton 2004), and floral visitation by *A. mellifera* may also exacerbate the negative impact(s) of landscape disturbance for particular plants (e.g., see Paton 2000).

- ***Genetics***

Finally, although not a direct study of pollination and plant reproduction per se, Starr and Carthew (1998) examined the genetics of the progeny of populations of the mixed-mating, insect-pollinated shrub *Hakea carinata* (Proteaceae) a plant species with a naturally patchy distribution throughout the SMLR. Populations of this species occur across a range of varying fragment types (e.g., roadside verges and conservation parks) and range in size from small (< 300 individuals) to large (> 1000 individuals) (Starr and Carthew 1998). However, although inbreeding was high across the sampled populations, and there was little evidence of gene flow among populations, this was due to populations being historically isolated rather than an impact of recent vegetation clearance (Starr and Carthew 1998). Thus, *H. carinata* proved to be relatively genetically robust to the impacts of landscape disturbance (Starr and Carthew 1998). Indeed, the genetic consequences of habitat loss and fragmentation, at least in the short-term, may not be particularly detrimental for plant species which already naturally exist as isolated populations with limited inter-population gene-flow via pollen or seed dispersal, and also regularly reproduce via self-pollination (Llorens et al. 2018b). Nevertheless, changes to processes which impact demography (e.g., frequency of fire) under landscape disturbance may still threaten plant populations within fragmented landscapes (Yates and Ladd 2005). More recently, Ottewell et al. (2016) also found that despite a patchy, localised, and highly fragmented distribution, there was limited detectable genetic differentiation among populations of the endangered, wind-pollinated shrub *Allocasuarina robusta* (Casuarinaceae) in the SMLR, suggesting the maintenance of genetic connectivity among fragmented populations of this plant species. Nevertheless, populations remain threatened due to a lack of recruitment (Ottewell et al. 2016).

- ***Summary***

In summary, although limited, studies of pollination and plant reproduction in the SMLR suggest that responses of plant species to landscape disturbance are species specific. Indeed, the results agree with the conclusion of Hobbs and Yates (2003), who highlight the idiosyncratic nature of plant species responses to landscape disturbance. The combined results of Faast (2009) particularly emphasise the importance of measuring temporal variability in reproduction among multiple plant populations, and the study of biotic interactions other than pollination (e.g., florivory, seed predation, etc.). Ultimately, more empirical studies are required to better understand how landscape disturbance has impacted the reproduction of current plant populations in the fragmented landscape of the SMLR.

1.5 Study Species

Three common plant species with contrasting pollination systems were chosen to examine the possible impacts of landscape disturbance on pollination and plant reproduction in the Adelaide Hills, allow informative comparisons between plant species in the current study, and further help to address gaps and biases (see section **1.3 Gaps and Biases in Landscape Disturbance Studies** above) by continuing to add to the literature on the impact(s) of landscape disturbance on common widespread plants with understudied traits (e.g., insect pre-dispersal seed predation and nocturnal moth pollination).

1.) *Pultenaea daphnoides* J.C.Wendl. (Fabaceae):

This species is a common perennial sclerophyllous shrub that grows within dry sclerophyll forests and woodlands of the SMLR. Armstrong et al. (2003) in their biodiversity survey of the SMLR found *P. daphnoides* in 30.00 % of quadrats (out of 1177) across the whole of the region, making *P. daphnoides* the nineteenth most commonly recorded perennial plant species. *Pultenaea daphnoides* is also a dominant understorey plant within sclerophyll forests and woodlands of the eastern states of Australia (de Kok and West 2002). Within the Adelaide Hills, *P. daphnoides* flowers profusely during spring and forms a dominant floral display (Pers. Obs.). The papilionaceous flowers are regularly visited by both generalist native bees (e.g., various species of *Lasioglossum*) and the invasive honeybee, *A. mellifera*, which possibly buffers plant reproduction against any declines in native pollinator abundance and diversity (e.g., see Gross 2001). Thus, reproduction of *P. daphnoides* may be relatively robust to landscape disturbance in the Adelaide Hills. Overall, *Pultenaea* is a diverse genus of the largest group of endemic Australian bush peas (Mirbelieae and Bossiaeeae), commonly termed “egg and bacon” peas (Barrett et al. 2021). Members of this group frequently form a dominant understorey component within sclerophyll forests and woodlands of southern Australia. However, there appears to be no published research examining the reproductive consequences of landscape disturbance to this group of plants.

2.) *Hibbertia exutiacies* N.A.Wakef. (Dilleniaceae):

This species is a common perennial sclerophyllous shrub that grows within dry sclerophyll forests and woodlands of the SMLR. Armstrong et al. (2003) found *H. exutiacies* in 31.00 % of quadrats (out of 1177) across the whole of the region, making *H. exutiacies* the eighteenth most commonly recorded perennial plant species in the SMLR. As for *P. daphnoides*, *H. exutiacies* flowers profusely during spring and forms a dominant floral display within the Adelaide Hills (Pers. Obs.). However, flowers seem to be visited rather sporadically by native bees (Pers. Obs.), as also observed for comparable species of *Hibbertia* (Schatral 1996). The flowers of *H. exutiacies* also do not provide nectar (Pers. Obs.), and the floral morphology matches that of other *Hibbertia* species whereby pollination is performed by native bees collecting pollen via thoracic vibrations (i.e., buzz-pollination; Buchmann 1983; Tucker and Bernhardt 2000). *Apis mellifera*, which is unable to perform buzz-pollination (Buchmann 1983), is not observed visiting flowers of *H. exutiacies* in the Adelaide Hills (Pers. Obs.). Thus, unlike for *P. daphnoides*, *A. mellifera* is unlikely to buffer plant reproduction against possible declines in native bee pollinators. Moreover, buzz-pollination represents a form of pollinator specialization by the plant as it restricts native bee pollinators to only those able to perform the behaviour. Thus, *H. exutiacies* may be more vulnerable to landscape disturbance due to this pollinator specialization. Seeds of *H. exutiacies* also suffer heavy attack by the insect larvae of pre-dispersal seed predators (Pers. Obs.), as do a number of *Hibbertia* species (Sweedman and Brand 2006 p. 187). Thus, landscape disturbance may alter reproductive output of *H. exutiacies* by also impacting levels of pre-dispersal seed predation, an understudied biotic interaction relative to pollination (Hobbs and Yates 2003). Currently, there appears to be no published research examining the reproductive consequences of landscape disturbance to any species of *Hibbertia*.

3.) *Stackhousia aspericocca* Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker (Celastraceae):

This taxon is a relatively common herbaceous perennial that grows within dry sclerophyll forests and woodlands of the Adelaide Hills (Barker 1977). Again, when in abundance, *S.*

aspericocca ssp. *Cylindrical inflorescence* can form a dominant floral display during spring (Pers. Obs.). Species of *Stackhousia* are often assumed to be pollinated by night-flying moths (e.g., Clarke and Lee 2019 p. 186). However, there appears to be no published research providing direct evidence of moth pollination in any species of *Stackhousia*. Nevertheless, nocturnal visitation by moths to flowers has been irregularly observed to some *Stackhousia* species (W.R. Barker Pers. Comms.), and their floral morphology is strongly suggestive of pollination by night-flying settling moths (i.e., a so-called phalenophilous pollination syndrome; Faegri and van der Pijl 1979; Willmer 2011). Nevertheless, nocturnal moth pollination in *Stackhousia* remains a working hypothesis. There also appears to be no published studies regarding levels of fruit and seed production in any species of *Stackhousia*, and no previous study appears to have examined the impact of variation in landscape characteristics on a nocturnally moth-pollinated plant in Australia.

1.6 Research Aim

The overarching aim of this research is to examine spatio-temporal variability in pollination and reproduction of three common plant species across a range of reserve areas protecting plant populations within a typically fragmented landscape and to test whether reserve area is a significant predictor of reproductive success. To achieve this, a mixture of pollen-limitation experiments and mensurative censuses of natural levels of plant reproduction (i.e., fruit and seed production) among conservation reserves of increasing area (~1.40 ha up to 1027.47 ha) were conducted between 2017-2019. Thus, the two main variables measured in this thesis are plant focused, with pollen-limitation assessing if there is a deficiency in the pollination process, either by changes in pollen quantity (e.g., as may occur via reduced pollinator visitation) and/or pollen quality (e.g., via greater receival of genetically incompatible pollen), and measures of natural plant reproduction assessing final reproductive output as moderated by a number of abiotic and biotic factors.

Specifically, and comparable to related studies (e.g., Aizen and Feinsinger 1994a; Cunningham 2000a, b; Donaldson et al. 2002; González-Varo et al. 2009; Hadley et al. 2014; Matesanz et al. 2015; Lázaro et al. 2020), the degree of pollen-limitation and reproduction of a similar number of plants were measured within sample sites located across a number of fragments, here defined by the boundary of conservation reserves. Thus, although the chosen methodology allowed for a more balanced experimental design, given that sampling is not proportional to the size of the sampled plant populations, measures of pollen-limitation and plant reproduction in larger populations may less accurately reflect population-level estimates because a smaller proportion of the population is sampled. Nevertheless, given the large areas of many of the chosen reserves, and the widespread distribution of the three common, abundant plant species throughout the reserves, sampling in proportion to population size was not always feasible. Moreover, the true population size(s) of the three common, widespread plant species may not be limited to the reserves themselves and could include individuals outside of reserve boundaries (e.g., growing along roadside corridors, adjacent private heritage agreements, etc.). Ultimately, given these constraints, the chosen experimental design allowed for a good assessment of spatial variation in the degree of pollen-limitation and reproductive output of the sampled plants across the landscape in relation to reserve area. Importantly, given conservation reserves represent spatially defined areas purposefully implemented to protect natural ecosystems, a negative effect of small reserve area on pollination and plant reproduction would be of conservation concern.

The following hypotheses were tested:-

1.) That the likelihood of successful pollination increased in reserves of greater area.
2.) That the relationship between reserve area and successful pollination was temporally consistent across years.

These hypotheses were tested for *P. daphnoides* (Chapter Two) and *H. exutiacies* (Chapter Three).

3.) That the level of plant reproduction was higher in reserves of greater area.
4.) That the relationship between reserve area and plant reproduction was temporally consistent across years.

The latter two hypotheses were tested for *P. daphnoides* (Chapter Two), *H. exutiacies* (Chapter Three), and *S. aspericocca* ssp. *Cylindrical inflorescence* (Chapter Five). In particular, for *H. exutiacies*, which suffers both high levels of pre-dispersal seed predation and seed abortion (as discovered in this study), studying the spatio-temporal dynamics of pollination and plant reproduction in this species included measuring both the spatio-temporal variability of pre-dispersal seed predation (Chapter Three and Four) and temporal variability in the abiotic resource of water availability (Chapter Four).

Ultimately, the findings of this research will help to address gaps and biases in our understanding of the impacts of landscape disturbance on pollination and plant reproduction by adding detailed studies to the relevant literature, and contribute to our knowledge on the current and possible future impacts of landscape disturbance on plant populations within the Adelaide Hills and in other fragmented habitats.

1.7 REFERENCES:

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980.

Aguilar R, Cristóbal-Pérez EJ, Balvino-Olvera FJ, de Jesús Aguilar-Aguilar M, Aguirre-Acosta N, Ashworth L, Lobo JA, Martén-Rodríguez S, Fuchs EJ, Sanchez-Montoya G, Bernardello G (2019) Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecology Letters 22:1163-1173.

Aizen MA, Feinsinger P (1994a) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75:330-351.

Aizen MA, Feinsinger P (1994b) Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine “Chaco Serrano”. Ecological Applications 4:378-392.

Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? Journal of Vegetation Science 13:885-892.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Barker WR (1977) Taxonomic studies in *Stackhousia* Sm. (Stackhousiaceae) in South Australia. Journal of the Adelaide Botanic Garden 1:69-82.

Barrett RL, Clugston JA, Cook LG, Crisp MD, Jobson PC, Lepschi BJ, Renner MA, Weston PH (2021) Understanding diversity and systematics in Australian Fabaceae tribe Mirbelieae. Diversity 13:391.

Bennett JM, Steets JA, Burns JH, Burkle LA, Vamosi JC, Wolowski M, Arceo-Gómez G, Burd M, Durka W, Ellis AG, Freitas L (2020) Land use and pollinator dependency drives global patterns of pollen limitation in the Anthropocene. Nature Communications 11:3999.

Biesmeijer JC, Roberts SP, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351-354.

Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London B 344:83-90.

Breed MF, Ottewell KM, Gardner MG, Marklund MH, Dormontt EE, Lowe AJ (2015) Mating patterns and pollinator mobility are critical traits in forest fragmentation genetics. Heredity 115:108-114.

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 73-133.

Buxton MN, Anderson BJ, Lord JM (2018) The secret service - analysis of the available knowledge on moths as pollinators in New Zealand. New Zealand Journal of Ecology 42:1-9.

Byrne M, Elliott CP, Yates C, Coates DJ (2007) Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. Molecular Ecology 16:1303-1314.

Campbell DR, Halama KJ (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74:1043-1051.

CaraDonna PJ, Burkle LA, Schwarz B, Resasco J, Knight TM, Benadi G, Blüthgen N, Dormann CF, Fang Q, Fründ J, Gauzens B (2021) Seeing through the static: the temporal dimension of plant-animal mutualistic interactions. Ecology Letters 24:149-161.

Cariveau DP, Bruninga-Socolar B, Pardee GL (2020) A review of the challenges and opportunities for restoring animal-mediated pollination of native plants. Emerging Topics in Life Sciences 4:99-109.

Celebrezze T, Paton DC (2004) Do introduced honeybees (*Apis mellifera*, Hymenoptera) provide full pollination service to bird-adapted Australian plants with small flowers? An experimental study of *Brachyloma ericoides* (Epacridaceae). Austral Ecology 29:129-136.

Chávez-Pesqueira M, Carmona D, Suárez-Montes P, Núñez-Farfán J, Aguilar R (2015) Synthesizing habitat fragmentation effects on plant-antagonist interactions in a phylogenetic context. Biological Conservation 192:304-314.

Clarke I, Lee H (2019) Name that flower. Melbourne University Press, Melbourne, Australia.

Crisp MD, Laffan S, Linder HP, Monro A (2001) Endemism in the Australian flora. Journal of Biogeography 28:183-198.

Cunningham SA (2000a) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Cunningham SA (2000b) Depressed pollination in habitat fragments causes low fruit set. Proceedings of the Royal Society of London B: Biological Sciences 267:1149-1152.

Delnevo N, van Etten EJ, Byrne M, Petraglia A, Carbognani M, Stock WD (2020) Habitat fragmentation restricts insect pollinators and pollen quality in a threatened Proteaceae species. Biological Conservation 252:108824.

Donaldson J, Nänni I, Zachariades C, Kemper J (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. Conservation Biology 16:1267-1276.

Duncan DH, Nicotra AB, Wood JT, Cunningham SA (2004) Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. Journal of Ecology 92:977-985.

Eckert CG, Kalisz S, Geber MA, Sargent R, Elle E, Cheptou PO, Goodwillie C, Johnston MO, Kelly JK, Moeller DA, Porcher E (2010) Plant mating systems in a changing world. Trends in Ecology and Evolution 25:35-43.

Faast R (2009) The reproductive ecology of two terrestrial orchids, *Caladenia rigida* and *Caladenia tentaculata*. Dissertation, University of Adelaide.

Faast R, Facelli JM (2009) Grazing orchids: impact of florivory on two species of *Caladenia* (Orchidaceae). Australian Journal of Botany 57:361-372.

Faast R, Farrington L, Facelli JM, Austin AD (2009) Bees and white spiders: unravelling the pollination syndrome of *Caladenia rigida* (Orchidaceae). Australian Journal of Botany 57:315-325.

Faast R, Facelli JM, Austin AD (2011) Seed viability in declining populations of *Caladenia rigida* (Orchidaceae): are small populations doomed? Plant Biology 13:86-95.

Faegri K, van der Pijl L (1979) The principles of pollination ecology. Pergamon Press, New York.

Fahrig L (2017) Ecological responses to habitat fragmentation per se. Annual Review of Ecology, Evolution, and Systematics 48:1-23.

Fahrig L (2019) Habitat fragmentation: a long and tangled tale. Global Ecology and Biogeography 28:33-41.

Farwig N, Bailey D, Bochud E, Herrmann JD, Kindler E, Reusser N, Schüepp C, Schmidt-Entling MH, (2009) Isolation from forest reduces pollination, seed predation and insect scavenging in Swiss farmland. Landscape Ecology 24:919-927.

Filazzola A, Cahill Jr JF (2021) Replication in field ecology: identifying challenges and proposing solutions. Methods in Ecology and Evolution 12:1780-1792.

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. Global Ecology and Biogeography 16:265-280.

Ford HA, Barrett GW, Saunders DA, Recher HF (2001) Why have birds in the woodlands of southern Australia declined? Biological Conservation 97:71-88.

Gaston KJ (2010) Valuing common species. Science 327:154-155.

Glémin S, Petit C, Maurice S, Mignot A (2008) Consequences of low mate availability in the rare self-incompatible species *Brassica insularis*. Conservation Biology 22:216-221.

González-Varo JP, Arroyo J, Aparicio A (2009) Effects of fragmentation on pollinator assemblage, pollen limitation and seed production of Mediterranean myrtle (*Myrtus communis*). Biological Conservation 142:1058-1065.

Goulson D, Lye GC, Darvill B (2008) Decline and conservation of bumble bees. Annual Review of Entomology 53:191-208.

Groom MJ (1998) Allee effects limit population viability of an annual plant. The American Naturalist 151:487-496.

Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. Biological Conservation 102:89-95.

Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). Biological Conservation 86:169-178.

Guerin GR, Biffin E, Baruch Z, Lowe AJ (2016) Identifying centres of plant biodiversity in South Australia. PLoS One 11:e0144779.

Hadley AS, Betts MG (2012) The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. Biological Reviews 87:526-544.

Hadley AS, Frey SJ, Robinson WD, Kress WJ, Betts MG (2014) Tropical forest fragmentation limits pollination of a keystone understory herb. Ecology 95:2202-2212.

Hahn M, Brühl CA (2016) The secret pollinators: an overview of moth pollination with a focus on Europe and North America. Arthropod-Plant Interactions 10:21-28.

Harris LF, Johnson SD (2004) The consequences of habitat fragmentation for plant-pollinator mutualisms. International Journal of Tropical Insect Science 24:29-43.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Holmes GD, James EA, Hoffmann AA (2008) Limitations to reproductive output and genetic rescue in populations of the rare shrub *Grevillea repens* (Proteaceae). Annals of Botany 102:1031-1041.

Jennersten OLA (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359-366.

Johnson SD, Collin CL, Wissman HJ, Halvarsson E, Ågren J (2004) Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. Biotropica 36:148-155.

Knight TM, Ashman TL, Bennett JM, Burns JH, Passonneau S, Steets JA (2018) Reflections on, and visions for, the changing field of pollination ecology. Ecology Letters 21:1282-1295.

de Kok RPJ, West JG (2002) A revision of *Pultenaea* (Fabaceae). 1. Species with ovaries glabrous and/or with tufted hairs. Australian Systematic Botany 15:81-113.

Krauss SL, Hermanutz L, Hopper SD, Coates DJ (2007) Population-size effects on seeds and seedlings from fragmented eucalypt populations: implications for seed sourcing for ecological restoration. Australian Journal of Botany 55:390-399.

Lamont BB, Klinkhamer PG, Witkowski ETF (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. Oecologia 94:446-450.

Lázaro A, Fuster F, Alomar D, Totland Ø (2020) Disentangling direct and indirect effects of habitat fragmentation on wild plants' pollinator visits and seed production. Ecological Applications 30:e02099.

Llorens TM, Yates CJ, Byrne M, Elliott CP, Sampson J, Fairman R, Macdonald B, Coates DJ (2018a) Altered soil properties inhibit fruit set but increase progeny performance for a foundation tree in a highly fragmented landscape. Frontiers in Ecology and Evolution 6:39.

Llorens TM, Ayre DJ, Whelan RJ (2018b) Anthropogenic fragmentation may not alter pre-existing patterns of genetic diversity and differentiation in perennial shrubs. Molecular Ecology 27:1541-1555.

Lothian A, Harris C (2015) Clearance of native vegetation in the Mount Lofty Ranges 1945-68. South Australian Geographical Journal 113:29-42.

Macgregor CJ, Pocock MJ, Fox R, Evans DM (2015) Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. Ecological Entomology 40:187-198.

Matesanz S, Gómez-Fernández A, Alcocer I, Escudero A (2015) Fragment size does not matter when you are well connected: effects of fragmentation on fitness of coexisting gypsophiles. Plant Biology 17:1047-1056.

McCallum KP, Breed MF, Lowe AJ, Paton DC (2019a) Plants, position and pollination: planting arrangement and pollination limitation in a revegetated eucalypt woodland. Ecological Management and Restoration 20:222-230.

McCallum KP, Breed MF, Paton DC, Lowe AJ (2019b) Clumped planting arrangements improve seed production in a revegetated eucalypt woodland. Restoration Ecology 27:638-646.

McIntyre S, Hobbs R (1999) A framework for conceptualizing human effects on landscapes and its relevance to management and research models. Conservation Biology 13:1282-1292.

Medel R, González-Browne C, Fontúrbel FE (2018) Pollination in the Chilean Mediterranean-type ecosystem: a review of current advances and pending tasks. Plant Biology 20:89-99.

Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology 13:266-273.

Neuschulz EL, Mueller T, Schleuning M, Böhning-Gaese K (2016) Pollination and seed dispersal are the most threatened processes of plant regeneration. Scientific Reports 6:29839.

Ollerton J (2017) Pollinator diversity: distribution, ecological function, and conservation. Annual Review of Ecology, Evolution, and Systematics 48:353-376.

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321-326.

Ottewell KM, Donnellan SC, Lowe AJ, Paton DC (2009) Predicting reproductive success of insect- versus bird-pollinated scattered trees in agricultural landscapes. Biological Conservation 142:888-898.

Ottewell KM, Bickerton DC, Byrne M, Lowe AJ (2016) Bridging the gap: a genetic assessment framework for population-level threatened plant conservation prioritization and decision-making. Diversity and Distributions 22:174-188.

Paton DC (1993) Honeybees in the Australian environment. Bioscience 43:95-103.

Paton DC (1997) Honey bees *Apis mellifera* and the disruption of plant-pollinator systems in Australia. Victorian Naturalist 114:23-29.

Paton DC (2000) Disruption of bird-plant pollination systems in southern Australia. Conservation Biology 14:1232-1234.

Paton DC, Rogers D, Harris W (2004) Birdscaping the environment: restoring the woodland system of the Mt Lofty region. In Lunney D (ed) Conservation of Australia’s forest fauna, 2nd edn. Royal Zoological Society of New South Wales, Mosman, NSW, Australia, pp 331-358.

Patrick KL, Johnson SD, Duffy KJ (2018) High levels of fecundity in small and isolated populations of a self-compatible *Aloe* pollinated by opportunistic birds and bees. Plant Biology 20:780-788.

Pauw A (2007) Collapse of a pollination web in small conservation areas. Ecology 88:1759-1769.

Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, Kunin WE (2010) Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution 25:345-353.

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rabasa SG, Gutiérrez D, Escudero A (2009) Temporal variation in the effects of habitat fragmentation on reproduction of the Mediterranean shrub *Colutea hispanica*. Plant Ecology 200:241-254.

Regan EC, Santini L, Ingwall-King L, Hoffmann M, Rondinini C, Symes A, Taylor J, Butchart SH (2015) Global trends in the status of bird and mammal pollinators. Conservation Letters 8:397-403.

Rodger JG, Bennett JM, Razanajatovo M, Knight TM, van Kleunen M, Ashman TL, Steets JA, Hui C, Arceo-Gómez G, Burd M, Burkle LA (2021) Widespread vulnerability of flowering plant seed production to pollinator declines. Science Advances 7:eabd3524.

Sánchez-Bayo F, Wyckhuys KA (2019) Worldwide decline of the entomofauna: a review of its drivers. Biological Conservation 232:8-27.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5:18-32.

Schatral A (1996) Floral predators, pollinators and seed set in Western Australian species of the genus *Hibbertia* (Dilleniaceae). In: Hopper SD, Chappill JA, Harvey MS, George AS (eds) Gondwanan heritage: past, present and future of the Western Australian biota. Surrey Beatty & Sons, Sydney, NSW, pp 203-211.

Schwarz MP, Hurst PS (1997) Effects of introduced honey bees on Australia's native bee fauna. Victorian Naturalist 114:7-12.

Simmons BI, Balmford A, Bladon AJ, Christie AP, De Palma A, Dicks LV, Gallego-Zamorano J, Johnston A, Martin PA, Purvis A, Rocha R (2019) Worldwide insect declines: an important message, but interpret with caution. Ecology and Evolution 9:3678-3680.

Skogen KA, Jogesh T, Hilpman ET, Todd SL, Rhodes MK, Still SM, Fant JB (2016) Land-use change has no detectable effect on reproduction of a disturbance-adapted, hawkmoth-pollinated plant species. American Journal of Botany 103:1950-1963.

Starr GJ, Carthew SM (1998) Genetic differentiation in isolated populations of *Hakea carinata* (Proteaceae). Australian Journal of Botany 46:671-682.

Steffan-Dewenter I (2003) Importance of habitat area and landscape context for species richness of bees and wasps in fragmented orchard meadows. Conservation Biology 17:1036-1044.

Steffan-Dewenter I, Tscharntke T (1999) Effects of habitat isolation on pollinator communities and seed set. Oecologia 121:432-440.

Sweedman L, Brand G (2006) Collection guidelines for common Australian families and genera. In: Sweedman L, Merritt D (eds) Australian seeds a guide to their collection, identification and biology. CSIRO Publishing, Collingwood, Victoria, pp 173-198.

Tennekes M (2018) tmap: thematic maps in R. Journal of Statistical Software 84:1-39.

Teixido AL, Fuzessy LF, Souza CS, Gomes IN, Kaminski LA, Oliveira PC, Maruyama PK (2022) Anthropogenic impacts on plant-animal mutualisms: a global synthesis for pollination and seed dispersal. Biological Conservation 266:109461.

Tucker SC, Bernhardt P (2000) Floral ontogeny, pattern formation, and evolution in *Hibbertia* and *Adrastaea* (Dilleniaceae). American Journal of Botany 87:1915-1936.

Vandepitte K, Honnay O, Jacquemyn H, Roldan-Ruiz I (2010) Effects of outcrossing in fragmented populations of the primarily selfing forest herb *Geum urbanum*. Evolutionary Ecology 24:1353-1364.

Van Zandt PA, Johnson DD, Hartley C, LeCroy KA, Shew HW, Davis BT, Lehnert MS (2020) Which moths might be pollinators? Approaches in the search for the flower-visiting needles in the Lepidopteran haystack. Ecological Entomology 45:13-25.

Vaughn KJ, Young TP (2010) Contingent conclusions: year of initiation influences ecological field experiments, but temporal replication is rare. Restoration Ecology 18:59-64.

Visscher PK, Seeley TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63:1790-1801.

Ward M, Johnson SD (2005) Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). Oikos 108:253-262.

Willmer P (2011) Pollination and floral ecology. Princeton University Press, Princeton, New Jersey.

Wolowski M, Ashman TL, Freitas L (2014) Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. PLoS One 9:e89498.

Yates CJ, Ladd PG (2005) Relative importance of reproductive biology and establishment ecology for persistence of a rare shrub in a fragmented landscape. Conservation Biology 19:239-249.

Yates CJ, Coates DJ, Elliott C, Byrne M (2007a) Composition of the pollinator community, pollination and the mating system for a shrub in fragments of species rich kwongan in south-west Western Australia. Biodiversity and Conservation 16:1379-1395.

Yates CJ, Elliott C, Byrne M, Coates DJ, Fairman R (2007b) Seed production, germinability and seedling growth for a bird-pollinated shrub in fragments of kwongan in south-west Australia. Biological Conservation 136:306-314.

Young AG, Pickup M (2010) Low S-allele numbers limit mate availability, reduce seed set and skew fitness in small populations of a self-incompatible plant. Journal of Applied Ecology 47:541-548.

CHAPTER TWO

2. REPRODUCTIVE OUTPUT OF A COMMON SCLEROPHYLLOUS SHRUB, *PULTENAEA DAPHNOIDES* J.C.WENDL. (FABACEAE) ACROSS A RANGE OF RESERVE SIZES.

Alex G. Blackall[1], Duncan A. Mackay[1], Molly A. Whalen[1]

[1]College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

2.1 ABSTRACT

Premise: Studies of landscape disturbance on plant reproduction have focused on rare and threatened plant species that are generally studied over one season. However, negative impact(s) on common species will have more wide-ranging implications, and responses may show temporal variability.

Methods: We used the common bee-pollinated sclerophyllous shrub, *Pultenaea daphnoides*, to examine if plant reproduction is positively related to reserve area within the fragmented landscape of the Adelaide Hills, a peri-urbanised area of southern Australia. This was achieved through a mix of pollen-limitation experiments (2017, 2019) and measures of natural reproduction (2017, 2018).

Results: We found no relationship between reserve area and the probability that a flower would produce a pod (either unfilled or filled). However, developing seeds in numerous pods per plant may be aborted. Thus, in 2017 and 2018, ~150-300 mature pods from 5-10 plants per sample site were opened to check for viable seeds. While no relationship between reserve area and the proportion of pods with viable seeds was evident in 2017, a significant negative relationship was apparent in 2018 when viable seed production was mostly limited to plants in the two smallest reserves studied (< 2 ha), versus an almost complete abortion of developing seeds for plants in larger reserves (> 80 ha). There was no strong evidence this result was due to pollen-limitation, and no measure of plant reproduction was significantly pollen-limited in 2017 or 2019. However, this striking result occurred in a year of historically low early spring rainfall throughout the Adelaide Hills.

Conclusion: The relationship between reserve area and plant reproduction was temporally variable between-years, highlighting the need for multi-year studies in understanding the spatio-temporal dynamics of plant reproduction within fragmented landscapes under variable rainfall. However there was no evidence that small reserve area alone negatively impacts the reproduction of *P. daphnoides* in the Adelaide Hills.

KEYWORDS: Fabaceae; Mirbelieae; *Pultenaea*; habitat loss; fragmentation; rainfall

2.2 INTRODUCTION

Habitat loss typically results in the subdivision of remnant native habitat into ever smaller and more isolated fragments, and both abiotic and biotic processes are often altered within smaller fragments relative to larger continuous areas of habitat (Saunders et al. 1991; McIntyre and Hobbs 1999; Fischer and Lindenmayer 2007). These changes can result in both positive and negative impacts to animals and plants (Haddad et al. 2015; see Fletcher et al. 2018 versus Fahrig et al. 2019). Nevertheless, both qualitative and quantitative reviews of pollination and plant reproduction generally find negative impacts of habitat loss and fragmentation (e.g., reviewed by Aizen et al. 2002; Hobbs and Yates 2003; Harris and Johnson 2004; Aguilar et al. 2006; Hadley and Betts 2012). However, our understanding of the impact(s) of landscape disturbance (here used to describe the combined effects of habitat loss and fragmentation and other interrelated processes (e.g., edge effects)) on pollination and plant reproduction are biased by the plant species typically chosen and the limited duration of their study. In particular, studies of rare and threatened plants, particularly of perennial herbs and self-incompatible trees, have been over-represented in the literature (as recognised by Hobbs and Yates 2003 and Aguilar et al. 2006; see Chapter One). However, negative impact(s) on common plant species, which generally participate in a greater number of plant animal-interactions (e.g., herbivory, pollination, etc.) and ecosystem functions, will have more wide-ranging implications (Broadhurst and Young 2007). Studies are also usually conducted over a single flowering season (Aguilar et al. 2006; see Chapter One), although the impact(s) of landscape disturbance on plant reproduction may fluctuate between years (Hobbs and Yates 2003). For example, Morgan (1999) found seed production of the endangered Australian grassland plant, *Rutidosis leptorrhynchoides* (Compositae), was positively related to population size in two of three years studied. However, low rainfall in one of the studied years masked the relationship between seed production and population size by reducing seed output in a larger population (Morgan 1999). Considering many regions globally are experiencing drier conditions, relative to historical records, and drier conditions are predicted to increase into the future (Dai 2013), the way in which reduced rainfall may interact with the current impact(s) of landscape disturbance on plant reproduction is of conservation concern (Matesanz et al. 2009). Lastly, many studies measure pollination and plant reproduction at the most severe scale(s) of disturbance (e.g., small vegetation patches under 5 ha in area (Donaldson et al. 2002); roadside verges of native vegetation (Lamont et al. 1993)). However, the impact of landscape disturbance on plant reproduction within larger fragments, with areas typical of many conservation reserves, is less studied (e.g., see Cunningham 2000a and Pauw 2007).

Here, we use the common sclerophyllous shrub, *Pultenaea daphnoides*, to observe the impact of landscape disturbance on pollination and plant reproduction within the fragmented landscape of the Adelaide Hills, a peri-urbanised section of the Southern Mount Lofty Ranges, Australia. This study was conducted over three consecutive years across a range of reserve areas (1.40-713.95 ha) currently conserving populations of *P. daphnoides* within the study region. Specifically, we tested the hypothesis that both successful pollination (measured via pollen-limitation experiments) and natural levels of plant reproduction would be lower in smaller conservation reserves than in larger reserves. Pollen-limitation studies were performed in the alternating years of 2017 and 2019, while a larger dataset on natural levels of reproduction was collected in 2018.

2.3 METHODS

2.3.1 Study species

Pultenaea daphnoides J.C.Wendl. (Fabaceae) is a common sclerophyllous shrub that grows within the dry sclerophyll forests and woodlands of the Adelaide Hills (Armstrong et al. 2003). Adult plants typically grow to a height of 1-3 m (Weber 1986; supplementary material (SM) Fig. 2-1a). Plants produce large floral displays over time of a few hundred to potentially over 7000 flowers (estimated from the number of flower buds: 3887.71 ± 1832.47 flower buds per plant; $n = 5$). In terms of floral morphology, *P. daphnoides* produces typical pea-flowers (i.e., papilionaceous), grouped in terminal heads of 5-11 flowers (Weber 1986; SM Fig. 2-1b). Flowers within each inflorescence differ in their timing of anthesis. Thus, although an individual plant may produce a large number of flowers, only a subset is available for pollination at any one time (SM Fig. 2-1b). The flowers themselves are predominantly yellow, with a scarlet keel and red nectar guides on the standard petal (Weber 1986; SM Fig. 2-1b). Whether flowers of *P. daphnoides* produce nectar is unknown. The level of nectar production is not constant across the genus, with undetectable quantities of nectar in flowers of *P. densifolia* (Gross 1992), versus measurable nectar production in flowers of *P. villosa* (Ogilvie et al. 2009). Using a hand-held refractometer (Atago Co. Ltd. (Type 500, 0-90 %)) and methods adapted from Gross (1992), no measurable quantities of nectar were detected in flowers of *P. daphnoides*. However, more sensitive methods (e.g., those used by Scaccabarozzi et al. 2020) may be required to detect more minute quantities. Observations of diurnal Lepidoptera foraging on the flowers of *P. daphnoides* suggest some nectar production occurs (Pers. Obs.). Nevertheless, native bees (e.g., species of the widespread speciose bee genus *Lasioglossum* Curtis (Fam. Halictidae) (Houston 2018)) likely function as the most effective native pollinators of *P. daphnoides*, being capable of parting the wings and depressing the keel petals of flowers upon visitation and contacting the exposed reproductive structures (Pers. Obs.; SM Fig. 2-1c). Although not native, the now invasive honeybee, *Apis mellifera* Linnaeus (Fam. Apidae), is also a frequent visitor to flowers of *P. daphnoides*, and also contacts the reproductive structures of flowers when foraging (Pers. Obs.; Paton 1993; SM Fig. 2-1d).

2.3.2 Sample sites

The Adelaide Hills is a peri-urbanised section of the Southern Mount Lofty Ranges (Fig. 2-1), a region representative of a typically fragmented landscape (a landscape where remaining native vegetation cover is between 10-60 %, as defined by McIntyre and Hobbs (1999)). Here, sample sites were located in conservation reserves of varying areas (Table 2-1), and all chosen reserves were managed by the Department for Environment and Water (DEW), excluding one private reserve (Nurrutti) managed by the National Trust of South Australia (Fig. 2-1). The chosen reserves ranged in area from 1.40 ha up to 713.95 ha (Table 2-1), and *P. daphnoides* was abundant within each reserve and distributed across most of their area (Pers. Obs.). However, the location(s) of sample sites within reserves was based not only on the presence of *P. daphnoides* but also accessibility, which was restricted due to terrain, and the avoidance of areas designated for prescribed burns. Thus, it was not possible to hold constant the distance from each sample site to the edge of their respective reserve (Fig. 2-1). Moreover, native vegetation also occurred outside reserve boundaries (Fig. 2-1). Nevertheless, reserve area is positively correlated with the amount of native vegetation surrounding sample sites. For example, at 1000 m from the approximate centre of each sample site, a distance used previously to define local landscapes in studies of bee diversity within both agricultural (Brown et al. 2020) and urban landscapes in Australia (Threlfall et al. 2015), there was a clear positive relationship between reserve area and the percentage area of native vegetation surrounding sample sites within each reserve (Spearman's rho (r_s) = 0.966, $p = 1.429 \times 10^{-6}$, n = 11). Thus, sample sites within smaller reserves are surrounded by less native vegetation in the neighbouring landscape (i.e., at a scale of 1000 m), versus sample sites within larger reserves (Fig. 2-1).

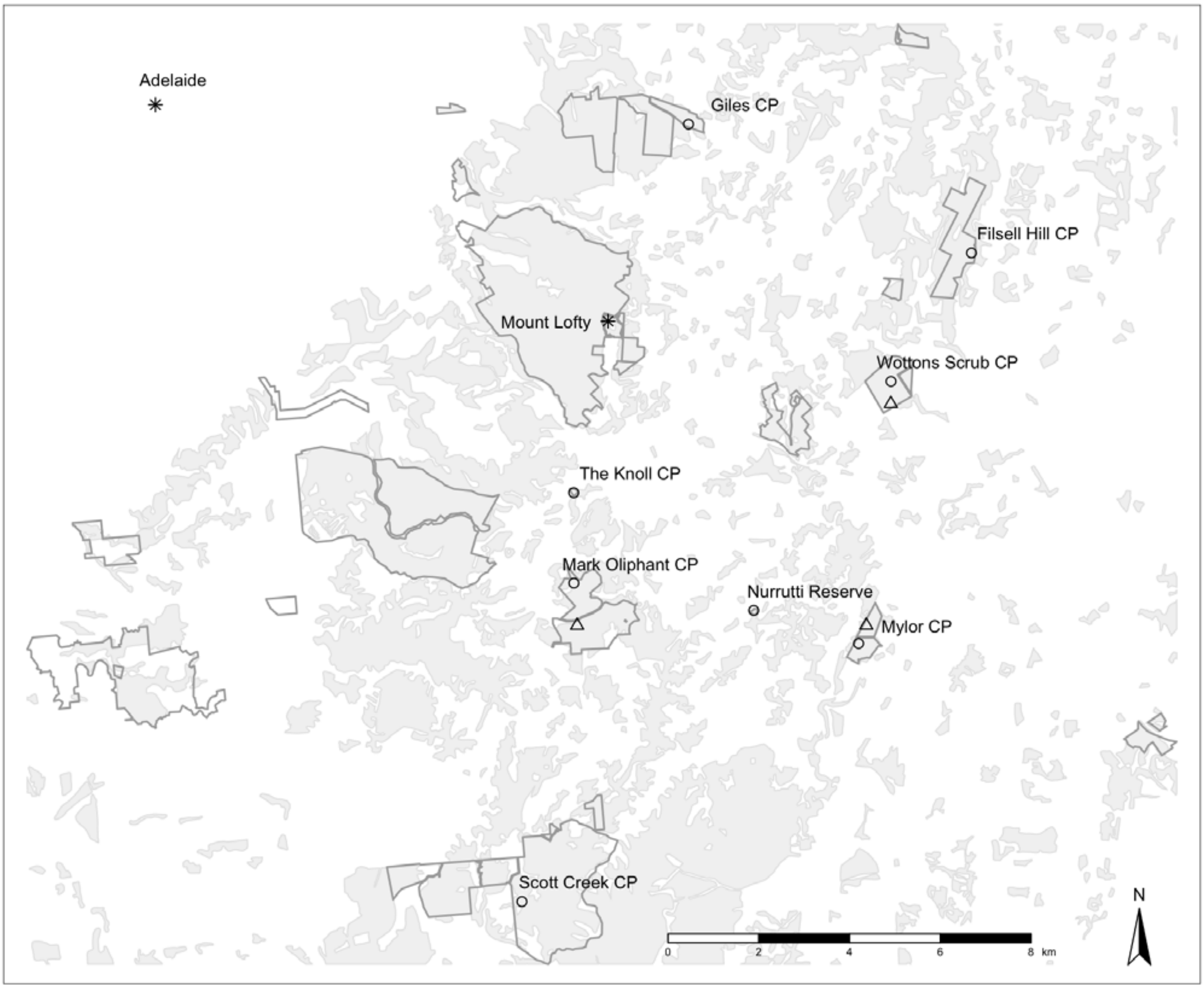


Fig. 2-1 Location of sample sites used to study the plant, *Pultenaea daphnoides*. All reserves managed by the Department for Environment and Water (DEW) and a private reserve (Nurrutti) managed by the National Trust of South Australia are outlined. Adelaide, the capital city of South Australia, and Mount Lofty, the highest point within the study region (720 m), are both marked by an asterisk. Triangles represent second sample sites located within the reserves of Mylor CP, Wottons Scrub CP, and Mark Oliphant CP, respectively. The scale bar represents a total distance of 8 km. Native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

Table 2-1 Location of sample sites within reserves, reserve area, data collected in each sample site across the years of 2017, 2018, and 2019, and the mean annual rainfall of the closest weather station to each sample site for this century. CP stands for Conservation Park.

Reserve	Sample sites	Latitude	Longitude	Reserve area (ha)	Data collected (year)[a]	Mean annual rainfall (mm)[b]
Nurrutti Reserve	-	35 ° 1.90’ S	138 ° 44.53’ E	1.40	**NR** (18), **SC** (17,18)	1107.7 ± 253.2
The Knoll CP	-	35 ° 0.45’ S	138 ° 41.96’ E	1.74	**PL** (17,19), **NR** (18), **SC** (17,18)	922.7 ± 185.7
Mylor CP	1	35 ° 2.32’ S	138 ° 46.04’ E	45.10	**SC** (17)	753.2 ± 193.3
	2	35 ° 2.11’ S	138 ° 46.15’ E	-	**SC** (17)	1107.7 ± 253.2
Wottons Scrub CP	1	34 ° 59.21’ S	138 ° 46.59’ E	82.50	**PL** (17,19), **NR** (18), **SC** (17,18)	1026.3 ± 104.1
	2	34 ° 59.48’ S	138 ° 46.58’ E	-	**SC** (17)	-
Giles CP	-	34 ° 56.09’ S	138 ° 43.74’ E	108.92	**PL** (17), **SC** (17)	1038.2 ± 249.1
Filsell Hill CP	-	34 ° 57.70’ S	138 ° 47.80’ E	128.67	**PL** (17,19), **NR** (18), **SC** (17,18)	998.7 ± 237.6
Mark Oliphant CP	1	35 ° 1.53’ S	138 ° 41.93’ E	189.96	**PL** (17), **NR** (18), **SC** (17)	1082.1 ± 214.3
	2	35 ° 2.03’ S	138 ° 41.97’ E	-	**PL** (17,19), **NR** (18), **SC** (17,18)	-
Scott Creek CP	-	35 ° 5.31’ S	138 ° 41.07’ E	713.95	**PL** (17), **NR** (18), **SC** (17,18)	774.6 ± 304.2

[a] **NR** = Data on natural levels of reproduction via tagged flowers in 2018, **PL** = Pollen-limitation data via tagged flowers in 2017 and 2019, **SC** = Seed collection from mature pods in 2017 and 2018.

[b] Mean annual rainfall was calculated from the closest weather station to each sample site with available data for the years of 2000 to 2016. However, due to missing monthly total rainfall for some months within years, which is common across weather stations in the study area, not all years from 2000 to 2016 could be included for each weather station. Weather stations may also have missed rainfall events localised to sample sites and, alternatively, also captured rainfall events which did not occur at sample sites. The closest weather station to each sample site varied in distance (mean of 1.88 ± 0.75 km, range: 0.85 - 2.89 km), and some sample sites are closer to each other than their respective closest weather stations (e.g., Mylor sample sites 1 and 2). Thus, there are inherent limitations in using weather station data as accurate rainfall records of the sample sites themselves, and these mean annual rainfall values should be viewed as coarse estimates. Weather station numbers followed by years of available data in parentheses: Nurrutti: 23817 (2000 - 2004, 2007, 2012 - 2013, 2015 - 2016), Knoll: 23873 (2002 - 2009, 2011 - 2016), Mylor - 1: 23911 (2007 - 2013, 2015 - 2016), Mylor - 2: 23817 (2000 - 2004, 2007, 2012 - 2013, 2015 - 2016), Wottons 1 and 2: 23707 (2000, 2007 - 2014), Giles: 23803 (2000 - 2003, 2007 - 2009, 2012, 2015 - 2016), Filsell: 23801 (2000 - 2002, 2004 - 2010, 2012 - 2016), Mark Oliphant 1 and 2: 23843 (2000, 2002 - 2005, 2010 - 2013, 2016), Scott Creek: 23921 (2014 - 2016).

2.3.3 Plant community

The Adelaide Hills possesses a temperate climate (hot, dry summers and cool, wet winters) with a mean annual rainfall of ~700-1000 mm (Bureau of Meteorology), suitable for *Eucalyptus* L'Hér. (Myrtaceae) forests and woodlands (Armstrong et al. 2003). The dominant vegetation type(s) at the chosen sample sites were dry sclerophyll forests and woodlands of messmate stringybark (*Eucalyptus obliqua* L'Hér.) and brown stringybark (*E. baxteri* (Benth.) Maiden & Blakely ex J.M.Black), a plant community characteristic of areas with nutrient-poor soils and higher rainfall throughout the Adelaide Hills (Specht and Perry 1948). Indeed, due to patterns of past vegetation clearance, most native vegetation in the study region is now composed of dry sclerophyll forest and woodlands (Armstrong et al. 2003). Thus, much of the remaining native vegetation within the landscape is of a type suitable for *P. daphnoides* (Fig. 2-1). The understorey of sample sites was predominantly composed of sclerophyllous legumes (mainly *Pultenaea daphnoides* and *Daviesia leptophylla* A.Cunn. ex G.Don), and species of *Hibbertia* Andrews (Dilleniaceae) (Pers. Obs.). In particular, the main native plant species that flower concurrently with *P. daphnoides* (September to early October) within the chosen sample sites were a combination of the legumes, *D. leptophylla* and *Platylobium obtusangulum* Hook., the large spreading shrub, *Hakea rostrata* F.Muell. ex Meisn. (Proteaceae), the small buzz-pollinated shrubs, *Hibbertia exutiacies* N.A.Wakef. and *Tetratheca pilosa* Labill. (Elaeocarpaceae), and the herbaceous moth-pollinated perennial, *Stackhousia aspericocca* Schuch. (Celastraceae) (Chapter Five; see Table 2-5 in Results). The herbaceous perennial, *Caesia calliantha* R.J.F.Hend. (Asphodelaceae), was particularly abundant in the sample site located in Scott Creek CP (Table 2-5).

2.3.4 Bee floral visitors - 2017 and 2018

Both video (~1 hour per plant) and personal observations (15-minute observations per plant) were used to measure visitation rates by bee visitors to the flowers of *P. daphnoides*. We recorded bee visitation because bees were the only floral visitors seen to successfully manipulate the flower of *P. daphnoides* (e.g., SM Fig. 2-1c-d). Due to the size of the floral display of individual plants, video recordings focused on only a subsection of each plants floral display. For playback, the number of bee visitors to flowers, which successfully manipulated the flower to expose the reproductive structures (i.e., anthers and stigma), were counted within 1-minute intervals every 5 minutes. We simply summed the number of recorded visits to each plant and did not use video data for statistical analysis. This was due to the inability to account for bee visitors moving in and out of the video frame but not leaving the plant itself. Videos were taken on sunny days between 09:00-15:00, from 20/09/2017 to 27/09/2017 (Mark

Oliphant CP - S1 (3 plants), Wottons Scrub CP - S2 (3 plants), and Filsell Hill CP (7 plants)). The mean ± standard deviation (SD) of the maximum temperature at Mount Lofty (Weather station number: 23842) on the four days of filming was 19.25 ± 3.42 ℃.

Personal observations were taken in temperatures above 15℃ (mean of 20.39 ± 2.83℃.). For personal observations, either the whole plant was observed, or again, only a subsection of the floral display of the plant was watched. All personal observations of floral visitors occurred in 2018, with observations of plants within each sample site occurring within a week of plants being tagged for measures of natural levels of reproduction (excluding Mark Oliphant CP - S2 where no observations were undertaken; see section *Natural levels of reproduction - 2017 and 2018* below). Here, floral visitation was measured as the number of bees visiting at least one flower (i.e., at least contacting part of the flower) over the 15-minute observation period per plant, divided by the number of inflorescences with at least one flower opened watched per plant. Whether or not each bee successfully manipulated at least one flower prior to departing the plant was also recorded. Sample sizes are given in Table 2-2 of the Results.

2.3.5 Measures of reproduction

Following successful fertilization, flowers of *P. daphnoides* produce a flat dehiscent ovoid pod containing either a single large seed, or less commonly, two smaller seeds (Fig. 2-2d; the flowers of *P. daphnoides* being 2-ovulate (Weber 1986)). Mature viable seeds are black in colouration, possessing a prominent white coloured elaiosome (Fig. 2-2h; mean seed mass ± SD: 5.61 ± 0.77 mg and elaiosome mass: 0.11 ± 0.02, $n = 20$). However, although flowers may produce a “pod”, seeds within these pods may not fully develop (Fig. 2-2a-c, f-g). Moreover, a gradient is evident regarding the size at which the development of seeds within pods terminates (Fig. 2-2a-c, f-g). Thus, we assessed reproduction using four measures. **1.) Pod-set**: this was measured as the proportion of flowers per plant which formed pods, regardless of seed viability within each pod. Thus, here, we consider the fruiting structure itself the pod, which includes both filled pods (i.e., visually swollen pods indicating pods contained either viable seeds or seeds which terminated later in their development; Fig. 2-2c-d, g-h) and unfilled pods (i.e., pods in which seeds terminated their development relatively early and little pod swelling is evident; Fig. 2-2a-b, f). **2.) Filled pod-set**: this was measured as the proportion of flowers per plant which formed pods which were visually swollen, indicating pods contained either viable seeds (Fig. 2-2d, h) or seeds which terminated relatively later in their development (Fig. 2-2c, g). These two measures were able to be recorded on plants in the field without pod dissection. **3.) Proportion of flowers producing pods with viable seeds**: this was measured as the proportion of flowers per plant which produced pods with viable seeds (i.e., the most typical measure of pod- or fruit-set; Fig. 2-2d, h). **4.) Proportion of pods with viable seeds**: this was measured as the proportion of pods (unfilled and filled) per plant which contained viable seeds (Fig. 2-2d, h). These two measure required pods to be collected and opened under a dissecting microscope.

Ultimately, the production of mature viable seeds (Fig. 2-2h) represents a successful outcome of flower fertilisation. However, considering plants can produce large numbers of pods in which seeds never fully develop (Fig. 2-2a-c, f-g), the production of these “incomplete” pods may represent a considerable resource drain. Thus, the four measures of pod-set, filled pod-set, the proportion of flowers producing pods with viable seeds, and the proportion of pods (unfilled and filled) with viable seeds, allowed us to study the extent to which flowers produced pods which resulted in the successful production of offspring (Fig. 2-2d, h), versus pods with seeds which were otherwise terminated (Fig. 2-2a-c, f-g). In particular, this combination of reproductive variables allows for a better understanding of possible factors restricting reproductive output. For example, high pod-set is unlikely if pollen quantity (i.e., low receival of conspecific pollen) limits reproduction. Similarly, high filled pod-set is unlikely if resources, whether abiotic or biotic, severely limit initial seed development. In contrast, if natural filled pod-set is high but the proportion of pods with viable seeds is low, this indicates that a process acting relatively later in seed development is restricting viable seed output (e.g., as may occur via pre-dispersal seed predation or a constraint on abiotic resources available for seed development later in the season).

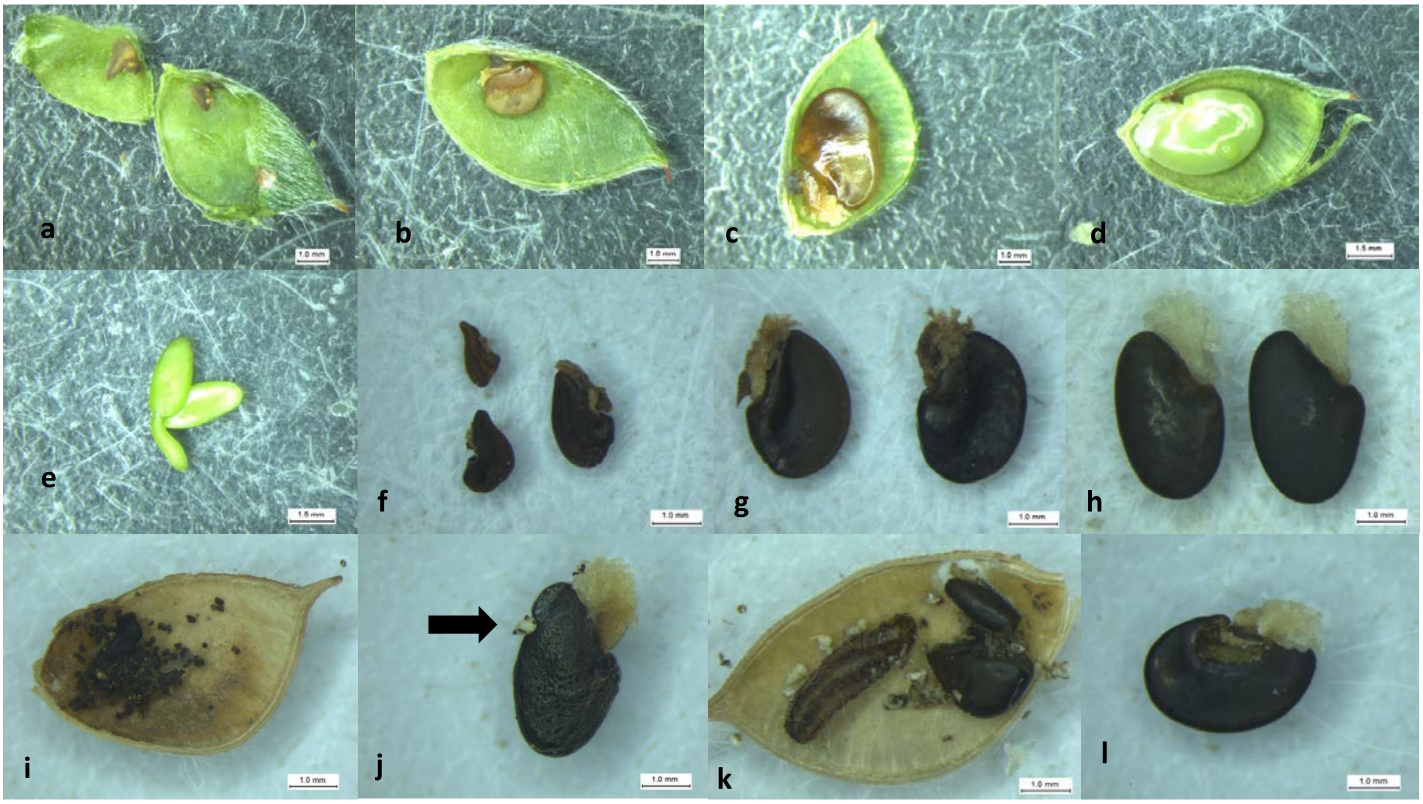


Fig. 2-2 (above) Pods and seeds of *Pultenaea daphnoides*. (**a**) Developing pod with minimal seed development prior to seed termination. (**b**) Developing pod containing a seed which has terminated at a relatively earlier stage of development. Pods in both (**a**) and (**b**) would be graded as unfilled. (**c**) Developing pod containing a seed which has terminated at a relatively later stage of development. (**d**) Developing pod containing a viable, still developing seed. Pods in both (**c**) and (**d**) would be graded as filled. Note that the developing pods shown in (**a-d**) are of the approximately the same age. (**e**) Developing embryo within prior viable seed. (**f**) Seeds within mature pods (i.e., collected during the period in which pods had begun to naturally dehisce and disperse their seeds) which terminated relatively early in their development. (**g**) Seeds within mature pods which terminated relatively later in their development. Note that the terminated seeds in (**f**) and (**g**) are all flat with thin brittle seed coats and poorly formed elaiosomes. (**h**) Enlarged viable seeds from mature pods, both bearing prominent white elaiosomes. (**i-l**) Range of pods and seeds exhibiting signs of pre-dispersal seed predation. Arrow in (**j**) indicates posterior end of larvae within a predated seed.

2.3.6 Autonomous self-pollination - 2017

To test whether *P. daphnoides* can produce pods and seeds via autonomous self-pollination white chiffon bags were used to prevent visitation by pollinators to a subset of flowers on individual plants in 2017. All treatments were conducted in the field. Following the end of flowering, bags were removed, and any subsequent pod and seed formation was recorded over the coming months. In total, for six plants across three sample sites (Mark Oliphant CP - S1, Wottons Scrub CP - S1, and Filsell Hill CP), pollinators were prevented from visiting 778 flowers. All experimental plants produced mature pods with viable seeds (Fig. 2-2h) on unbagged branches.

2.3.7 Self-pollination via geitonogamy - 2019

To test whether reproduction can occur via facilitated self-pollination through geitonogamy, three treatments were applied to individual plants in the field in 2019 (early October). Firstly, bagged virgin flowers were hand-pollinated with outcross pollen taken from three conspecifics a minimum of 10 m from the recipient plant. Secondly, bagged virgin flowers were hand-pollinated with self-pollen taken from the anthers of other flowers within the same bag (i.e., measures of geitonogamy). Thirdly, unbagged flowers were left open to pollinators to measure levels of reproduction resulting from open-pollination. Pollen was applied by depressing the keel of each flower to expose its reproductive structures (i.e., anthers and stigma), followed by pressing dehisced anthers onto the exposed stigma. For outcross pollination, a minimum of three anthers (one each from a separate donor plant) were used until ample pollen could be observed on the stigma (confirmed with 10-40 × jewellery loupe). Bags were removed following the abscission of flowers. To reduce the risk of mature pods from tagged flowers rapidly dehiscing and dispersing seeds before being censused, all pods were collected in late November when developing pods were close to maturity, and seeds had developed to the point that viable seeds could be recognised (Fig. 2-2d-e). Seed maturity and natural pod dehiscence occur from mid-December onwards. All pods collected close to maturity in late November to early December are hereafter termed developing pods.

Seeds within developing pods were examined and graded as either **1.) Viable**: enlarged seed(s) containing a healthy-looking embryo (Fig. 2-2d-e). **2.) Terminated**: discoloured small to large seeds, flatter than viable seeds, and unfilled with a poorly formed or non-existent embryo (Fig. 2-2a-c); or seeds which had suffered pre-dispersal seed predation, as recognized by a combination of the presence of insect larvae (or their remains), frass (excrement of insect larvae), and entry or exit holes in the pod wall or seed coat (Fig. 2-2i-l). Typically, in late November to early December, pods with seeds which had suffered complete predation were dry and brown in colouration (Fig. 2-2i), while other developing pods were still green and fleshy (Fig.2a-d).

In total, six plants were treated at Mark Oliphant CP - S2 in 2019, with 26 flowers outcrossed, 15 flowers self-pollinated, and 36 flowers left open to pollinators. Both outcross- and self-pollination treatments of flowers occurred on separate inflorescences contained within the same bag. A small number of pods (4/36) suffered attack from pre-dispersal seed predators. Since we were only interested in measuring the effect of outcross- versus self-pollen on viable seed production, flowers and developing pods with predated seeds were not included in calculations of the proportion of flowers producing pods with viable seeds and the proportion of pods (unfilled and filled) with viable seeds.

2.3.8 Pollen-limitation of reproduction - 2017 and 2019

Pollen-limitation of reproduction was measured by artificially pollinating flowers with outcross pollen taken from conspecific donors, following the methods used to pollinate flowers with outcross pollen in the section *Self-pollination via geitonogamy - 2019*. However, here, pollen supplemented flowers were left open to additional pollination via pollinator visitation. Two controls were used to account for the possibility of resource reallocation between flowers on the same plant confounding the effects of pollen addition (Knight et al. 2006). First, an internal control was allocated by tagging an equal number of flowers on the same plant that received the artificial pollination treatments. Second, an equal number of flowers were tagged on a similar sized conspecific, growing within the local vicinity (≤ 10 m) of the treatment plant. This additional control represented the external control. Thus, each treatment plant (i.e., a tagged plant receiving pollen additions) was paired with an external control plant. A statistically lower level of reproduction in the internal versus the external control is considered to indicate the presence of resource reallocation among the flowers of treatment plants (Knight et al. 2006). In both 2017 and 2019, flowers were tagged for pollen-limitation experiments in mid-September to early October.

In 2017, both the internal control and treatment flowers (i.e., pollen additions) were tagged on the same inflorescence, with 1-2 flowers of each treatment per tagged inflorescence. Treatments within inflorescences were separated by different coloured cotton thread tied at the base of the calyx, a standard technique used to tag flowers inconspicuously (Kearns and Inouye 1993 p. 35). Moreover, both native bees and the introduced *A. mellifera* were seen to visit tagged flowers and even visited flowers on inflorescences when being manipulated (e.g., tags being applied, pollen addition treatments occurring), suggesting tagging had no adverse effect on floral visitation. Overall, in 2017, across seven sample sites in six reserves (Table 2-1), a total of 164 flowers over 27 plants (mean ± SD: 6.07 ± 1.50 flowers per treatment per plant; 3-5 plants per treatment per sample site) were supplemented with pollen, with replicates for both the internal and external control. Thus, the number of experimental plant pairings used, both within and pooled across sample sites, was comparable to other studies assessing the pollen-limitation of similar legumes in Australia (Gross 1996 and 2001; Eakin-Busher et al. 2020), and also comparable to other studies testing for an effect of fragment area (Cunningham 2000b) or population size (Delnevo et al. 2020) on the pollen-limitation of plant reproduction. A further statistical analysis of sampling power was not undertaken. In 2017, filled pod-set was the response variable measured because the majority of pods were swollen, indicating pods contained either viable seeds (Fig. 2-2d, h) or seeds which terminated later in their development (Fig. 2-2c, g), and this reproductive measure was able to be quickly assessed in the field across the seven sample sites used (Table 2-1). Filled pod-set was recorded for developing pods in late November to early December.

In 2019, following the results of 2017, which suggested no resource reallocation between flowers on the same inflorescence, internal control flowers were marked on a close but separate inflorescence. Because of the difficulty tagging individual flowers with cotton twine due to their tightly packed inflorescences, flowers were instead marked with black permanent ink on the base of the calyx, a standard technique used when flowers are difficult to tag with cotton twine, jewellers tags, etc. (Kearns and Inouye 1993 p. 35). A small pilot study of a single plant in 2017 suggested no adverse impact on filled pod-set (filled pod-set = 3/4 for tagged flowers and 9/10 for ink marked flowers). Treatments were separated by different coloured cotton threads tied at the base of the inflorescences. Overall, in 2019, across four sample sites in four reserves (Table 2-1), a total of 64 flowers over 13 plants (mean ± SD: 4.95 ± 0.32 flowers per treatment per plant; 3-4 plants per treatment per sample site) were supplemented with pollen, with replicates for both the internal and external control. Note that only four reserves were used in 2019 (versus six in 2017) due to time limitations, and the reserves used were a subset of the six sampled in 2017 and ranged from the smallest sampled (The Knoll) to the second largest (Mark Oliphant) in area (Table 2-1). Moreover, because of the widespread abortion of developing seeds in 2018 (see Results), we also opened developing pods to grade seeds as viable or terminated in 2019. Thus, we measured pod-set, the proportion of flowers producing pods with viable seeds, and the proportion of pods (unfilled and filled) with viable seeds in 2019, which allowed for a more detailed study of pollen-limitation. Pod-set was recorded in late November to early December. Developing pods were then collected, opened, and developing seeds within graded as viable (Fig. 2-2d-e) or terminated (Fig. 2-2a-c, i-l) following methods described above under *Self-pollination via geitonogamy - 2019*. Again, a small number of pods (8/118) suffered attack from pre-dispersal seed predators. Since we were only interested in measuring the effect of pollen addition on viable seed production, flowers and developing pods with predated seeds were not included in calculations of the proportion of flowers producing pods with viable seeds and the proportion of pods (unfilled and filled) with viable seeds.

2.3.9 Natural levels of reproduction - 2017 and 2018

The relationship between natural levels of reproduction and reserve area was measured across two years (2017 and 2018), with the largest dataset coming from 2018. This was due to the time required to conduct pollen-limitation experiments on plants in 2017. Note that because of only four reserves being sampled in 2019 (versus six in 2017 and 2018, respectively) the relationship between reserve area and natural levels of reproduction was not assessed (although see SM Fig. 2-3, 2-4, and 2-5 for plots of external-control plants in each reserve in 2019).

In 2017, estimates of filled pod-set were made from the external controls of pollen-limitation experiments (see section *Pollen-limitation of reproduction - 2017 and 2019* above). Note that in contrast to the breeding system and pollen-limitation experiments both flowers and pods with predated seeds were included in the calculation of these reproductive variables, given we were interested in measuring natural levels of reproduction. Additional haphazard sampling of mature pods (i.e., collected during the period in which pods had begun to naturally dehisce and disperse their seeds) was also undertaken from plants across sample sites in mid-December in 2017 (Table 2-1). However, not all sample sites used for pollen-limitation experiments in 2017 were able to be sampled for mature pods because plants in some sample sites (e.g., Scott Creek) had already mostly dispersed their seeds. Due to this, we added additional samples sites both within and across reserves (Table 2-1). This later sample of mature pods was used to measure the proportion of pods with viable seeds only, since flowers were not tagged prior to the pod collection. For mature pods, seeds were graded as viable when enlarged (i.e., not flat) and with a hard seed coat and prominent elaiosome (Fig. 2-2h). Terminated seeds were graded as per the methods described above under *Self-pollination via geitonogamy - 2019* (Fig. 2-2f-g, i-l). Mature pods were sampled by haphazardly selecting five fruiting inflorescences, bearing a minimum of five mature but non-dehisced pods (i.e., pods on the plant had dried and turned brown but not yet dispersed their seeds), from each plant.

In 2018, due to lengthy pollen-limitation experiments not being undertaken, a larger sample size was obtained for measures of natural reproduction by tagging the flowers of ten plants per sample site (except for Mark Oliphant - S2, where only 5 plants were tagged due to time constraints) across seven sample sites in six reserves (Table 2-1), comparable to other similar studies of legumes in Australia (Cunningham 2000a; Broadhurst and Young 2006). Five inflorescences were tagged per plant, with the calyxes of at least two flowers per inflorescence marked with black permanent ink. In total, 719 flowers on 350 inflorescences were tagged. Flowers were marked from late September to early October. In comparison to 2017, most pods on plants appeared to be unfilled, indicating seed development had terminated relatively early. Thus, both pod-set and filled pod-set were recorded in early December for developing pods. As for 2017, mature pods were also collected in the following weeks of December, this time from tagged plants when possible, and from additional haphazardly chosen plants when not enough mature pods were available on tagged plants within sample sites. Five inflorescences were haphazardly sampled from each plant, regardless of the number of pods per inflorescence, due to the overall lower number of mature pods produced by plants compared to 2017, when a cut-off of at least five mature pods per inflorescence was used. Seeds within mature pods were graded as viable (Fig. 2-2h) or terminated (Fig. 2-2f-g, i-l).

Additional variables measured on tagged plants in 2018 included the height and width of the floral display, the number of open flowers per plant at the time of tagging, the number of conspecifics within 2 m of tagged plants, the number of flowering conspecifics within 2 m, and the number of open flowers on conspecifics within 2 m (see Table 2-5 in Results). These variables were statistically compared between sample sites and related to differences in pod and seed production. We also counted the number of plants other than *P. daphnoides* flowering within 2 m, excluding wind-pollinated species, and the number of open flowers on plants other than *P. daphnoides* within 2 m. Transects were also used to estimate these two variables (Table 2-5). We present the three most numerically abundant plant species in flower (i.e., number of individuals), and the three species with the largest floral displays (i.e., number of flowers), to assess both the floral dominance of *P. daphnoides*, and qualitatively examine differences between sample sites in their dominant flowering species.

2.3.10 Statistical analysis

All statistical models were run within the program R (R Core Team 2020). Generalised linear mixed-effect models (GLMM) were constructed within the package 'lme4' (Bates et al. 2015). In some instances, the variance of specified random effects within models was singular (i.e., ~ 0). When this occurred, a partially Bayesian method was used to help prevent singular fits via the 'b(g)lmer' function in the package 'blme' (Chung et al. 2013). Diagnostic checks of mixed-effect models were performed within the package 'DHARMa' (Hartig 2021). Statistical significance of predictors in GLMM's were tested via an Analysis of Deviance, using a Type II Wald Chi-square test (implemented in package 'car'; Fox and Weisberg 2019). Multiple comparisons were performed via the package 'multcomp' (Hothorn et al. 2008), with a Bonferroni correction used to account for inflated Type I error. Figures were constructed via the packages 'ggplot2' (Wickham 2016) and 'tmap' (Tennekes 2018).

2.3.11 Bee floral visitors - 2017

A one-way ANOVA was used to analyse whether the mean number of bee visits per inflorescence per plant during 15-min observations differed among sample sites in 2018. Assumptions of normality and equality of variance were checked via plots of model residuals (i.e., Q-Q plots, S-L plots). Following this, a square root transformation was applied to the response variable to improve equality of variance.

2.3.12 Pollen-limitation of reproduction - 2017 and 2019

A Binomial GLMM was used to examine whether the pollen-addition treatment interacted with reserve area (e.g., if pollen-limitation declined with increasing reserve area) for the response variable of filled pod-set in 2017. Treatment and reserve area, which was log transformed prior to analysis to reduce right skewness, were considered fixed effects. Reserve and plant pair nested within reserve were treated as random effects. Plant pair was explicitly accounted for in the model as it is equivalent to a blocking factor within the experimental design. Note that the main effect of reserve area was not of particular interest and was considered separately for models including only external controls used to measure levels of natural reproduction (see statistical models in section *Natural levels of reproduction - 2017 and 2018* below).

Only four reserves were used in 2019 (versus six in 2017) and this smaller sample size, combined with the confounding effect of an autumnal prescribed burn (06/05/2019) across approximately half of the smallest reserve sampled in 2019 (The Knoll), precluded an analysis of the interaction between reserve area and pollen-limitation (although see SM Fig. 2-3, 2-4, 2-5 for plots of the external- and internal-control and pollen-addition plants in each reserve in 2019). Thus, Binomial GLMMs of pod-set, the proportion of flowers producing pods with viable seeds, and the proportion of pods (unfilled and filled) with viable seeds in 2019 included the fixed effect of treatment only. Reserve and plant pair nested within reserve were treated as random effects.

2.3.13 Natural levels of reproduction - 2017 and 2018

Binomial GLMM's were used to analyse the relationship between the response variables of pod-set (2018), filled pod-set (2017 and 2018), and the proportion of pods (unfilled and filled) with viable seeds (2017 and 2018), and the predictor of reserve area. Reserve was treated as a random effect to account for the inherent pseudo-replication between individual plants within sample sites in reserves. An observation-level random intercept term was also included to account for over-dispersion when present (Warton and Hui 2011). Where more than one sample site was present within a reserve (e.g., Mark Oliphant in 2017), data were pooled across the respective sample sites for analysis. The independent predictor of reserve area was again log transformed prior to analysis to reduce right skewness. Note that flowers and pods which suffered seed predation were not excluded from analysis.

We also statistically tested whether sample sites differed in the additional variables measured for tagged plants in 2018 (i.e., width of the floral display, number of conspecifics within 2 m of tagged plants, etc. See Table 2-5 in Results). This was done by a one-way ANOVA. However, following checks of normality and equality of variance (i.e., Q-Q plots, S-L plots) some data transformations were required. This included a square root transformation on both the number of conspecifics within 2 m of tagged plants and the number of flowering conspecifics within 2 m, and a cube root transformation on both the width of the floral display and the number of open flowers on conspecifics within 2 m. For the number of open flowers per plant a fourth root transformation improved normality. However, heterogeneity of variance remained, so a James Second Order test (Quinn and Keough 2002 p. 195) was conducted via the R package ‘onewaytests’ (Dag et al. 2018).

2.4 RESULTS

2.4.1 Bee floral visitors - 2017 and 2018

In 2017, across 13 plants, and a total of 2 hours and 36 minutes of video footage, 20 observations of native bees visiting and successfully manipulating the flowers of *P. daphnoides* were recorded, as well as 86 observations of *A. mellifera*. Due to difficulties in keeping the camera in focus, and clearly seeing bees among the inflorescences of *P. daphnoides*, identification of native bees was difficult, but included a number of white-banded bees most likely species of *Lasioglossum* (e.g., *L.* (*Chilalictus*) *lanarium* (Smith)). No individuals of the genus *Trichocolletes* Cockerell, species of which are mostly legume specialists (Houston 2018), were recorded visiting flowers of *P. daphnoides*, although they were seen visiting flowers of *Daviesia leptophylla* in Mark Oliphant and Wottons Scrub throughout filming.

In comparison, fifteen-minute observations of 31 plants in 2018 resulted in a total of 86 floral visits by native bees observed to plants of *P. daphnoides*, and 29 visits by *A. mellifera*. Of the native bees observed in 2018, 73 out of 84 depressed the keel and contacted the reproductive structures (i.e., anthers and stigma) of at least one flower before leaving the plant (note that bee behaviour on the flower was unobserved for two individuals). Native bees caught foraging on flowers of *P. daphnoides* were predominantly species of *Lasioglossum* including *Lasioglossum* (*Chilalictus*) *lanarium* and *L.* (*Chilalictus*) *erythrurum* (Cockerell), both widespread across Australia (Atlas of Living Australia). *Lasioglossum* (*Chilalictus*) *lanarium* in particular is common throughout anthropogenically modified landscapes in southern Australia (Brown et al. 2020; Neave et al. 2020), and forages on a wide range of flowering plants (Howard et al. 2021). Unidentified species of *Lasioglossum* (*Parasphecodes*) Smith and *Leioproctus* Smith (Fam. Colletidae) were also seen visiting flowers. Again, no individuals of *Trichocolletes* were seen during observations, although they were haphazardly seen occasionally visiting flowers of *P. daphnoides* during fieldwork. All individuals of *A. mellifera* contacted the reproductive structures of at least one flower prior to departing. There was no statistically significant difference between sample sites in the mean number of bee visits per inflorescence per plant during 15-min observations ($F_{5,25} = 1.487$, $p = 0.230$; Table 2-2). This was also true when excluding visitation by native bees which did not successfully manipulate at least one flower prior to departing the plant ($F_{5,25} = 1.395$, $p = 0.260$).

Table 2-2 The mean ± standard deviation of the number of bee visits per inflorescence of *Pultenaea daphnoides* plants during 15-min observations in 2018. S1 stands for sample site 1.

Sample site	Date of observation	No. plants observed	Visits per inflorescence[b] - native bees and *Apis mellifera*	Visits per inflorescence[b] - native bees	Visits per inflorescence[b] - *A. mellifera*
Nurrutti Reserve	26/9/2018	5	0.22 ± 0.21	0.16 ± 0.21	0.06 ± 0.07
The Knoll CP	12/10/2018	5	0.12 ± 0.10	0.09 ± 0.09	0.03 ± 0.03
Wottons Scrub CP - S1	7/10/2018, 11/10/2018[a]	5	0.09 ± 0.08	0.06 ± 0.07	0.02 ± 0.03
Filsell Hill CP	13/10/2018	5	0.05 ± 0.04	0.05 ± 0.04	0.00 ± 0.00
Mark Oliphant CP - S1	8/10/2018	6	0.08 ± 0.10	0.08 ± 0.10	0.00 ± 0.00
Scott Creek CP	6/10/2018	5	0.06 ± 0.02	0.00 ± 0.00	0.06 ± 0.02

[a] Three plants observed on 07/10/2018 and two on 11/10/2018. The two plants observed on 11/10/2018 had no floral visitation.

[b] Inflorescences with min. one open flower.

2.4.2 Autonomous self-pollination - 2017

Tests of autonomous self-pollination demonstrated that floral visitation is required to initiate high levels of pod production. Of 778 flowers bagged, only 19 developed into pods (2.44 %), and of these, none ultimately developed mature viable seeds.

2.4.3 Self-pollination via geitonogamy - 2019

Pod-set (i.e., formation of a pod (either unfilled or filled) and regardless of seed viability within each pod) was similar between outcross- (12 pods/26 flowers - 46.15 %), self- (7/15 - 46.67 %), and open-pollinated flowers (17/36 - 47.22 %; Pearson's Chi-squared tests: χ_2^2 = 0.007, *p* = 0.997). However, when these developing pods were opened and seeds within examined, pods derived from self-pollinated flowers were less likely to contain viable seeds. Thus, the proportion of self-pollinated flowers producing pods with viable seeds (2 pods with viable seeds/15 flowers - 13.33 %) was approximately half that of outcrossed flowers (6/25 - 24.00 %), and a third that of open-pollinated flowers (13/33 - 39.39 %). Nevertheless, statistically significant differences were not evident between treatments (Fisher's Exact Test: *p* = 0.152). The proportion of pods (unfilled and filled) with viable seeds was also lower for self-pollinated flowers (2 pods with viable seeds/7 pods - 28.57 %) versus outcrossed (6/11 - 54.54 %) and open-pollinated flowers (13/14 - 92.86 %), and there was a statistically significant association between the proportion of pods with viable seeds and treatment (Fisher's Exact Test: *p* = 0.008). However, only open-pollinated (92.86 %) versus self-pollinated (28.57 %) flowers were significantly different from one another (Fisher's Exact Test: *p* = 0.017; *p*-value Bonferroni corrected). The difference between outcross-pollinated (54.54 %) and self-pollinated (28.57 %) flowers approached significance (Fisher's Exact Test: *p* = 0.056) prior to a Bonferroni correction (*p* = 0.168).

2.4.4 Pollen limitation of reproduction - 2017 and 2019

There was no statistically significant interaction effect between reserve area and treatment on filled pod-set (i.e., proportion of flowers producing visually swollen pods indicating pods contained either viable seeds or seeds which terminated later in their development) in 2017 (Table 2-3; SM Fig. 2-2), and there was no statistically significant main effect of treatment (Table 2-3). Thus, the effect of the pollen addition treatment was not significantly dependent on reserve area and, overall, the probability of a flower becoming a filled pod was not limited by pollen receipt (Fig. 2-3a).

In 2019, due to four reserves (versus six in 2017) being sampled, the main effect of treatment was examined only. There was no statistically significant effect of treatment on pod-set (Table 2-3), and thus the probability of a flower becoming a pod (unfilled and filled) was not limited by pollen receipt (Fig. 2-3b). In 2019, developing pods were also opened and their seeds examined. There was no statistically significant effect of treatment on the proportion of flowers producing pods with viable seeds (Table 2-3). However, the probability that a flower produced a pod with a viable seed was still overall higher for the pollen addition treatment versus both controls in 2019 (Fig. 2-3c), and the treatment effect approached statistical significance (p = 0.072) suggesting some possible pollen-limitation (also see SM Fig. 2-4). There was no statistically significant effect of treatment on the proportion of pods (unfilled and filled) with viable seeds (Table 2-3), and thus the probability that a pod contained a viable seed was not significantly limited by pollen receipt (Fig. 2-3d). However, like the proportion of flowers producing pods with viable seeds (Fig. 2-3c), the probability that a pod contained a viable seed was still overall higher for the pollen addition treatment versus both controls (Fig. 2-3d).

Table 2-3 Models of the effect of pollen-limitation treatment (2017 and 2019) and their interaction with reserve area (2017 only) on the reproduction of *Pultenaea daphnoides*.

Year	Model	Response	Predictors	Test Statistic	*p-value*
2017	Binomial[1]	Filled pod-set	Log(reserve area (ha))[3]	χ^2_1 = 2.025	0.155
			Treatment	χ^2_2 = 1.857	0.395
			Log(reserve area (ha)) × Treatment	χ^2_2 = 3.600	0.165
2019	Binomial[1]	Pod-set	Treatment	χ^2_2 = 2.534	0.282
2019	Binomial[1]	Prop. flowers producing pods viable seeds[2]	Treatment	χ^2_2 = 5.260	0.072
2019	Binomial[1]	Prop. pods with viable seeds[2]	Treatment	χ^2_2 = 4.054	0.132

[1] Partially Bayesian method used to prevent singular fits via the ‘bglmer’ function in the package ‘blme’ (Chung et al. 2013).

[2] Developing pods were collected, opened, and developing seeds graded close to maturity. Flowers and pods with predated seeds were excluded.

[3] Six reserves of varying area were sampled.

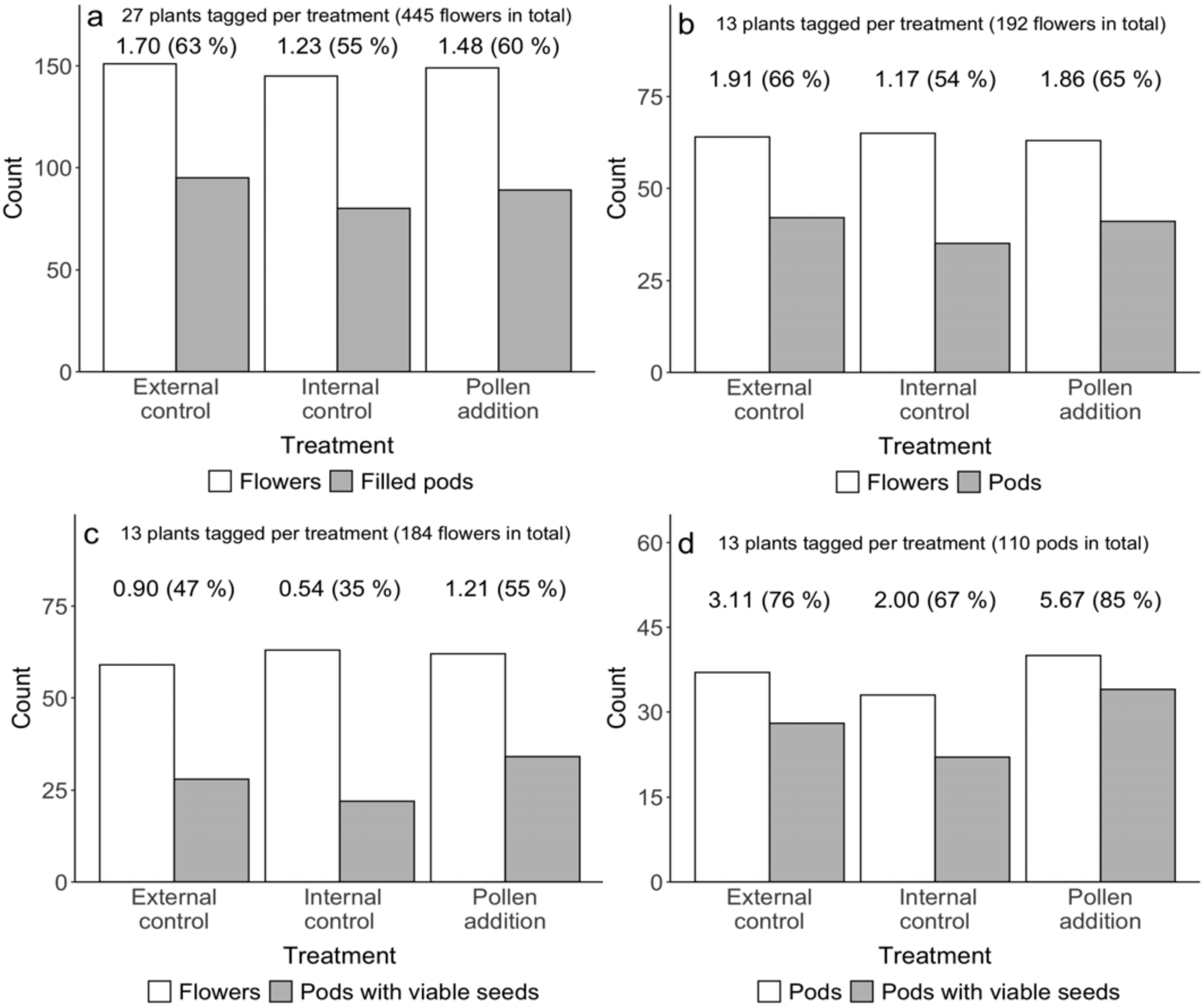


Fig. 2-3 Effects of pollen addition treatment on the production of (**a**) filled pods relative to tagged flowers in 2017, (**b**) pods (unfilled and filled) relative to tagged flowers in 2019, (**c**) pods with viable seeds relative to tagged flowers in 2019, and (**d**) pods with viable seeds relative to the number of pods (unfilled and filled) produced by tagged flowers in 2019. The odds of (**a**) a filled pod, (**b**) a pod (unfilled and filled), and (**c**, **d**) pods with viable seeds per treatment are given above each of the respective group of columns in each plot. The associated percentages (rounded to the nearest whole number) are provided in parentheses. Note that for (**c**) and (**d**) developing pods were collected, opened, and developing seeds graded close to maturity, and flowers and pods with predated seeds were excluded. The sample size of tagged flowers in (**a-c**) is also less than the original number of flowers tagged. This is because some tags were lost between the tagging of flowers and developing pods being censused.

2.4.5 Natural levels of reproduction - 2017 and 2018

In 2017, filled pod-set of external controls ranged from 54.70 % in Mark Oliphant (S1 and S2 combined) to 93.80 % in Filsell Hill. However, across the six reserves sampled no significant relationship was evident between the filled pod-set of external controls and reserve area (Table 2-4; SM Fig. 2-2).

A much larger sample size was obtained in 2018. Pod-set ranged from 45.40 % in Mark Oliphant (S1 and S2 combined) to 64.20 % in The Knoll. Similarly, filled pod-set ranged from 17.00 % in Mark Oliphant (S1 and S2 combined) to 45.30 % in The Knoll. Nevertheless, there was no statistically significant relationship between reserve area and pod-set or reserve area and filled pod-set in 2018 across the six sampled reserves (Table 2-4; SM Fig. 2-6 and 2-7). Taken together, these results suggest the probability of a flower forming a pod (either filled or unfilled) is unrelated to reserve area.

In 2017 and 2018 mature pods were sampled from plants and opened to check for the successful development of mature viable seeds (Fig. 2-2h). In 2017, a total of 1157 mature fruits, from 45 plants (mean ± SD: 34.60 ± 9.28 pods per plant), were collected and opened. Of these, 751 pods (48.23 %) contained viable seeds, and of the 1582 seeds examined, 759 seeds were viable, while 823 were terminated. Of the terminated seeds, only 97 showed signs of predation. There was no statistically significant relationship between the proportion of pods with viable seeds per plant and reserve area across the six sampled reserves in 2017 (Table 2-4). Given we sampled two separate sample sites within three of the six reserves for mature pods (Table 2-1), we also analysed whether there were differences in the proportion of pods with viable seeds per plant between sample sites. There were significant differences between sample sites in the odds of a pod containing a viable seed (Quasibinomial GLM: $F_{8,36} = 4.885$, $p = 3.906 \times 10^{-4}$). Pairwise comparisons showed fewer pods contained viable seeds in Filsell Hill versus Nurrutti, Mylor - S1, Giles, and Mark Oliphant - S2 (Fig. 2-4a). However, each of the separate sample sites within the reserves of Mylor, Wottons Scrub, and Mark Oliphant did not differ from one another (Fig. 2-4a).

In contrast to 2017, few of the mature pods sampled in 2018 produced viable seeds (Fig. 2-4b). From a total of 1706 mature pods, sampled from 60 plants (mean ± SD: 28.43 ± 8.88 pods per plant), only 230 pods (13.48 %) contained viable seeds. Moreover, of the 1761 seeds examined, 241 seeds were viable, while 1520 were terminated. Of the terminated seeds, 361 showed signs of predation. The production of viable seeds was also mostly limited to plants within the two smallest reserves of Nurrutti and The Knoll, and the proportion of pods with viable seeds was highest in the smallest reserve of Nurrutti (Fig. 2-4b). Thus, in 2018 there was a statistically significant negative relationship between the proportion of pods with viable seeds per plant and reserve area across the six sampled reserves (Table 2-4).

The additional variables measured for tagged plants in 2018 did not obviously explain why viable seed production was mostly limited to Nurrutti and The Knoll. For example, although there were statistically clear differences between sample sites in some variables (Table 2-5), both Nurrutti and The Knoll were not similarly higher or lower in any of these variables compared to sample sites in larger reserves. *Pultenaea daphnoides* was also florally dominant within all sample sites in 2018, at least within the vicinity of tagged plants (Table 2-5), suggesting that reproduction was not limited by a relative lack of flowering by *P. daphnoides* in larger reserves. However, Nurrutti and The Knoll were the only sample sites where weed species were also florally dominant (Table 2-5). Weeds included introduced species of the genus *Erica* (Ericaceae), in both Nurrutti and The Knoll, and the introduced species, *Pittosporum undulatum* Vent. (Pittosporaceae), in The Knoll only. Clearly, the presence of these introduced species did not negatively impact reproduction of *P. daphnoides* in 2018.

Table 2-4 Models of the effect of reserve area on pod-set (2018 only), filled pod-set, and the proportion of pods (unfilled and filled) with viable seeds for open-pollinated plants of *Pultenaea daphnoides* in 2017 and 2018. The random intercept terms (nested from top to bottom) for each model are presented along with their standard deviations. The intercept and slope on the scale of the link function for each model are provided along with their 95 % confidence intervals (Wald) in brackets. Significant results highlighted in bold.

Year	Response	Predictor[2]	Random Effect(s)	Intercept	Slope	Model	Test Statistic	*p-value*
2017	Filled pod-set	Reserve area (log transformed)	Reserve: 0.234	0.543 (-0.47, 1.56)	0.008 (-0.20, 0.22)	Binomial	χ_1^2 = 0.005	0.943
	Prop. of pods with viable seeds[1]	Reserve area (log transformed)	Reserve: 0.692 Individual: 1.317	0.851 (-1.03, 2.73)	-0.253 (-0.69, 0.19)	Binomial	χ_1^2 = 1.260	0.262
2018	Pod-set	Reserve area (log transformed)	Reserve: 0.299 Individual: 0.884	0.432 (-0.32, 1.19)	-0.055 (-0.22, 0.11)	Binomial[3]	χ_1^2 = 0.421	0.516
	Filled pod-set	Reserve area (log transformed)	Reserve: 0.155 Individual: 0.842	-0.649 (-1.29, -0.01)	-0.114 (-0.25, 0.03)	Binomial	χ_1^2 = 2.544	0.111
	Prop. of pods with viable seeds[1]	Reserve area (log transformed)	Reserve: 1.055 Individual: 2.027	-0.471 (-2.41, 1.47)	-1.054 (-1.60, -0.51)	Binomial[3]	χ_1^2 = 14.196	**1.647×10^{-04}**

[1] Pods were collected, opened, and seeds graded at maturity.

[2] For each reproductive variable six reserves of varying area were sampled. However, the reserves used differed between 2017 and 2018 and between filled pod-set and the proportion of pods (unfilled and filled) with viable seeds in 2017 (Table 2-1).

[3] Partially Bayesian method used to prevent singular fits via the ‘bglmer’ function in the package ‘blme’ (Chung et al. 2013).

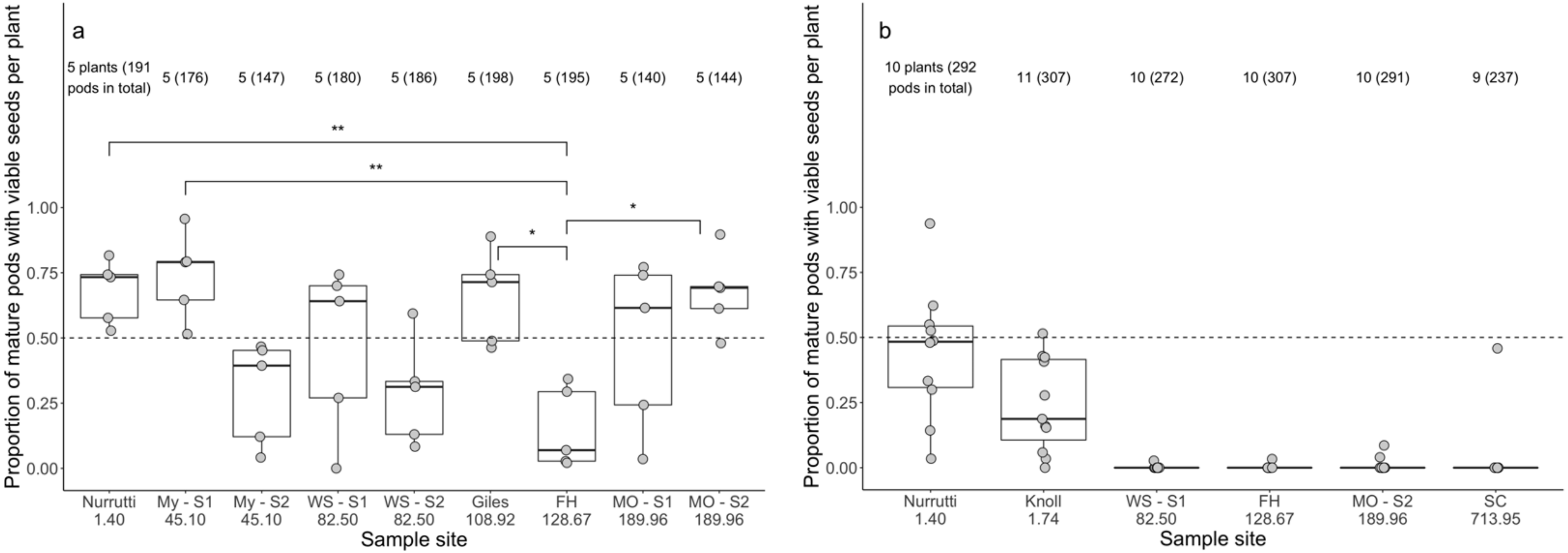


Fig. 2-4 Boxplots of the proportion of mature pods with viable seeds per plant of *Pultenaea daphnoides* collected within sample sites in (**a**) 2017 and (**b**) 2018. Pods were collected from open-pollinated plants. Individual data points (grey circles) are values for each plant within a sample site. The total number of plants and mature pods (in parenthesis) collected per sample site are provided above their respective boxplots. Sample sites are plotted in ascending order of their reserve area (ha), provided below sample site names. My = Mylor, WS = Wottons Scrub, FH = Filsell Hill, MO = Mark Oliphant, SC = Scott Creek. S1 and S2 stand for sample site 1 and 2, respectively. Boxplots connected above by square brackets in (**a**) are statistically significantly different from one another. * $p < 0.05 > 0.01$, ** $p < 0.01 > 0.001$.

Table 2-5 Attributes of plants of *P. daphnoides* tagged in 2018, and floral abundance measures of flowering plants within sample sites in 2018. Values given as mean ± standard deviation. Mean values within columns that share different lowercase letters are statistically significantly different from one another. S1 and S2 stand for sample site 1 and 2, respectively. Note, NA refers to the fact no transects were done in Mark Oliphant CP - S2.

Sample site	Open flowers[1]	Height floral display	Width floral display	Conspecifics within 2 m	Flowering conspecifics within 2 m	No. open flowers on conspecifics within 2m	Three most abundant flowering species within 2m	Three most abundant open flowers within 2m	Three most abundant flowering species along transects[2]	Three most abundant open flowers along transects[2]
Nurrutti Reserve	440.6 ± 488.9(**a**,**b**)	1.94 ± 0.43(**a**)	1.27 ± 0.71(**a**)	2.0 ± 2.45(**a**)	1.2 ± 1.32(**a**)	60.6 ± 121.16(**a**)	***Pultenaea daphnoides***, *Tetratheca pilosa*, *Stackhousia aspericocca*	***P. daphnoides***, *Erica sp.*[3], *T. pilosa*	*T. pilosa*, ***P. daphnoides***, *H. rostrata*	*Erica sp.*[3], ***P. daphnoides***, *H. rostrata*
The Knoll CP	244.8 ± 68.9(**b**)	1.66 ± 0.18(**a**)	1.11 ± 0.39(**a**)	5.0 ± 4.59(**a**)	4.4 ± 4.45(**a**,**b**)	192.9 ± 288.10(**a**)	***P. daphnoides***, *T. pilosa*, *Hibbertia exutiacies*	***P. daphnoides***, *T. pilosa*, *Pittosporum undulatum*[4]	***P. daphnoides***, *T. pilosa*, *Erica sp.*[3]	***P. daphnoides***, *Erica sp.*[3], *T. pilosa*
Wottons Scrub CP - S1	325.4 ± 300.4(**a**,**b**)	1.73 ± 0.16(**a**)	0.97 ± 0.24(**a**)	2.2 ± 1.62(**a**)	1.5 ± 0.97(**a**,**b**)	184.6 ± 275.62(**a**)	*H. exutiacies*, ***P. daphnoides***, *T. pilosa*	***P. daphnoides***, *H. exutiacies*, *T. pilosa*	*H. exutiacies*, *T. pilosa*, ***P. daphnoides***,	***P. daphnoides***, *T. pilosa*, *H. exutiacies*
Filsell Hill CP	219.1 ± 65.9(**b**)	1.88 ± 0.27(**a**)	0.89 ± 0.28(**a**)	14.0 ± 6.27(**b**)	5.9 ± 2.81(**b**)	153.4 ± 155.89(**a**)	***P. daphnoides***, *H. exutiacies*, *T. pilosa*	***P. daphnoides***, *Hakea rostrata*, *H. exutiacies*	***P. daphnoides***, *H. exutiacies*, *Platylobium obtusangulum*	***P. daphnoides***, *H. exutiacies*, *H. rostrata*
Mark Oliphant CP - S1	374.2 ± 211.7(**b**)	1.96 ± 0.25(**a**)	1.42 ± 0.41(**a**)	4.0 ± 2.83(**a**)	2.9 ± 2.51(**a**,**b**)	83.9 ± 82.19(**a**)	*Drosera sp.*, *H. exutiacies*, ***P. daphnoides***	*H. exutiacies*, ***P. daphnoides***, *Pimelea linifolia*	*H. exutiacies*, *T. pilosa*, *S. aspericocca*	*S. aspericocca*, *Daviesia leptophylla*, *T. pilosa*
Mark Oliphant CP - S2	93.6 ± 22.8(**a**)	2.14 ± 0.34(**a**)	1.14 ± 0.18(**a**)	6.0 ± 3.87(**a**,**b**)	4.4 ± 2.88(**a**,**b**)	90.8 ± 81.07(**a**)	*H. exutiacies*, ***P. daphnoides***, *Drosera sp.*	***P. daphnoides***, *P. linifolia*, *H. exutiacies*	NA	NA
Scott Creek CP	196.6 ± 146.3(**a**,**b**)	2.06 ± 0.63(**a**)	1.31 ± 0.51(**a**)	6.2 ± 4.10(**a**,**b**)	4.6 ± 3.17(**a**,**b**)	98.6 ± 83.22(**a**)	*Caesia calliantha*, ***P. daphnoides***, *T. pilosa*	***P. daphnoides***, *T. pilosa*, *C. calliantha*	*C. calliantha*, *H. exutiacies*, *P. obtusangulum*	*C. calliantha*, ***P. daphnoides***, *P. obtusangulum*

[1] Estimated by calculating the mean number of open flowers on five haphazardly chosen inflorescences with at least one open flower and multiplying this value by the total number of inflorescences with at least one open flower for each plant.

[2] The number of plants in flower (excluding wind-pollinated species) on either side of two haphazardly placed 20 m long 1.5 m transects was counted, along with numbers of open flowers. Open flowers were directly counted per plant or estimated by counting open flowers on a portion of the plant and extrapolating across the remainder.

[3] Non-native (to Australia) introduced species.

[4] Non-native (to South Australia) introduced species.

2.5 DISCUSSION

Pultenaea daphnoides is a prominent member of the largest group of endemic Australian bush peas (~750 species), commonly termed "egg and bacon" peas, consisting of the tribes Mirbelieae and Bossiaeeae (Barrett et al. 2021). Members of this group frequently form a dominant understorey component within sclerophyll forests and woodlands of southern Australia (e.g., see Table 2-5). Despite their abundance, there have been a limited number of studies on the pollination biology, breeding system(s), and the degree to which reproduction is constrained by abiotic and biotic resources for members of this widespread group of plants. In particular, no study has analysed spatial variability in plant reproduction in relation to reserve area. Here, we discuss the results of this study across these broad contexts, highlighting the importance of studies across multiple years in systems with large temporal variability in abiotic resources (e.g., precipitation).

2.5.1 Floral visitation

Pultenaea daphnoides forms a conspicuous and abundant floral display from September-October within the Adelaide Hills (e.g., see Table 2-5). Flowers of *P. daphnoides* are mostly visited by native bees of the widespread, speciose genus *Lasioglossum*. Bees of the genus *Lasioglossum* are also frequent visitors to flowers of other similar "egg and bacon" peas within Australia (Armstrong 1979; Gross 2001; Ogilvie et al. 2009; Lomov et al. 2010). The introduced honeybee, *A. mellifera*, was likewise a frequent floral visitor (Table 2-2). A greater number of foraging individuals of *A. mellifera*, relative to native bees, were observed via video observations in 2017 versus personal observations in 2018. However, it is difficult to compare the two methods of visitor observation to ascertain if such a result reflects true differences between years. In addition, observations in 2018 occurred later in the flowering period of *P. daphnoides*, versus 2017. Nevertheless, floral visitation by *A. mellifera* was not always observed upon the day of bee visitor observations in 2018 (Table 2-2). Thus, pollination of *P. daphnoides* may be performed exclusively by native bees at particular times throughout the flowering period.

Larger bees effectively parted the wings and depressed the keel petals of flowers upon visitation (Pers. Obs.). However, smaller bees of the genus *Lasioglossum* (*Chilalictus*), such as *L.* (*Chilalictus*) *erythrurum*, appeared unable to expose the reproductive structures of flowers via obviously depressing the keel petal and were otherwise observed to pry the tips of the keel petals open to access anthers (Pers. Obs.). Similar observations of smaller bee visitors have been observed for the congeneric *P. villosa* visited by small bees of *Trigona*

(*Heterotrigona*) *carbonaria* (Ogilvie et al. 2009). Given a relative lack of spatial separation between the anthers and stigma within flowers of *P. daphnoides*, as also observed for the congeners *P. densifolia* (Gross 1996) and *P. villosa* (Ogilvie et al. 2009), such manipulation may still facilitate pollination. Nevertheless, smaller sized bees may act more as pollen thieves if the stigma is not readily contacted during foraging on anthers, as suggested for other "egg and bacon" peas (Scaccabarozzi et al. 2020).

Similar to the results of Gross (2001), who studied the "egg and bacon" pea, *Dillwynia juniperina*, within a fragmented landscape dominated by *A. mellifera* in New South Wales (NSW); bees of the genus *Trichocolletes*, species of which were present within sample sites (Pers. Obs.), visited the flowers of *P. daphnoides* infrequently (Pers. Obs.). In comparison, a species of *Trichocolletes* was the predominant pollinator within populations of the congeneric *P. densifolia*, located in a semi-arid region to the east of the Adelaide Hills, within the rain shadow of the Southern Mount Lofty Ranges (Gross 1996). Here, within the Adelaide Hills, *Trichocolletes* was frequently observed visiting flowers of both early and co-flowering *Daviesia* species (i.e., *Daviesia brevifolia*, *D. leptophylla*, and *D. ulicifolia*; Pers. Obs.). This is similar to the results of Scaccabarozzi et al. (2020), who found *Trichocolletes* to be the predominant native pollinator(s) of *Daviesia* species in Western Australia.

Overall, pollination of *P. daphnoides* flowers is specialised in the sense that only floral visitors able to part the wing petals and depress the keel petals upon visitation may act as pollinators (Pers. Obs.). However, a relatively wide range of native bees, including common species such as *L.* (*Chilalictus*) *lanarium*, and the pervasive introduced bee, *A. mellifera*, seem to be effective pollinators. Although further experimental tests are required to directly confirm the pollinating abilities of different bee species, this diversity of visitors may help to reduce the chances that *P. daphnoides* will experience reproductive limitation due to a scarcity of pollinators. In addition, given honeybees are able to forage over several kilometres from their nests (Visscher and Seeley 1982), versus a flight range of a few hundred meters for most native bees in Australia (Schwarz and Hurst 1997), *A. mellifera* may also effectively disperse pollen among fragmented populations of *P. daphnoides*, thus helping to maintain pollination and plant reproduction across the landscape, although this requires further study.

2.5.2 Breeding system

Regarding the breeding system of *P. daphnoides*, negligible (2.44 %) pod-set (i.e., formation of a pod (either unfilled or filled) and regardless of seed viability within each pod) via autonomous self-pollination was observed, suggesting successful fertilization is highly reliant on floral visitation, whether reproduction occurs via self- or cross-pollination. Moreover, no apparent difference was evident between pod-set resulting from geitonogamous self- (46.68 %), outcross- (46.15 %), and open-pollination (47.22 %). Thus, pollen quality does not obviously limit the initial production of pods (unfilled and filled) from pollinated flowers. However, the proportion of self-pollinated flowers producing pods with viable seeds was approximately half that of outcrossed flowers (13.33 % versus 24.00 %), and a third that of open-pollinated flowers (13.33 % versus 39.39 %). Similarly, pods resulting from self-pollination were also less likely to contain viable seeds (28.57 % via self-pollination versus 54.54 % and 92.86 % via outcross- and open-pollination, respectively), suggesting some self-sterility of reproduction, although the precise cause(s) remain ambiguous (e.g., inbreeding depression, late-acting self-incompatibility; Gibbs 2014).

Gross (1990) observed low levels of fruit-set, defined as the proportion of flowers producing pods with viable seeds, from self-pollinations in the congeneric *P. densifolia* (6.06 % fruit-set via facilitated autogamy and 7.54 % fruit-set via geitonogamy). Similar results were also found for *Pultenaea villosa*, with fruit-set below 5.00 % via autonomous and facilitated autogamy, and geitonogamy, versus ~20.00 % fruit-set via out-crossing (Ogilvie et al. 2009). In contrast, it was noted that greenhouse grown plants of the vulnerable, *Pultenaea parrisiae*, produced seeds in the absence of pollinators, although no quantitative measures were provided (Briggs and Crisp 1994). Thus, it appears at least some limited seed production via self-pollination is possible across tested species of *Pultenaea*.

Of other “egg and bacon” peas tested, Gross (1990) found lower fruit-set via facilitated autogamy and geitonogamy for *Dillwynia hispida* (1.35 % and 3.51 %) and *D. uncinata* (0.54 % and 0.00 %). Young and Brown (1998) found fruit-set via facilitated autogamy was less than 1.00 % for *Daviesia mimosoides* and *D. suaveolens*, respectively. No fruits with seeds were formed via facilitated autogamy and geitonogamy for *Dillwynia juniperina* (Gross 2001), via autonomous self-pollination in the congeneric *D. sieberi* (Lomov et al. 2010), or for *Jacksonia sericea* via geitonogamy (Eakin-Busher et al. 2020). However, Rymer et al. (2002) observed ~30-40 % fruit-set via autonomous and facilitated autogamy across plants of *Dillwynia tenuifolia*. Nevertheless, this was still roughly less than half the fruit-set of outcrossed and open-pollinated flowers (Rymer et al. 2002). Clearly, given the low number of species examined relative to the diversity of such bush-peas (~750 species), the extent to which high self-sterility is typical of such legumes is unclear and requires further study.

2.5.3 Pod production and seed viability

In the breeding systems and pollen limitation experiments and in the observations of natural reproduction, most terminated seeds appeared to be a result of abortion rather than pre-dispersal seed predation. Moreover, there was a clear range of sizes at which seeds terminated their development within pods of *P. daphnoides* (Fig. 2-2a-d, f-h), possibly suggesting early acting inbreeding depression, although this requires further study (Husband and Schemske 1996; Hao et al. 2012). Excess flower production and abortion of developing seeds is also consistent with conditions required for selective embryo abortion (Janzen 1977; Melser and Klinkhamer 2001; Korbecka et al. 2002). Burd (2004) observed abortion of developing pods, which had noticeably begun to swell, in plants of *Pultenaea gunnii* ssp. *gunnii*. Moreover, Burd (2004) found some evidence of selective embryo abortion in naturally pollinated plants. However, fitness returns, as measured via seed mass and seedling height, were minimal compared with increasing excess flower production by the parental plant. Thus, the results of Burd (2004) suggest a limited role of selective embryo abortion in selecting for larger floral displays in *P. gunnii* ssp. *gunnii*. Nevertheless, it is evident *P. daphnoides* produces a floral display in excess of the number of seeds which are able to be produced. Similar observations are common for many plant species (Stephenson 1981), including other Australian “egg and bacon” peas (Hansen et al. 1991, 1992). Future studies would benefit from examining the trade-off(s) between the number of flowers produced by plants of *P. daphnoides* versus viable seeds produced and fathered.

2.5.4 Spatial variability in plant reproduction - 2017 and 2018

No significant positive relationship was evident between our measures of plant reproduction and reserve area across 2017 and 2018 (Table 2-4). Thus, there was no support for the hypothesis that natural levels of plant reproduction would be lower in smaller conservation reserves than in larger reserves. In contrast, viable seed production was almost limited to the two smallest reserves in 2018, resulting in a significant negative relationship between reserve area and the proportion of mature pods with viable seeds per plant (Table 2-4). Indeed, plants within the smallest reserve of Nurrutti, the most successful at producing viable seeds in 2018 (Fig. 2-4b), were also highly successful at producing viable seeds in 2017 (Fig. 2-4a). Nevertheless, reserve area was not a consistent predictor of reproductive success across years, and plants within both small and large reserves were both successful at producing viable seeds in 2017, when there was no significant relationship between reserve area and the proportion of mature pods with viable seeds per plant (Table 2-4).

There are no other studies of “egg and bacon” peas which measure reproduction in relation to reserve or fragment area. However, Lomov et al. (2010) compared reproduction of *Dillwynia sieberi* in sites of remnant and revegetated native woodland in NSW. Although plants in remnant and revegetated woodland differed in their rates of pollinator visitation, number of flowers, and seeds per inflorescence, overall seed production per plant showed no difference between remnant and revegetated sites (Lomov et al. 2010). Thus, pollination and reproduction were quickly re-established for *D. sieberi* plants within abandoned pastureland revegetated less than 20 years prior (Lomov et al. 2010). Moreover, the pollinator assemblage of *D. sieberi* was dominated by *A. mellifera*, and the pervasiveness of this introduced floral visitor may have helped re-establish and maintain pollination of this plant within revegetated and remnant areas, respectively (Lomov et al. 2010). Similarly, honeybees are now a regular visitor to flowers of *P. daphnoides* throughout the Adelaide Hills and may buffer reproduction against possible declines in native pollinators, although this remains to be tested. While we measured plant reproductive output only, altered mating patterns under landscape disturbance may also negatively impact population persistence. For example, increased production of full-sibling progeny in smaller populations has the potential to increase inbreeding between relatives within a population, reducing genetic diversity over time (Young et al. 1996; Aguilar et al. 2019). As such, Young and Brown (1998) found offspring within smaller populations of the common widespread bush pea, *Daviesia mimosoides*, had higher measures of correlated paternity. A similar relationship may occur across differently sized *P. daphnoides* populations in the Adelaide Hills.

In contrast to “egg and bacon” peas, there have been studies of other Australian legumes (i.e., not from the tribes of Mirbelieae and Bossiaeeae) in relation to landscape disturbance. For example, Buza et al. (2000) observed that smaller populations of the endangered legume, *Swainsona recta*, had reduced genetic diversity via a loss of rare alleles and increased inbreeding across fragmented grasslands of NSW. Moreover, percentage seed germination declined under high inbreeding, demonstrating negative impacts to plant fitness (Buza et al. 2000). Regarding plant reproduction, for the common, self-compatible (breeding system given in Aguilar et al. 2006), buzz-pollinated legume, *Senna artemisioides*, plant fecundity (i.e., total seed output) showed a marginally significant increase in linear strips (e.g., road verges) of mallee woodland relative to larger reserves (26-800 ha) in central NSW (Cunningham 2000a). However, in comparison, fecundity of the common, self-incompatible (Aguilar et al. 2006), generalist insect-pollinated plant, *Acacia brachybotrya*, declined within the same linear strips versus larger reserves (Cunningham 2000a). A follow-up study demonstrated a lack of pollen receipt within these linear strips limited fruit production of *A. brachybotrya*, suggesting a possible lack of pollinators restricted reproduction (Cunningham 2000b). Similarly, small, fragmented populations of the self-incompatible *Acacia dealbata* in the southern tablelands of NSW suffered poor seed production relative to larger populations (Broadhurst and Young 2006). However, this was largely due to limited mate availability within smaller populations (Broadhurst et al. 2008). Thus, although plant responses are variable, for two common Australian legumes with relatively un-specialised pollination systems, plants within smaller fragments and populations exhibited decreased reproduction.

In comparison, there was limited evidence that reproduction of *P. daphnoides* was negatively impacted by smaller reserve area, and the combined findings of 2017 and 2018 suggest that the seed production of this common understorey plant is not overall negatively affected by small reserve area. Furthermore, the smallest reserves of Nurrutti and The Knoll were the only sample sites where weed species were florally dominant along with *P. daphnoides* (Table 2-5). In particular, there are a number of abundant weeds in The Knoll (e.g., weeds listed in Table 2-5, *Ulex europaeus* L. (Fabaceae), *Asphodelus fistulosus* L. (Asphodelaceae)), which is a relatively disturbed reserve (Pers. Obs.). Nevertheless, this did not evidently negatively impact plant reproduction (Fig. 2-4). This compares to the findings of Broadhurst and Young (2006), the authors of which found plants of *A. dealbata* in populations with fewer exotic species had a greater probability of producing fruits from their inflorescences than plants in populations with more exotic species. Thus, for this common legume, both small population size and the presence of weeds negatively impacted plant reproduction (Broadhurst and Young 2006). In contrast, there is no strong evidence that small reserve area combined with the presence of florally dominant weed species negatively impacts reproduction of *P. daphnoides* in the Adelaide Hills. Ultimately, given the small number of studies which have examined the reproduction of Australian legumes in relation to aspects of landscape disturbance, a greater number of studies are required to better allow for generalisations to be made about the possible impact(s) of landscape disturbance on this important group of plants.

2.5.5 Pollen-limitation

The termination of many pods and their developing seeds is inconsistent with widespread pollen-limitation of reproduction, at least from a pollen quantity perspective (Burd 2004). Thus, we found no evidence that the production of filled pods and pods (unfilled and filled) was limited by pollen receipt in 2017 and 2019, respectively (Table 2-3). In comparison, the probability of flowers producing pods with viable seeds was overall higher for the pollen-addition treatment versus both controls in 2019 (Fig. 2-3c), and the effect of treatment approached statistical significance (Table 2-3). Thus, the proportion of flowers producing pods with viable seeds may have been limited to some extent by pollen receipt in 2019, and a more intensive examination and larger sample size may reveal pollen quality as an important factor limiting viable seed output.

Statistically significant evidence of pollen-limitation has been found in only a single species of “egg and bacon” pea. Gross (1996) observed pollen-limitation of reproduction within populations of *Dillwynia hispida* in a semi-arid area to the east of Adelaide Hills, although it was not consistent across sample sites or years. Neither pollinator density nor low visitation rates to flowers explained pollen-limitation, rather Gross (1996) considered intraspecific competition between plants for outcross pollen as important in contributing to pollen-limitation. In this system, reproduction of *D. hispida* was obligately reliant, predominantly, on a single species of *Trichocolletes* bee (Gross 1992, 1996). Similarly, an overall positive effect of pollen supplementation on fruit-set, as based on the pollen-limitation index of Larson and Barrett (2000), was observed for the “egg and bacon” pea, *Jacksonia sericea*, within an urban fragment of vegetation in Western Australia (WA), although a separate Binomial GLMM failed to find a statistically significant effect (Eakin-Busher et al. 2020). Flowers of *J. sericea* were visited by a sole species of native bee from the bee family Megachilidae (Eakin-Busher et al. 2020). Although data are available for only two studies, the results are at least partly consistent with the general notion that the degree of pollen-limitation increases with increasing reliance on a smaller subset of pollinators (Bond 1994; Wolowski et al. 2014; Bennett et al. 2020). Nevertheless, a greater number of studies are required before any definitive conclusion can be drawn as to the role of pollinator specialisation in this group of plants.

In comparison to the preceding studies, no evidence of pollen-limitation was found for two populations of *Dillwynia juniperina* located in highly fragmented woodlands on the Northern Tablelands of NSW (Gross 2001). Here, *A. mellifera* was a prominent pollinator of *D. juniperina*, effectively supplementing pollination by native bees (Gross 2001). The invasive *A. mellifera* is also a common visitor to flowers of *P. daphnoides* in the Adelaide Hills and may likewise substitute for native pollinators and buffer plant reproduction. Nevertheless, the quality of pollen delivered, and subsequent fitness of offspring, may differ between flowers pollinated by native bees versus *A. mellifera*. For example, compared to native bees, Gross (2001) observed honeybees at one study site foraged at significantly more flowers per visit to *D. juniperina*, and Lomov et al. (2010) observed that honeybees spend longer foraging at individuals of *D. sieberi*. Similar behaviour by *A. mellifera* toward *P. daphnoides* may lead to greater rates of self-pollination, although this remains to be tested.

2.5.6 Temporal variability in plant reproduction - 2017 versus 2018

Temporal variability between years was a clear factor influencing natural reproductive output, with almost total loss of successful viable seed production in 2018, excluding the two smallest reserves (Fig. 2-4b). Large temporal variability in reproductive output has also been observed across years for the Australian "egg and bacon" peas *Bossiaea aquifolium* (Hansen et al. 1991) and *Gompholobium marginatum* (Hansen et al. 1992) within sclerophyll forests of WA. In particular, for *B. aquifolium*, relatively poorer seasons of reproduction occurred in years of lower rainfall during the principal seed filling months of September-November (Hansen et al. 1991), while reproduction of *G. marginatum* responded positively to spring rainfall (Hansen et al. 1992).

The beginning of spring 2018 (i.e., September) saw historically low rainfall throughout the Adelaide Hills (e.g., September rainfall Mount Lofty weather station (Station number: 23842): 31.4 mm versus a mean of 113.5 mm from 2010-2018; see SM Fig. 2-8), with South Australia experiencing its third driest September on record (Bureau of Meteorology). Moreover, total spring rainfall (September-November) was generally below average throughout the Adelaide Hills in 2018 (Bureau of Meteorology). In comparison, rainfall was generally average to above-average in the Adelaide Hills during September 2017 (e.g., 140.0 mm at Mount Lofty; SM Fig. 2-8), and about average for the period September-November 2017 (Bureau of Meteorology). Thus, low rainfall across spring, particularly the month of September, is a strong candidate for the almost complete absence of viable seed produced by pods across sample sites in 2018. Seed abortion was also high for the co-flowering common shrub, *Hibbertia exutiacies*, further suggesting a negative impact of low spring rainfall on plant reproduction across the Adelaide Hills in 2018 (Chapter Three). However, low rainfall does not explain why viable seed production was mostly limited to the two smallest reserves of Nurrutti and The Knoll in 2018 (Fig. 2-4b).

In 2017, when rainfall was average to above-average throughout spring (e.g., see SM Fig. 2-8), the overall percentage of pods with viable seeds in Nurrutti was 69.63 % (133/191), similar to the percentage of pods with viable seeds in the larger reserves of Giles (63.13 % (125/198)) and Mark Oliphant (57.39 % (163/284)). In comparison, the overall percentage of pods with viable seeds in Nurrutti in 2018 was considerably lower at 43.84 % (128/292), suggesting a possible decline in viable seed production in Nurrutti relative to the same reserve in 2017 (also compare Fig. 2-4a-b). However, given almost no sampled plants produced viable seeds in the four larger reserves of Wottons Scrub (82.50 ha), Filsell Hill (128.67 ha), Mark Oliphant - S2 (189.96 ha), and Scott Creek (713.95 ha) in 2018, the production of viable seeds mostly restricted to plants in the smallest reserves of Nurrutti (1.40 ha) and The Knoll (1.74 ha) is striking (Fig. 2-4b). Potentially, localised rainfall events occurred at these two small reserves and helped to maintain viable seed production. However, when examining the rainfall recorded at the three nearest weather stations to each sample site, rainfall was not evidently greater for Nurrutti or The Knoll, respectively, although rainfall events localised to either sample site may not have been recorded (Unpublished Data). Thus, the reason(s) as to why viable seed production was maintained in these two small reserves remains ambiguous.

2.5.7 Summary

Overall, reproduction of *P. daphnoides* does not appear to be pollen-limited, at least by pollen quantity. However, pollen quality may limit viable seed production and deserves further investigation, particularly considering the widespread abortion of developing seeds across years (including in the wetter spring of 2017). Regarding landscape disturbance, larger reserve area was not a significant predictor of reproductive success, and plants within both small and large reserves were both successful at producing viable seeds in 2017. However, viable seed production was almost limited to the two smallest reserves in 2018, resulting in a negative relationship between reserve area and the proportion of pods with viable seeds per plant. This occurred in a year of historically low early spring rainfall (September), and low spring rainfall (September-November) more generally. Importantly, from a conservation perspective, average spring rainfall in the Adelaide Hills is modelled to decline by 17.0-25.2 % by the year 2070 (Charles and Fu 2015). Such declines in rainfall may lead to decreased reproductive output relative to historical levels, irrespective of any current impact(s) of landscape disturbance on *P. daphnoides*. Thus, it would seem prudent to determine why viable seed production was at least partially maintained in two of the smallest reserves within the study region (< 2 ha), versus the almost complete absence of viable seed production in larger reserves (> 80 ha), possibly in response to lower spring rainfall.

2.6 REFERENCES:

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980.

Aguilar R, Cristóbal-Pérez EJ, Balvino-Olvera FJ, de Jesús Aguilar-Aguilar M, Aguirre-Acosta N, Ashworth L, Lobo JA, Martén-Rodríguez S, Fuchs EJ, Sanchez-Montoya G, Bernardello G (2019) Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecology Letters 22:1163-1173.

Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? Journal of Vegetation Science 13:885-892.

Armstrong JA (1979) Biotic pollination mechanisms in the Australian flora - a review. New Zealand Journal of Botany 17:467-508.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Atlas of Living Australia website. Species page (*Lasioglossum* (*Chilalictus*) *erythrurum*): https://bie.ala.org.au/species/https://biodiversity.org.au/afd/taxa/95e060d3-c732-465c-a401-fa588cfda704. Accessed 19 October 2022.

Atlas of Living Australia website. Species page (*Lasioglossum* (*Chilalictus*) *lanarium*): https://bie.ala.org.au/species/https://biodiversity.org.au/afd/taxa/8634ccfb-2dea-48d4-8853-6f91018440f2. Accessed 19 October 2022.

Barrett RL, Clugston JA, Cook LG, Crisp MD, Jobson PC, Lepschi BJ, Renner MA, Weston PH (2021) Understanding diversity and systematics in Australian Fabaceae tribe Mirbelieae. Diversity 13:391.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Bennett JM, Steets JA, Burns JH, Burkle LA, Vamosi JC, Wolowski M, Arceo-Gómez G, Burd M, Durka W, Ellis AG, Freitas L (2020) Land use and pollinator dependency drives global patterns of pollen limitation in the Anthropocene. Nature Communications 11:3999.

Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London B: Biological Sciences 344:83-90.

Briggs JD, Crisp MD (1994) *Pultenaea parrisiae* (Fabaceae: Mirbelieae), a new species from south-eastern Australia. Telopea 5:647-655.

Broadhurst LM, Young AG (2006) Reproductive constraints for the long-term persistence of fragmented *Acacia dealbata* (Mimosaceae) populations in southeast Australia. Biological Conservation 133:512-526.

Broadhurst L, Young A (2007) Seeing the wood and the trees - predicting the future for fragmented plant populations in Australian landscapes. Australian Journal of Botany 55:250-260.

Broadhurst LM, Young AG, Forrester R (2008) Genetic and demographic responses of fragmented *Acacia dealbata* (Mimosaceae) populations in southeastern Australia. Biological Conservation 141:2843-2856.

Brown J, Barton PS, Cunningham SA (2020) Flower visitation and land cover associations of above ground- and below ground-nesting native bees in an agricultural region of south-east Australia. Agriculture, Ecosystems and Environment 295:106895.

Burd M (2004) Offspring quality in relation to excess flowers in *Pultenaea gunnii* (Fabaceae). Evolution 58:2371-2376.

Buza L, Young A, Thrall P (2000) Genetic erosion, inbreeding and reduced fitness in fragmented populations of the endangered tetraploid pea *Swainsona recta*. Biological Conservation 93:177-186.

Charles SP, Fu G (2015) Statistically downscaled projections for South Australia. Goyder Institute for Water Research Technical Report Series No. 15/1, Adelaide, South Australia.

Chung Y, Rabe-Hesketh S, Dorie V, Gelman A, Liu J (2013) A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. Psychometrika 78:685-709.

Cunningham SA (2000a) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Cunningham SA (2000b) Depressed pollination in habitat fragments causes low fruit set. Proceedings of the Royal Society of London B: Biological Sciences 267:1149-1152.

Dag O, Dolgun A, Konar NM (2018). onewaytests: an R package for one-way tests in independent groups designs. The R Journal 10:175-199.

Dai A (2013) Increasing drought under global warming in observations and models. Nature Climate Change 3:52-58.

Delnevo N, van Etten EJ, Byrne M, Petraglia A, Carbognani M, Stock WD (2020) Habitat fragmentation restricts insect pollinators and pollen quality in a threatened Proteaceae species. Biological Conservation 252:108824.

Donaldson J, Nänni I, Zachariades C, Kemper J (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. Conservation Biology 16:1267-1276.

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

Fahrig L, Arroyo-Rodríguez V, Bennett JR, Boucher-Lalonde V, Cazetta E, Currie DJ, Eigenbrod F, Ford AT, Harrison SP, Jaeger JAG, Koper N, Martin AE, Martin JL, Metzger JP, Morrison P, Rhodes JR, Saunders DA, Simberloff D, Smith AC, Tischendorf L, Vellend M, Watling JI (2019) Is habitat fragmentation bad for biodiversity? Biological Conservation 230:179-186.

Fischer J, Lindenmayer DB (2007) Landscape modification and habitat fragmentation: a synthesis. Global Ecology and Biogeography 16:265-280.

Fletcher Jr RJ, Didham RK, Banks-Leite C, Barlow J, Ewers RM, Rosindell J, Holt RD, Gonzalez A, Pardini R, Damschen EI, Melo FPL, Ries L, Prevedello JA, Tscharntke T, Laurance WF, Lovejoy T, Haddad NM (2018) Is habitat fragmentation good for biodiversity? Biological Conservation 226:9-15.

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks, California.

Gibbs PE (2014) Late-acting self-incompatibility - the pariah breeding system in flowering plants. New Phytologist 203:717-734.

Gross CL (1990) The breeding systems of three cooccurring legumes: *Dillwynia hispida*, *Dillwynia uncinata* and *Pultenaea densifolia* (Leguminosae, Papilionoideae). Australian Journal of Botany 38:207-215.

Gross CL (1992) Floral traits and pollinator constancy: foraging by native bees among three sympatric legumes. Austral Ecology 17:67-74.

Gross CL (1996) Is resource overlap disadvantageous to three sympatric legumes? Australian Journal of Ecology 21:133-143.

Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. Biological Conservation 102:89-95.

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD, Lovejoy TE, Sexton JO, Austin MP, Collins CD, Cook WM (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances 1:1-9.

Hadley AS, Betts MG (2012) The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. Biological Reviews 87:526-544.

Hansen A, Pate JS, Hansen AP (1991) Growth and reproductive performance of a seeder and a resprouter species of *Bossiaea* as a function of plant age after fire. Annals of Botany 67:497-509.

Hansen A, Pate JS, Hansen AP (1992) Growth, reproductive performance and resource allocation of the herbaceous obligate seeder *Gompholobium marginatum* R. Br. (Fabaceae). Oecologia 90:158-166.

Hao YQ, Zhao XF, She DY, Xu B, Zhang DY, Liao WJ (2012) The role of late-acting self-incompatibility and early-acting inbreeding depression in governing female fertility in monkshood, *Aconitum kusnezoffii*. PLoS One 7:e47034.

Harris LF, Johnson SD (2004) The consequences of habitat fragmentation for plant-pollinator mutualisms. International Journal of Tropical Insect Science 24:29-43.

Hartig F (2021) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Houston T (2018) A guide to native bees of Australia. CSIRO Publishing, Victoria, Australia.

Howard SR, Prendergast K, Symonds MRE, Shrestha M, Dyer AG (2021) Spontaneous choices for insect-pollinated flower shapes by wild non-eusocial halictid bees. Journal of Experimental Biology 224:jeb242457.

Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression in plants. Evolution 50:54-70.

Janzen DH (1977) A note on optimal mate selection by plants. The American Naturalist 111:365-371.

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.

Knight TM, Steets JA, Ashman TL (2006) A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. American Journal of Botany 93:271-277.

Korbecka G, Klinkhamer PGL, Vrieling K (2002) Selective embryo abortion hypothesis revisited - a molecular approach. Plant Biology 4:298-310.

Lamont BB, Klinkhamer PG, Witkowski ETF (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. Oecologia 94:446-450.

Larson BM, Barrett SC (2000) A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society. 69:503-520.

Lomov B, Keith DA, Hochuli DF (2010) Pollination and plant reproductive success in restored urban landscapes dominated by a pervasive exotic pollinator. Landscape and Urban Planning 96:232-239.

Matesanz S, Escudero A, Valladares F (2009) Impact of three global change drivers on a Mediterranean shrub. Ecology 90:2609-2621.

McIntyre S, Hobbs R (1999) A framework for conceptualizing human effects on landscapes and its relevance to management and research models. Conservation Biology 13:1282-1292.

Melser C, Klinkhamer PG (2001) Selective seed abortion increases offspring survival in *Cynoglossum officinale* (Boraginaceae). American Journal of Botany 88:1033-1040.

Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology 13:266-273.

Neave MJ, Brown J, Batley M, Rao S, Cunningham SA (2020) Phenology of a bee (Hymenoptera: Apoidea) community over a 10 year period in south-eastern Australia. Austral Entomology 59:602-611.

Ogilvie JE, Zalucki JM, Boulter SL (2009) Pollination biology of the sclerophyllous shrub *Pultenaea villosa* Willd. (Fabaceae) in southeast Queensland, Australia. Plant Species Biology 24:11-19.

Paton DC (1993) Honeybees in the Australian environment. Bioscience 43:95-103.

Pauw A (2007) Collapse of a pollination web in small conservation areas. Ecology 88:1759-1769.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, United Kingdom.

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rymer PD, Morris EC, Richardson BJ (2002) Breeding system and population genetics of the vulnerable plant *Dillwynia tenuifolia* (Fabaceae). Austral ecology 27:241-248.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5:18-32.

Scaccabarozzi D, Dixon KW, Tomlinson S, Milne L, Bohman B, Phillips RD, Cozzolino S (2020) Pronounced differences in visitation by potential pollinators to co-occurring species of Fabaceae in the Southwest Australian biodiversity hotspot. Botanical Journal of the Linnean Society 194:308-325.

Schwarz MP, Hurst PS (1997) Effects of introduced honey bees on Australia's native bee fauna. Victorian Naturalist 114:7-12.

Specht RL, Perry RA (1948) The plant ecology of part of the Mount Lofty Ranges (1). Transactions of the Royal Society of South Australia 72:91-139.

Stephenson AG (1981) Flower and fruit abortion: proximate causes and ultimate functions. Annual Review of Ecology and Systematics 12:253-279.

Tennekes M (2018) tmap: thematic maps in R. Journal of Statistical Software 84:1-39.

Threlfall CG, Walker K, Williams NS, Hahs AK, Mata L, Stork N, Livesley SJ (2015) The conservation value of urban green space habitats for Australian native bee communities. Biological Conservation 187:240-248.

Visscher PK, Seeley TD (1982) Foraging strategy of honeybee colonies in a temperate deciduous forest. Ecology 63:1790-1801.

Warton DI, Hui FK (2011) The arcsine is asinine: the analysis of proportions in ecology. Ecology 92:3-10.

Weber JZ (1986) Leguminosae subfamily - Papilionoideae. In: Jessop JP, Toelken HR (eds) Flora of South Australia, part 2, Leguminosae-Rubiaceae, 4th edn. South Australian Government Printing Division, Adelaide, pp 569-709.

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

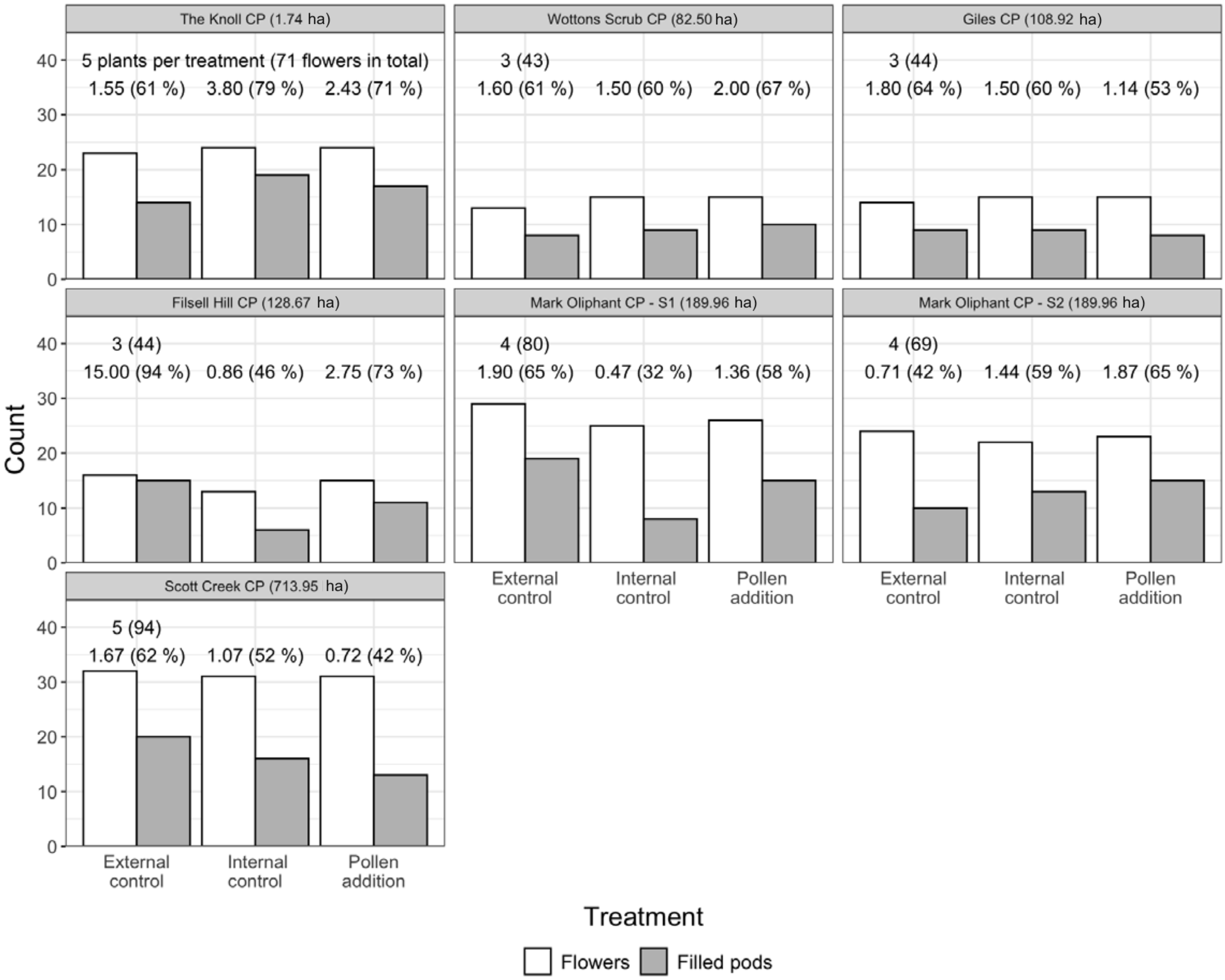
Wolowski M, Ashman TL, Freitas L (2014) Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. PLoS One 9:e89498.

Young AG, Brown AHD (1998) Comparative analysis of the mating system of the rare woodland shrub *Daviesia suaveolens* and its common congener *D. mimosoides*. Heredity 80:374-381.

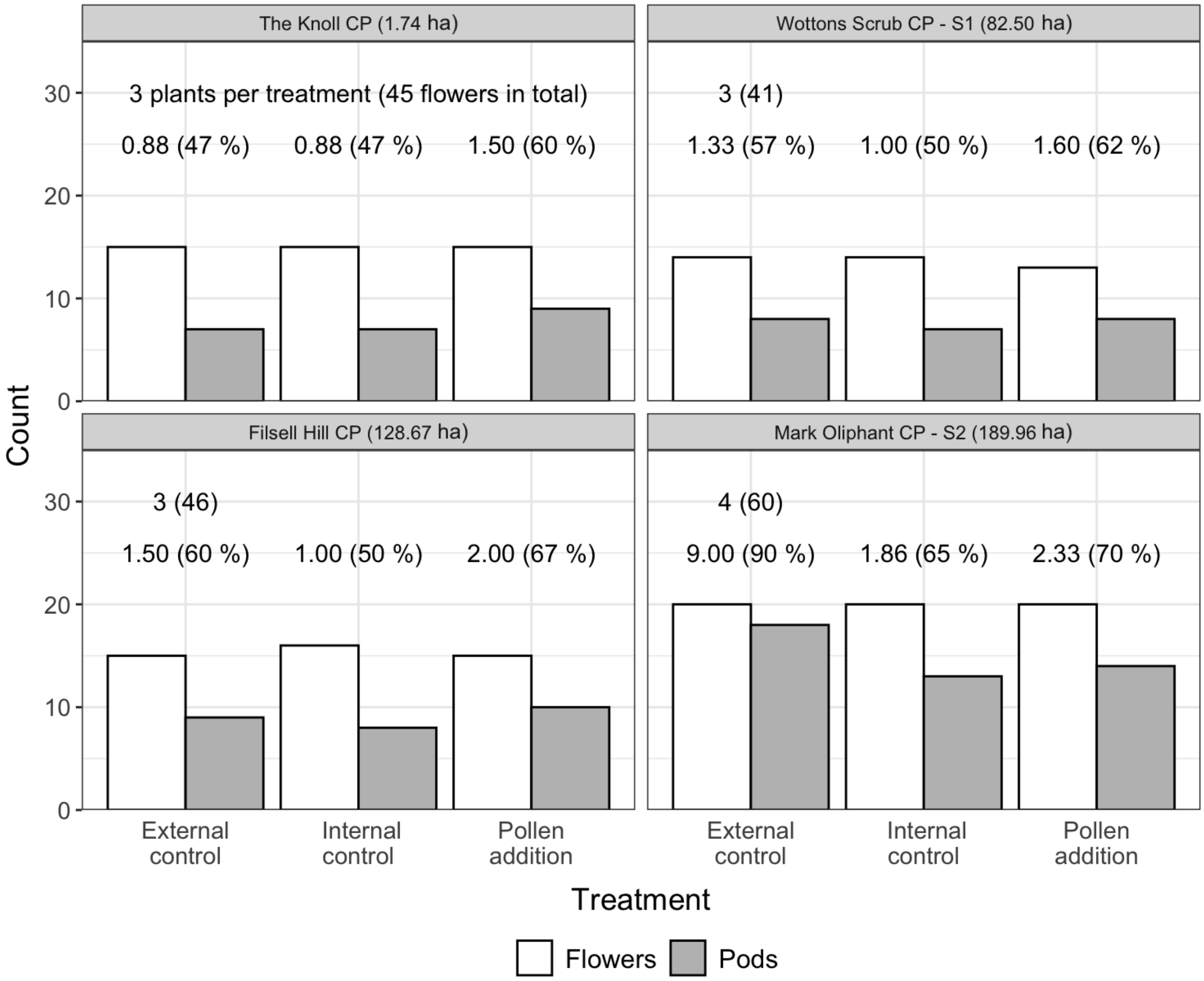
Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends in Ecology and Evolution 11:413-418.

2.7 SUPPLEMENTARY MATERIAL

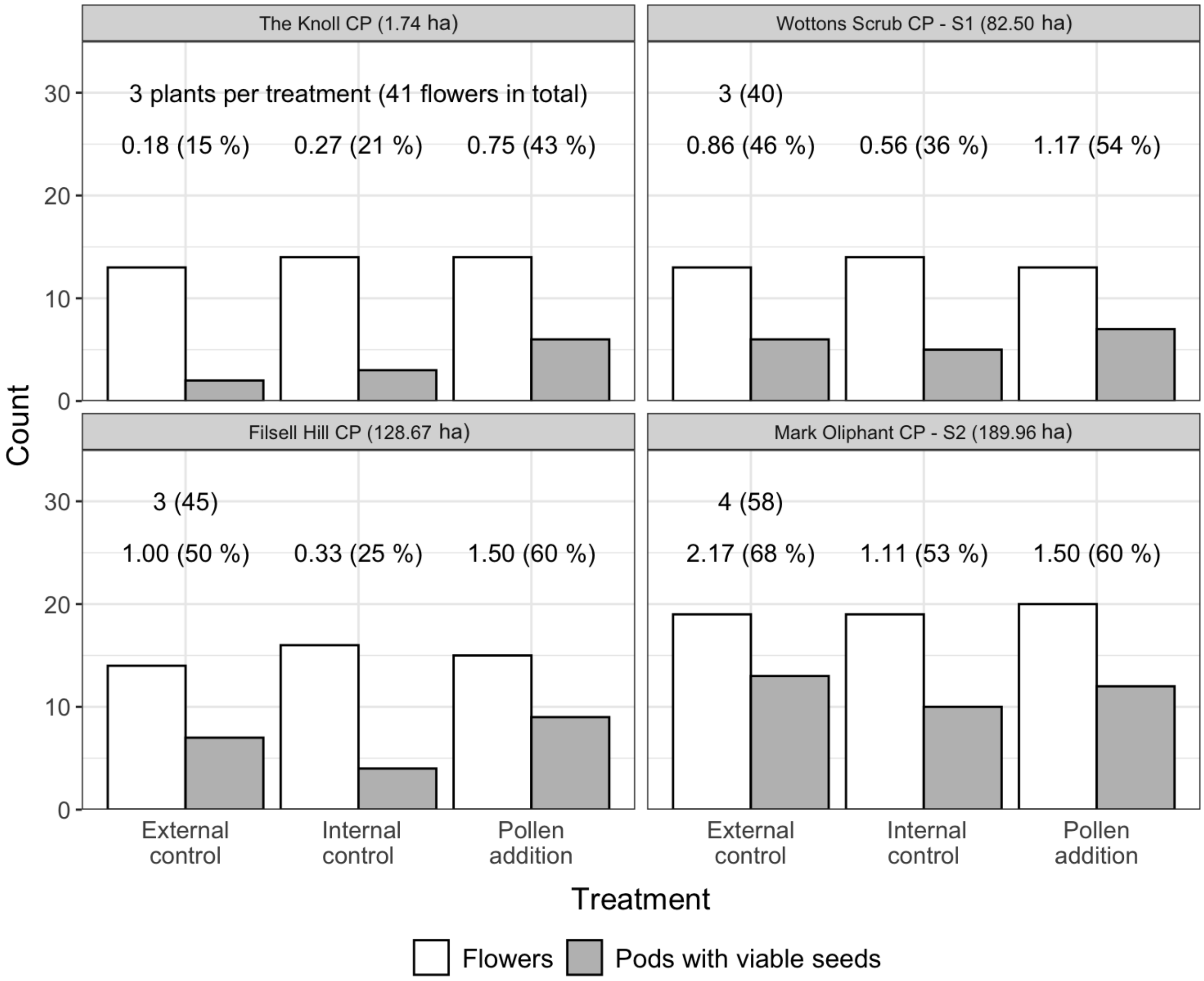
SM Fig. 2-1 (**a**) Adult flowering plant of *Pultenaea daphnoides*. (**b**) Open flowers and flower buds of *P. daphnoides*. (**c**) Native bee of the genus *Lasioglossum* (Fam. Halictidae), visiting and successfully manipulating a flower of *P. daphnoides* (arrow). (**d**) Introduced honeybee, *Apis mellifera* (Fam. Apidae), visiting and successfully manipulating a flower of *P. daphnoides*.



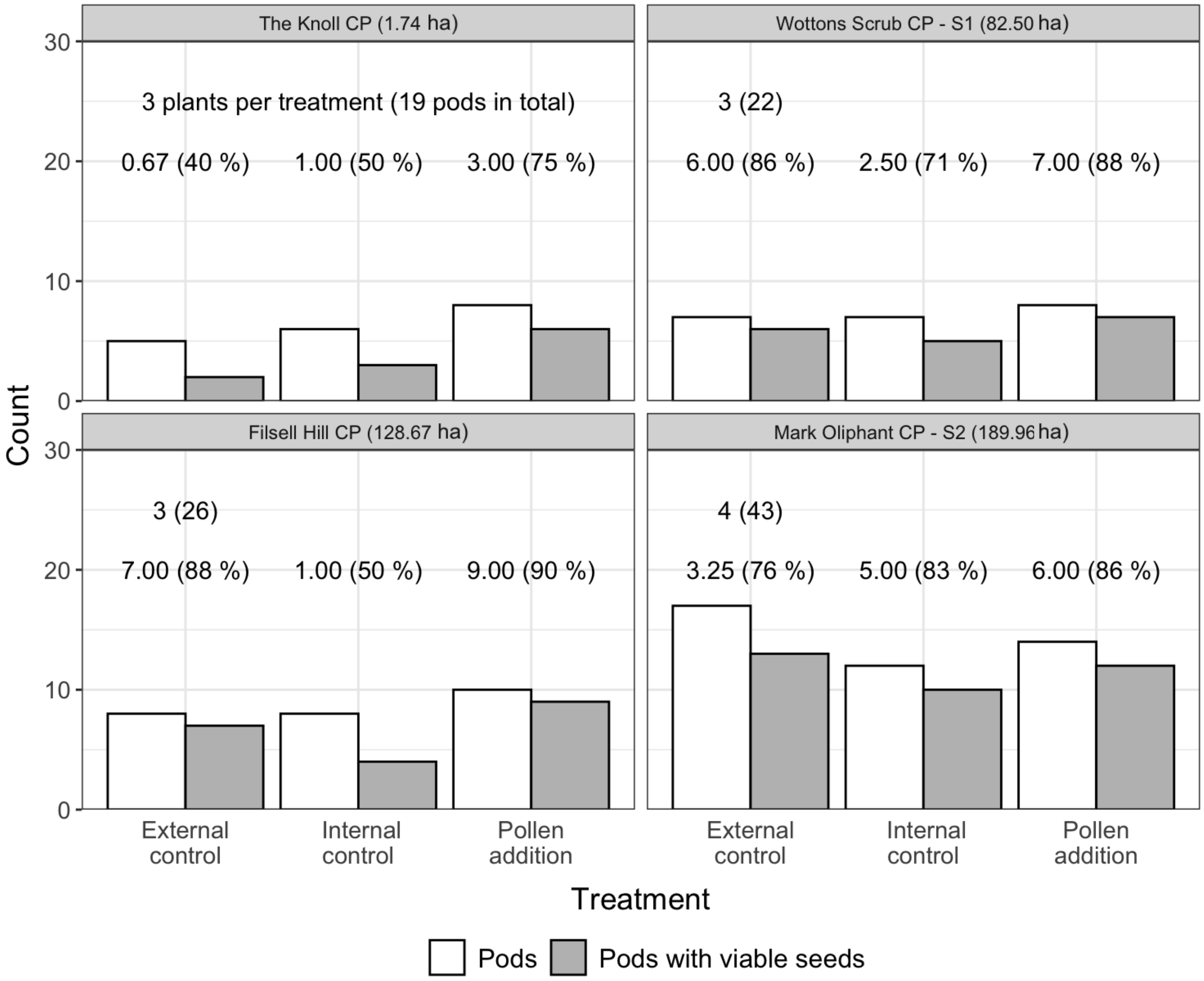
SM Fig. 2-2 Effect of the pollen addition treatment on the production of filled pods per sample site in 2017. Plotted are the number of flowers tagged per treatment relative to the number of filled pods (i.e., visually swollen pods indicating pods contained either viable seeds or seeds which terminated later in their development) which were subsequently produced, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per treatment per sample site, followed by the total number of flowers tagged across the three treatments per sample site in parentheses, is provided within each plot. The odds of a filled pod being produced per treatment are given above each of the respective groups of columns per sample site. Percentage filled pod-set (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that the sample size of tagged flowers is less than the original number of flowers tagged. This is because some tags were lost between the tagging of flowers and developing pods being censused.



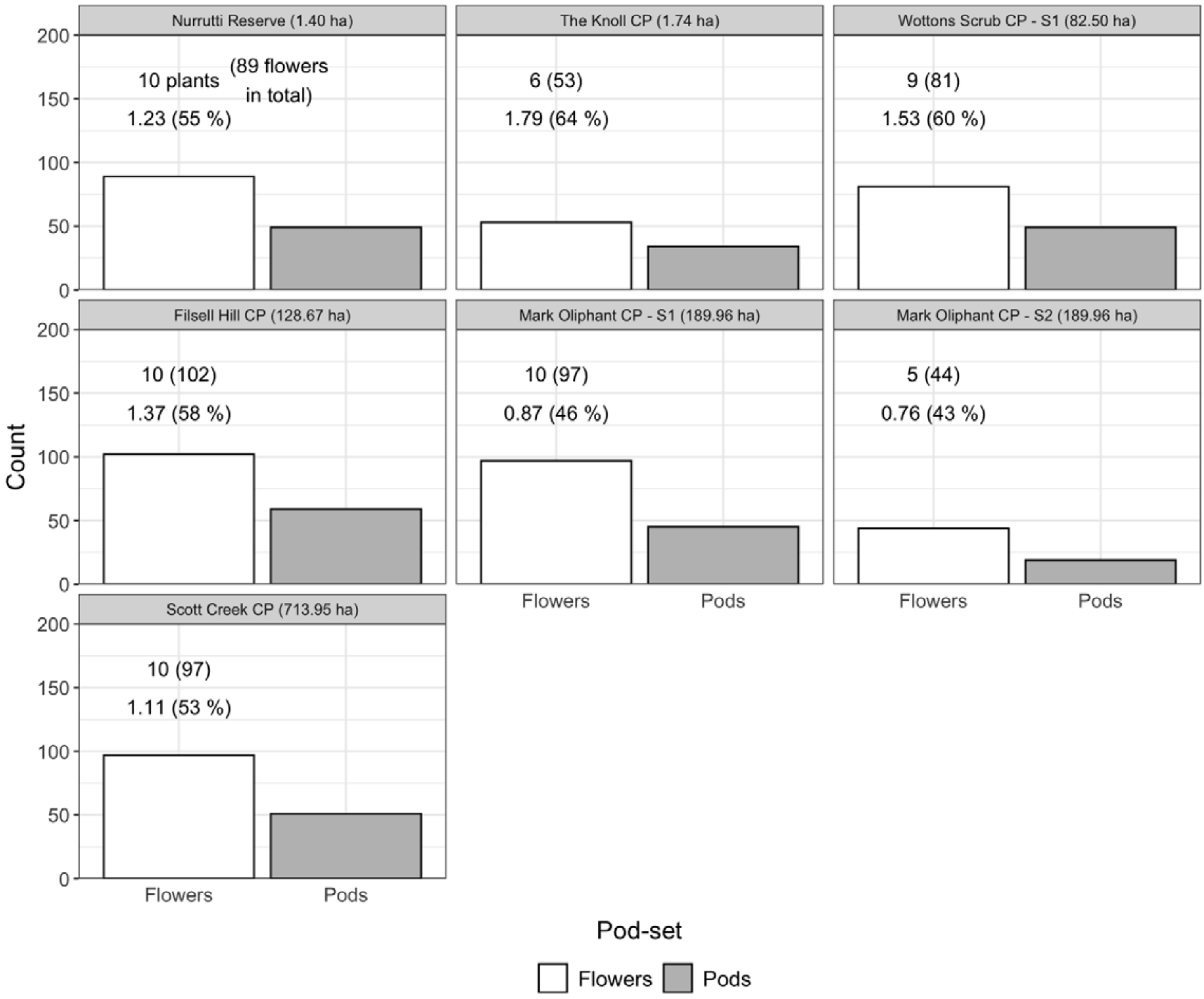
SM Fig. 2-3 Effect of the pollen addition treatment on the production of pods per sample site in 2019. Plotted are the number of flowers tagged per treatment relative to the number of pods (unfilled and filled) which were subsequently produced, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per treatment per sample site, followed by the total number of flowers tagged across the three treatments per sample site in parentheses, is provided within each plot. The odds of a pod being produced per treatment are given above each of the respective groups of columns per sample site. Percentage pod-set (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that the sample size of tagged flowers is less than the original number of flowers tagged. This is because some tags were lost between the tagging of flowers and developing pods being censused.



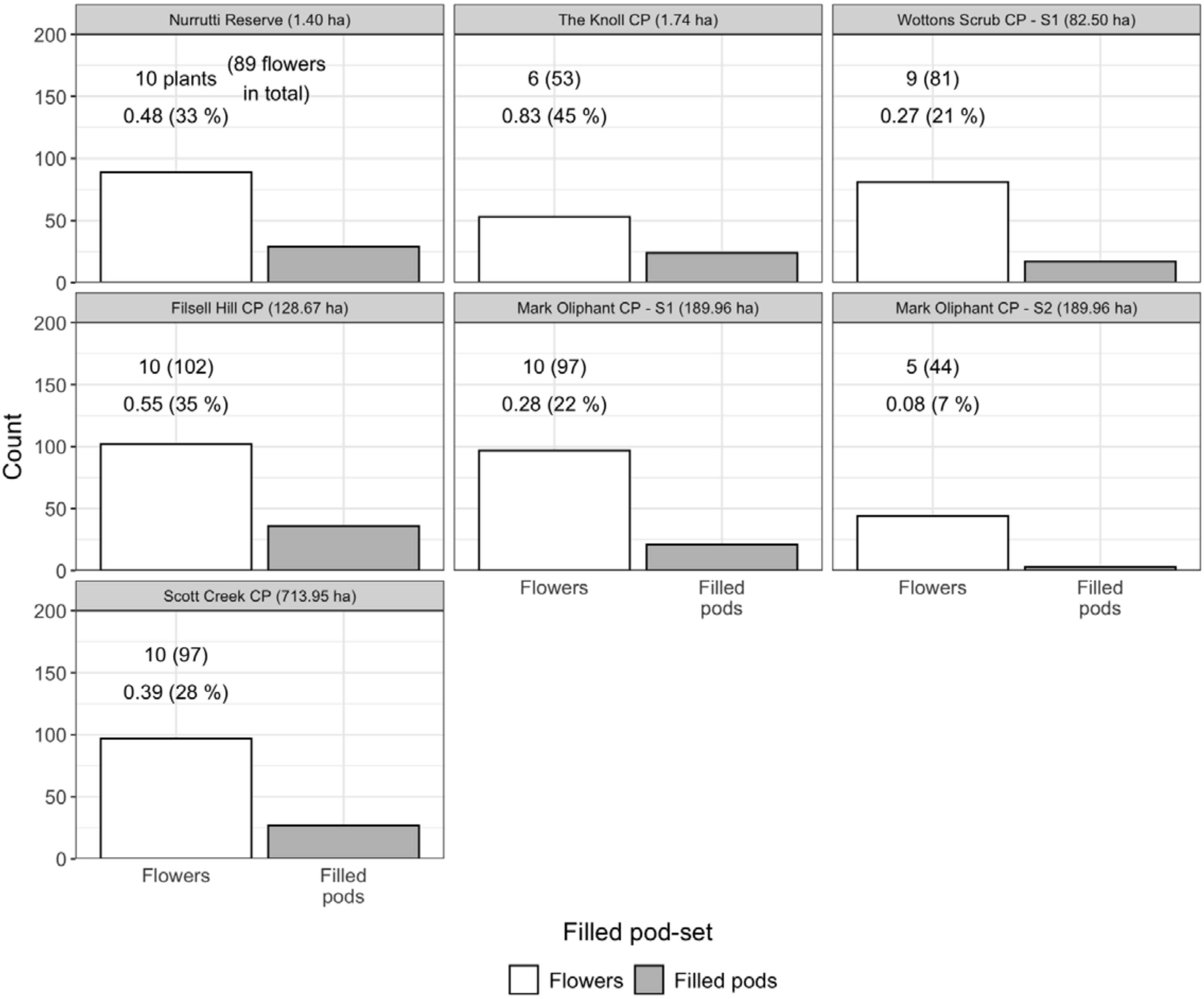
SM Fig. 2-4 Effect of the pollen addition treatment on the production of pods with viable seeds (relative to flowers) per sample site in 2019. Plotted are the number of flowers tagged per treatment relative to the number of pods with viable seeds which were subsequently produced, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per treatment per sample site, followed by the total number of flowers tagged across the three treatments per sample site in parentheses, is provided within each plot. The odds of a pod containing a viable seed per treatment are given above each of the respective groups of columns per sample site. The percentage of pods with viable seeds (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that developing pods were collected, opened, and developing seeds graded close to maturity. Flowers and pods with predated seeds were excluded. Also note that the sample size of tagged flowers is less than the original number of flowers tagged. This is because some tags were lost between the tagging of flowers and developing pods being censused.



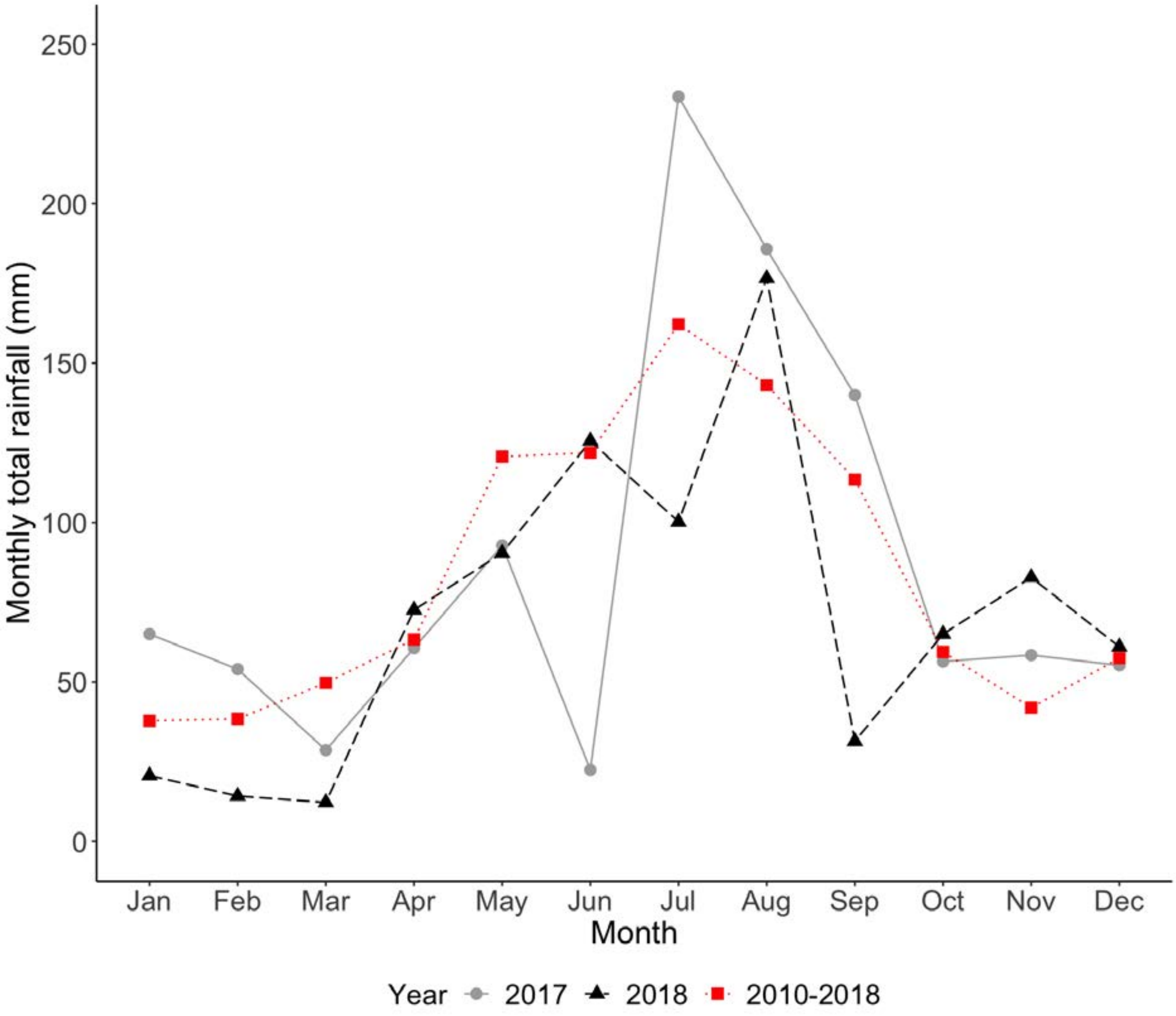
SM Fig. 2-5 Effect of the pollen addition treatment on the production of pods with viable seeds (relative to pods (unfilled and filled)) per sample site in 2019. Plotted are the number of pods (unfilled and filled) produced from tagged flowers per treatment relative to the number of these pods with viable seeds, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per treatment per sample site, followed by the total number of pods (unfilled and filled) produced across the three treatments per sample site in parentheses, is provided within each plot. The odds of a pod containing a viable seed per treatment are given above each of the respective groups of columns per sample site. The percentage of pods with viable seeds (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that developing pods were collected, opened, and developing seeds graded close to maturity. Pods with predated seeds were excluded.



SM Fig. 2-6 Pod-set of open-pollinated plants in 2018. Plotted are the number of flowers tagged relative to the number of pods (unfilled and filled) which were subsequently produced, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per sample site, followed by the total number of flowers tagged across plants per sample site in parentheses, is provided within each plot. Below, the odds of a pod (unfilled and filled) being produced are given, and the percentage pod-set (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that the sample size of tagged plants and flowers is less than the original number of plants and flowers tagged. This is because some tagged plants and flowers were lost between the tagging of plants and flowers and developing pods being censused.



SM Fig. 2-7 Filled pod-set of open-pollinated plants in 2018. Plotted are the number of flowers tagged relative to the number of filled pods (i.e., visually swollen pods indicating pods contained either viable seeds or seeds which terminated later in their development) which were subsequently produced, pooled across individual plants per sample site, for the study species, *Pultenaea daphnoides*. The number of plants tagged per sample site, followed by the total number of flowers tagged across plants per sample site in parentheses, is provided within each plot. Below, the odds of a filled pod being produced are given, and the percentage filled pod-set (rounded to the nearest whole number) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that the sample size of tagged plants and flowers is less than the original number of plants and flowers tagged. This is because some tagged plants and flowers were lost between the tagging of plants and flowers and developing pods being censused.



SM Fig. 2-8 Monthly total rainfall (mm) recorded at the Mount Lofty weather station (Station number: 23842) in the years of 2017, 2018, and averaged for the years 2010-2018.

CHAPTER THREE

3. SPATIAL AND TEMPORAL VARIABILITY IN POLLEN-LIMITATION AND PRE-DISPERSAL SEED PREDATION OF A COMMON SCLEROPHYLLOUS SHRUB WITHIN A FRAGMENTED LANDSCAPE.

Alex G. Blackall[1], Duncan A. Mackay[1], Molly A. Whalen[1]

[1]College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

3.1 ABSTRACT

Premise: Studies of the impact(s) of landscape disturbance on pollination and plant reproduction are biased toward rare and threatened plants and single reproductive episodes. Thus, additional studies of common plant species across multiple years are still required to allow for a greater understanding of the possible effect(s) of landscape disturbance.

Methods: We used the common bee-pollinated shrub, *Hibbertia exutiacies*, to examine the impact(s) of landscape disturbance on pollination and reproduction across two consecutive years (2017 and 2018). To do this we conducted pollen-limitation experiments and measured natural levels of reproduction across conservation reserves of increasing area, while also considering native vegetation within the landscape more generally, via a scale-of-effect analysis.

Results: We observed an impact of landscape disturbance on reproduction in 2017. Specifically, pre-dispersal seed predation declined, and viable seed production increased for fruits of plants within sample sites surrounded by a greater amount of native vegetation (within a distance of ~2400 up to 4000 m). We also observed temporal variability in the efficiency of the pollination process, with pollen-limitation of fruit-set in 2017 but not 2018. However, in contrast to 2017, the majority of developing seeds within fruits were aborted in 2018 and, ultimately, no viable seeds were produced. Thus, there was no spatial variability in viable seed production. This was likely due to exceptionally low rainfall in the early spring of 2018 constraining reproduction equally across the fragmented plant populations.

Conclusion: Ultimately, although fruit-set was pollen-limited in 2017, spatial variability of viable seed production in 2017 appeared to be largely due to pre-dispersal seed predation, emphasising the need to study biotic interactions other than animal-pollination in relation to the impacts of landscape disturbance. However, between-year variability was significant, and almost all developing seeds were aborted in the dry spring 2018. Thus, multi-year studies are essential in understanding the different roles of abiotic and biotic factors in limiting plant reproduction in fragmented landscapes.

KEYWORDS: buzz-pollination; *Hibbertia*; landscape disturbance; reserve area; scale-of-effect; pollen-limitation.

3.2 INTRODUCTION

Given the majority of the world's flowering plants are pollinated by animals (Ollerton et al. 2011), recent declines in important groups of pollinators, potentially in conjunction with the plants they pollinate, are of conservation and economic concern (Biesmeijer et al. 2006; Anderson et al. 2011; Regan et al. 2015). Landscape disturbance (here used to describe the combined effects of habitat loss and fragmentation and other interrelated processes (e.g., edge effects)) is considered one of the major drivers of this decline (e.g., reviewed by Hobbs and Yates 2003; Harris and Johnson 2004; Aguilar et al. 2006). Although many patch-level studies (those studies predominantly focused on measures of patch area and isolation) have not explicitly partitioned the independent effects of habitat loss and fragmentation (i.e., fragmentation per se; Fahrig 2017), studies at the scale of individual fragments have clearly demonstrated the detrimental effects of landscape disturbance on pollination and plant reproduction. Moreover, studies that have examined fragmentation per se also suggest negative consequences to plants and their pollinators via fragmentation alone (reviewed by Hadley and Betts 2012).

Conservation reserves form an important network of remaining native vegetation within fragmented landscapes (Margules and Pressey 2000), although they are not a conservation panacea (e.g., see Pressey and Tully 1994). It is frequently hypothesized that the negative consequences of landscape disturbance for pollination and plant reproduction are greater in smaller vegetation fragments, and as isolation of fragments from other such habitat increases (an extrapolation of the theory of island biogeography; see Fahrig 2019). This interpretation initially led to an early belief that larger reserves are necessarily of greater conservation importance than smaller reserves (Diamond 1975), an overly simplistic view of reserve values (Volenec and Dobson 2020; Wintle et al. 2019). More recently, it has been argued that the amount of habitat in the surrounding local landscape, as determined by the characteristics of the species studied (see Jackson and Fahrig 2012, 2015) can better explain the ecological response(s) of species within a sample site, independently of the combined variables of patch area and isolation (i.e., the habitat amount hypothesis; see Fahrig 2013). These alternative hypotheses relate to the relative influences of habitat composition versus configuration on biodiversity, and support has been found for both habitat amount (Melo et al. 2017; Watling et al. 2020) and independent effects of habitat configuration, such as patch size and connectivity (Hadley et al. 2018; Horvath et al. 2019), on various biodiversity measures (e.g., species richness). Thus, debate continues over how to appropriately measure ecological responses at the patch scale and upward to that of the landscape, particularly in regard to the effects of fragmentation (e.g., Fletcher et al. 2018 versus Fahrig et al. 2019). Nevertheless, at the patch-scale, conservation reserves represent spatially defined areas purposefully implemented to protect natural ecosystems and a negative effect of small reserve areas on ecological processes would be of conservation concern.

Currently, gaps and biases remain in our understanding of the impact(s) of landscape disturbance on pollination and plant reproduction. First, studies of rare and threatened plants, which may be more vulnerable to landscape disturbance to begin with, are over-represented in the literature (as recognised by Hobbs and Yates 2003 and Aguilar et al. 2006; see Chapter One). Common species, abundant across both small and large fragments, may be less impacted by landscape disturbance. Second, studies are typically conducted over a single flowering season (Aguilar et al. 2006; see Chapter One), essentially ignoring the role of temporal variability in pollination and plant reproduction in mediating the impact(s) of landscape disturbance (Hobbs and Yates 2003). This is true, even though the detrimental effects of landscape disturbance may be masked in certain years, due to temporal variation in overriding factors, such as rainfall (e.g., see Morgan 1999). Thus, there is still a need to study the impact(s) of landscape disturbance on the pollination and reproduction of common plants, preferably over more than a single reproductive episode.

The Southern Mount Lofty Ranges (SMLR), South Australia, is an often-overlooked region of floristic diversity within Australia (Crisp et al. 2001). With 13 % of the original native vegetation remaining, the present plant communities occur in fragments of various sizes, isolation, and levels of degradation (Armstrong et al. 2003). Thus, the SMLR is representative of a typically fragmented landscape (a landscape where remaining native vegetation cover is between 10-60 %, as defined by McIntyre and Hobbs (1999)). Accordingly, it may be expected that pollination and plant reproduction have declined in response to habitat loss and fragmentation and, as a result, both pollination and plant reproduction are lower in smaller vegetation fragments than larger ones. Here, we use the common sclerophyllous bee-pollinated shrub, *Hibbertia exutiacies*, to examine the impact(s) of landscape disturbance on pollination and plant reproduction within the fragmented landscape of the Adelaide Hills, a peri-urbanised section of the SMLR. The hypotheses tested was that both successful pollination (measured via pollen-limitation experiments) and plant reproduction (measured via fruit and seed production) would increase in reserves of greater area. The importance of native vegetation surrounding sample sites more generally (i.e., the local landscape) was also considered via a scale-of-effect analysis (e.g., see Holland et al. 2004).

3.3 METHODS

3.3.1 Study species

Hibbertia exutiacies N.A.Wakef. (Dilleniaceae) is a common sclerophyllous shrub growing to a height of 30-50 cm within the dry sclerophyll forests and woodlands of the Adelaide Hills (Armstrong et al. 2003). Solitary sessile to subsessile nectarless, odourless, yellow flowers (~10-15 mm diameter) are produced along the length of branches in spring (Jessop 1986; supplementary materiel (SM) Fig. 3-1a-b). Each flower contains typically 4-8 stamens with anthers opening by terminal pores and lateral slits (Jessop 1986; SM Fig. 3-1c-d). Anthers are functionally poricidal in comparable species of *Hibbertia* (Tucker and Bernhardt 2000). The two lateral carpels typically each contain four ovules (SM Fig. 3-1e), with the style of each recurving to flank opposite sides of the androecium (Jessop 1986). The calyx is persistent (SM Fig. 3-1g). Each carpel may form a follicle after fertilisation and when both carpels produce seeds, the fruit may be considered to consist of a pair of follicles (Clarke and Lee 2019; SM Fig. 3-1f). Mature seeds of *H. exutiacies* (mean ± SD of 2.87 ± 0.56 mg, n = 10) range in colour from black to brownish-red and are enclosed by a white-coloured elaiosomes (SM Fig. 3-1h). Seeds contain abundant endosperm, while the embryo is minute (Horn 2007; SM Fig. 3-1i-j). However, production of viable seed is low for many *Hibbertia* species (Fox et al. 1987 p. 86; Schatral and Fox 1994; Cochrane 2002). Likewise, many seeds contain malformed or no endosperm (Schatral and Fox 1994; Schatral et al. 1994; Cochrane 2002; Erickson et al. 2016 p. 85; SM Fig. 3-1n).

3.3.2 Sample sites

All chosen reserves were managed by the Department for Environment and Water (DEW), excluding one private reserve (Malcolm Wicks) owned by the National Trust of South Australia

(Fig. 3-1). Reserve area ranged from 1.74 ha up to 1027.47 ha (Table 3-1). The chosen reserves represented the range of reserve areas currently conserving populations of *H. exutiacies* within the study region. Due to their suitability for field work, two sample sites were used in Mark Oliphant, while all other reserves contained a single sample site (Fig. 3-1). Sample sites within each of the reserves were located based on the presence of the required plant species, as well as accessibility. In all but the smallest reserve (The Knoll), plants within sample sites were located a minimum of 40 m from the edge of the reserve (a delimitation used by Cunningham 2000). However, it should be noted that the closest edge of the reserve, with respect to a sample site within the reserve itself, was not necessarily the closest edge to the surrounding matrix, defined as all other landscape types excluding native vegetation. This was due to the presence of native vegetation outside of reserve boundaries (Fig. 3-1). However, site suitability was given priority over holding distance to edge constant, which was not possible across all reserves due to factors such as terrain and frequent prescribed burning around reserve boundaries. Thus, if edge effects exist, they may be operating more in some sample sites but not others. Nevertheless, at 1000 m from the approximate centre of each sample site, a distance used previously to define local landscapes in studies of bee diversity within both agricultural (Brown et al. 2020) and urban landscapes in Australia (Threlfall et al. 2015), there was a clear positive relationship between reserve area and the percentage area of native vegetation surrounding individual sample sites (Spearman's rho (r_s) = 0.954, $p = 1.788 \times 10^{-5}$, $n = 10$). Thus, reserve area is reflective of the amount of native vegetation present in the “local landscape” (here defined within a distance of 1000 m) surrounding sample sites. Given the strong association between measures of isolation and habitat amount within the surrounding landscape (Bender et al. 2003; Fahrig 2003), sample sites within reserves of smaller area are more isolated than sample sites within larger reserves (i.e., within a distance of 1000 m).

3.3.3 Plant community

Due to the relatively high rainfall (~700-1000 mm (Bureau of Meteorology)) and temperate climate of the Adelaide Hills, *Eucalyptus* L'Hér. (Myrtaceae) forests and woodlands dominate (Armstrong et al. 2003). Specifically, the vegetation type(s) at the chosen sample sites were dry sclerophyll forests and woodlands of messmate stringybark (*Eucalyptus obliqua* L'Hér.) and brown stringybark (*E. baxteri* (Benth.) Maiden & Blakely ex J.M.Black), an association typical of vegetation occupying areas of nutrient-poor soils and higher rainfall (Specht and Perry 1948). The understorey of sample sites was predominantly composed of sclerophyllous legumes (mainly *Pultenaea daphnoides* J.C.Wendl. and *Daviesia leptophylla* A.Cunn. ex G.Don) and the study species *H. exutiacies*. Notably, given savanna-type woodlands were preferentially cleared throughout the Adelaide Hills, the majority of remnant vegetation is composed of dry sclerophyll forest and woodlands (Armstrong et al. 2003). Thus, remaining native vegetation within the landscape is predominantly of a type suitable for *H. exutiacies* (Fig. 3-1).

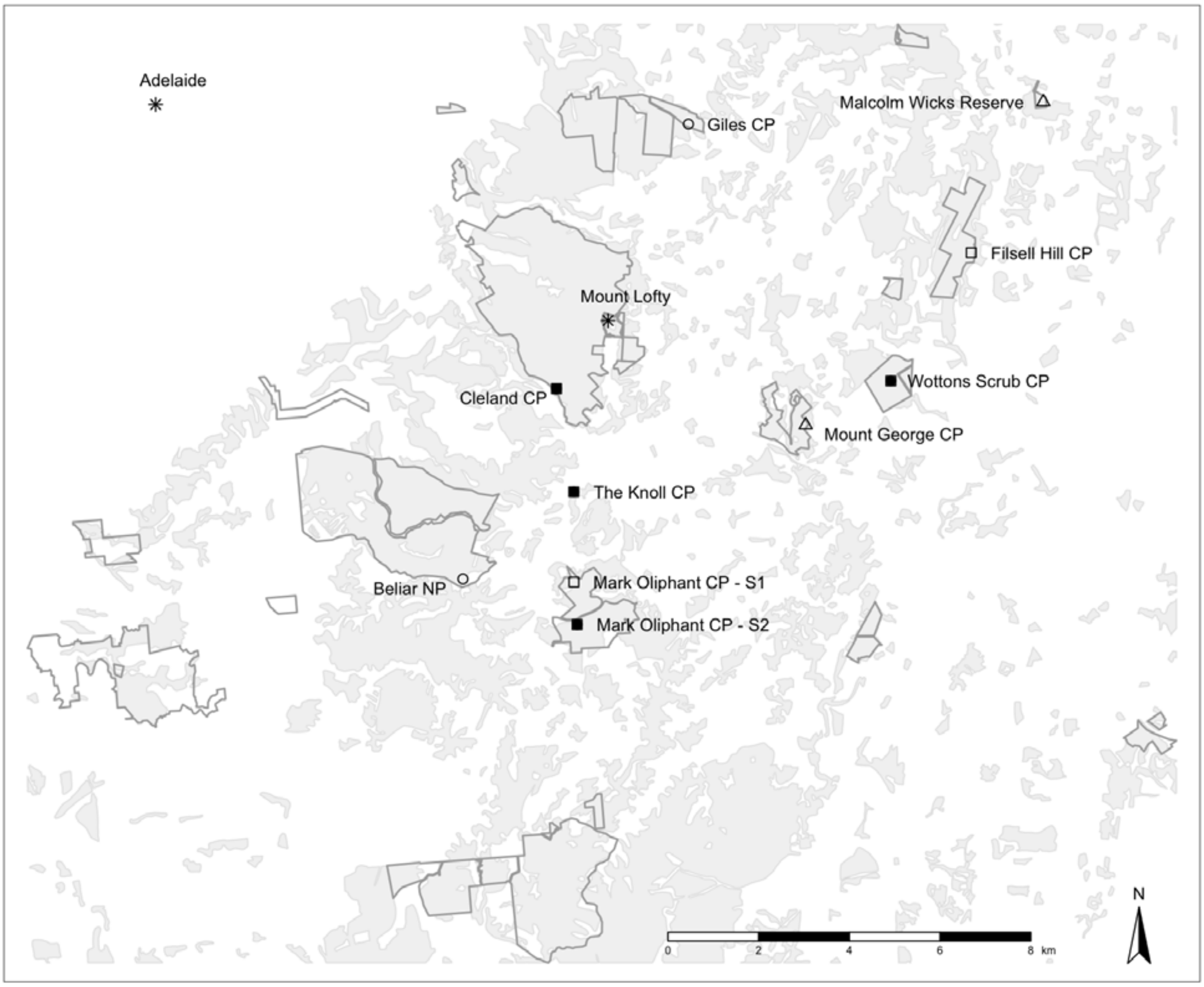


Fig. 3-1 Location of sample sites used to study the plant, *Hibbertia exutiacies*. All reserves managed by the Department for Environment and Water (DEW) and a private reserve (Malcolm Wicks) owned by the National Trust of South Australia are outlined. Adelaide, the capital city of South Australia, and Mount Lofty (720 m), the highest point within the study region, are both marked by an asterisk. Sample sites marked with squares had pollen-limitation experiments undertaken in 2017. Natural reproduction (open-pollination) of additional unmanipulated plants was also measured in these sample sites in 2017. Filled in squares represent sample sites where pollen-limitation experiments were replicated in 2018. Sample sites marked by triangles represent additional samples sites used for pollen-limitation experiments in 2018. Sample sites marked by circles had measures of natural reproduction (open-pollination) of unmanipulated plants undertaken in 2017, but no measures of pollen-limitation. S1 and S2 stand for sample site 1 and 2, respectively. The scale bar represents a total distance of 8 km. Native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

Table 3-1 Location of sample sites within reserves, reserve area, and data collected in each sample site across the years of 2017 and 2018. S1 and S2 stand for sample site 1 and 2, respectively. CP stands for Conservation Park.

Reserve	Latitude	Longitude	Reserve area (ha)	Data collected (year)[a]
The Knoll CP	35° 0.45' S	138° 41.96' E	1.74	**PL** (17,18), **NR** (17,18)
Malcolm Wicks Reserve	34° 55.92' S	138° 48.89' E	7.90	**PL** (18), **NR** (18)
Wottons Scrub CP	34° 59.21' S	138° 46.59' E	82.50	**PL** (17,18), **NR** (17,18)
Mount George CP	34° 59.72' S	138° 45.34' E	84.89	**PL** (18), **NR** (18)
Giles CP	34° 56.09' S	138° 43.74' E	108.92	**NR** (17)
Filsell Hill CP	34° 57.70' S	138° 47.80' E	128.67	**PL** (17), **NR** (17)
Mark Oliphant CP - S1	35° 1.53' S	138° 41.93' E	189.96	**PL** (17), **NR** (17)
Mark Oliphant CP - S2	35° 2.03' S	138° 41.97' E	189.96	**PL** (17,18), **NR** (17,18)
Belair NP	35° 1.46' S	138° 40.33' E	837.54	**NR** (17)
Cleland CP	34° 59.21' S	138° 41.75' E	1027.47	**PL** (17,18), **NR** (17,18)

[a] **NR** = Data on natural levels of reproduction (note that measures of natural reproduction (open-pollination) in 2018 came from the external controls of pollen-limitation experiments only), **PL** = Pollen-limitation data.

3.3.4 Pollinators

There are no published reports on the pollinators of *H.* exutiacies, however, the floral morphology matches that of *Hibbertia* species whereby pollination is performed by native bees collecting pollen via thoracic vibrations (i.e., buzz-pollination (Buchmann 1983); see Tucker and Bernhardt (2000) for a discussion of putative pollination syndromes in *Hibbertia*). Other floral visitors to species of *Hibbertia* with bee-pollinated pollination syndromes are considered, at best, incidental pollinators. For example, Bernhardt (1986) observed a *Tapeigaster* (Heleomyzidae) fly foraging on spilled pollen left by visiting bees on the flowers of *H. fasciculata*, but no contact with the stigma(s) was observed. Here, similar behaviour has been seen for hoverflies (Syrphidae) visiting flowers of *H. exutiacies* (SM Fig. 3-1v). Pollen feeding beetles (e.g., *Diphucephala*) may affect minor pollination in some bee-pollinated *Hibbertia* species (Keighery 1975) but typically decrease reproduction due to floral damage (see Hawkeswood 1989 and Schatral 1996). Floral visitation also appears infrequent to *H. exutiacies* within the study region (Pers. Obs.), as is true of *Hibbertia* species elsewhere (e.g., see Schatral 1996 and Hingston 1999).

Video cameras (Panasonic HC-VX980M) were initially used to film floral visitation in the spring of 2017. Cameras were placed 1-2 m from individual plants and several flowers were filmed per plant (mean ± SD of 35.10 ± 33.35 flowers per video; 351 flowers in total). Filming lasted an average of 1 hour and 23 minutes per plant (13 hours and 51 minutes in total; *n* = 10 plants). In 2018, 15-minute personal observations of flowering *H. exutiacies* were undertaken (*n* = 11 plants). Filming and observations (2017-2018) were undertaken during field work (September-October) on relatively warm sunny days (temp. ≥ 15℃). Given surveys were conducted to simply confirm the behaviour of native bees on flowers of *H. exutiacies* (i.e., buzzing of anthers by bees to transmit pollen); results are presented without regard to sample site and date of observation (details in SM Table 3-1).

The following year (November 2019) six native bees were opportunistically caught visiting *H. exutiacies* flowers during unrelated fieldwork. These bees were killed in kill jars containing ethyl acetate and placed in individual specimen tubes before being frozen. Sampled bees were subsequently swabbed for pollen with a small cube of fuchsin jelly (Beattie 1971) to confirm presence of pollen grains of *H. exutiacies*. Pollen grains were extracted from the anthers of *H. exutiacies* flowers prior to anthesis for comparison (SM Fig. 3-1A-B). Bees were identified using Houston (2018) and Smith (2018).

3.3.5 Pollination and reproduction

Two manipulative experiments and one mensurative census were performed regarding the pollination and reproduction of *H. exutiacies*. Details for these experiments are provided below. However, beforehand, we outline definitions and general methodology used to measure fruit and seed production. We used proportional fruit-set per plant as a measure of fruit production, hereafter simply termed fruit-set. Fruit-set was measured by tagging a subset of flowers per plant, which were subsequently monitored for fruit development following flowering. Fruits were separable from persistent calyxes (SM Fig. 3-1f-g). Here, the term fruit is used whether one or both carpels per flower contained developing or developed seeds (i.e., a fruit may consist of one or two follicles). Specifically, we considered a flower to have developed into a fruit based on the presence of swollen carpel(s) containing developing seed(s), which demonstrated initial seed development, without regard to the viability of seeds within fruits, which was considered separately. Thus, although carpels may noticeably swell due to seed development, fruits may produce only inviable seeds due to pre-dispersal seed predation or abortion of developing seeds (SM Fig. 3-1k-n). Fruits of *Hibbertia* are known to mature, dehisce, and drop their seeds quickly in response to hot weather, making timing of collection for mature seeds difficult to estimate (Fox et al. 1987 p. 86; Cochrane 2002; Pers. Obs.). Thus, fruits were retrieved prior to the seeds within reaching full maturity (SM Fig. 3-1f, i), with fruits collected approximately ~1-1.5 months after initially being tagged as flowers.

Fruits were kept under refrigeration in paper envelopes until examination of their developing seeds (~4℃).

Although fruits were retrieved prior to full maturity (occurring from mid-December onwards), developing seeds within could be graded into three categories. First, seeds were considered as viable if they contained firm white endosperm filling most of the seed (SM Fig. 3-1i-j). Second, seeds containing insect larvae (or their remains), frass (excrement of insect larvae), showing entry or exit holes within the seed coat, and the majority of endosperm consumed or not present were recognised as predated (SM Fig. 3-1k-m, p). Seed predation was wholly due to the larvae of insect seed predators. Third, seeds were classed as unfilled if they were empty or contained a small amount of poorly formed often shrivelled endosperm not filling the seed (SM Fig. 3-1n). Unfilled seeds were considered to have aborted during maturation. The number of inviable seeds per fruit was the combination of predated seeds and unfilled seeds. For estimates of total seed production per fruit, viable, predated, and unfilled seeds were combined into a single category, termed developing seeds. The number of developing seeds within a fruit is considered to represent the number of viable seeds a fruit would have produced (out of the number of seeds initiated) in the absence of seed predation and abortion.

3.3.6 Breeding system

To confirm whether *H. exutiacies* can produce fruits via autonomous self-pollination, white chiffon bags were used to prevent visitation by pollinators to a subset of flowers on individual plants in the field in 2017. A total of 200 flower buds across 7 plants were excluded from pollinator visitation (SM Table 3-2). Following the end of flowering, bags were removed, and subsequent fruit formation was recorded. All plants produced fruits on unbagged branches.

To confirm whether reproduction can occur via self-fertilization, experimental treatments were applied to individual plants in 2017 and 2019. First, bagged virgin flowers were hand-pollinated with outcross pollen taken from three conspecifics a minimum of 10 m from the recipient plant. Second, bagged virgin flowers were hand-pollinated with self-pollen taken from the anthers of additional flowers within the same bag (geitonogamy). Third, unbagged flowers were left open to pollinators to measure natural levels of reproduction resulting from open-pollination. For both the outcross- and self-pollination treatments, pollen was applied by pressing anthers, which had been manually opened with forceps to allow the pollen held within to become exposed, onto the stigmas of the recipient flower. A minimum of three anthers were used until ample pollen could be observed on both stigmas (confirmed with 10-40 × jewellery loupe). A total of 15 flowers across 6 plants were outcrossed, 16 flowers across seven plants were self-pollinated, and 35 flowers across the seven plants served as open-pollinated controls (SM Table 3-2). Bags remained on each plant until the collection of fruits with developing seeds.

3.3.7 Pollen-limitation of fruit-set and seed production

Pollen-limitation of fruit-set and seed production in *H. exutiacies* was measured by artificially pollinating flowers with outcross pollen taken from three conspecific donors, following the methods used in the breeding system experiment above. Flowers receiving pollen addition were then left open to further pollination via natural pollinator visitation. Two controls were required to account for the possibility of resource reallocation between flowers on the treatment plant confounding the result (Knight et al. 2006). First, an internal control was allocated by tagging an equal number of flowers on the same plant that received the artificial pollination treatment. Second, an external control was allocated by tagging an equal number of flowers on a similar sized conspecific plant growing within the local vicinity of the treatment plant (≤ 10 m). Lower fruit-set or seed production in the internal control versus the external control is thought to indicate the presence of resource reallocation among flowers or fruits of the treatment plant (Knight et al. 2006). In such cases, the pollen addition treatment should be compared to the external rather than internal control. In 2017, across six sample sites, a total of 112 flowers, over 23 plants, were supplemented with pollen from mid-October to early November (SM Table 3-3). In 2018, across six sample sites, a total of 120 flowers, over 24 plants, were supplemented with pollen from mid to late October (SM Table 3-3).

3.3.8 Natural levels of fruit-set and seed production

Across eight sample sites, a total of 401 flowers, over 72 plants, were tagged from mid-October to early November 2017 to measure natural levels of fruit-set and seed production (SM Table 3-3). This total included those plants used as external controls in the pollen-limitation experiments. Flowering of *H. exutiacies* was noticeably reduced in 2018 compared to 2017 (Pers. Obs.), and natural levels of reproduction were estimated from the external controls of pollen-limitation experiments only, as a larger sample size could not readily be obtained.

3.3.9 Statistical analysis

Here, models used to analyse fruit-set and seed production for pollen-limitation experiments and natural reproduction are detailed. All statistical models were run within the program R (R Core Team 2020). Linear and generalised linear mixed-effect models (LMM and GLMM, respectively) were constructed within the packages 'lme4' (Bates et al. 2015) and 'glmmTMB' (Brooks et al. 2017). However, in some instances, the variance of specified random effects within models was singular (i.e., ~ 0). In these cases, the model was either further simplified (i.e., averaging the response to reduce nested levels of random effects), or a partially Bayesian method was used to help prevent singular fits via the 'b(g)lmer' function in the package 'blme' (Chung et al. 2013). Diagnostic checks of mixed-effect models were performed within the package 'DHARMa' (Hartig 2021). Statistical significance of predictors in GLMM's were tested via an Analysis of Deviance, using a Type II Wald Chi-square test (implemented in package 'car'; Fox and Weisberg 2019). Statistical significance of predictors in LMM's were tested via an Analysis of Deviance, using a Type II Wald F tests with Kenward-Roger corrected degrees of freedom (implemented in package 'car'). Multiple comparisons were performed via the package 'multcomp' (Hothorn et al. 2008a), with a Bonferroni correction used to account for inflated Type I error. Bonferroni-corrected *p*-values are presented (*p*-value × no. of comparisons). The package 'MuMIn' was used to calculate small-sample-size corrected Akaike information criterion (AICc) values (Bartoń 2020). Figures were constructed via the packages 'ggplot2' (Wickham 2016), 'tmap' (Tennekes 2018), and 'sjPlot' (Lüdecke 2021).

3.3.10 Pollen-limitation of fruit-set - 2017 and 2018

To examine whether the pollen addition treatment interacted with reserve area (e.g., if pollen-limitation declined with increasing reserve area), a Binomial GLMM was constructed to test for an interaction between treatment and reserve area on the response variable fruit-set. Treatment and reserve area were considered fixed effects, while reserve and plant pair nested within reserve were treated as random effects. Plant pair was explicitly accounted for in the model as it is equivalent to a blocking factor within the experimental design. The independent predictor of reserve area was log transformed prior to analysis to reduce right skewness. When the treatment effect was significant, but not reserve area or its interaction with treatment, a reduced model with treatment as the only predictor was used for post-hoc comparisons between treatment levels (i.e., pollen addition versus internal control, etc.). The main effect of reserve area was not of particular interest and was considered separately for models including only the external controls and additional tagged plants (2017) used to measure levels of natural reproduction (see statistical models in section *Natural reproduction - 2017 and 2018* below). Note that for Mark Oliphant reserve, which contained two sample sites in 2017 (Fig. 3-1), data were pooled across the respective sample sites for analysis, therefore, data for both sample sites were used as a measure of pollen-limitation for the reserve overall. This was appropriate given the two sample sites within Mark Oliphant never statistically differed from one another in any measure of natural reproduction (see Results) and, for measures of pollen-limitation, only two plant parings were available in Mark Oliphant - Sample Site 1 (see SM Table 3-3).

To visualize an effect of reserve area on the magnitude of pollen-limitation of fruit-set, the pollen-limitation index (pl-index) of Larson and Barrett (2000) was calculated for each treatment plant. The pl-index was calculated as 1 - ($FS_{Ctr} \div FS_{PA}$), where FS_{Ctr} is the fruit-set of the control treatment, and FS_{PA} is the fruit set of the pollen addition treatment, per plant. The pl-index is constrained to be lower bounded by 0, when no pollen-limitation of reproduction occurs, and reaches a maximum value of 1, when all flowers produce fruit for the pollen addition treatment plant and no flowers produce fruit on the respective control (100 % pollen-limitation). The results of the GLMM, discussed above, determined which of the two controls to use (e.g., resource reallocation would mean the use of the external rather than internal controls). Thus, resource reallocation, if present, was considered to occur across all combinations of sample sites and plants. The pl-index for each plant was subsequently plotted against reserve, in order of ascending reserve area. Kendall's correlation was used to analyse if a relationship existed between the mean pl-index per plant for each reserve and reserve area.

3.3.11 Pollen-limitation of seed production - 2017 and 2018

Since seeds within fruits were categorised into viable, predated, and unfilled classes, several separate models were constructed. To avoid confusion, we use the term "seed-set" when referring to models using seeds per fruit as the response, with random effects to account for multiple fruits per plant, and "mean seed-set" when the number of seeds per fruit were averaged for each individual plant prior to analysis (i.e., the response variable is already averaged at the plant-level).

In 2017, developing seed-set (viable + predated + unfilled seeds per fruit) was modelled by a truncated Conway-Maxwell-Poisson (CMP) distribution. The CMP distribution effectively handles both over- and under-dispersion in count models (Brooks et al. 2019). The distribution was zero truncated as fruits were defined as possessing at least one developing seed. For this model, the parameters of reserve, plant pair nested within reserve, and treatment nested within each plant pair were included as random effects. The hierarchical nature of the random effects in the model was required to account for the inherent pseudo-replication among multiple fruits examined for each treatment by plant combination. Due to a singular fit for the lowest level of the random effect structure in 2018, developing seed-set was averaged per plant (mean developing seed-set) and modelled with a linear mixed-effect model (LMM). Reserve and plant pair nested within reserve were included as random effects.

Inviable seed-set and its components predated and unfilled seed-set were analysed by a non-truncated CMP distribution (i.e., zero values are possible). For these models, reserve, plant pair nested within reserve, and treatment nested within plant pair were included as random effects. There were very few unfilled seeds in 2017 and GLMM's were difficult to construct (e.g., false convergences, large standard errors, poor estimates, etc.). Instead, a Fisher-Pitman permutation test (10 000 permutations) was used to examine if a significant difference existed between mean unfilled seed-set for plants across treatments, with data pooled across reserves (implemented in R package 'coin'; Hothorn et al. 2008b). In 2018, all seeds were inviable and, therefore, inviable seed number was already modelled by mean developing seed-set (see above). In 2018, there were too few predated seeds to reliably model, and a Fisher-Pitman permutation test was implemented on mean predated seed-set for plants across treatments.

As no viable seeds were produced in 2018, models of viable seed-set were only constructed for 2017. However, in 2017, most seeds were graded as inviable and, at the level of individual fruits, the presence of viable seeds was largely dichotomous (i.e., majority of fruits contained either no viable seeds or only viable seeds). Moreover, the number of viable seeds per fruit was low (overall mean 0.59), and for those fruits with viable seeds, 96.92 % of them contained only 1-2 seeds. Due to this low mean and range, viable seed-set was instead modelled as the presence versus absence of viable seeds per fruit (i.e., a binary variable). Thus, a Binomial GLMM was used for analysis. This model returns estimates of the probability of a fruit having viable seeds, conditional on the random effects. Thus, we refer to this variable as "the probability of fruits having viable seeds". Reserve, plant pair nested within reserve, and treatment nested within plant pair were included as random effects.

The proportion of viable seeds per fruit was modelled by a Binomial GLMM. This model returns estimates of the probability of seeds being viable per fruit, conditional on the random effects. However, to avoid confusion with the model above, we refer to this variable as "the proportion of viable seeds per fruit". Reserve, plant pair nested within reserve, and treatment nested within plant pair were included as random effects.

Lastly, for 2017, a model also considered the number of viable plus predated seeds per fruit (i.e., viable + predated seed-set). The number of viable plus predated seeds per fruit is an alternative measure of the production of viable seeds, assuming that predated seeds would have otherwise been viable if not predated. However, because so few unfilled seeds were produced in 2017, this response variable is effectively equivalent to developing seed-set. Nonetheless, we present results for viable + predated seed-set in 2017, as modelled via a CMP GLMM. Reserve, plant pair nested within reserve, and treatment nested within plant pair were included as random effects.

In 2017, data were also only available for 19 of 23 plant pairs due to the initial mislabelling of seeds for four plant pairs (see SM Table 3-4). Consequently, data for the two plant pairs in Mark Oliphant - Sample Site 1 were not used.

3.3.12 Natural reproduction - 2017 and 2018

We first define models used on the larger dataset obtained in 2017, before discussing issues with the smaller dataset obtained in 2018. In particular, although the number of response variables assessed in 2017 is large, the various models of fruit-set and seed production, which included models of the three categories of viable, predated, and unfilled seeds and their derivations, allowed for the detailed study of the various possible factors limiting natural levels of reproduction (e.g., conversion of flowers to fruits, pre-dispersal seed predation, etc.). Across these models, each response variable was regressed against the predictor of reserve area to test the hypothesis that reproduction would increase with reserve area. The independent predictor of reserve area was log transformed prior to analysis to reduce the right skewness of this predictor for all models.

Fruit-set was analysed via a Beta-Binomial GLMM. The Beta-Binomial distribution was used to account for over-dispersion (Harrison et al. 2015). Reserve was included as a random effect. Developing seed-set was averaged per plant (mean developing seed-set) and then analysed by a LMM with reserve as the random effect. A fourth root transformation was used on the response variable to improve normality of residuals. Inviable seed-set, predated seed-set, and viable + predated seed-set were also averaged per plant and analysed by separate LMM's. Viable seed-set was again analysed as a binary response (Binomial GLMM). For this model, reserve and individual plants nested within reserve, were included as random effects. Individual plants were treated as a random effect to account for multiple fruits opened per plant. Similarly, a Binomial GLMM was used to analyse the proportion of viable seeds per fruit against reserve area with the same random effect structure.

In addition, to examine whether plant reproduction differed between the sample sites chosen (i.e., treating the effect of sample site within each reserve as fixed and independent, and irrespective of reserve area), models were constructed to test whether the measured reproductive variables differed between sample sites. In the case of fruit-set, no random effects were required, and the effect of sample site was tested via a quasibinomial GLM using a Type II F-test. In contrast, a truncated CMP GLMM was used for developing seed-set, a Binomial GLMM for the probability of fruits having viable seeds, a CMP GLMM for inviable and predated seed-set, and a Binomial GLMM for the proportion of viable seeds per fruit. For these models, individual plants (nested implicitly within sample sites) were treated as random effects.

Due to the striking results obtained in 2018 (i.e., zero viable seed production which precluded models analysing viable seed-set), subsets of the above models were used on external control plants to examine trends in relation to reserve area. However, sample sizes per sample site were smaller relative to 2017 (e.g., 2-5 plants versus 5-11 plants per sample site for seed-set models). Additionally, most fruits in 2018 contained only one seed versus two or more seeds per fruit in 2017. Thus, the range of seeds per fruit was low. Moreover, there was no or little variability between plants within sample sites which resulted in poor coefficient estimates. When this occurred, data for these sample sites were removed from the analysis (noted in the Results). Nonetheless, model results should be treated with caution.

We also compared fruit-set (Binomial GLMM) and developing seed-set (Truncated CMP GLMM) between years, with year as the predictor and sample site (fruit- and developing seed-set models), and individual plants nested within sample site (developing seed-set model only) treated as random effects. Note that a nested sample site by year random factor was not included due to not all sample sites being used in both 2017 and 2018.

3.3.13 Scale-of-effect - 2017

When there was evidence of reserve area being a significant predictor of any measure of natural reproduction in 2017, the influence of native vegetation surrounding sample sites more generally (i.e., both within and outside reserve boundaries) was explored via a scale-of-effect analysis, as described in Fig. 3-2. In particular, the scale-of-effect analysis allows for the further assessment of the possible influence of native vegetation in the landscape surrounding sample sites within each of the reserves. Here, the two sample sites of Mark Oliphant were treated as individual datapoints, given the percentage area of surrounding native vegetation was calculated independently for each sample site. Model comparisons and resulting plots were constructed via the ‘multifit’ function of Huais (2018). Percentage area of native vegetation was calculated by using the R package ‘sf’ (Pebesma 2018). Calculations utilised the native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898). Although of potential concern in such analyses (Zuckerberg et al. 2020), there was little evidence of spatial autocorrelation of model residuals across the compared models (as assessed via residual plots and DHARMa Moran's I (although the ability to detect statistically significant spatial autocorrelation with eight sample sites may be somewhat limited)). For model comparisons via AICc, LMM's were first fitted by maximum likelihood, then restricted maximum likelihood was used to estimate parameters of the final model selected. For LMM's, transformations of the response variable were used when required. Scale-of-effect analyses were not considered for the reproductive data collected in 2018 due to the lower number of plants and sample sites available for analysis.

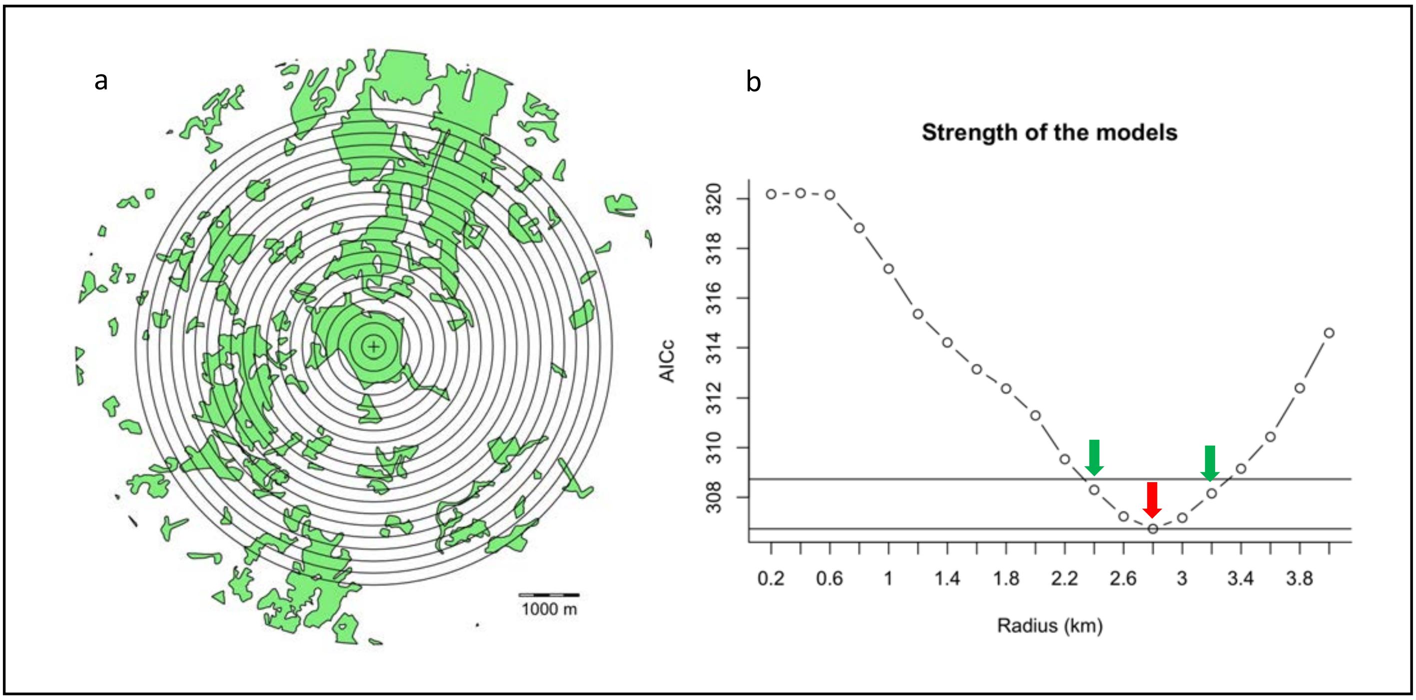


Fig. 3-2 Method and interpretation of the scale-of-effect analysis in 2017. (**a**) The percentage area of native vegetation within concentric circles, starting at an initial radius of 200 m, and increasing by 200 m to a maximum radius of 4000 m (20 measurements in total), was calculated from the centre of each sample site, respectively (here presented for the sample site located within Wottons Scrub CP). The percentage area of native vegetation surrounding sample sites within each radius (200-4000 m) was then included as the predictor variable of a response (e.g., viable seed-set) in separate models (20 models per response variable). (**b**) Following this, the small-sample-size corrected Akaike information criterion (AICc) values of each model was plotted against the respective distance category of the model's predictor (i.e., 200 m, 400 m, etc.). A scale-of-effect was identified where a distinct peak in model adequacy was present, suggesting the response variable responded most strongly to native vegetation within the distance category of the best supported model, identified as the model with the lowest AICc value (central (red) arrow). Models within a Δ AICc ≤ 2 from the model with the greatest support are also considered to exhibit substantial support for their estimated scale-of-effect (models within lateral (green) arrows). The horizontal reference line represents a Δ AICc ≤ 2 from the best supported model. When all models occur within Δ AICc ≤ 2 from the best supported model, no scale-of-effect is considered to exist within the range evaluated.

3.4 RESULTS

3.4.1 Pollinators

Seventeen visits by native bees to the flowers of *H. exutiacies* were filmed in 2017. In 14 of these visits the bees interacted with the anthers of at least one flower prior to departing (i.e., either moving outside of the video frame or leaving the plant itself), while in the remaining visits bees simply landed on the petals of one or more flowers before departing. Nine of these visitors were small-medium sized bees (~5-9 mm (estimated from video recordings)) able to contact the stigmatic tips of the two lateral styles and the central anthers simultaneously. These bees were likely species of *Lasioglossum* (*Chilalictus*) Michener (SM Fig. 3-1q-r, w), considered an important group of pollinators within southern Australia (Dorey 2018 p. 69). Bees grasped the central anthers, removing pollen via a mixture of thoracic vibration and scraping and milking of the anthers. Bees would buzz anthers in spurts followed by either grooming of the released pollen or rotating their position on the flower before buzzing the anthers once more. Either of the stigmatic tips of the two lateral styles contacted the ventral surface of the bee during this process (SM Fig. 3-1r). Although audible buzzes could not be heard via the video recordings, field observations confirmed thoracic vibration of anthers by these bees (Pers. Obs.). Similar foraging behaviour has been observed for species of *Lasioglossum* (*Chilalictus*) foraging on flowers of *H. stricta* (Bernhardt 1984).

Unidentified species of smaller bees (~3-4 mm) appeared to extract pollen via thoracic vibrations, but often spent longer scraping the anthers, and did not always contact the stigmatic tips of the two lateral styles (SM Fig. 3-1u). For this reason, larger bees are likely more effective pollinators (SM Fig. 3-1q-r versus SM Fig. 3-1u). This conclusion was also reached by Bernhardt (1984) for bee visitors to *H. stricta*. Size constraints to the pollination efficiency of visiting bees is probably a general feature of buzz-pollinated flowers with spatially separated anthers and stigma(s) (i.e., herkogamy; Mesquita-Neto et al. 2021).

In 2018, five native bees were seen visiting *H. exutiacies* flowers, however, only two of these buzzed the anthers of visited flowers. Opportunistically, six native bees visiting flowers of *H. exutiacies* were caught in 2019. These bees belonged to the genera *Exoneura* Smith (*n* = 4), and *Lasioglossum* (*Chilalictus*) (*n* = 2; SM Fig. 3-1x-z). Species in each genus visit and buzz the flowers of other *Hibbertia* species throughout Australia, such as *H. stricta* in eastern Australia (Bernhardt 1984) and *H. hypericoides* in southwest Western Australia (Schatral 1996). All sampled bees carried pollen of *H. exutiacies* (SM Fig. 3-1s-t). As for other species of *Hibbertia* (see Bernhardt 1984 and Schatral 1996), the pollen of *H. exutiacies* is sticky and covered in oil droplets (i.e., pollenkitt; SM Fig. 3-1B). Notably, this differs from the typically dry pollen grains of other buzz-pollinated plants (Buchmann 1983), including the co-flowering buzz-pollinated species, *Tetratheca pilosa* ssp. *pilosa* Labill. (Elaeocarpaceae), within the same sample sites (SM Fig. 3-1C-D).

Though a more systematic study of pollinators was not undertaken, it is clear floral visitation to *H. exutiacies* is infrequent (SM Table 3-1) and pollinators likely vary in their effectiveness (SM Fig. 3-1q-r versus SM Fig. 3-1u). Thus, both pollen quantity and quality may limit reproduction.

3.4.2 Breeding system

Tests of autonomous self-pollination demonstrated floral visitation is at least required to initiate fruit and seed production (0 fruits/200 flowers). The breeding system was highly outcrossing, with self-pollination producing 6.25 % fruit-set (1/16), outcrossing producing 66.67 % fruit-set (10/15), and open-pollination producing 42.86 % fruit-set (15/35). The single fruit produced via self-pollination contained both a viable and a predated seed. For seeds produced via outcrossing, 61.90 % (13/21) were graded as viable, the remainder suffering from pre-dispersal seed predation. For fruits resulting from open-pollination, the percentage of seeds graded as viable was lower at 17.65 % (6/34). Of the remaining seeds produced via open-pollination, 73.53 % (25/34) were predated while 8.82 % (3/34) were unfilled. However, in terms of developing seed-set there was no statistically clear difference between the overall mean number of developing seeds per fruit of outcrossed and open-pollinated flowers (2.10 ± 0.88 versus 2.27 ± 1.22 developing seeds per fruit (pooled across plants); Wilcoxon test: $W = 71.5$, $p = 0.863$). Thus, there was no evidence that outcross-pollinated flowers initiated the development of more or less seeds per fruit than flowers receiving open-pollination. The higher percentage of viable seeds produced by outcrossed flowers is a possible artifact of the experimental design, whereby bagging of outcross-pollinated flowers and fruits restricted access to seed predators, particularly considering bags remained in place from prior to flowering to fruit collection.

3.4.3 Pollen-limitation of fruit-set in 2017

There was no statistically significant interaction between treatment and reserve area for plants of *H. exutiacies* in 2017 (SM Fig. 3-2), indicating that the effect of the pollen addition treatment on fruit-set did not vary with reserve area, and the main effect of reserve area was not significant. Thus, results are presented for models with treatment as the only predictor (Table 3-2). There was a significant treatment effect on fruit-set (Fig. 3-3a; Table 3-2) and post-hoc analysis showed the odds of a flower producing fruit were lower for the internal control compared to the external control (z = -2.809, p = 0.015; Fig. 3-3b), suggesting resource reallocation was occurring between flowers on plants containing the internal control and pollen addition treatment. However the odds of a flower producing fruit within the pollen addition treatment was also greater compared to the external control (z = 3.678, $p = 7.065 \times 10^{-4}$; Fig. 3-3b), suggesting pollen-limitation of fruit-set was also present. There was no relationship between the mean pl-index per plant for each reserve and reserve area (τ = -0.2, n = 5, p = 0.817; Fig. 3-3c), further corroborating the absence of an interaction between pollen-limitation and reserve area.

3.4.4 Pollen-limitation of seed production in 2017

From a total of 36 plants, 148 fruits were opened yielding 88 viable seeds (33.33 %) and 176 inviable (predated and unfilled) seeds (66.67 %). Most inviable seeds examined had suffered predation (159/176), with few graded as unfilled (Table 3-3). Overall, 62.16 % (92/148) of fruits contained two or more developing seeds. Nevertheless, in terms of the viability of seeds within fruits, the data was quite binary, with 56.08 % (83/148) of fruits containing no viable seeds, 30.41 % (45/148) of fruits containing only viable seeds, and 13.51 % (20/148) of fruits containing a mixture of viable and inviable seeds. Only 5.41 % (8/148) fruits contained just unfilled seeds, and 9.46 % (14/148) of fruits contained one or more unfilled seeds. Thus, abortion of seeds within fruits was uncommon across those plants used in pollen-limitation experiments in 2017.

There was no statistically significant interaction between treatment and reserve area, or a significant main effect of reserve area, for any of the six remaining response variables that measured various aspects of seed-set. Thus, results are presented for models with treatment as the only predictor (Table 3-2). When broken down by treatment, and pooled across sample sites, the mean number of developing seeds per fruit per plant was generally lower in the internal control versus both the external control and pollen addition treatment (Table 3-4). However, there was no significant effect of treatment on developing seed-set (Table 3-2; SM Fig. 3-3a). Thus, pollen addition did not increase the overall number of seeds which at least initiated development per fruit for plants of *H. exutiacies*. There was also no significant treatment effect on the probability of fruits having viable seeds, the proportion of viable seeds per fruit, or viable + predated seed-set for plants of *H. exutiacies* (Table 3-2; SM Fig. 3-3b-d). Thus, there was no support for pollen-limitation restricting the number of viable seeds produced within fruits. Likewise, there was no significant treatment effect on inviable and predated seed-set (Table 3-2; SM Fig. 3-3e-f), or the mean unfilled seed-set for plants across treatments (Fisher-Pitman permutation test: χ^2_2 = 1.190, *p* = 0.576; SM Fig. 3-3g).

Nevertheless, although the number of viable seeds per fruit was unrelated to treatment, the addition of pollen to the flowers of *H. exutiacies* did result in an overall greater number of viable seeds produced (Fig. 3-4a), due to the increased number of fruits produced by plants across the pollen addition treatments. Ultimately, due to the greater number of fruits produced, the pollen addition treatment produced the greatest numbers of predated and unfilled seeds, respectively (Fig. 3-4a).

3.4.5 Natural levels of fruit-set and reserve area in 2017

In 2017, overall fruit-set was 46.13 % (185/401) pooled across plants and sample sites with fruit-set ranging from 31.48 to 65.11 % between sample sites (SM Fig. 3-4). In two of eight sample sites fruit-set was greater than 50 %. This occurred in the smallest reserve (The Knoll) and the second to largest reserve (Belair), respectively, so unsurprisingly there was no significant relationship between fruit-set and reserve area in 2017 (Table 3-5). Thus, fruit-set was not greater for plants within reserves of larger area. There was also no significant difference between sample sites in fruit-set irrespective of reserve area (Quasibinomial GLM: $F_{7,64}$ = 1.464, *p* = 0.196). Thus, mean fruit-set of *H. exutiacies* showed no clear spatial variability among sample sites in 2017.

3.4.6 Natural levels of seed production in 2017

From a total of 60 plants, 176 fruits were opened yielding 108 viable seeds (34.39 %) and 206 inviable (predated and unfilled) seeds (65.61 %). Most inviable seeds examined had suffered predation (190/206), with few graded as unfilled (Table 3-3). Overall, 52.84 % (93/176) of fruits contained two or more developing seeds and the data were quite binary, with 58.52 % (103/176) of fruits containing no viable seeds, 28.41 % (50/176) of fruits containing only viable seeds, and 13.07 % (23/176) of fruits containing a mixture of viable and inviable seeds. Only 3.98 % (7/176) of fruits contained just unfilled seeds, and 6.82 % (12/176) fruits contained one or more unfilled seeds. Thus, abortion of seeds within fruits was uncommon across open-pollinated plants used in the mensurative census in 2017.

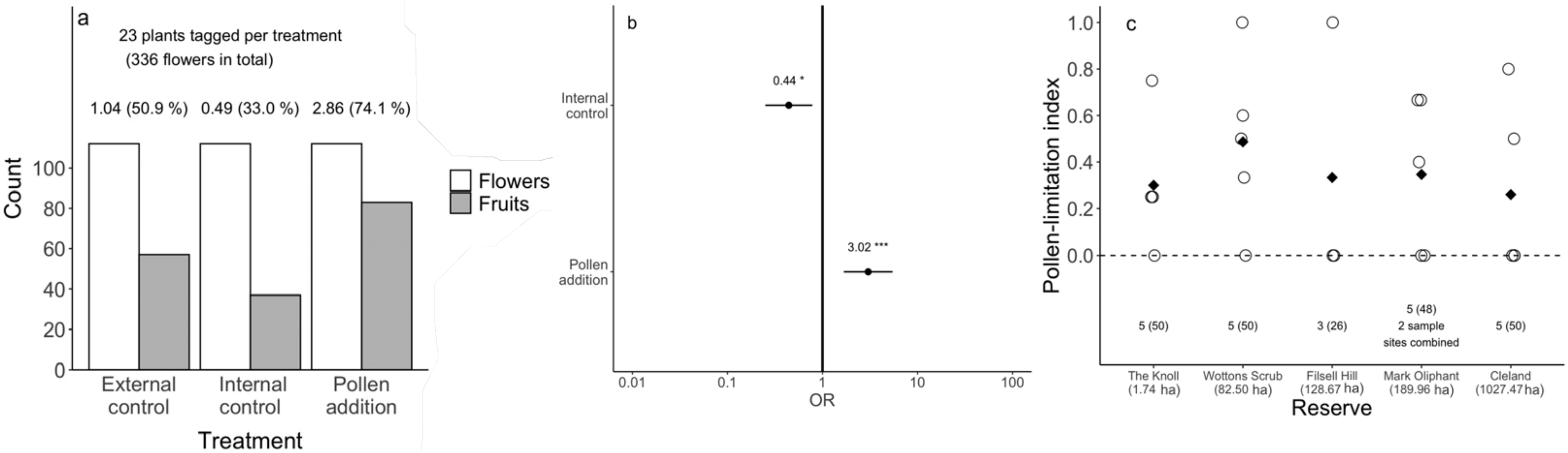


Fig. 3-3 Effect of the pollen addition treatment on the fruit-set of *Hibbertia exutiacies* in 2017. (**a**) The number of flowers tagged per treatment relative to the number of fruits which were subsequently produced, pooled across plants and sample sites. The odds of a fruit being produced per treatment are given above each of the respective group of columns. Percentage fruit-set (rounded to one decimal place) is provided in parentheses. (**b**) Exponentiated odds ratios (OR), ± 95 % confidence interval, of the fruit-set of the internal and external control and the pollen addition treatment and external control as estimated from a Binomial GLMM testing for an effect of treatment (see Methods for further statistical detail). (**c**) The pollen-limitation index per plant (open circles) plotted against reserve, in ascending order of reserve area. The mean pollen-limitation index per plant for each reserve is also plotted (solid diamonds). Provided below are the number of plants used per treatment (i.e., pollen addition and external control) and the total number of flowers tagged across treatments. * $p < 0.05 > 0.01$, *** $p < 0.001$.

Table 3-2 Models of the effect of pollen-limitation treatment on the reproduction of *Hibbertia exutiacies* in 2017 and 2018. The random intercept terms (nested from top to bottom) for each model are presented along with their standard deviations. For the fixed model parameters (on the scale of the link function), the intercept (Int) represents the estimated response of the external control, with IC the estimated difference (i.e., slope) between the internal and external control and PA the difference between the pollen addition treatment and external control. 95 % confidence intervals (Wald) are provided in brackets. Significant results highlighted in bold. * $p < 0.05 > 0.01$, *** $p < 0.001$, denote differences between IC or PA and the intercept (Int).

Year	Response	Predictor	Random effect(s)	Fixed parameters	Model	Test Statistic	*p-value*
2017	Fruit-set	Treatment	Reserve: 0.297 Plant Pair: 0.674	Int: 0.047 (-0.50, 0.59) IC: -0.816 (-1.39, -0.25)* PA: 1.107 (0.52, 1.70)***	Binomial[1]	χ^2_2 = 37.52	**7.122×10^{-9}**
	Developing seed-set	Treatment	Reserve: 0.115 Plant Pair: 0.150 Treatment: 0.063	Int: 0.567 (0.39, 0.74) IC: -0.102 (-0.30, 0.09) PA: 0.041 (-0.11, 0.20)	Tr. CMP[2]	χ^2_2 = 2.269	0.322
	Viable + predated seed-set	Treatment	Reserve: 0.195 Plant Pair: 0.130 Treatment: 0.102	Int: 0.527 (0.30, 0.76) IC: -0.076 (-0.30, 0.15) PA: 0.004 (-0.18, 0.18)	CMP[3]	χ^2_2 = 0.612	0.736
	Prob. fruits having viable seeds	Treatment	Reserve: 0.546 Plant Pair: 0.420 Treatment: 0.520	Int: -0.478 (-1.35, 0.39) IC: 0.205 (-0.85, 1.26) PA:0.204 (-0.68, 1.09)	Binomial[1]	χ^2_2 = 0.239	0.887
	Inviable seed-set	Treatment	Reserve: 0.233 Plant Pair: 0.204 Treatment: 0.103	Int: 0.210 (-0.13, 0.55) IC: -0.206 (-0.61, 0.19) PA: -0.008 (-0.32, 0.30)	CMP[3]	χ^2_2 = 1.228	0.541
	Predated seed-set	Treatment	Reserve: 0.344 Plant Pair: 0.197 Treatment: 0.257	Int: 0.092 (-0.34, 0.53) IC: -0.163 (-0.62, 0.30) PA: -0.063 (-0.43, 0.31)	CMP[3]	χ^2_2 = 0.480	0.787
	Prop. viable seeds per fruit	Treatment	Reserve: 0.512 Plant Pair: 0.081 Treatment: 0.733	Int: -0.953 (-1.75, -0.16) IC: 0.275 (-0.70, 1.25) PA: 0.104 (-0.71, 0.92)	Binomial	χ^2_2 = 0.308	0.857
2018	Fruit-set	Treatment	Reserve: 0.035 Plant Pair: 0.444	Int: 0.175 (-0.24, 0.59) IC: 0.214 (-0.31, 0.74) PA: 0.630 (0.09, 1.17)	Binomial	χ^2_2 = 5.367	0.068
	(Mean developing seed-set)$^{0.25}$	Treatment	Reserve: 0.039 Plant Pair: 0.027	Int: 1.067 (1.02, 1.11) IC: 0.044 (0.00, 0.09) PA: 0.029 (-0.01, 0.07)	Gaussian	$F_{(2, 45.37)}$ = 2.275	0.114
	Unfilled seed-set	Treatment	Reserve: 0.211 Plant Pair: 0.175 Treatment: 0.076	Int: 0.014 (-0.23, 0.26) IC: 0.263 (0.07, 0.46)* PA: 0.261 (0.07, 0.45)*	CMP[3]	χ^2_2 = 8.960	**0.011**

[1] Partially Bayesian method used to prevent singular fits via the ‘bglmer’ function in the package ‘blme’ (Chung et al. 2013).

[2] Truncated Conway-Maxwell-Poisson.

[3] Conway-Maxwell-Poisson.

Table 3-3 The number of fruits and seeds examined, pooled across individuals of *Hibbertia exutiacies*, used to measure pollen-limitation of reproduction and natural levels of reproduction (open-pollination) in 2017 and 2018. The number of plants represents only those plants which produced at least one fruit from tagged flowers. No viable seeds were produced in 2018.

Year	Dataset	Treatment	No. plants	No. fruits	No. developing seeds	No. viable seeds	No. inviable seeds[b]	No. predated seeds	No. unfilled seeds
2017	Pollen-limitation	Pollen addition	19	69	129	42	87	77	10
		Internal control	15	31	51	19	32	30	2
		External control	17	48	84	27	57	52	5
	Open-pollination[a]	Open-pollination	60	176	314	108	206	190	16
2018	Pollen-limitation	Pollen addition	24	82	130	-	-	11	119
		Internal control	23	71	113	-	-	11	102
		External control	24	65	93	-	-	18	75

[a] Includes both the external controls and additional plants used to measure natural levels of reproduction (open-pollination) in 2017.

[b] Equivalent to developing seeds in 2018 as all examined seeds were inviable.

Table 3-4 The mean number of seeds per fruit per plant (pooled across sample sites), ± SD, for *Hibbertia exutiacies* used to measure pollen-limitation of reproduction and natural levels of reproduction (open-pollination) in 2017 and 2018. No viable seeds were produced in 2018.

Year	Dataset	Treatment	Mean no. developing seeds fruit^{-1} plant^{-1}	Mean no. viable seeds fruit^{-1} plant^{-1}	Mean no. inviable[b] seeds fruit^{-1} plant^{-1}	Mean no. predated seeds fruit^{-1} plant^{-1}	Mean no. unfilled seeds fruit^{-1} plant^{-1}	Mean prop. viable seeds fruit^{-1} plant^{-1}
2017	Pollen-limitation	Pollen addition	1.83 ± 0.47	0.62 ± 0.44	1.21 ± 0.66	1.05 ± 0.78	0.15 ± 0.32	0.38 ± 0.27
		Internal control	1.58 ± 0.46	0.57 ± 0.58	1.01 ± 0.74	0.95 ± 0.78	0.06 ± 0.15	0.39 ± 0.41
		External control	1.80 ± 0.69	0.55 ± 0.46	1.26 ± 0.88	1.13 ± 0.93	0.13 ± 0.29	0.34 ± 0.30
Open-pollination[a]		Open-pollination	1.75 ± 0.63	0.60 ± 0.66	1.14 ± 0.74	1.03 ± 0.73	0.12 ± 0.28	0.35 ± 0.34
2018	Pollen-limitation	Pollen addition	1.52 ± 0.43	-	-	0.143 ± 0.34	1.38 ± 0.56	-
		Internal control	1.62 ± 0.56	-	-	0.184 ± 0.36	1.44 ± 0.68	-
		External control	1.37 ± 0.42	-	-	0.306 ± 0.56	1.07 ± 0.55	-

[a] Includes both the external controls and additional plants used to measure natural levels of reproduction (open-pollination) in 2017.

[b] Equivalent to developing seeds in 2018 as all examined seeds were inviable.

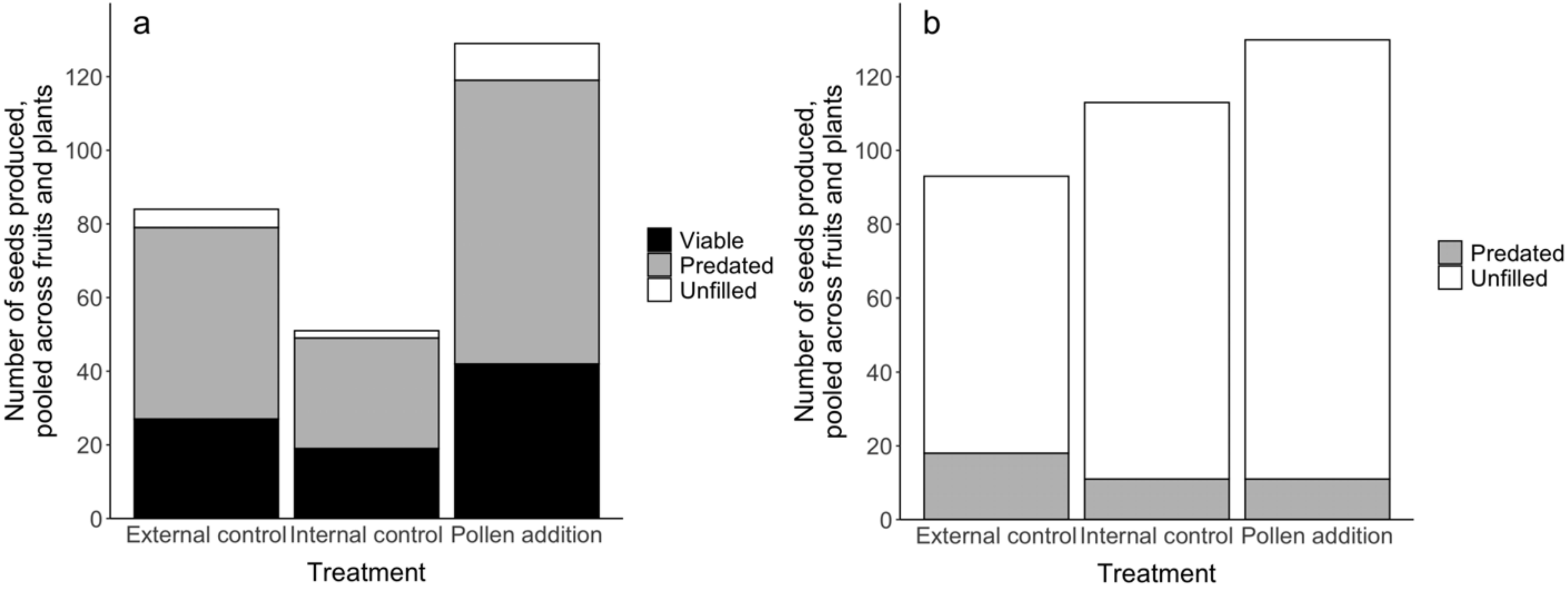


Fig. 3-4 Effect of the pollen addition treatment on the total number of developing seeds (viable + predated + unfilled) produced, pooled across fruits and plants, for the study species *Hibbertia exutiacies* in (**a**) 2017 and (**b**) 2018. Developing seeds were graded into three categories termed viable, predated, and unfilled. Note that no viable seeds were produced for any treatment group in 2018. Each treatment initially started with the same number of plants and flowers tagged within each year.

3.4.7 Natural levels of developing seed-set and reserve area in 2017

There was no statistically significant effect of reserve area on mean developing seed-set (Table 3-5) and hence there was no evidence that the number of seeds per fruit which initiated development increased for plants of *H. exutiacies* within larger reserves (SM Fig. 3-5). There was, however, a significant difference between sample sites in developing seed-set irrespective of reserve area (Truncated CMP GLMM: χ^2_7 = 14.712, p = 0.040). Post-hoc analysis showed developing seed-set was greater for plants within the sample site of Wottons Scrub versus Mark Oliphant - S2 (z = -3.345, p = 0.023; SM Fig. 3-6a). Thus, although reserve area had no significant impact on developing seed-set, unknown factors resulted in increased numbers of developing seeds per fruit for plants within Wottons Scrub relative to Mark Oliphant - S2.

3.4.8 Natural levels of viable seed production and reserve area in 2017

The probability of fruits having viable seeds increased for plants of *H. exutiacies* with increasing reserve area (Fig. 3-5a), although statistically the relationship was marginally non-significant (Table 3-5). A similar marginally non-significant positive relationship was evident for the proportion of viable seeds per fruit (Table 3-5), although model residuals showed deviations from uniformity. However, inspection of the data suggested that the lack of a statistically significant effect of reserve area was largely due to the influence of the smallest reserve The Knoll (Fig. 3-5b), and removal of The Knoll reserve resulted in an adequately fitted model and a statistically significant positive relationship with reserve area (Table 3-5). Similarly, removal of The Knoll reserve from the model of the probability of fruits having viable seeds resulted in a statistically significant positive relationship with reserve area (Table 3-5).

Comparisons of sample sites disregarding reserve area revealed significant differences between sample sites in the probability of fruits having viable seeds (Binomial GLMM: χ^2_7 = 22.136, p = 0.002) and the proportion of viable seeds per fruit for plants of *H. exutiacies* (Binomial GLMM: χ^2_7 = 22.248, p = 0.002). Post-hoc analysis showed that the probability of fruits having viable seeds was greater for plants in Belair versus Wottons Scrub, The Knoll, Filsell Hill, and Mark Oliphant - S2 ($z = \geq 3.466$, $p = \leq 0.015$; SM Fig. 3-6c). While the proportion of viable seeds per fruit was greater for plants in Belair versus The Knoll, Mark Oliphant - S2, Filsell Hill, Giles, and Wottons Scrub ($z = \geq 3.312$, $p = \leq 0.026$; SM Fig. 3-6d). Thus, the fruits of plants in Belair were clearly more successful in producing viable seeds than plants from a number of other sample sites. The alternative measure of viable plus predated seed-set was unrelated to reserve area (Table 3-5), with similar differences between sample sites as developing seed-set (SM Fig. 3-6b).

Given that the strength of the relationship between reserve area and measures of reproductive output such as the proportion of viable seeds per fruit appeared to be quite dependent on whether particular study sites were included in the analysis, a further “scale-of-effect” analysis was conducted to investigate the effect of the amount of native vegetation surrounding sample sites in each reserve (see *Scale-of-effect - 2017* in the Methods above). A scale-of-effect was apparent, with the percentage area of native vegetation at 2800 m being the best model according to a comparison of model AICc for both the probability of fruits having viable seeds and the proportion of viable seeds per fruit (Fig. 3-6a-b). Combined, the scale-of-effect analyses suggested the percentage area of native vegetation surrounding sample sites within a radius of 2400-3400 m was a strong predictor of viable seed production (Fig. 3-6a-b). Models of the chosen scale-of-effect (2800 m for both response variables) were significant, showing a positive effect of surrounding native vegetation on viable seeds per fruit for plants of *H. exutiacies* (Fig. 3-6d-e; Table 3-5). Both models included data for The Knoll reserve and removal of The Knoll from the chosen models of the scale of effect (2800 m) did not qualitatively alter statistical significance ($p < 0.001$). None of the compared models exhibited evidence of spatial autocorrelation (Moran's I $p > 0.05$).

3.4.9 Natural levels of inviable seed-set and reserve area in 2017

There was no significant effect of reserve area on mean inviable seed-set (Table 3-5). Thus, the number of inviable seeds per fruit showed no relation to reserve area. Again, however, when the smallest reserve The Knoll was excluded, there was a clear negative effect of reserve area on mean inviable seed-set (Fig. 3-5c; Table 3-5).

A test for difference between sample sites in inviable seed-set irrespective of reserve area was significant (CMP GLMM: $\chi^2_7 = 26.307$, $p = 4.438 \times 10^{-4}$), with the number of inviable seeds per fruit fewer for plants in the sample site of Belair versus Giles, Filsell Hill, and Wottons Scrub ($z = \leq -3.545$, $p = < 0.011$; SM Fig. 3-6e). Given the majority of inviable seeds across all sample sites was due to pre-dispersal seed predation, and no unfilled seeds were observed in fruits sampled from Belair, fruits of plants in Belair were clearly more successful at escaping pre-dispersal seed predation than plants from other sample sites. In support, a model of mean predated seed-set excluding The Knoll reserve showed a qualitatively similar negative effect of reserve area (Table 3-5), and there were comparable differences among sample sites irrespective of reserve area (Belair < Filsell Hill and Wottons Scrub; SM Fig. 3-6f).

Given the significant relationship between mean inviable seed-set and reserve area (excluding The Knoll), a scale-of-effect analysis was conducted. A scale-of-effect was apparent, with the percentage area of native vegetation at 2600 m the best model according to a comparison of model AICc (Fig. 3-6c). The analysis suggested the percentage area of native vegetation surrounding sample sites within 2400-3200 m was a strong predictor of square root transformed mean inviable seed-set per plant (Fig. 3-6c). The model of the chosen scale-of-effect (2600 m) was significant, showing a clear negative relationship between the percentage area of native vegetation surrounding sample sites and square root transformed mean inviable seed-set for plants of *H. exutiacies* (Fig. 3-6f; Table 3-5). Removal of The Knoll from this model did not qualitatively alter statistical significance ($p < 0.05$). None of the compared models exhibited evidence of spatial autocorrelation (Moran's I $p > 0.05$).

Although models of mean predated seed-set were not statistically significant for reserve area (Table 3-5), the model excluding The Knoll reserve was marginally non-significant and the confidence interval of the slope did not include zero, and thus we ran a scale-of-effect analysis on this response variable. Similar evidence of a scale-of-effect was evident for mean predated seed-set, although the range of selected models was larger at 2600-4000 m (SM Fig. 3-7). The best selected model was at 3800 m (Intercept = 2.733 (95 % CI: 1.85, 3.62), Slope = -0.041 (-0.06, -0.02), $F_{(1, 5.70)} = 15.548$, $p = 8.417 \times 10^{-3}$). There was some evidence of positive spatial autocorrelation for models including the percentage area of native vegetation between 200-2800 m (Moran's I $p < 0.05$). However, the best selected model at 3800 m showed no evidence of spatial autocorrelation.

Lastly, although not considered in detail, models of the alternative response variables of the probability of fruits having predated seeds and the proportion of predated seeds per fruit were also significantly negatively related to reserve area following exclusion of The Knoll reserve (SM Fig. 3-8a-b), and a scale-of-effect analysis on both these variables identified the percentage area of native vegetation surrounding sample sites within 2600-3600 m as a strong predictor of seeds being predated (SM Fig. 3-8c-d), with both variables significantly negatively related to the amount of native vegetation surrounding sample sites (SM Fig. 3-8e-f). In particular, because a small number of seeds were unfilled in 2017 and most fruits contained only viable or only predated seeds, the probability of fruits having predated seeds and the proportion of predated seeds per fruit are effectively the inverse of the probability of fruits having viable seeds and the proportion of viable seeds per fruit, respectively (e.g., compare Fig. 3-5a-b and Fig. 3-6a-b, d-e to that of SM Fig. 3-8), and hence we only further discuss the probability of fruits having viable seeds and the proportion of viable seeds per fruit of open-pollinated plants in 2017.

Table 3-5 Models of the relationships between either reserve area (log transformed), or the percentage area of native vegetation within a defined radius of sample sites (2600 m or 2800 m), and variables used to measure natural levels of reproduction (open-pollination) of *Hibbertia exutiacies* in 2017. The random intercept terms for each model (nested from top to bottom) are presented along with their standard deviations. The intercept and slope values on the scale of the link function are provided. 95 % confidence intervals (Wald) are provided in brackets. Significant results highlighted in bold.

Response	Predictor	Random effect(s)	Model	Intercept	Slope	Test statistic	*p-value*
Fruit-set	Reserve area	Reserve: 0.145	Beta-Binomial	0.097 (0.65, 0.85)	-0.054 (-0.20, 0.09)	χ^2_1 = 0.523	0.470
(Mean developing seed-set)$^{0.25}$	Reserve area	Reserve: 0.047	Gaussian	1.123 (1.01, 1.24)	0.005 (-0.02, 0.03)	$F_{(1,\ 4.76)}$ = 0.186	0.685
Mean viable + predated seed-set	Reserve area	Reserve: 0.355	Gaussian	1.493 (0.68, 2.31)	0.044 (-0.11, 0.20)	$F_{(1,\ 4.79)}$ = 0.303	0.607
Prob. fruits having viable seeds	Reserve area	Reserve: 0.739 Plant: 0.584	Binomial	-1.913 (-3.70, -0.13)	0.318 (-0.03, 0.66)	χ^2_1 = 3.274	0.070
Prob. fruits having viable seeds	Reserve area (Knoll removed)	Reserve: 0.520 Plant: 0.567	Binomial	-5.213 (-8.85, -1.58)	0.898 (0.25, 1.55)	χ^2_1 = 7.378	**0.007**
Mean inviable seed-set	Reserve area	Reserve: 0.451	Gaussian	1.565 (0.57, 2.56)	-0.080 (-0.27, 0.11)	$F_{(1,\ 4.83)}$ = 0.678	0.449
Mean inviable seed-set	Reserve area (Knoll removed)	Reserve: 0.237	Gaussian	3.509 (1.95, 5.06)	-0.425 (-0.70, -0.15)	$F_{(1,\ 4.16)}$ = 9.323	**0.033**
Mean predated seed-set	Reserve area	Reserve: 0.466	Gaussian	1.402 (0.38, 2.42)	-0.067 (-0.27, 0.13)	$F_{(1,\ 4.84)}$ = 0.462	0.528
Mean predated seed-set	Reserve area (Knoll removed)	Reserve: 0.296	Gaussian	3.311 (1.59, 5.04)	-0.405 (-0.71, -0.10)	$F_{(1,\ 4.27)}$ = 6.899	0.054
Prop. viable seeds per fruit	Reserve area	Reserve: 0.667 Plant: 1.106	Binomial	-2.413 (-4.17, -0.65)	0.320 (-0.02, 0.66)	χ^2_1 = 3.453	0.063
Prop. viable seeds per fruit	Reserve area (Knoll removed)	Reserve: 0.532 Plant: 1.144	Binomial[1]	-6.654 (-10.57, -2.73)	1.062 (0.37, 1.75)	χ^2_1 = 9.034	**0.003**
Prob. fruits having viable seeds	% Native veg. 2800 m	Reserve: 0.097 Plant: 0.777	Binomial	-4.236 (-6.25, -2.23)	0.088 (0.04, 0.13)	χ^2_1 = 15.282	**9.261×10^{-5}**
(Mean inviable seed-set)$^{0.5}$	% Native veg. 2600 m	Reserve: 0.144	Gaussian[1]	1.894 (1.32, 2.47)	-0.021 (-0.03, -0.01)	$F_{(1,\ 5.70)}$ = 10.907	**0.018**
Prop. viable seeds per fruit	% Native veg. 2800 m	Reserve: 0.371 Plant: 1.214	Binomial[1]	-4.968 (-7.27, -2.66)	0.094 (0.04, 0.14)	χ^2_1 = 13.330	**2.612×10^{-4}**

[1] Partially Bayesian method used to prevent singular fits via the 'b(g)lmer' function in the package 'blme' (Chung et al. 2013).

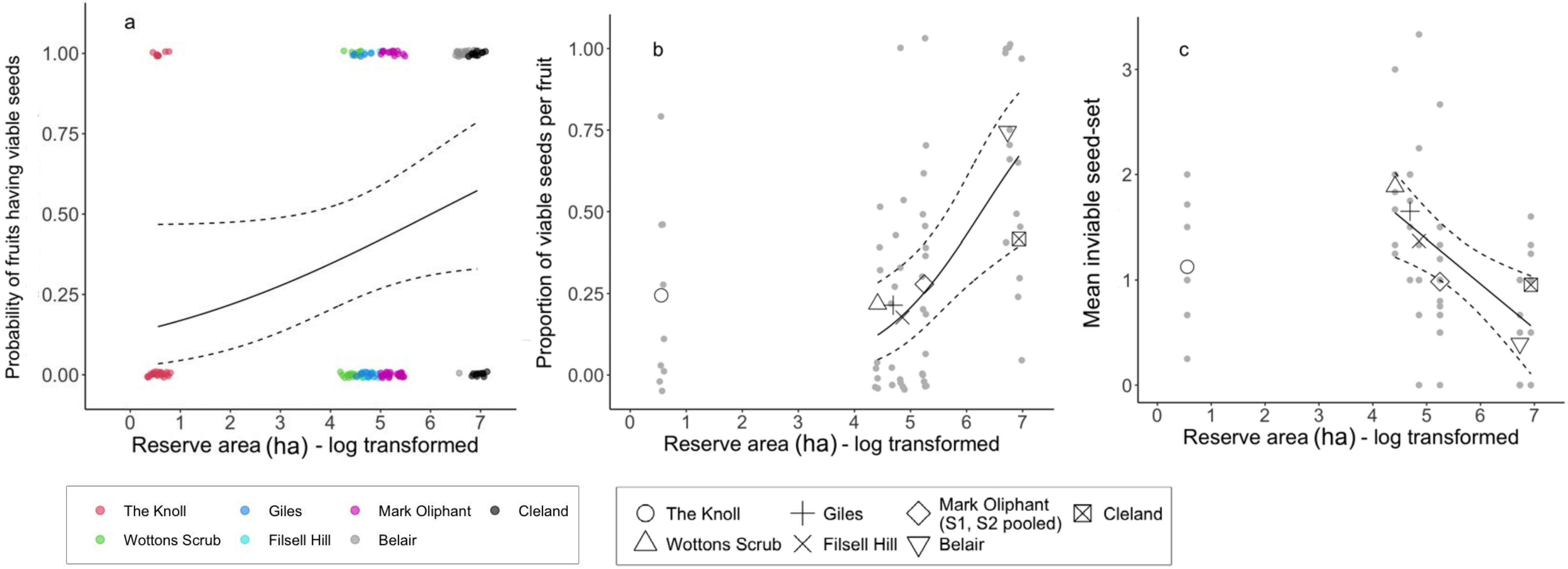


Fig. 3-5 Plots of the relationships between reserve area (log transformed) and three variables used to measure natural levels of seed production (open-pollination) of *Hibbertia exutiacies* in 2017. (**a**) The probability of fruits having viable seeds. The trend line plotted is the predicted probability of fruits having viable seeds for plants across reserves, including The Knoll reserve. (**b**) The proportion of viable seeds per fruit. Large symbols show the overall proportion of seeds graded as viable per reserve (i.e., population proportion). The overall proportion of seeds graded as viable for each individual plant are also plotted (grey data points). The trend line plotted is the predicted probability of seeds being viable per fruit (i.e, “the proportion of viable seeds per fruit”) for plants across reserves, excluding The Knoll reserve. (**c**) Mean inviable seed-set. Large symbols show reserve averages of mean inviable seed-set across plants. The mean inviable seed-set for each individual plant are also plotted (grey data points). The trend line plotted is the predicted mean inviable seed-set for plants across reserves, excluding The Knoll reserve. All trend lines are population-level predictions (i.e., setting all random effects to zero). Dashed lines represent 95 % confidence intervals. S1 and S2 stand for sample site 1 and 2, respectively. Points in plots have been randomly jittered to aid visualisation.

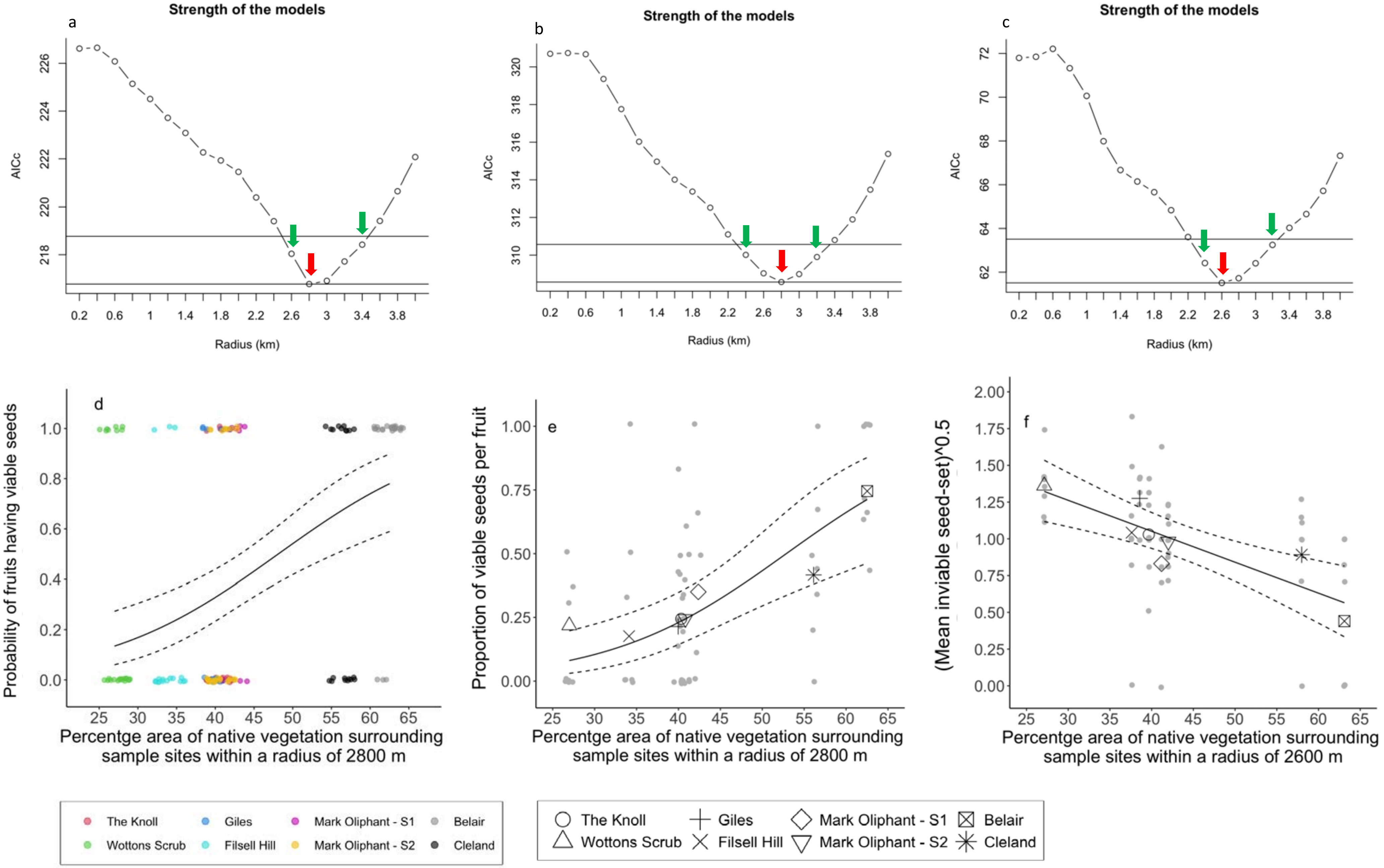

a
Strength of the models
AICc
226
224
222
220
218
0.2
0.6
1
1.4
1.8
2.2
2.6
3
3.4
3.8
Radius (km)
b
Strength of the models
AICc
320
318
316
314
312
310
Radius (km)
c
Strength of the models
AICc
72
70
68
66
64
62
Radius (km)
d
Probability of fruits having viable seeds
1.0
0.8
0.6
0.4
0.2
0.0
25
30
35
40
45
50
55
60
65
Percentge area of native vegetation surrounding sample sites within a radius of 2800 m
e
Proportion of viable seeds per fruit
1.00
0.75
0.50
0.25
0.00
Percentge area of native vegetation surrounding sample sites within a radius of 2800 m
f
(Mean inviable seed-set)^0.5
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
Percentge area of native vegetation surrounding sample sites within a radius of 2600 m
The Knoll
Giles
Mark Oliphant - S1
Belair
Wottons Scrub
Filsell Hill
Mark Oliphant - S2
Cleland
The Knoll
Giles
Mark Oliphant - S1
Belair
Wottons Scrub
Filsell Hill
Mark Oliphant - S2
Cleland

Fig. 3-6 (above) Results of scale-of-effect analyses on three variables used to measure natural levels of seed production (open-pollination) of *Hibbertia exutiacies* in 2017. Shown are the comparisons of model support for the percentage area of native vegetation within a defined radius of sample sites (200-4000 m) on (**a**) the probability of fruits having viable seeds, (**b**) the proportion of viable seeds per fruit, and (**c**) square root transformed mean inviable seed-set. A total of 20 models were constructed and compared via their small-sample-size corrected Akaike information criterion (AICc) values. The chosen scale-of-effect was identified as the model with the best support, identified as the model with the lowest AICc value (central (red) arrow). Models within a Δ AICc $\leq$ 2 from the model with the greatest support are also considered to exhibit substantial support for their estimated scale-of-effect (models within lateral (green) arrows). The horizontal reference line represents a Δ AICc $\leq$ 2 from the best supported model. (**d-f**) Plots displaying the chosen scale-of-effect for each of the three response variables. (**d**) The probability of fruits having viable seeds, plotted against the percentage area of native vegetation surrounding sample sites within a radius of 2800 m. The trend line plotted is the predicted probability of fruits having viable seeds for plants of *H. exutiacies*. (**e**) The proportion of viable seeds per fruit. Large symbols show the overall proportion of seeds graded as viable per sample site (i.e., population proportion), plotted against the percentage area of native vegetation surrounding sample sites within a radius of 2800 m. The overall proportion of seeds graded as viable for each individual plant are also plotted (grey data points). The trend line plotted is the predicted probability of seeds being viable per fruit (i.e, “the proportion of viable seeds per fruit”) for plants of *H. exutiacies*. (**f**) Square root transformed mean inviable seed-set. Large symbols show sample site averages of square root transformed mean inviable seed-set across plants, plotted against the percentage area of native vegetation surrounding sample sites within a radius of 2600 m. The square root transformed mean inviable seed-set for each individual plant are also plotted (grey data points). The trend line plotted is the predicted square root transformed mean inviable seed-set for plants of *H. exutiacies*. All trend lines are population-level predictions (i.e., setting all random effects to zero). Dashed lines represent 95 % confidence intervals. S1 and S2 stand for sample site 1 and 2, respectively. Points in plots (**d-f**) have been randomly jittered to aid visualisation.

3.4.10 Pollen-limitation of fruit-set in 2018

There was no statistically significant interaction between the pollen addition treatment and reserve area for levels of fruit-set of plants of *H. exutiacies* in 2018 (SM Fig. 3-9), and the main effect of reserve area was not significant. Thus, results are presented for models with treatment as the only predictor (Table 3-2). There was no effect of treatment on fruit-set (Fig. 3-7a; Table 3-2). Thus, there was no evidence of either resource reallocation or pollen-limitation of fruit-set in 2018 (Fig. 3-7b), in contrast with the results of 2017 (Fig. 3-3a-b). There was also no relationship between the mean pl-index per plant for each reserve and reserve area (τ = -0.067, *n* = 6, *p* = 1.00; Fig. 3-7c).

3.4.11 Pollen-limitation of seed production in 2018

Across those plants which produced fruits, a total of 218 fruits were opened, with no viable seeds found (Fig. 3-4b; Table 3-3). Thus, although the initial development of fruits and their seeds was not pollen-limited in 2018, ultimately, this had no impact on production of offspring. Of the seeds examined, 11.90 % (40/336) showed evidence of pre-dispersal seed predation (Table 3-3). The remaining seeds examined in 2018 did not show evidence of predation, being predominantly unfilled and possessing a thin brittle seed coat. Of these unfilled seeds, 17.91 % (53/296) contained minute larvae which appeared to have starved to death, given there was no evidence of endosperm having filled the seed (SM Fig. 3-1o). Thus, in contrast to 2017, when unfilled seeds were uncommon (6.44 % of seeds examined in the pollen-limitation experiment) and most seeds suffered from pre-dispersal seed predation (60.23 %), unfilled seeds were the most common seed type found in 2018 (Fig. 3-4). This result indicates abortion of developing seeds was extensive across the study region for plants of *H. exutiacies* in 2018 (also see SM File 3-1).

There was no statistically significant interaction between treatment and reserve area, or a significant main effect of reserve area, for any of the response variables considered. Thus, results are presented for models with treatment as the only predictor (Table 3-2). There was no significant treatment effect on mean developing seed-set (Table 3-2; SM Fig. 3-10a). In contrast, there was a significant treatment effect on unfilled seed-set (Table 3-2). However, unfilled seed-set was higher in both the pollen addition treatment (*z* = 2.720, *p* = 0.020) and internal control (*z* = -2.656, *p* = 0.024) versus the external control (Table 3-2 and 3-4; SM Fig. 3-10b). Thus, if there is a treatment effect then pollen addition equally increased the unfilled seed-set of internal control flowers on the same plant, making an interpretation of cause difficult to establish. Further experimental manipulations would be needed to ascertain if the statistically significant result represents a truly biological phenomenon, rather than a spurious result (type I error). There was no statistically significant evidence that mean predated seed-set differed for plants between treatments (Fisher-Pitman permutation test: χ^2_2 = 3.160, p = 0.206; SM Fig. 3-10c-d).

3.4.12 Natural levels of fruit-set and reserve area in 2018

Pooled across plants and sample sites overall fruit-set of external controls was 54.17 % (65/120) in 2018, with fruit-set ranging from 20.00 to 72.00 % between sample sites. Models with reserve area as the predictor fitted poorly, however, there was a significant difference between sample sites in fruit-set irrespective of reserve area (Binomial GLM: χ^2_5 = 15.678, p = 0.008; SM Fig. 3-11). Post-hoc analysis showed that the fruit-set of plants was greater in the smallest and second largest reserves of The Knoll and Mark Oliphant versus the largest reserve of Cleland, respectively (z = -2.972, p = 0.044 (same z and p values for both comparisons)). Thus, there was no clear evidence of an effect of reserve area on fruit-set.

3.4.13 Natural levels of seed production and reserve area in 2018

Models of mean developing seed-set (LMM: $F_{(1,\ 4.16)}$ = 2.391, p = 0.122) and mean unfilled seed-set (LMM: $F_{(1,\ 3.34)}$ = 0.502, p = 0.525) showed no influence of reserve area. There was also no difference between sample sites in developing seed-set (CMP GLMM: χ^2_4 = 2.119, p = 0.714; Cleland removed from analysis) or unfilled seed-set (CMP GLMM: χ^2_5 = 7.110, p = 0.213). Likewise, there was no evidence of differences in mean predated seed-set between sample sites (Fisher-Pitman permutation test: χ^2_5 = 2.761, p = 0.824). This lack of difference was also evident from plots of the data (SM Fig. 3-12).

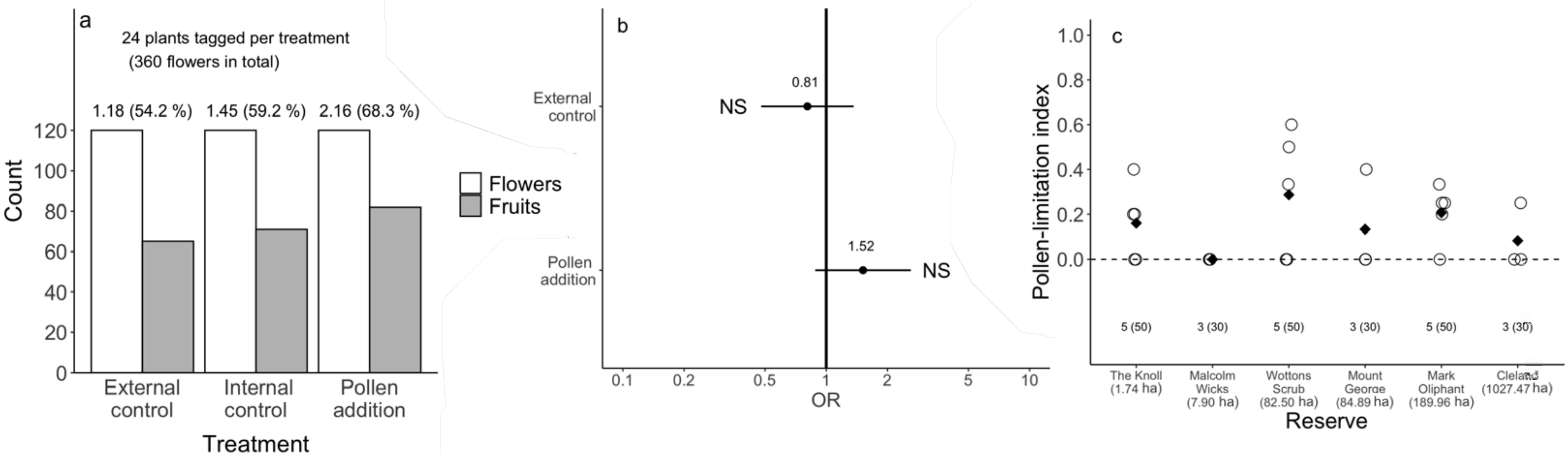


Fig. 3-7 Effect of the pollen addition treatment on the fruit-set of *Hibbertia exutiacies* in 2018. (**a**) The number of flowers tagged per treatment relative to the number of fruits which were subsequently produced, pooled across plants and sample sites. The odds of a fruit being produced per treatment are given above each of the respective group of columns. Percentage fruit-set (rounded to one decimal place) is provided in parentheses. (**b**) Exponentiated odds ratios (OR), ± 95 % confidence interval, of the fruit-set of the external and internal control and the pollen addition treatment and internal control as estimated from a Binomial GLMM testing for an effect of treatment (see Methods for further statistical detail). (**c**) The pollen-limitation index per plant (open circles) plotted against reserve, in ascending order of reserve area. The mean pollen-limitation index per plant for each reserve is also plotted (solid diamonds). Provided below are the number of plants used per treatment (i.e., pollen addition and internal control) and the total number of flowers tagged across treatments. The sample site used in Mark Oliphant in 2018 was Mark Oliphant - Sample Site 2. NS $p > 0.05$.

3.4.14 Natural levels of fruit-set - 2017 versus 2018

Given fruit-set per plant showed no evidence of pollen-limitation in 2018 but did in 2017, it may be expected that natural levels of fruit-set would be greater in 2018 relative to 2017, due to the lifting of pollen receipt as a resource constraint in 2018. However, there was no clear difference in the fruit-set of open-pollinated plants between 2017 and 2018 (Binomial GLMM: χ_1^2 = 1.884, *p* = 0.170; Fig. 3-3a versus Fig. 3-7a). Thus, although pollen receipt apparently did not significantly constrain fruit-set in 2018, natural levels of fruit-set showed no difference between years for plants of *H. exutiacies*.

3.4.15 Natural levels of seed production - 2017 versus 2018

There was a statistically significant difference between years in the developing seed-set of open-pollinated plants (Truncated CMP GLMM: χ_1^2 = 4.810, *p* = 0.029; Fig. 3-8a). The mean number of developing seeds per fruit per plant for open-pollinated plants in 2017 was 1.75 ± 0.63 versus 1.37 ± 0.42 in 2018 (Fig. 3-8b; Table 3-4). Thus, the number of seeds per fruit per plant that initiated development was lower in 2018. However, the fate of these seeds was also vastly different in 2018 versus 2017 and, ultimately, from a plant fitness perspective, pollen addition did not result in an increased production of viable seeds in 2018, as was clearly the case in 2017 (Fig. 3-4).

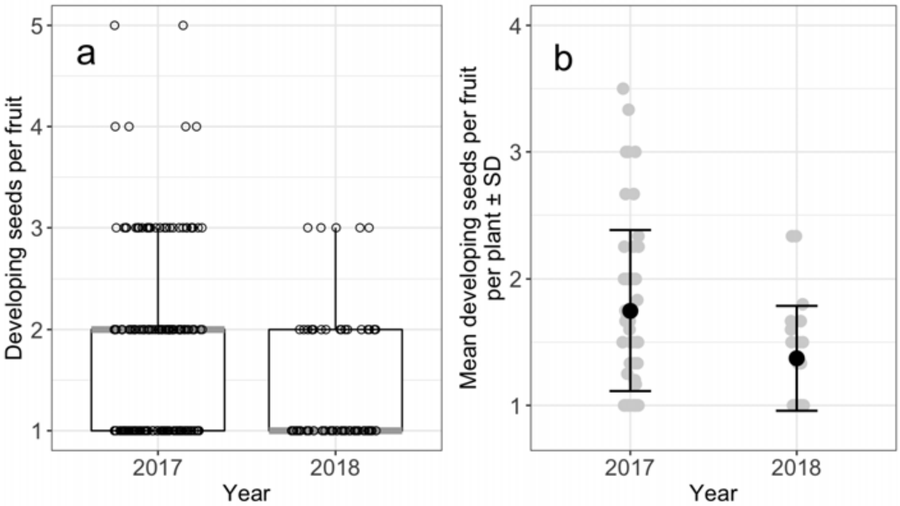


Fig. 3-8 (**a**) Boxplots of the number of developing seeds per fruit (i.e., developing seed-set) of open-pollinated plants of *Hibbertia exutiacies* in 2017 versus 2018. (**b**) The mean number of developing seeds per fruit per plant ± SD of open-pollinated plants in 2017 versus 2018. The mean number of developing seeds per fruit (i.e., mean developing seed-set) for each individual plant are also plotted (grey data points).

3.5 DISCUSSION

We observed marked spatio-temporal variability in the pollination and reproduction of *H. exutiacies* across two consecutive years. There was evidence for an effect of landscape structure on viable seed production in 2017, a difference in pollen-limitation of fruit-set between years, and extreme abortion of developing seeds in one of the years studied.

3.5.1 Pollination

Hibbertia exutiacies was sporadically visited by native bees (*Exoneura* and *Lasioglossum*) that collect pollen via thoracic vibrations (i.e., buzz-pollination). Thus, pollination of *H. exutiacies* is comparable to species of *Hibbertia* with similar floral morphology (Tucker and Bernhardt 2000). Anthers that require buzzing by bees to transmit pollen represent a form of pollinator specialization by the plant, as it restricts pollinators to those floral visitors capable of performing the sonicating behaviour (Buchmann 1983). Honeybees (*Apis mellifera*), introduced to Australia and now a prevalent if not dominant floral visitor to many Australian plants (Paton 1993), cannot buzz-pollinate (Buchmann 1983); and are unable to effectively substitute for the required native pollinators of buzz-pollinated plants. This is in contrast to other non-buzz-pollinated bee-pollinated Australian plants (e.g., *Dillwynia juniperina* (Fabaceae); Gross 2001). Gross and Mackay (1998), studying the buzz-pollinated *Melastoma affine* (Melastomataceae) in tropical Australia found *A. mellifera* was not only a poor pollinator, as may be expected, but that it also reduced fruit-set by actively collecting pollen from the stigmas of already pollinated flowers. It was observed that *A. mellifera* also disrupted the foraging of native bees on *M. affine* (Gross and Mackay 1998). Here, *A. mellifera* was never observed foraging on the flowers of *H. exutiacies*, one of the most abundant floral resources across sample sites. Thus, there was no obvious competitive interaction between the introduced *A. mellifera* and native bees on the flowers of *H. exutiacies*. Thus, pollen-limitation of fruit-set in 2017 does not appear to be caused via negative impacts of floral visitation by *A. mellifera*.

Specialization by a plant on its pollinator(s) is hypothesized to increase a plant's vulnerability to landscape disturbance (Bond 1994). Inherent in such hypotheses is that trade-offs in specialization on different pollinators exist, such that increasing specialization on one pollinator type leads to a reduction in the pollinator effectiveness of other floral visitors (Aigner 2006). Thus, plants become increasingly reliant on the presence of their most effective pollinator(s). In terms of buzz-pollination, prior studies do not suggest such specialization makes plants necessarily more vulnerable to landscape disturbance. For example, although

Cunningham (2000) found that fruit-set of the buzz-pollinated Australian plant *Dianella revoluta* (Asphodelaceae) was less in linear strips (e.g., road verges) of native vegetation than larger woodland reserves (26-800 ha), fruit-set of another buzz-pollinated plant, *Senna artemisioides* (Fabaceae), significantly increased in the same sites (Cunningham 2000). Donaldson et al. (2002) observed no difference in fruit-set of the buzz-pollinated *Cyanella lutea* (Tecophilaeaceae) between large and small fragments of native shrubland vegetation in South Africa. However, fruit-set did decline as vegetation fragments became more distant from other larger fragments, a result Donaldson et al. (2002) suggested may be related to the relative specialization of buzz-pollination. Similarly, within Australia, Duncan et al. (2004a) observed decreased receipt of outcross pollen to the flowers of *D. revoluta* with increasing isolation from a source population. However, pollinator visitation appeared unaltered, and access to compatible mates (i.e., mate availability) limited reproduction to a greater extent in the studied fragments than access to pollinators (Duncan et al. 2004a). Thus, although pollen-limitation of plant reproduction is evidence of deficiency in the pollination process, it does not necessarily relate to pollinator availability and may be a result of other processes (e.g., mate availability, pollinator behaviour etc.).

3.5.2 Breeding system

Few studies have been conducted to determine the breeding systems of *Hibbertia* species. Schatral (1996) studied the reproduction of three Western Australian (WA) *Hibbertia* species (*H. amplexicaulis*, *H. commutata*, and *H. hypericoides*), and observed negligible fruit-set via autonomous self-pollination. In *H. hypericoides*, outcross-pollen significantly increased fruit-set relative to self-pollen (Schatral 1996). Likewise, Keighery (1975) comments that the WA species of *H. hypericoides*, *H. glabella*, and *H. volubilis* do not reproduce via autonomous self-pollination. *Hibbertia exutiacies* also does not appear to produce fruits via autonomous self-pollination, meaning successful fertilization requires floral visitation, whether reproduction occurs via self- or cross-pollination. However, the reproduction of *H. exutiacies* is also highly reliant on the receipt of outcross-pollen from floral visitors, with self-pollination producing less than 10 % fruit-set (relative to > 60 % fruit-set via outcrossing).

3.5.3 Pollen-limitation

The reliance of *H. exutiacies* on pollinators to effect pollination and the significant increase of total seed production with outcrossing suggests reproduction is susceptible to changes in both the quantity and quality of pollen received. The fruit-set of *H. exutiacies* (as defined by the presence of developing seeds) was found to be pollen-limited in 2017 (Fig. 3-3) but not 2018

(Fig. 3-7). Thus, there was evident deficiency in the pollination process in at least one of the years studied. However, in contrast to fruit-set, pollen-limitation had no clear impact on mean seed production per fruit in both 2017, when fruit-set was pollen-limited, and 2018, when fruit-set was not pollen-limited. Thus, access to effective pollination did not limit the number of seeds at the level of individual fruits. Nonetheless, the addition of pollen to the flowers of *H. exutiacies* in 2017 did result in an overall greater number of viable seeds produced, relative to control treatments, due to the greater number of fruits produced by plants within the pollen addition treatments (Fig. 3-4a). Thus, in 2017, overall production of viable seeds was constrained by access to efficient pollination, because access to efficient pollination limited the number of fruits produced per plant.

A relatively low rate of floral visitation may suggest that a lack of pollinators and reduced quantity of pollen receipt were the causes of pollen-limitation of fruit-set in *H. exutiacies*. This was also posited by Schatral (1996) for the WA species, *H. hypericoides*, for which floral visitation by native bees was infrequent, and seed production increased following pollen supplementation relative to open-pollinated flowers. However, pollen-limitation due to pollen quality (e.g., interreference of outcross pollen by self-pollen, ovule discounting by low quality pollen, early abortion of developing seeds due to inbreeding depression) cannot be disregarded. Duncan et al. (2004b) demonstrated significant movement of self-pollen during buzz-pollination of the predominantly self-incompatible *D. revoluta*, providing evidence reproduction was limited by effective access to outcross pollen. Similarly, reproductive output was limited by movement of self-pollen, via pollinators, to the stigma of the Australian bulbine lily, *Bulbine bulbosa* (Asphodelaceae) (Vaughton and Ramsey 2010). Transfer of self-pollen to the flowers of *H. exutiacies* may occur via facilitated autogamy or geitonogamy, given foraging bees often move between multiple flowers on an individual plant (Pers. Obs.). The likely presence of resource reallocation in 2017 implies *H. exutiacies* also preferentially shifts resources toward those more adequately pollinated flowers (e.g., possibly those flowers with higher quality pollen). Nevertheless, the number of developing seeds produced by control flowers was not lower than that produced by flowers receiving added pollen. Thus, pollen quality did not obviously limit the number of seeds which at least initiated development per fruit (i.e., those flowers which successfully become fruits have similar numbers of seeds regardless of treatment). Although not explicitly comparable, the difference between the fruit-set of bagged outcrossed virgin flowers versus unbagged open-pollinated flowers from the breeding system experiments (2017 and 2019) is similar to that between unbagged external controls versus unbagged pollen supplemented flowers in 2017 (~20 %; see Results and Fig. 3-3a). This may suggest any prior pollinator visitation does not overtly impact fruit-set, for example, by moving poor quality pollen (e.g., self-, or hetero-specific pollen) to stigmas and blocking subsequent cross-pollination (Ramsey and Vaughton 2000). Further manipulative studies are required to partition the effects of pollen quantity versus quality on the reproduction of *H. exutiacies*.

3.5.4 Reserve area and scale-of-effect

Pollen-limitation of fruit-set, and natural levels of fruit-set, showed no clear relationship with reserve area in 2017. Measures of seed-set from open-pollinated plants (natural reproduction) also did not exhibit significant associations with reserve area when tested across seven reserves in 2017 (Table 3-5). Inspection of these trends (see Fig. 3-5) suggested that The Knoll reserve had a markedly higher overall proportion of viable seeds and a markedly lower mean number of inviable seeds than expected from its reserve area which was only ca. 2 % of that of the next largest reserve (Wottons Scrub).

A re-analysis of these trends excluding The Knoll reserve indicated that reserve area was positively related to the probability of fruits having viable seeds and the proportion of viable seeds per fruit for plants of *H. exutiacies* in 2017 (Fig. 3-5a-b; Table 3-5). This positive relationship with reserve area was not simply due to spatial variation in the pollination process, given both variables were not pollen-limited in 2017. The positive relationship between viable seed production and reserve area may largely result from spatial variation in pre-dispersal seed predation. In 2017, 65.61 % of all developing seeds from open-pollinated plants were graded as inviable and of these, 92.23 % had experienced pre-dispersal seed predation. Given mean developing seed-set was unrelated to reserve area (Table 3-5; SM Fig. 3-5), an increase in viable seed production with reserve area is most likely a result of reduced levels of pre-dispersal seed predation. In support, both mean inviable seed-set and mean predated seed-set were negatively related to reserve area following the exclusion of The Knoll reserve (Fig. 3-5c; Table 3-5). Nevertheless, the results of The Knoll reserve suggest site-specific factors not directly related to reserve area also likely impact pre-dispersal seed predation and in turn viable seed production within sample sites.

To further investigate why the strength of the above trends were so dependent on the presence or absence of The Knoll reserve and to determine the importance of native vegetation surrounding sample sites more generally, a scale-of-effect analysis was conducted. This analysis of natural reproduction, which included data for The Knoll, suggested that the probability of fruits having viable seeds, and the proportion of viable seeds per fruit, were positively related to the percentage area of native vegetation within 2400-3400 m of sample sites (Fig. 3-6a-b, d-e). The related measure of mean inviable seed-set was negatively related to the percentage area of native vegetation within 2400-3200 m of sample sites (Fig. 3-6c, f). Similarly, mean predated seed-set was negatively related to the percentage area of native vegetation between 2600-4000 m (SM Fig. 3-7). Given the strong association between measures of isolation and habitat amount within the surrounding landscape (Bender et al. 2003; Fahrig 2003), the results of the scale-of-effect analysis suggest numbers of inviable seeds and hence pre-dispersal seed predation (the principal cause of inviable seeds in 2017) increases for plants in more isolated sample sites.

Notably, at increasing distances, sample sites within smaller reserves are not necessarily surrounded by less native vegetation than sample sites within larger reserves (see The Knoll versus Wottons Scrub in Fig. 3-9a-b). Consequently, the strength of the positive relationship between reserve area and the percentage area of native vegetation surrounding sample sites decreases as habitat amount is considered at greater distances from the sample sites themselves (e.g., r_s between reserve area and percentage area of native vegetation is 0.954 at 1000 m versus 0.675 at 3000 m). Thus, the positive effect of reserve area on viable seed production is likely due to the generally positive association between reserve area and amount of native vegetation surrounding sample sites. As such, larger reserve area is important in as much as it provides habitat within the landscape surrounding sample sites (see Belair in Fig. 3-9c). Further design and analysis aspects are required to successfully partition the independent effects of reserve area and habitat amount (Fahrig 2013), which are generally positively correlated within this study system. Nevertheless, reserve area is clearly a poorer predictor of viable seed production than the percentage area of native vegetation within 2400-3400 m of sample sites (Fig. 3-5a-b versus Fig. 3-6d-e). This is due to fact that for some sample sites (e.g., The Knoll) reserve area does not strongly reflect the amount of native vegetation in the surrounding landscape (e.g., within a distance of 2800 m, see Fig. 3-9).

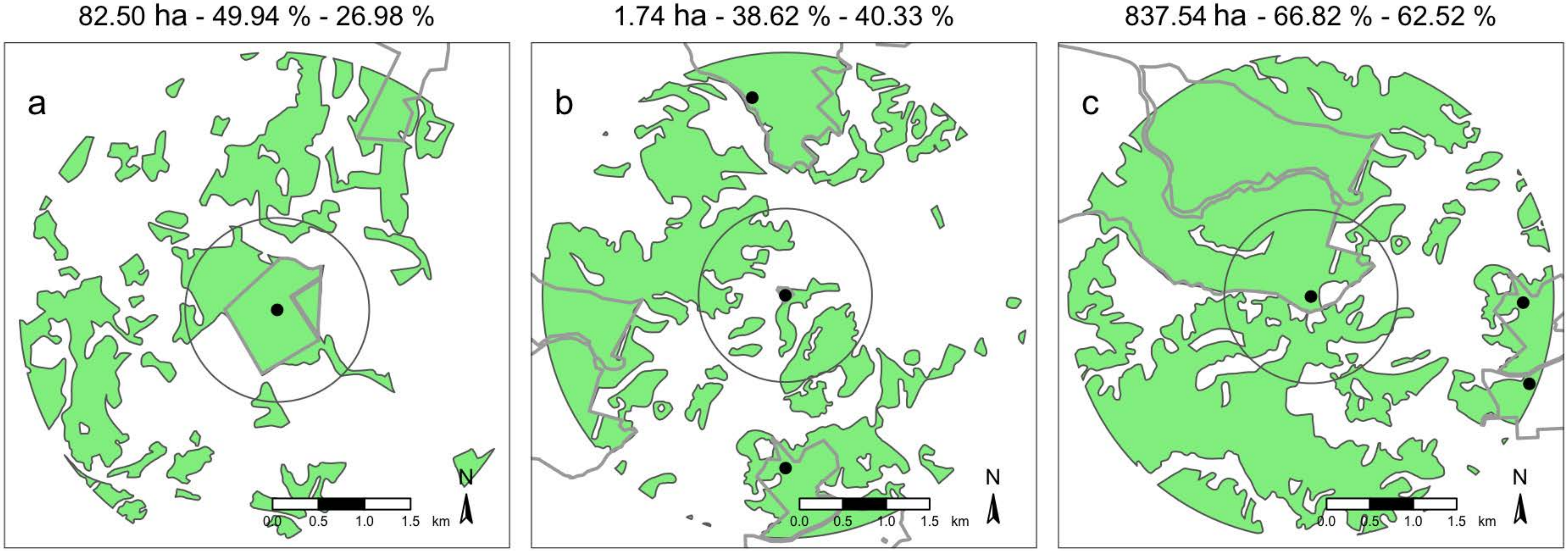


Fig. 3-9 Native vegetation, highlighted in green, within a 2800 m radius of sample sites located in the reserves of (**a**) Wottons Scrub, (**b**) The Knoll, and (**c**) Belair. The sample site around which native vegetation was highlighted is at the centre of each plot (central black dot). Sample sites located in other reserves falling within 2800 m of the central sample site are also marked (see **b** and **c**). The borders of all reserves within the 2800 m radius are highlighted in grey. The inner circle represents a distance of 1000 m from the central sample site. Values above each of the plots represent, firstly, the area of the reserve (ha) within which the central sample site is located, the percentage area of native vegetation within 1000 m of this sample site, and the percentage area of native vegetation within 2800 m of this sample site, respectively. The scale bar represents a distance of 1.5 km for each plot. Native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

3.5.5 Pre-dispersal seed predation

Pre-dispersal seed predation has been understudied relative to pollination with respect to its potential impacts under landscape disturbance (Hobbs and Yates 2003). Here, although fruit-set was generally pollen-limited across populations in 2017 (Fig. 3-3), spatial variability in reproduction appeared to be largely due to pre-dispersal seed predation. Without knowledge of the identity and ecology of the pre-dispersal seed predators, it is difficult to hypothesize as to the cause of this relationship. Adult wasps of the Chalcidoidea genus *Megastigmus* Dalman, the larvae of which are typically phytophagous within developing seeds (Janšta et al. 2018), emerged from mature seeds of *H. exutiacies* (SM Fig. 3-1E). Adult wasps of other seed feeding chalcids are considered weak flying with limited dispersal capacity (Chung and Waller 1986). Thus, limitations to the dispersal capacity of seed predators (i.e., *Megastigmus* adults) may result in higher seed predation in isolated fragments if such predators are unable to effectively disperse to other areas of flowering host plants within the local landscape (putatively within a distance of ~2400-4000 m). Natural predators of pre-dispersal seed predators may also be reduced in isolated fragments (Zabel and Tscharntke 1998), resulting in a greater abundance of seed predators. Similar hypotheses have been proposed by Matesanz et al. (2015), who found increased insect pre-dispersal seed predation in isolated fragments, irrespective of area, for the semiarid Mediterranean composite, *Centaurea hyssopifolia* (although the identity of the seed predators was not provided). Certainly, the exceptionally high level of pre-dispersal seed predation in *H. exutiacies* relative to many other plants (Moles et al. 2003), and that of *Hibbertia* generally (Sweedman and Brand 2006 p. 187), deserves further investigation (as also advocated by Schatral 1996), particularly the extent to which this apparently high level of pre-dispersal seed predation limits population growth (Katz 2016).

3.5.6 Temporal variability in reproduction - 2017 versus 2018

We observed marked variability in the reproductive behaviour of *H. exutiacies* between the two years of this study. In contrast to the results of 2017, fruit-set of *H. exutiacies* was not pollen-limited in 2018, suggesting pollen receipt was not limiting reproduction. Flowering of *H. exutiacies*, as well as other co-flowering plants, was noticeably reduced in 2018 compared to 2017 (Pers. Obs.). Such an observation may suggest intraspecific competition for effective pollination, which may be greater in years of better flowering when assuming pollinator numbers remain relatively stable, was greater in 2017 relative to 2018. Consequently, pollination services were adequate in 2018, and supplementary pollination did not increase fruit-set. Such intraspecific competition was posited by Gross (1996) as a main determinant of pollen-limitation of fruit-set in populations of the bee-pollinated legume, *Dillwynia hispida* located to the east of the Adelaide Hills. However, the lack of a clear difference between the fruit-set of external controls in 2017 and 2018 suggests natural levels of fruit-set were not impacted. This implies that resource(s) available to plants in 2017, which allowed for fruit-set to increase with supplementary pollination, were reduced in 2018 and constrained reproduction. Certainly, the reduced number of seeds which initiated development per fruit in 2018, followed by the almost total abortion of developing seeds within fruits, suggests plants were under increased stress in 2018 relative to 2017. Moreover, there was no evidence of resource reallocation, implying plants were not actively shifting resources among flowers, as occurred in 2017. This result points toward an abiotic constraint strongly constraining reproduction across the whole of the study region. In particular, although a number of abiotic factors may have functioned to limit viable seed production (e.g., soil chemistry, sun exposure, etc.), the available data on rainfall across the whole of the study region suggests that this is an important factor.

Rainfall is typically a strong determinant of reproductive output for plants within sclerophyll communities across Australia, with studies of a number of species observing reduced reproduction in years of lower rainfall (e.g., Hansen et al. 1991; Hansen et al. 1992). Reduced plant reproduction in response to low rainfall may also mask impacts of landscape disturbance in particular years, which are otherwise evident in years of better rainfall (see Morgan 1999). The beginning of spring in 2018 saw exceptionally low rainfall throughout the Adelaide Hills, with South Australia experiencing its third driest September on record (September rainfall Mount Lofty weather station (Station number: 23842): 31.4 mm; see SM Fig. 3-13). In contrast, rainfall was generally average to above-average in the Adelaide Hills during September 2017 (140.0 mm). Thus, *H. exutiacies* likely experienced severe water-stress during the start of spring 2018, which potentially prevented increased fruit-set with supplemental pollination and caused the frequent abortion of developing seeds within fruits. Rainfall appears to be an important determinant of fruit-set for the generalist pollinated orchid, *Caladenia rigida*, within the study region (Faast 2009) although, in contrast to the results here, increased fruit-set with hand-pollination for *C. rigida* during the studied drought year (2006) suggested reproduction was constrained by the absence of pollinators, which most likely declined in response to the lack of rainfall; rather than the abiotic resource of rainfall per se from the plant's perspective (Faast 2009). Fruit-set of *H. exutiacies* did not increase with pollen addition in 2018, suggesting the abiotic resource of rainfall itself may have constrained reproduction (i.e., water-limitation). The hypothesis that water stress limits reproduction of *H. exutiacies* has subsequently been tested via a manipulative field experiment, with additional watering of *H.*

exutiacies plants in the field leading to greater fruit-set for watered plants versus controls plants receiving natural rainfall only (Chapter Four).

3.5.7 Summary

Reproduction of *H. exutiacies* was not fully realized across the two sampled years, being constrained by the biotic interactions of pollination (mutualistic) and pre-dispersal seed predation (antagonistic) in 2017 and possibly by the abiotic resource of rainfall in 2018. Spatial variability in reproduction in 2017 appeared to be largely due to pre-dispersal seed predation. In particular, a scale-of-effect analysis of various measures of seed-set suggested that the composition of the landscape surrounding populations of *H. exutiacies* (i.e., the amount of native vegetation within a distance of ~2400 up to 4000 m of sample sites) is important in regulating levels of pre-dispersal seed predation and in turn viable seed production. Thus, the reproductive viability of populations of *H. exutiacies* in both small and large reserves should be viewed in the context of their surrounding landscapes, and not only in the context of their host reserve areas. However, plant reproduction may also significantly decline across fragmented plant populations in certain years, possibly due to periods of lower-than-average rainfall. Given average spring rainfall in the Adelaide Hills is modelled to decline by 17.0-25.2 % by the year 2070 (Charles and Fu 2015), decreasing rainfall may also lead to reduced reproductive output relative to historical levels, irrespective of effects of current landscape disturbance on the biotic factors of pollination and pre-dispersal seed predation.

3.6 REFERENCES:

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980.

Aigner PA (2006) The evolution of specialized floral phenotypes in a fine-grained pollination environment. In: Waser NM, Ollerton J (eds) Plant-pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago, pp 23-46.

Anderson SH, Kelly D, Ladley JJ, Molloy S, Terry J (2011) Cascading effects of bird functional extinction reduce pollination and plant density. Science 331:1068-1071.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Bartoń K (2020) MuMIn: multi-model inference. R package version 1.43.17.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Beattie AJ (1971) A technique for the study of insect-borne pollen. The Pan-Pacific Entomologist 47:82.

Bender DJ, Tischendorf L, Fahrig L (2003) Using patch isolation metrics to predict animal movement in binary landscapes. Landscape Ecology 18:17-39.

Bernhardt P (1984) The pollination biology of *Hibbertia stricta* (Dilleniaceae). Plant Systematics and Evolution 147:267-277.

Bernhardt P (1986) Bee-pollination in *Hibbertia fasciculata* (Dilleniaceae). Plant Systematics and Evolution 152:231-241.

Biesmeijer JC, Roberts SP, Reemer M, Ohlemüller R, Edwards M, Peeters T, Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, Kunin WE (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science 313:351-354.

Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London B 344:83-90.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378-400.

Brooks ME, Kristensen K, Darrigo MR, Rubim P, Uriarte M, Bruna E, Bolker BM (2019) Statistical modeling of patterns in annual reproductive rates. Ecology 100:e02706.

Brown J, Barton PS, Cunningham SA (2020) Flower visitation and land cover associations of above ground- and below ground-nesting native bees in an agricultural region of south-east Australia. Agriculture, Ecosystems and Environment 295:106895.

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 73-133.

Charles SP, Fu G (2015) Statistically downscaled projections for South Australia. Goyder Institute for Water Research Technical Report Series No. 15/1, Adelaide, South Australia.

Chung Y, Rabe-Hesketh S, Dorie V, Gelman A, Liu J (2013) A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. Psychometrika 78:685-709.

Chung JC, Waller DM (1986) Patterns of insect predation on seeds of smooth sumac (*Rhus glabra* L.). American Midland Naturalist 116(2):315-322.

Clarke I, Lee H (2019) Name that flower. Melbourne University Press, Melbourne, Australia.

Cochrane A (2002) Seed notes for Western Australia. No. 17 *Hibbertia*. Wildflower Society of Western Australia.

Crisp MD, Laffan S, Linder HP, Monro A (2001) Endemism in the Australian flora. Journal of Biogeography 28:183-198.

Cunningham SA (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Diamond JM (1975) The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. Biological Conservation 7:129-146.

Donaldson J, Nänni I, Zachariades C, Kemper J (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. Conservation Biology 16:1267-1276.

Dorey J (2018) Bees of Australia: a photographic exploration. CSIRO Publishing, Victoria, Australia.

Duncan DH, Nicotra AB, Wood JT, Cunningham SA (2004a) Plant isolation reduces outcross pollen receipt in a partially self-compatible herb. Journal of Ecology 92:977-985.

Duncan DH, Nicotra AB, Cunningham SA (2004b) High self-pollen transfer and low fruit set in buzz-pollinated *Dianella revoluta* (Phormiaceae). Australian Journal of Botany 52:185-193.

Erickson TE, Barrett RL, Symons DR, Turner SR, Merritt DJ (2016) An atlas to the plants and seeds of the Pilbara region. In: Erickson TE, Barrett RL, Merritt DJ, Dixon KW (eds) Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Clayton South, Victoria, pp 43-256.

Faast R (2009) The reproductive ecology of two terrestrial orchids, *Caladenia rigida* and *Caladenia tentaculata*. Dissertation, University of Adelaide.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. Annual Review of Ecology, Evolution, and Systematics 34:487-515.

Fahrig L (2013) Rethinking patch size and isolation effects: the habitat amount hypothesis. Journal of Biogeography 40:1649-1663.

Fahrig L (2017) Ecological responses to habitat fragmentation per se. Annual Review of Ecology, Evolution, and Systematics 48:1-23.

Fahrig L (2019) Habitat fragmentation: a long and tangled tale. Global Ecology and Biogeography 28:33-41.

Fahrig L, Arroyo-Rodríguez V, Bennett JR, Boucher-Lalonde V, Cazetta E, Currie DJ, Eigenbrod F, Ford AT, Harrison SP, Jaeger JAG, Koper N, Martin AE, Martin JL, Metzger JP, Morrison P, Rhodes JR, Saunders DA, Simberloff D, Smith AC, Tischendorf L, Vellend M, Watling JI (2019) Is habitat fragmentation bad for biodiversity? Biological Conservation 230:179-186.

Fletcher Jr RJ, Didham RK, Banks-Leite C, Barlow J, Ewers RM, Rosindell J, Holt RD, Gonzalez A, Pardini R, Damschen EI, Melo FPL, Ries L, Prevedello JA, Tscharntke T, Laurance WF, Lovejoy T, Haddad NM (2018) Is habitat fragmentation good for biodiversity? Biological Conservation 226:9-15.

Fox J, Dixon B, Monk D (1987) Germination in other plant families. In: Langkamp P (ed) Germination of Australian native plant seed. Inkata Press, Melbourne and Sydney, pp 83-97.

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks, California.

Gross CL (1996) Is resource overlap disadvantageous to three sympatric legumes? Australian Journal of Ecology 21:133-143.

Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. Biological Conservation 102:89-95.

Gross CL, Mackay D (1998) Honeybees reduce fitness in the pioneer shrub *Melastoma affine* (Melastomataceae). Biological Conservation 86:169-178.

Hadley AS, Betts MG (2012) The effects of landscape fragmentation on pollination dynamics: absence of evidence not evidence of absence. Biological Reviews 87:526-544.

Hadley AS, Frey SJK, Robinson WD, Betts MG (2018) Forest fragmentation and loss reduce richness, availability, and specialization in tropical hummingbird communities. Biotropica 50:74-83.

Hansen A, Pate JS, Hansen AP (1991) Growth and reproductive performance of a seeder and a resprouter species of *Bossiaea* as a function of plant age after fire. Annals of Botany 67:497-509.

Hansen A, Pate JS, Hansen AP (1992) Growth, reproductive performance and resource allocation of the herbaceous obligate seeder *Gompholobium marginatum* R. Br. (Fabaceae). Oecologia 90:158-166.

Harris LF, Johnson SD (2004) The consequences of habitat fragmentation for plant-pollinator mutualisms. International Journal of Tropical Insect Science 24:29-43.

Harrison XA (2015) A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology and evolution. PeerJ 3:e1114.

Hartig F (2021) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1.

Hawkeswood TJ (1989) Notes on *Diphucephala affinis* (Coleoptera: Scarabaeidae) associated with flowers of *Hibbertia* and *Acacia* in Western Australia. Plant Systematics and Evolution 168:1-5.

Hingston AB (1999) Affinities between southern Tasmania plants in native bee visitor profiles. Australian Journal of Zoology 47:361-384.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Holland JD, Bert DG, Fahrig L (2004) Determining the spatial scale of species' response to habitat. Bioscience 54:227-233.

Horn JW (2007) Dilleniaceae. In: Kubitzki K (ed) The families and genera of vascular plants, vol 9, flowering plants, eudicots, 1st edn. Springer, Berlin, Heidelberg, pp 132-154.

Horváth Z, Ptacnik R, Vad CF, Chase JM (2019) Habitat loss over six decades accelerates regional and local biodiversity loss via changing landscape connectance. Ecology Letters 22:1019-1027.

Hothorn T, Bretz F, Westfall P (2008a) Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2008b) Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28:1-23.

Houston T (2018) A Guide to Native Bees of Australia. CSIRO Publishing, Victoria, Australia.

Huais PY (2018) Multifit: an R function for multi-scale analysis in landscape ecology. Landscape Ecology 33:1023-1028.

Jackson HB, Fahrig L (2012) What size is a biologically relevant landscape? Landscape Ecology 27:929-941.

Jackson HB, Fahrig L (2015) Are ecologists conducting research at the optimal scale? Global Ecology and Biogeography 24:52-63.

Janšta P, Cruaud A, Delvare G, Genson G, Heraty J, Křížková B, Rasplus JY (2018) Torymidae (Hymenoptera, Chalcidoidea) revised: molecular phylogeny, circumscription and reclassification of the family with discussion of its biogeography and evolution of life-history traits. Cladistics 34:627-651.

Jessop JP (1986) Dilleniaceae. In: Jessop JP, Toelken HR (eds) Flora of South Australia, part 1, Lycopodiaceae-Rosaceae, 4th edn. South Australian Government Printing Division, Adelaide, pp 354-358.

Katz DS (2016) The effects of invertebrate herbivores on plant population growth: a meta-regression analysis. Oecologia 182:43-53.

Keighery GJ (1975) Pollination of *Hibbertia hypericoides* (Dilleniaceae) and its evolutionary significance. Journal of Natural History 9:681-684.

Knight TM, Steets JA, Ashman TL (2006) A quantitative synthesis of pollen supplementation experiments highlights the contribution of resource reallocation to estimates of pollen limitation. American Journal of Botany 93:271-277.

Larson BM, Barrett SC (2000) A comparative analysis of pollen limitation in flowering plants. Biological Journal of the Linnean Society 69:503-520.

Lüdecke D (2021) sjPlot: data visualization for statistics in social science. R package version 2.8.7.

Margules CR, Pressey RL (2000) Systematic conservation planning. Nature 405:243-253.

Matesanz S, Gómez-Fernández A, Alcocer I, Escudero A (2015) Fragment size does not matter when you are well connected: effects of fragmentation on fitness of coexisting gypsophiles. Plant Biology 17:1047-1056.

McIntyre S, Hobbs R (1999) A framework for conceptualizing human effects on landscapes and its relevance to management and research models. Conservation Biology 13:1282-1292.

Melo GL, Sponchiado J, Cáceres NC, Fahrig L (2017) Testing the habitat amount hypothesis for South American small mammals. Biological Conservation 209:304-314.

Mesquita-Neto JN, Vieira ALC, Schlindwein C (2021) Minimum size threshold of visiting bees of a buzz-pollinated plant species: consequences for pollination efficiency. American Journal of Botany 108:1006-1015.

Moles AT, Warton DI, Westoby M (2003) Do small-seeded species have higher survival through seed predation than large-seeded species? Ecology 84:3148-3161.

Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology 13:266-273.

Ollerton J, Winfree R, Tarrant S (2011) How many flowering plants are pollinated by animals? Oikos 120:321-326.

Paton DC (1993) Honeybees in the Australian environment. Bioscience 43:95-103.

Pebesma E (2018) Simple features for R: standardized support for spatial vector data. The R Journal 10:439-446.

Pressey RL, Tully SL (1994) The cost of ad hoc reservation: a case study in western New South Wales. Australian Journal of Ecology 19:375-384.

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsey M, Vaughton G (2000) Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). American Journal of Botany 87:845-852.

Regan EC, Santini L, Ingwall-King L, Hoffmann M, Rondinini C, Symes A, Taylor J, Butchart SHM (2015) Global trends in the status of bird and mammal pollinators. Conservation Letters 8:397-403.

Schatral A (1996) Floral predators, pollinators and seed set in Western Australian species of the genus *Hibbertia* (Dilleniaceae). In: Hopper SD, Chappill JA, Harvey MS, George AS (eds) Gondwanan heritage: past, present and future of the Western Australian biota. Surrey Beatty & Sons, Sydney, NSW, pp 203-211.

Schatral A, Fox JED (1994) Quality and viability of seeds in the genus *Hibbertia*. Seed Science and Technology 22:273-284.

Schatral A, Kailis SG, Fox JED (1994) Seed dispersal of *Hibbertia hypericoides* (Dilleniaceae) by ants. Journal of the Royal Society of Western Australia 77:81-85.

Smith TJ (2018) The Australian bee genera: an annotated, user-friendly key. The Rader Community Ecology Lab, University of New England, Armidale, Australia.

Specht RL, Perry RA (1948) The plant ecology of part of the Mount Lofty Ranges (1). Transactions of the Royal Society of South Australia 72:91-139.

Sweedman L, Brand G (2006) Collection guidelines for common Australian families and genera. In: Sweedman L, Merritt D (eds) Australian seeds a guide to their collection, identification and biology. CSIRO Publishing, Collingwood, Victoria, pp 173-198.

Tennekes M (2018) tmap: thematic maps in R. Journal of Statistical Software 84:1-39.

Threlfall CG, Walker K, Williams NSG, Hahs AK, Mata L, Stork N, Livesley SJ (2015) The conservation value of urban green space habitats for Australian native bee communities. Biological Conservation 187:240-248.

Tucker SC, Bernhardt P (2000) Floral ontogeny, pattern formation, and evolution in *Hibbertia* and *Adrastaea* (Dilleniaceae). American Journal of Botany 87:1915-1936.

Vaughton G, Ramsey M (2010) Floral emasculation reveals pollen quality limitation of seed output in *Bulbine bulbosa* (Asphodelaceae). American Journal of Botany 97:174-178.

Volenec ZM, Dobson AP (2020) Conservation value of small reserves. Conservation Biology 34:66-79.

Watling JI, Arroyo-Rodríguez V, Pfeifer M, Baeten L, Banks-Leite C, Cisneros LM, Fang R, Hamel-Leigue AC, Lachat T, Leal IR, Lens L, Possingham HP, Raheem DC, Ribeiro DB, Slade EM, Urbina-Cardona JN, Wood EM, Fahrig L (2020) Support for the habitat amount hypothesis from a global synthesis of species density studies. Ecology Letters 23:674-681.

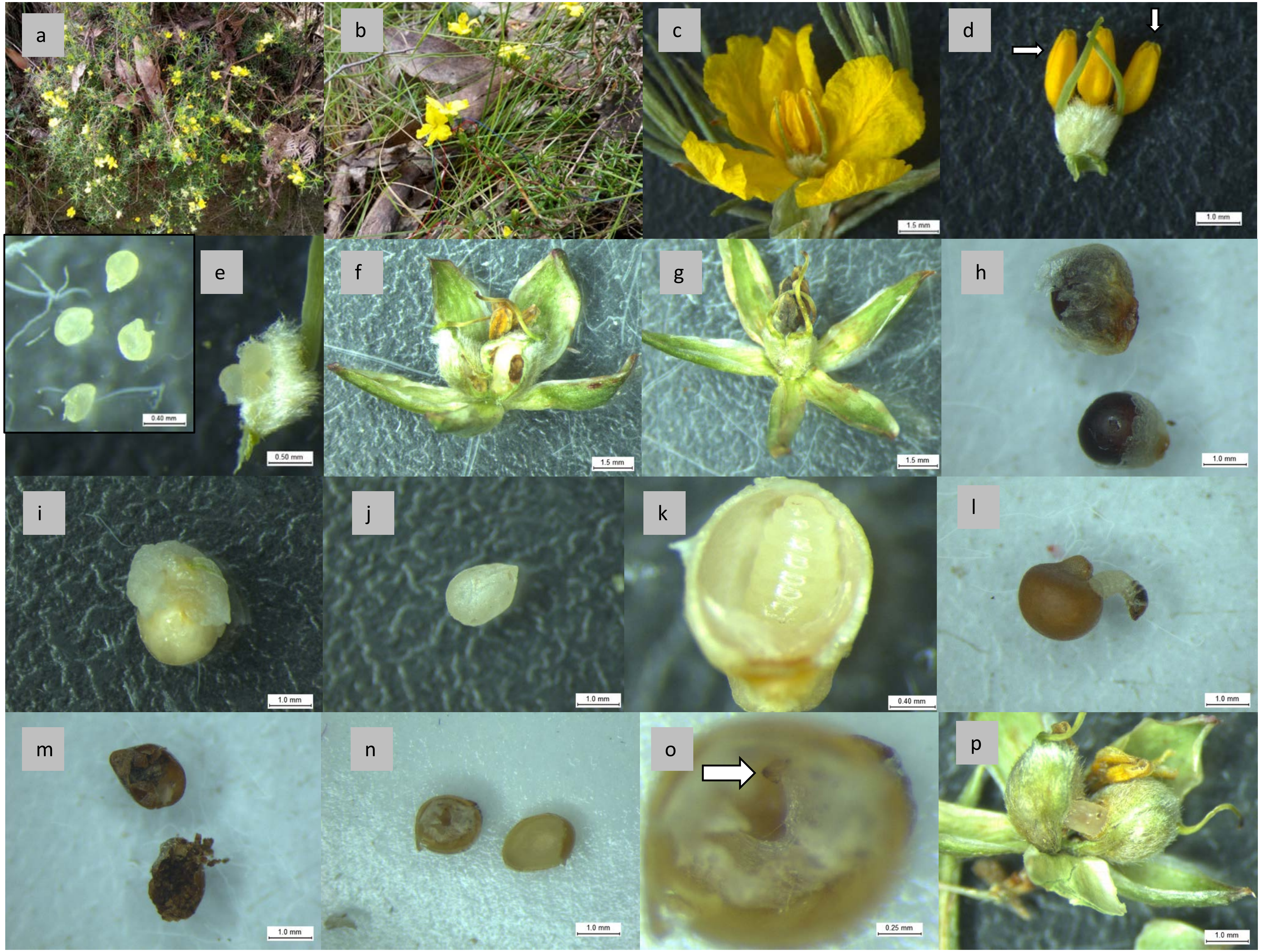
Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Wintle BA, Kujala H, Whitehead A, Cameron A, Veloz S, Kukkala A, Moilanen A, Gordon A, Lentini PE, Cadenhead NCR, Bekessy SA (2019) Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. Proceedings of the National Academy of Sciences 116:909-914.

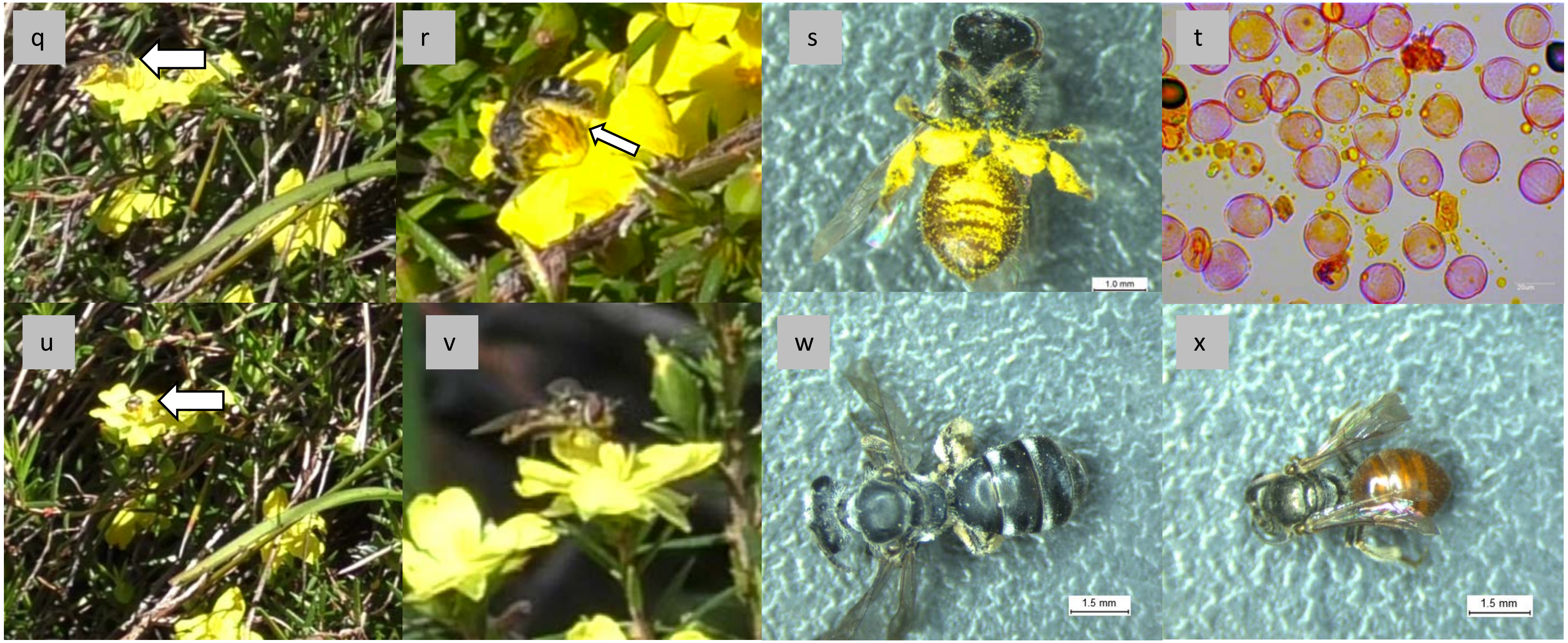
Zabel J, Tscharntke T (1998) Does fragmentation of *Urtica* habitats affect phytophagous and predatory insects differentially? Oecologia 116:419-425.

Zuckerberg B, Cohen JM, Nunes LA, Bernath-Plaisted J, Clare JDJ, Gilbert NA, Kozidis SS, Nelson SBM, Shipley AA, Thompson KL, Desrochers A (2020) A review of overlapping landscapes: pseudoreplication or a red herring in landscape ecology? Current Landscape Ecology Reports 5:140-148.

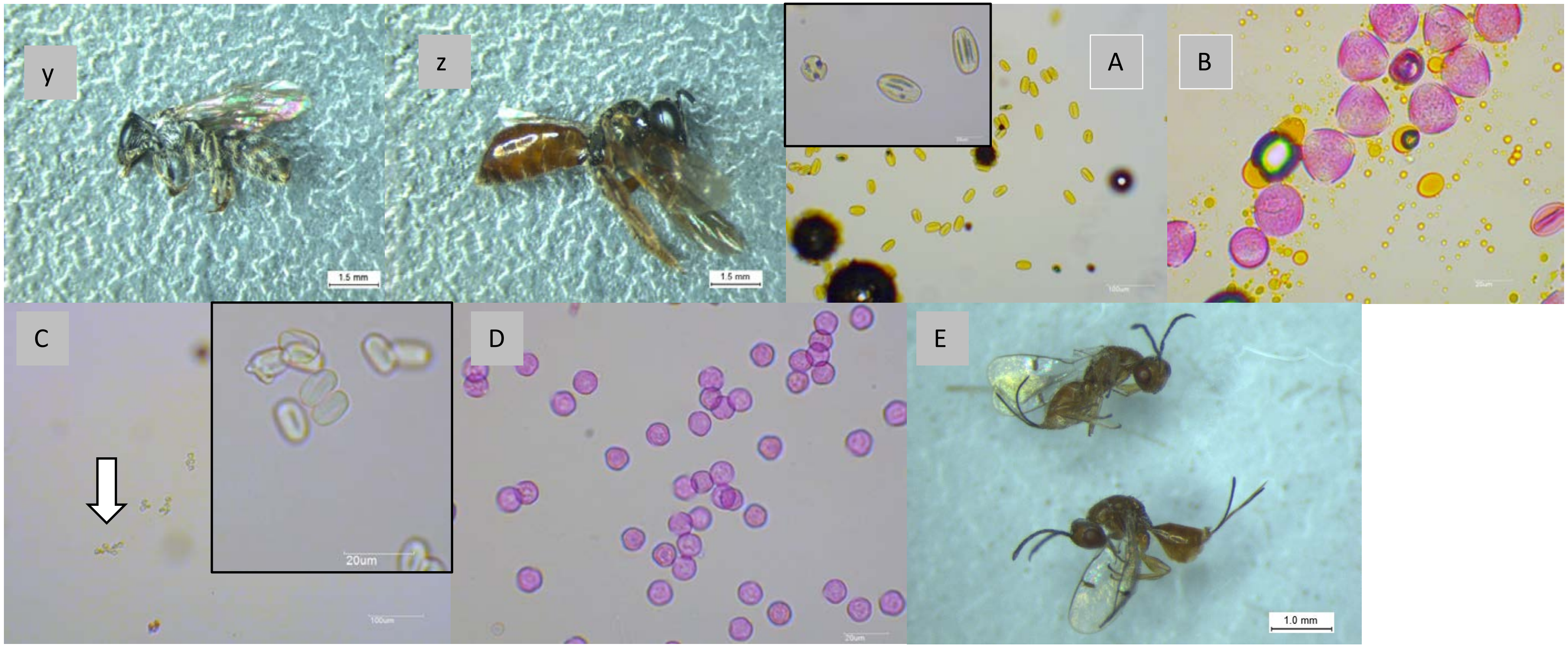
3.7 SUPPLEMENTARY MATERIAL



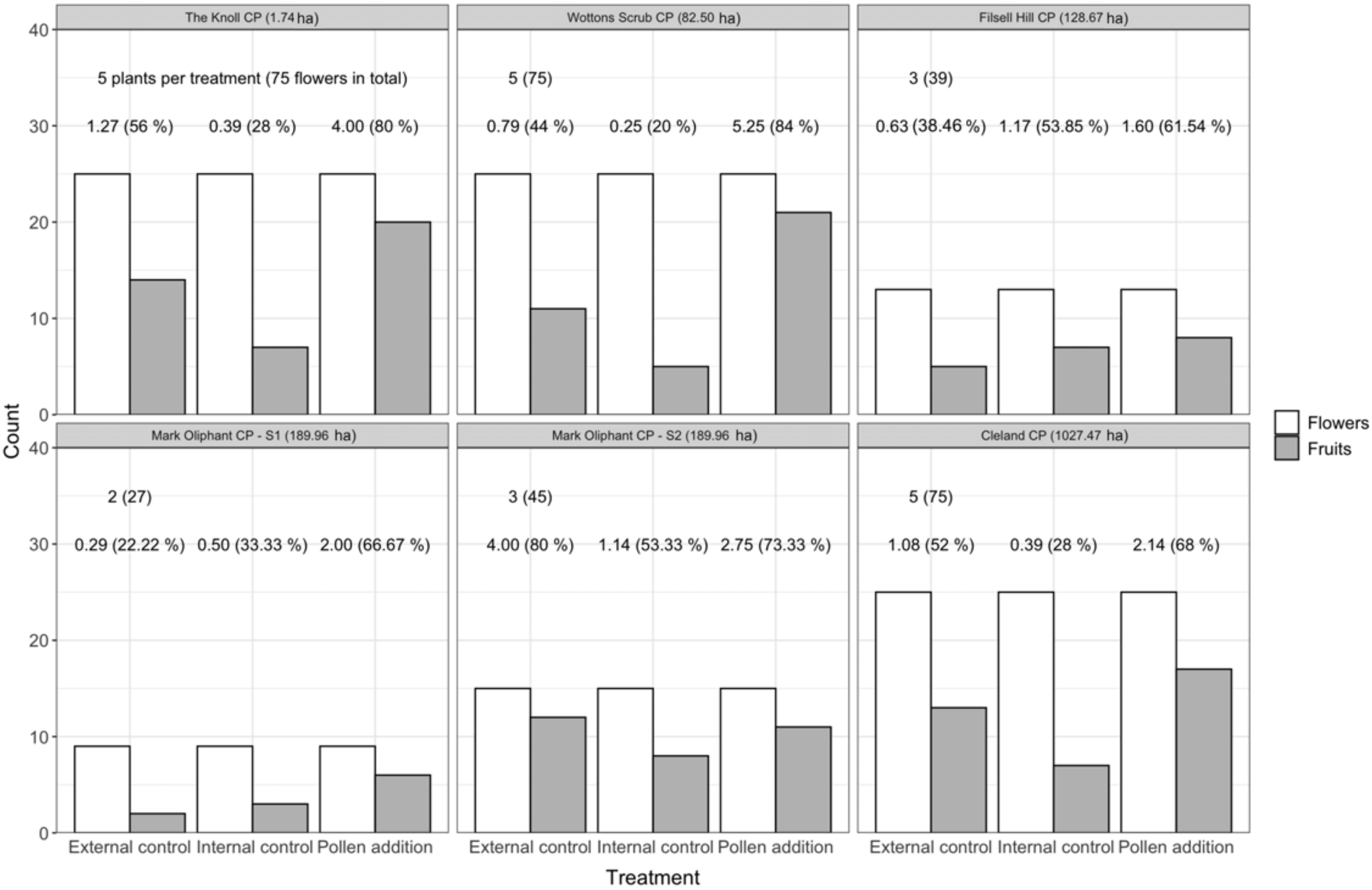
SM Fig. 3-1 (above) (**a**) Flowering plant of *Hibbertia exutiacies*. (**b**) Flowers of *H. exutiacies* tagged for pollen-limitation experiments in 2017. (**c**) Flower of *H. exutiacies* taken from a frozen specimen. (**d**) Excised reproductive organs from a flower bud of *H. exutiacies* just prior to anthesis, the anthers open by terminal pores (vertical arrow) and lateral slits (horizontal arrow). Note that this flower only possessed three anthers. (**e**) Dissected carpel from a flower bud of *H. exutiacies* just prior to anthesis. Inset shows excised ovules. (**f**) Fruit of *H. exutiacies* showing swollen ovaries, a developing seed (white in colouration) is visible inside the right-hand carpel (which has been split opened), with a brown coloured non-fertilised or otherwise early aborted fertilised ovule also showing. (**g**) Persistent calyx of *H. exutiacies* showing no enlargement of ovaries. (**h**) Mature viable seeds of *H. exutiacies*. (**i**) Seed of *H. exutiacies* removed from a fruit sampled prior to maturity and classified as viable. (**j**) Endosperm contained within the prior seed. (**k-l**) Predated seeds of *H. exutiacies* containing insect larvae. (**m**) Predated seed of *H. exutiacies* containing insect frass. (**n**) Two halves of an unfilled seed of *H. exutiacies*. The papery inner part of the seed coat can be seen in the left half of the cut unfilled seed. (**o**) Unfilled seed of *H. exutiacies* with minute larvae (arrow). (**p**) Fruit of *H. exutiacies* with larvae moving between follicles. **SM Fig. 3-1** continues below.



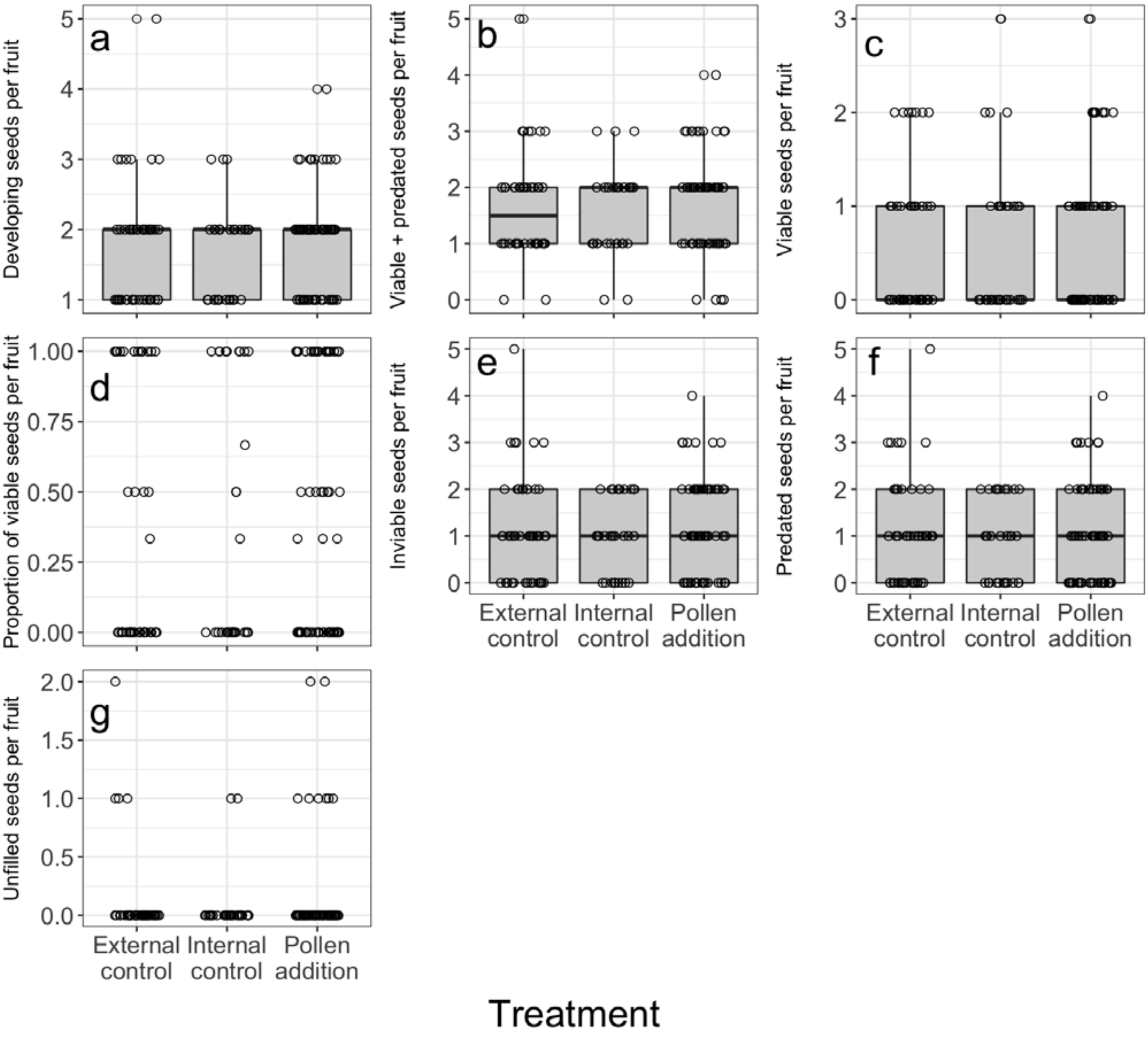
SM Fig. 3-1 - continued (above) (**q**) Small-medium sized native bee (~5-9 mm) of *Lasioglossum* (*Chilalictus*) visiting a flower of *H. exutiacies*. (**r**) *Lasioglossum* (*Chilalictus*) sp. collecting pollen via thoracic vibrations from a flower of *H. exutiacies*. Note how one of the stigmatic tips of the two lateral styles contacts the ventral side of the abdomen (arrow) as the bee rotates its position on the flower. (**s**) Captured native bee, *Lasioglossum* (*Chilalictus*) *erythrurum*, carrying pollen of *H. exutiacies*. (**t**) Pollen carried by prior *L.* (*Chilalictus*) *erythrurum* stained in fuchsin jelly (400× magnification, scalebar = 20 µm). (**u**) Smaller sized unidentified native bee (~3-4 mm) visiting a flower of *H. exutiacies* (arrow), note that this is the same flower as in (**q**). (**v**) Hoverfly (Syrphidae) scavenging pollen from a flower of *H. exutiacies*. (**w**) Native bee, *Lasioglossum* (*Chilalictus*) sp., captured visiting flowers of co-flowering *Pultenaea daphnoides*. This bee was sampled as a representative specimen of otherwise alike bees seen visiting flowers of *H. exutiacies* (e.g., see (**q**) and (**r**)) but were not able to be captured on *H. exutiacies* itself. (**x**) Native bee, *L.* (*Chilalictus*) *erythrurum*, captured visiting flowers of *H. exutiacies*. Note that this is the same specimen as shown in (**s**). **SM Fig. 3-1** continues below.



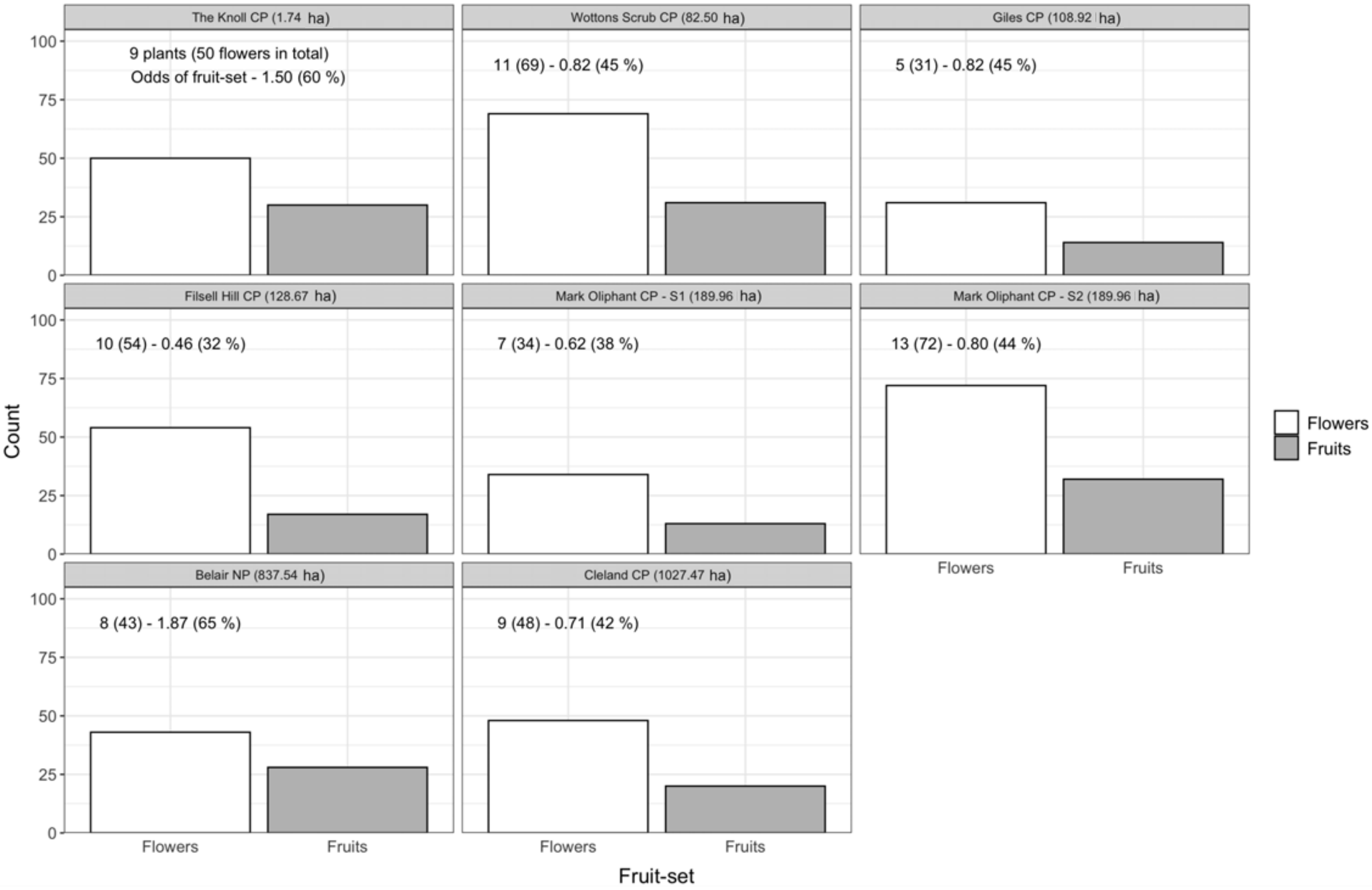
SM Fig. 3-1 - continued (above) (**y**) Native bee, *Lasioglossum* (*Chilalictus*) sp., captured visiting flowers of *H. exutiacies*. (**z**) Native bee, *Exoneura* sp., captured visiting flowers of *H. exutiacies*. (**A**) Pollen grains, suspended in corn syrup, extracted from the anthers of *H. exutiacies* (100× magnification, scalebar = 100 μm). Inset shows pollen grains at 400× magnification with a scalebar = 20 μm. (**B**) Pollen grains of *H. exutiacies* suspended in fuchsin jelly (400× magnification, scalebar = 20 μm). Note the expansion of pollen grains and abundant lipid residues. (**C**) Pollen grains (arrow), suspended in corn syrup, extracted from the anthers of *Tetratheca pilosa* ssp. *pilosa* (100× magnification, scalebar = 100 μm). Inset shows pollen grains at 400× magnification with a scalebar = 20 μm. (**D**) Pollen grains of *T. pilosa* ssp. *pilosa* suspended in fuchsin jelly (400× magnification, scalebar = 20 μm). Lipid residues like *H. exutiacies* were never observed in pollen of *T. pilosa* ssp. *pilosa*. (**E**) Individuals of the wasp genus *Megastigmus* which emerged from mature seeds of *H. exutiacies*.



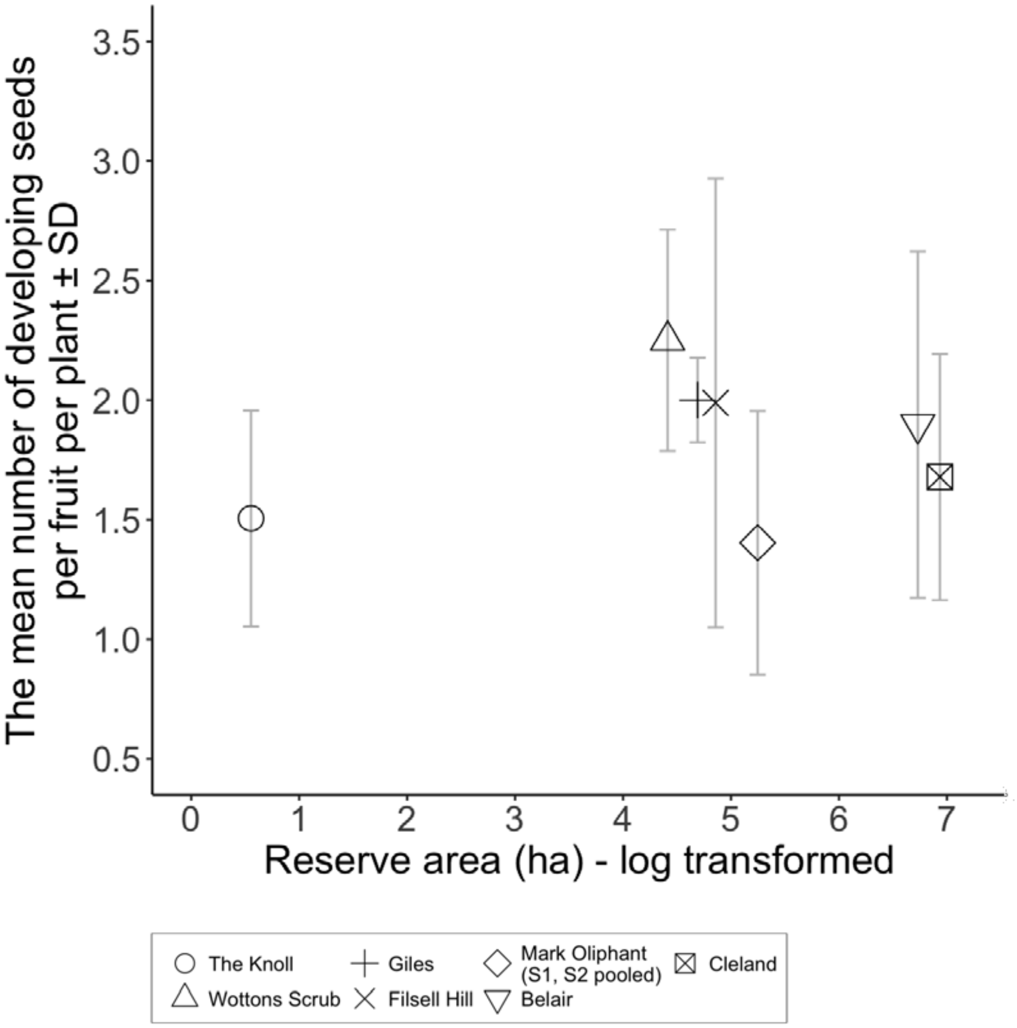
SM Fig. 3-2 Effect of the pollen addition treatment on the fruit-set of *Hibbertia exutiacies* per sample site in 2017. Plotted are the number of flowers tagged per treatment relative to the number of fruits which were subsequently produced, pooled across individual plants per sample site. The number of plants tagged per treatment per sample site, followed by the total number of flowers tagged across the three treatments per sample site in parentheses, is provided within each plot. The odds of a fruit being produced per treatment are given above each of the respective groups of columns per sample site. Percentage fruit-set (rounded to two decimal places) is provided in parenthesis. S1 and S2 stand for sample site 1 and 2, respectively. Note that originally Mark Oliphant CP - S1 contained an additional experimental plant pair (i.e., a pollen addition and external control plant). However, data was not obtained from this pairing due to what appeared to be seed release prior to data collection.



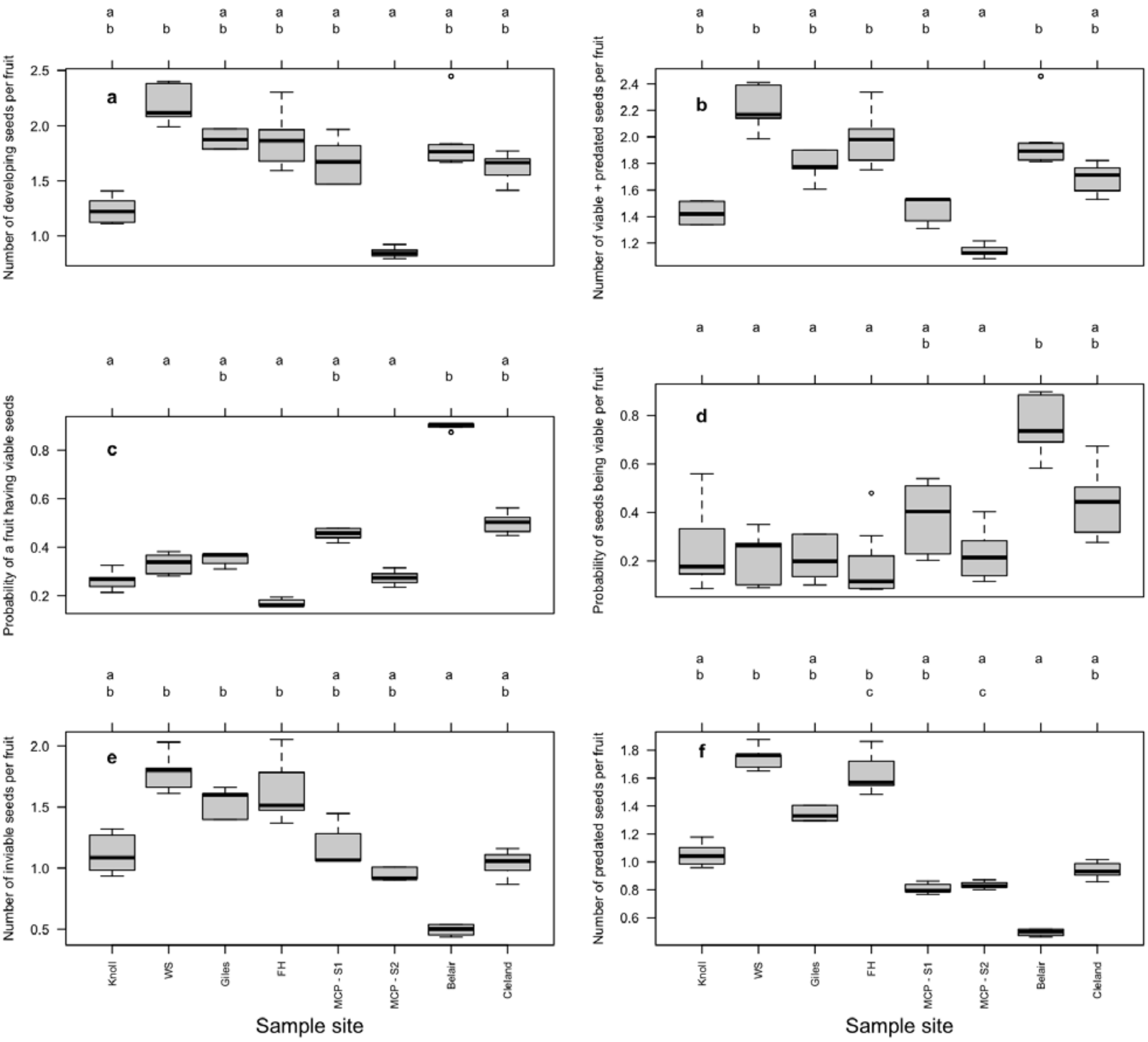
SM Fig. 3-3 Effect of the pollen addition treatment on the production of viable, predated, and unfilled seeds of *Hibbertia exutiacies* in 2017. Plotted are boxplots, along with raw datapoints (or only raw datapoints in the case of (**d**) and (**g**)) pooled across plants per treatment for (**a**) the number of developing seeds (viable + predated + unfilled) per fruit (i.e., developing seed-set), (**b**) the number of viable + predated seeds per fruit (i.e., viable + predated seed-set), (**c**) the number of viable seeds per fruit (i.e., viable seed-set), (**d**) the proportion of viable seeds per fruit (boxplots were not displayed due to the dichotomous nature of the data), (**e**) the number of inviable seeds per fruit (i.e., inviable seed-set), (**f**) the number of predated seeds per fruit (i.e., predated seed-set), and (**g**) the number of unfilled seeds per fruit (i.e., unfilled seed-set) (boxplots were not displayed due to the predominance of fruits with no unfilled seeds). Data was displayed at the level of individual fruits to show the distribution of seed numbers within fruits (see Table 3-4 for averages per fruit per plant).



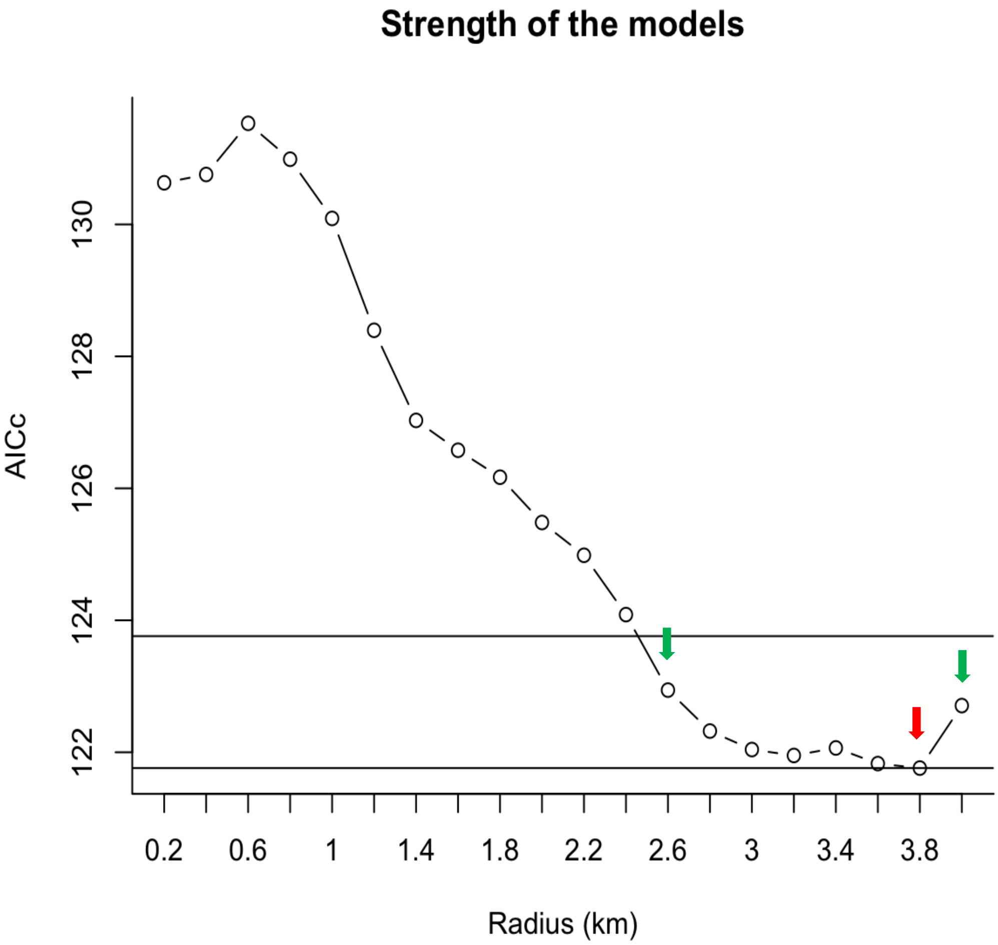
SM Fig. 3-4 Fruit-set of open-pollinated plants of *Hibbertia exutiacies* per sample site in 2017. Plotted are the number of flowers tagged relative to the number of fruits which were subsequently produced, pooled across individual plants per sample site. The number of plants tagged per sample site, followed by the total number of flowers tagged across individual plants per sample site in parentheses, is provided within each plot. The odds of a fruit being produced per sample site is also given above each of the respective groups of columns. Percentage fruit-set (rounded to the nearest whole number) is provided in parentheses. S1 and S2 stand for sample site 1 and 2, respectively. The percentage fruit-set for plants within Mark Oliphant CP - S1 and Mark Oliphant CP - S2 combined is 42.45 %.



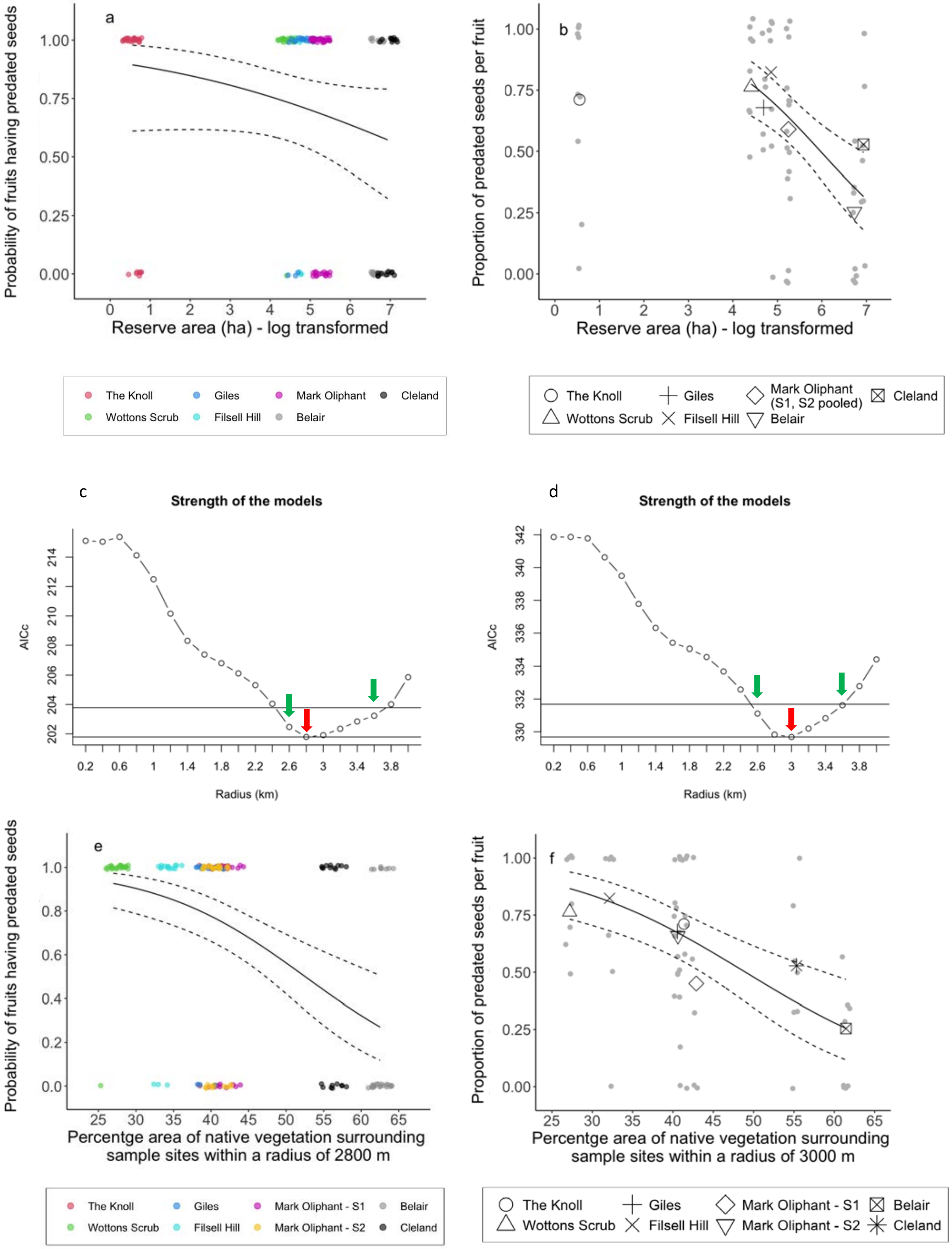
SM Fig. 3-5 Reserve averages of the mean number of developing seeds per fruit per plant (i.e., the average of mean developing seed-set across plants), ± SD, plotted against reserve area (log transformed). S1 and S2 stand for sample site 1 and 2, respectively. The mean number of developing seeds per fruit per plant for Mark Oliphant - S1 and Mark Oliphant - S2 was 1.60 ± 0.89 and 1.31 ± 0.33, respectively. Data are from open-pollinated plants of *Hibbertia exutiacies* in 2017.



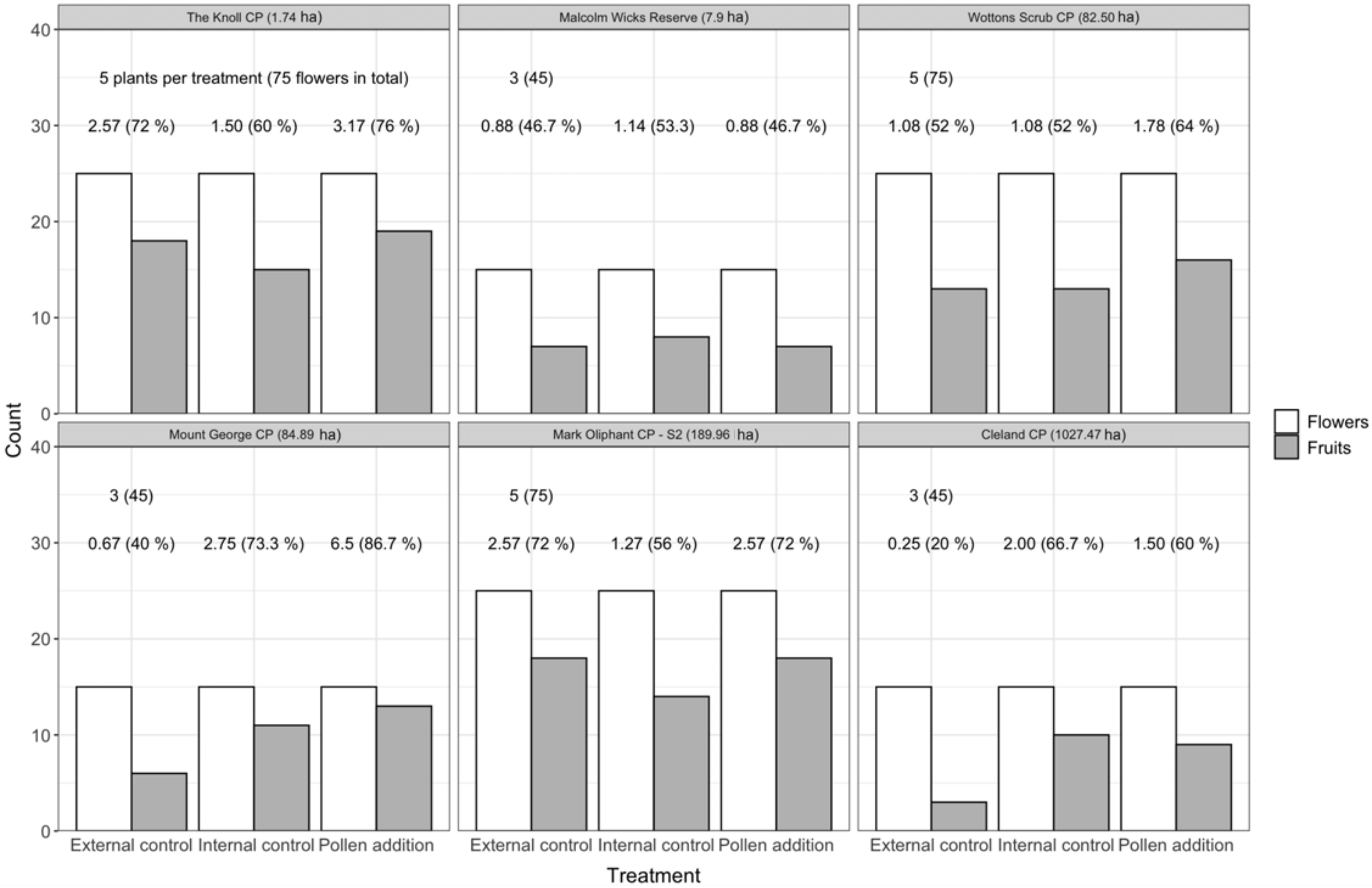
SM Fig. 3-6 Plots of model estimates (i.e., not the raw data) of natural levels of seed production of open-pollinated plants of *Hibbertia exutiacies* per sample site in 2017. Plotted are boxplots displaying the mean number of (**a**) developing seeds per fruit (i.e., developing seed-set), (**b**) viable + predated seeds per fruit (i.e., viable + predated seed-set), (**c**) the probability of a fruit having viable seeds, (**d**) the probability of seeds being viable per fruit (i.e., "the proportion of viable seeds per fruit"), the mean number of (**e**) inviable seeds per fruit (i.e., inviable seed-set), and (**f**) predated seeds per fruit (i.e., predated seed-set), as estimated for each plant within sample sites. See Methods (and below) for statistical details on models used to estimate response variables. Sample sites sharing the same letter (located above each plot) are not statistically different from one another for each response variable. S1 and S2 stand for sample site 1 and 2, respectively. Model results for viable + predated seed-set modelled by a Conway-Maxwell-Poisson (CMP) GLMM: $\chi^2_7 = 26.583$, $p = 3.961 \times 10^{-4}$. Model results for predated seed-set modelled by a CMP GLMM: $\chi^2_7 = 29.034$, $p = 1.426 \times 10^{-4}$. WS = Wottons Scrub, FH = Filsell Hill, MCP - S1 = Mark Oliphant - Sample Site 1, MCP - S2 = Mark Oliphant - Sample Site 2.



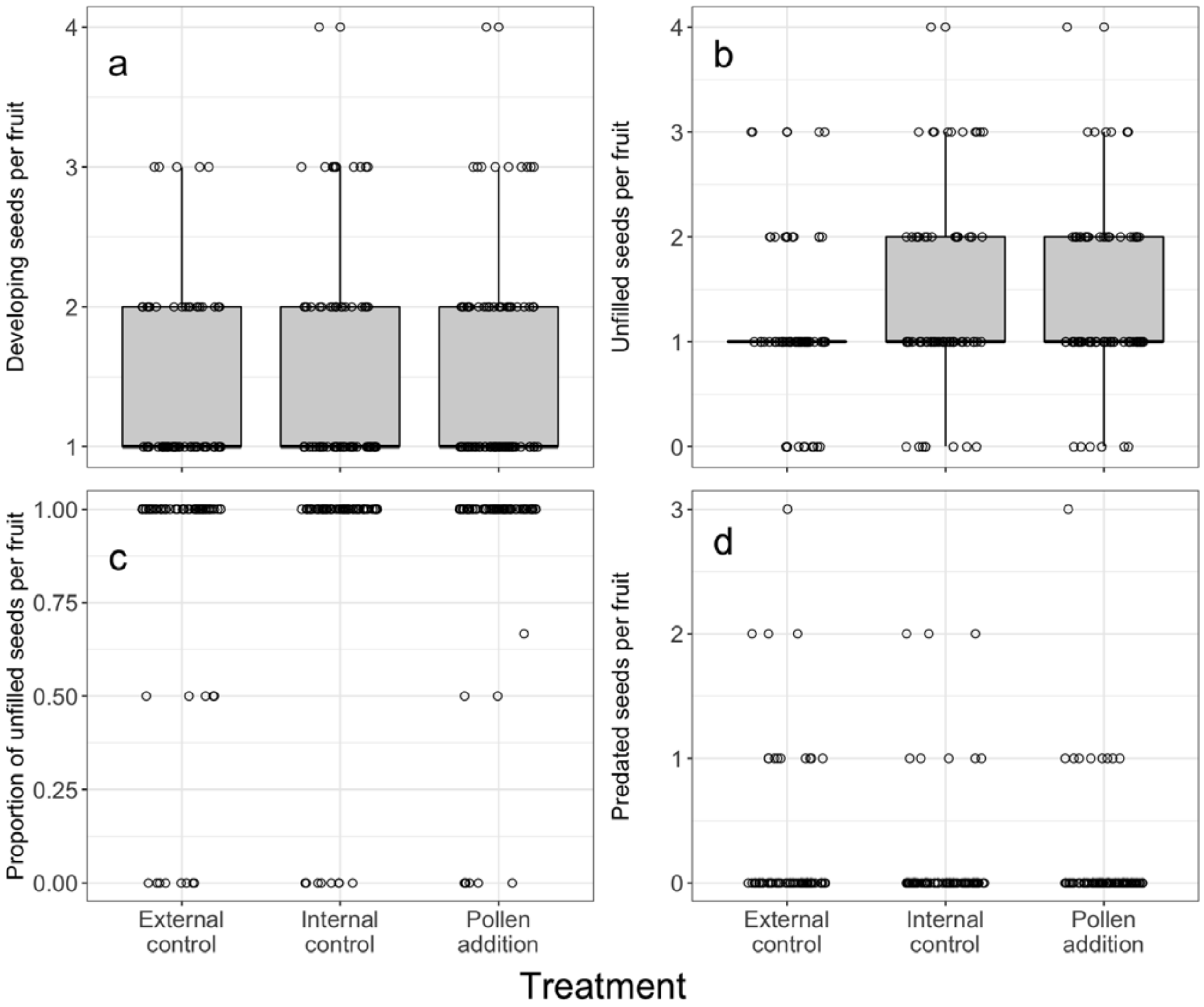
SM Fig. 3-7 Scale-of-effect analysis for mean predated seed-set of open-pollinated plants of *Hibbertia exutiacies* in 2017. Shown is the comparison of model support for the percentage area of native vegetation within a defined radius of sample sites (200-4000 m) on mean predated seed-set. A total of 20 models were constructed and compared via their small-sample-size corrected Akaike information criterion (AICc) values. The chosen scale-of-effect was identified as the model with the best support, identified as the model with the lowest AICc value (central (red) arrow). Models within a Δ AICc ≤ 2 from the model with the greatest support are also considered to exhibit substantial support for their estimated scale-of-effect (models within lateral (green) arrows). The horizontal reference line represents a Δ AICc ≤ 2 from the best supported model.


a
Probability of fruits having predated seeds
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6 7
Reserve area (ha) - log transformed
The Knoll
Giles
Mark Oliphant
Cleland
Wottons Scrub
Filsell Hill
Belair
b
Proportion of predated seeds per fruit
1.00
0.75
0.50
0.25
0.00
0 1 2 3 4 5 6 7
Reserve area (ha) - log transformed
The Knoll
Giles
Mark Oliphant (S1, S2 pooled)
Cleland
Wottons Scrub
Filsell Hill
Belair
c
Strength of the models
AICc
202 204 206 208 210 212 214
0.2 0.6 1 1.4 1.8 2.2 2.6 3 3.4 3.8
Radius (km)
d
Strength of the models
AICc
330 332 334 336 338 340 342
0.2 0.6 1 1.4 1.8 2.2 2.6 3 3.4 3.8
Radius (km)
e
Probability of fruits having predated seeds
1.0
0.8
0.6
0.4
0.2
0.0
25 30 35 40 45 50 55 60 65
Percentge area of native vegetation surrounding sample sites within a radius of 2800 m
The Knoll
Giles
Mark Oliphant - S1
Belair
Wottons Scrub
Filsell Hill
Mark Oliphant - S2
Cleland
f
Proportion of predated seeds per fruit
1.00
0.75
0.50
0.25
0.00
25 30 35 40 45 50 55 60 65
Percentge area of native vegetation surrounding sample sites within a radius of 3000 m
The Knoll
Giles
Mark Oliphant - S1
Belair
Wottons Scrub
Filsell Hill
Mark Oliphant - S2
Cleland

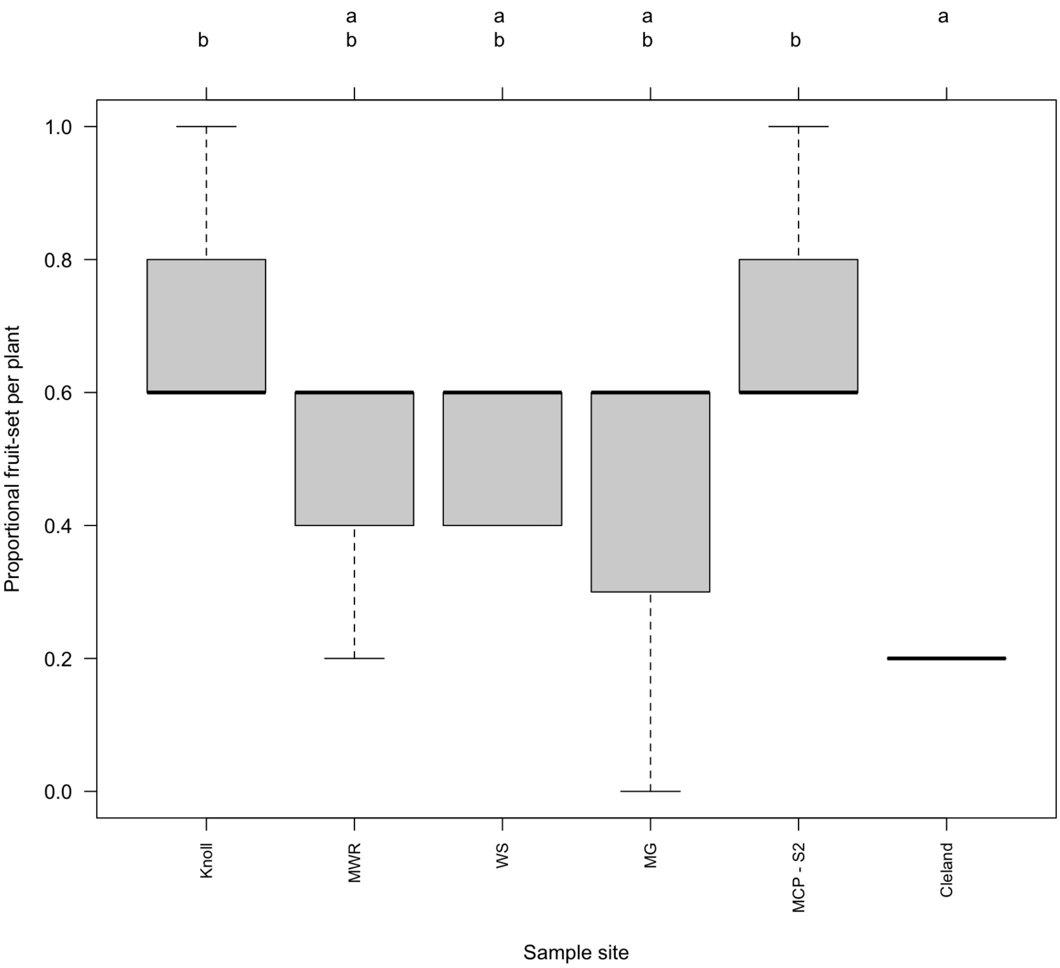
SM Fig. 3-8 (above) Plots of the relationships between reserve area (log transformed) and the probability of fruits having predated seeds, and reserve area (log transformed) and the proportion of predated seeds per fruit of open-pollinated plants of *Hibbertia exutiacies* in 2017, accompanied by plots of associated scale-of-effect analyses. (**a**) The probability of fruits having predated seeds plotted against reserve area (log transformed). The model results of a Binomial GLMM of the probability of fruits having predated seeds against reserve area (log transformed): Intercept = 2.293 (95 % CI: 0.43, 4.16), Slope = -0.289 (-0.64, 0.07), χ_1^2 = 2.539, p = 0.111. Model results excluding The Knoll reserve: Intercept = 6.260 (95 % CI: 3.18, 9.34), Slope = -0.988 (-1.52, -0.46), χ_1^2 = 13.363, p = 2.566 $\times 10^{-4}$. The trend line plotted is the predicted probability of fruits having predated seeds for plants across reserves, including The Knoll reserve. (**b**) The proportion of predated seeds per fruit. Large symbols show the overall proportion of seeds graded as predated per reserve (i.e., population proportion) plotted against reserve area (log transformed). The overall proportion of seeds graded as predated for each individual plant are also plotted (grey data points). The model results of a Beta-Binomial GLMM of the proportion of predated seeds per fruit against reserve area (log transformed): Intercept = 1.802 (95 % CI: 0.34, 3.26), Slope = -0.273 (-0.56, 0.01), χ_1^2 = 3.606, p = 0.058. Model results excluding The Knoll reserve: Intercept = 4.743 (95 % CI: 2.27, 7.21), Slope = -0.795 (-1.24, -0.35), χ_1^2 = 12.470, p = 4.135 $\times 10^{-4}$. The trend line plotted is the predicted probability of seeds being predated per fruit (i.e., "the proportion of predated seeds per fruit") for plants across reserves, excluding The Knoll reserve. (**c**-**d**) Comparisons of model support for the percentage area of native vegetation within a defined radius of sample sites (200-4000 m) on (**c**) the probability of fruits having predated seeds and (**d**) the proportion of predated seeds per fruit (see *Scale-of-effect - 2017* in the Methods and Fig. 3-6 for details on interpretation). (**e**-**f**) Plots displaying the chosen scale-of-effect for each of the two response variables. (**e**) The probability of fruits having predated seeds, plotted against the percentage area of native vegetation surrounding sample sites within a radius of 2800 m. The model results of a Binomial GLMM of the probability of fruits having predated seeds against the percentage area of native vegetation surrounding sample sites within a radius of 2800 m: Intercept = 5.216 (95 % CI: 2.87, 7.56), Slope = -0.099 (-0.15, -0.05), χ_1^2 = 14.805, p = 1.192 $\times 10^{-4}$. The trend line plotted is the predicted probability of fruits having predated seeds for plants of *H. exutiacies*. (**f**) The proportion of predated seeds per fruit. Large symbols show the overall proportion of seeds graded as predated per sample site (i.e., population proportion), plotted against the percentage area of native vegetation surrounding sample sites within a radius of 3000 m. The overall proportion of seeds graded as predated for each individual plant are also plotted (grey data points). The model results of a Binomial GLMM of the proportion of predated seeds per fruit against the percentage area of native vegetation surrounding sample sites within a radius of 3000 m: Intercept = 4.203 (95 % CI: 2.19, 6.21), Slope = -0.086 (-0.13, -0.04), χ_1^2 = 13.878, p = 1.950 $\times 10^{-4}$. The trend line plotted is the predicted probability of seeds being predated per fruit (i.e., "the proportion of predated seeds per fruit") for plants of *H. exutiacies*. All trend lines are population-level predictions (i.e., setting all random effects to zero). Dashed lines represent 95 % confidence intervals. In (**a**-**b**) and (**e**-**f**) S1 and S2 stand for sample site 1 and 2, respectively. Points in plots (**a**-**b**) and (**e**-**f**) have been randomly jittered to aid visualisation. Note that the above models of the probability of fruits having predated seeds and the proportion of predated seeds per fruit have the same random effect structure as the equivalent models of the probability of fruits having viable seeds and the proportion of viable seeds per fruit of open-pollinated plants in 2017 (see *Natural reproduction - 2017 and 2018* and *Scale-of-effect - 2017* in the Methods).



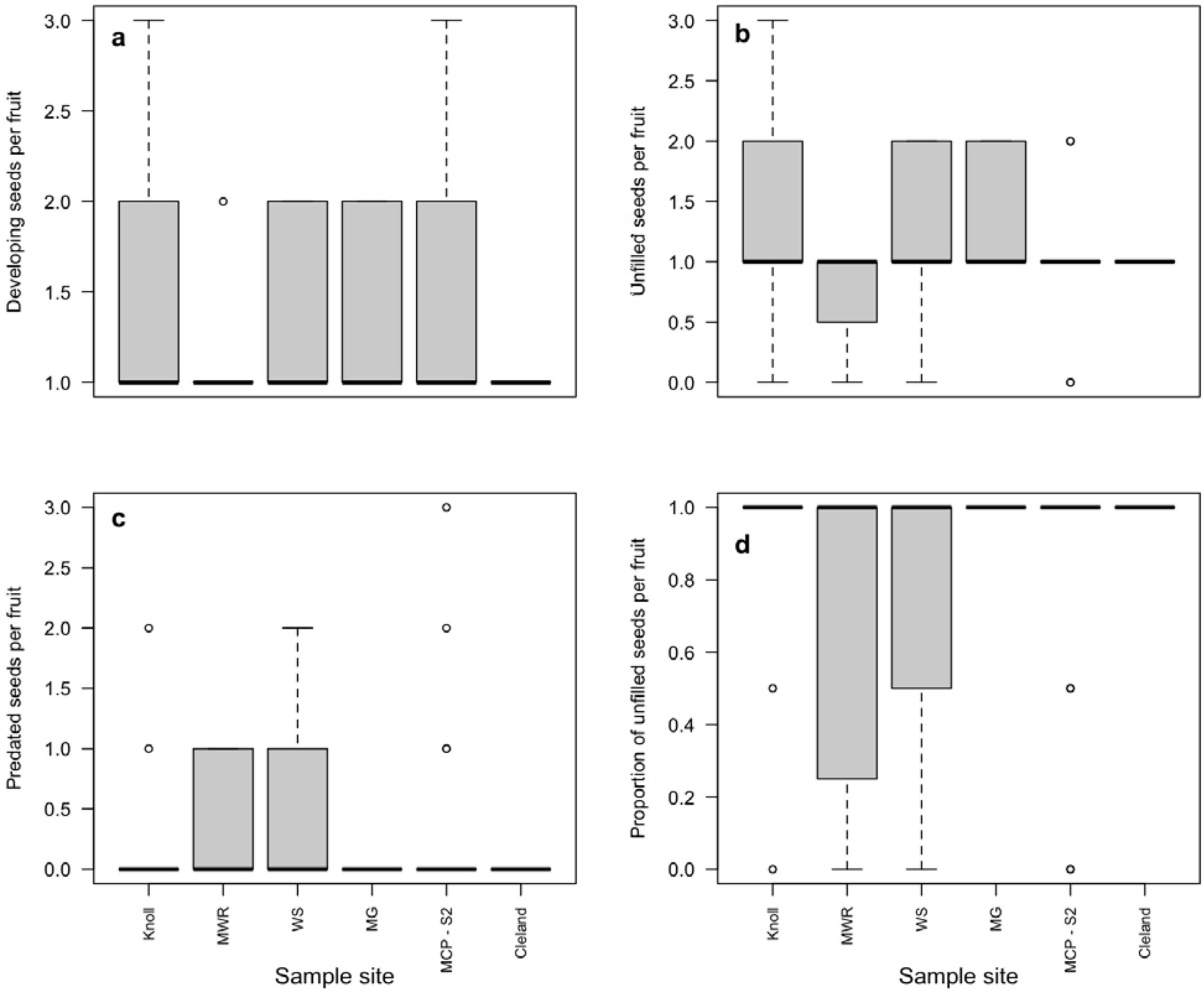
SM Fig. 3-9 Effect of the pollen addition treatment on the fruit-set of *Hibbertia exutiacies* per sample site in 2018. Plotted are the number of flowers tagged per treatment relative to the number of fruits which were subsequently produced, pooled across individual plants per sample site. The number of plants tagged per treatment per sample site, followed by the total number of flowers tagged across the three treatments per sample site in parentheses, is provided within each plot. The odds of a fruit being produced per treatment are given above each of the respective groups of columns per sample site. Percentage fruit-set (rounded to one decimal place) is provided in parenthesis. S2 stands for sample site 2.



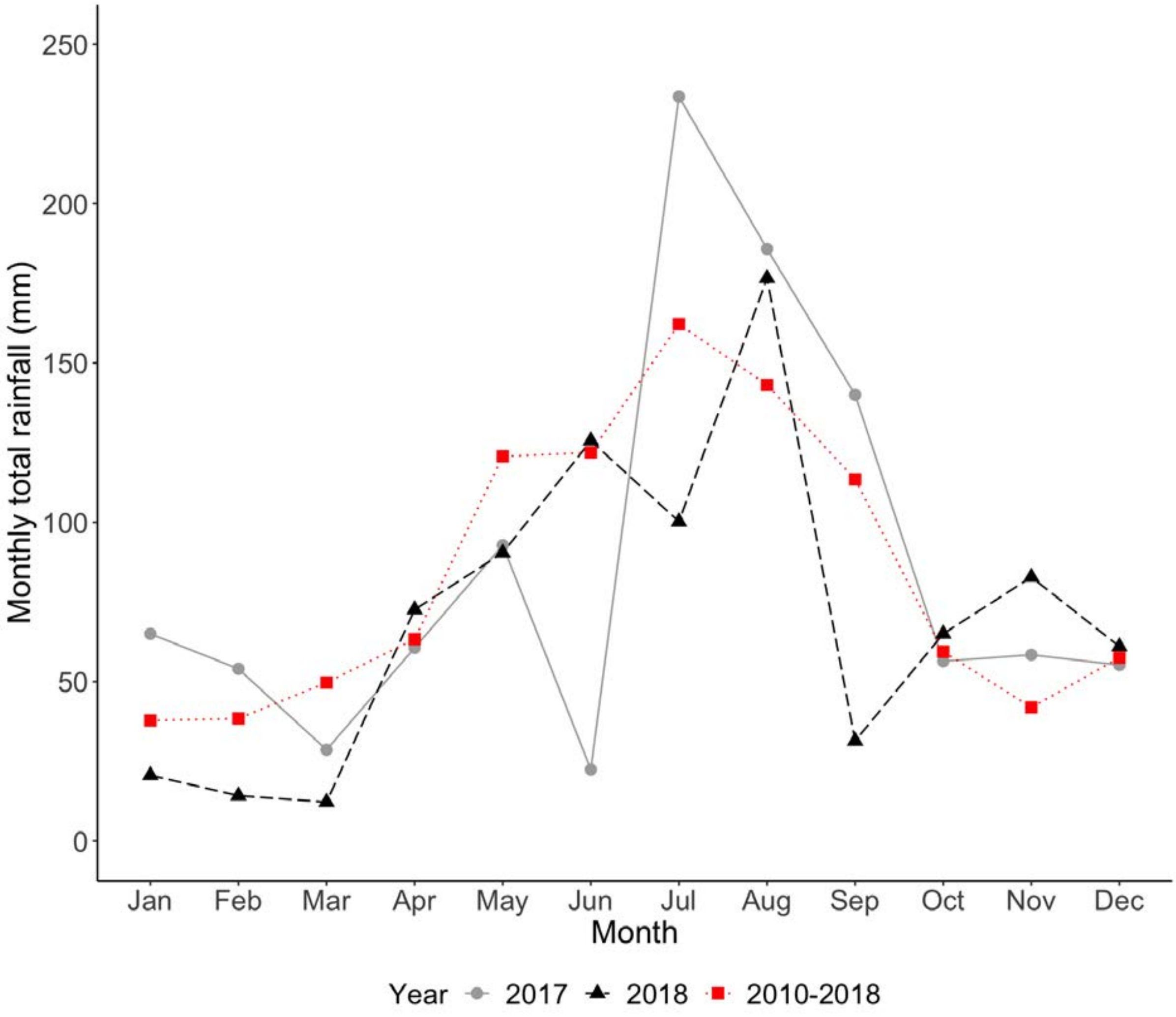
SM Fig. 3-10 Effect of the pollen addition treatment on the production of predated and unfilled seeds of *Hibbertia exutiacies* in 2018. Plotted are boxplots, along with raw datapoints (or only raw datapoints in the case of (**c**) and (**d**)) pooled across plants per treatment for (**a**) the number of developing seeds (predated + unfilled) per fruit (i.e., developing seed-set), (**b**) the number of unfilled seeds per fruit (i.e., unfilled seed-set), (**c**) the proportion of unfilled seeds per fruit (boxplots were not displayed due to the predominance of fruits with 100 % unfilled seeds), and (**d**) the number of predated seeds per fruit (i.e., predated seed-set) (boxplots were not displayed due to the predominance of fruits with no predated seeds). Data was displayed at the level of individual fruits to show the distribution of seed numbers within fruits (see Table 3-4 for averages per fruit per plant). Note that no viable seeds were produced by tagged plants in 2018.



SM Fig. 3-11 Fruit-set of open-pollinated plants of *Hibbertia exutiacies* per sample site in 2018. Sample sites sharing the same letter (located above the plot) are not statistically different from one another. MWR = Malcolm Wicks Reserve, WS = Wottons Scrub, MG = Mount George, MCP - S2 = Mark Oliphant - Sample Site 2.



SM Fig. 3-12 Natural levels of seed production of open-pollinated plants of *Hibbertia exutiacies* per sample site in 2018. Plotted are boxplots displaying the number of (**a**) developing seeds (predated + unfilled) per fruit (i.e., developing seed-set), (**b**) unfilled seeds per fruit (i.e., unfilled seed-set), (**c**) predated seeds per fruit (i.e., predated seed-set), and (**d**) the proportion of unfilled seeds per fruit between sample sites. MWR = Malcolm Wicks Reserve, WS = Wottons Scrub, MG = Mount George, MCP - S2 = Mark Oliphant - Sample Site 2. Data was displayed at the level of individual fruits to show distributions of seed numbers within fruits. Note that no viable seeds were produced by tagged plants in 2018.



SM Fig. 3-13 Monthly total rainfall (mm) recorded at the Mount Lofty weather station (Station number: 23842) in the years of 2017, 2018, and averaged for the years 2010-2018.

SM Table 3-1 Details related to observations of visiting native bees to flowers of *Hibbertia exutiacies*. Provided is the sample site in which the observation occurred, the date of observation, the type of observation (video or personal (Pers. Obs.)), the start time of the observation (24-hour clock), the total time of the observation (minutes), the number of flowers watched or filmed per observation, the number of native bees visiting flowers per observation (i.e., at least contacting part of a flower), the number of these visiting bees which interacted with the anthers of at least one flower (i.e., buzzing, scraping, milking of anthers), and the number of these bees which were large enough to contact the stigmatic tips of the two lateral styles and the central anthers simultaneously while collecting pollen (~5-9 mm). S1 and S2 stand for sample site 1 and 2, respectively.

Sample site	Date	Observation type	Start time	Total time observation	No. flowers	No. bees	No. bees interacting anthers	No. larger bees interacting anthers (~5-9 mm)
Mark Oliphant CP - S1	20/09/2017	Video	12:02	60 minutes	14	0	-	-
Filsell Hill CP	22/09/2017	Video	12:39	63 minutes	7	0	-	-
Filsell Hill CP	22/09/2017	Video	14:25	49 minutes	2	0	-	-
Filsell Hill CP	27/09/2017	Video	10:06	66 minutes	10	1	0	-
Filsell Hill CP	27/09/2017	Video	14:54	65 minutes	19	0	-	-
Mark Oliphant CP - S1	23/10/2017	Video	11:15	167 minutes	25	13	12	7
Wottons Scrub CP	24/10/2017	Video	11:14	140 minutes	59	0	-	-
Wottons Scrub CP	25/10/2017	Video	11:34	75 minutes	50	0	-	-
Wottons Scrub CP	25/10/2017	Video	12:58	75 minutes	109	0	-	-
Wottons Scrub CP	25/10/2017	Video	14:29	71 minutes	56	3	2	2
Mark Oliphant CP - S2	22/10/2018	Pers. Obs.	10:22	15 minutes	29	0	-	-
Mark Oliphant CP - S2	22/10/2018	Pers. Obs.	11:00	15 minutes	88	0	-	-
Mark Oliphant CP - S2	22/10/2018	Pers. Obs.	11:47	15 minutes	51	0	-	-
Mark Oliphant CP - S2	22/10/2018	Pers. Obs.	14:11	15 minutes	60	0	-	-
Mark Oliphant CP - S2	22/10/2018	Pers. Obs.	14:37	15 minutes	34	0	-	-
The Knoll CP	26/10/2018	Pers. Obs.	10:25	15 minutes	150	1	1	1
The Knoll CP	26/10/2018	Pers. Obs.	12:09	15 minutes	67	1	0	-
The Knoll CP	26/10/2018	Pers. Obs.	14:24	15 minutes	126	1	0	-
Wottons Scrub CP	27/10/2018	Pers. Obs.	12:33	15 minutes	21	1	1	1
Wottons Scrub CP	27/10/2018	Pers. Obs.	13:47	15 minutes	21	0	-	-
Wottons Scrub CP	27/10/2018	Pers. Obs.	14:49	15 minutes	14	0	-	-

SM Table 3-2 Details regarding breeding system experiments in 2017 and 2019 for the study species, *Hibbertia exutiacies*. Geographical coordinates for sample sites, located in conservation reserves, used in 2017 and 2019 are given. Sample sizes for the number of plants and flowers used to determine reproduction via autonomous self-pollination in 2017, and sample sizes for the number of plants and flowers pollinated with outcross-pollen, self-pollen, and left open to natural pollination (open-pollination) are provided for breeding system experiments undertaken in 2017 and 2019, respectively. S1 stands for sample site 1.

Sample site	Latitude	Longitude	Autonomous self-pollination (2017)	Breeding system (2017)	Breeding system (2019)
Wottons Scrub CP	34° 59.21' S	138° 46.59' E	1 plant used; 13 flower buds bagged	1 plant used; 2 flowers outcross-pollinated, 3 flowers self-pollinated, 5 flowers tagged for open-pollination	NA
Mark Oliphant CP - S1	35° 1.53' S	138° 41.93' E	7 plants used; 161 flower buds bagged in total	2 plants used; 4 flowers outcross-pollinated[a], 8 flowers self-pollinated, 10 flowers tagged for open-pollination in total	4 plants used[b]; 8 flowers outcross-pollinated, 5 flowers self-pollinated, 20 flowers tagged for open-pollination in total
Filsell Hill CP	34° 57.70' S	138° 47.80' E	1 plant used; 26 flower buds bagged	NA	NA

[a] A bagged replicate containing 3 outcross flowers was lost on one of the plants due to death of the branch.

[b] Due to the difficulty of bagging branches in *H. exutiacies*, both outcrossed and self-pollinated flowers were in the same bag.

SM Table 3-3 Details of the number of plants and flowers tagged in pollen-limitation experiments in 2017 and 2018, and for measures of natural levels of reproduction (open-pollination) in 2017 for the study species, *Hibbertia exutiacies*. Geographical coordinates for sample sites used in 2017 and 2018 are given. The area of the respective reserve in which the sample site was located is provided. Sample sizes for the number of plants and flowers used in pollen-limitation experiments conducted in 2017 and 2018 are provided. Specifically, sample sizes are given for these variables for the external controls (EC), internal controls (IC), and pollen addition treatments (PA) per sample site. Sample sizes per sample site of plants and flowers tagged to measure natural levels of reproduction (open-pollination) in 2017 are also provided. S1 and S2 stand for sample site 1 and 2, respectively.

Reserve	Latitude	Longitude	Reserve area (ha)	Pollen-limitation experiments (2017)	Pollen-limitation experiments (2018)	Natural levels of reproduction (2017[a])
The Knoll	35° 0.45' S	138° 41.96' E	1.74	EC - 5 plants (25 flowers) IC - 5 plants (25 flowers) PA - 5 plants (25 flowers)	EC - 5 plants (25 flowers) IC - 5 plants (25 flowers) PA - 5 plants (25 flowers)	9 plants (50 flowers)
Malcolm Wicks Reserve	34° 55.92' S	138° 48.89' E	7.90	NA	EC - 3 (15) IC - 3 (15) PA - 3 (15)	NA
Wottons Scrub CP	34° 59.21' S	138° 46.59' E	82.50	EC - 5 (25) IC - 5 (25) PA - 5 (25)	EC - 5 (25) IC - 5 (25) PA - 5 (25)	11 (69)
Mount George CP	34° 59.72' S	138° 45.34' E	84.89	NA	EC - 3 (15) IC - 3 (15) PA - 3 (15)	NA
Giles CP	34° 56.09' S	138° 43.74' E	108.92	NA	NA	5 (31)
Filsell Hill CP	34° 57.70' S	138° 47.80' E	128.67	EC - 3 (13) IC - 3 (13) PA - 3 (13)	NA	10 (54)
Mark Oliphant CP - S1	35° 1.53' S	138° 41.93' E	189.96	EC - 2 (9) IC - 2 (9) PA - 2 (9)	NA	7 (34)

Mark Oliphant CP - S2	35° 2.03' S	138° 41.97' E	189.96	EC - 3 (15) IC - 3 (15) PA - 3 (15)	EC - 5 (25) IC - 5 (25) PA - 5 (25)	13 (72)
Belair NP	35° 1.46' S	138° 40.33' E	837.54	NA	NA	8 (43)
Cleland CP	34° 59.21' S	138° 41.75' E	1027.47	EC - 5 (25) IC - 5 (25) PA - 5 (25)	EC - 3 (15) IC - 3 (15) PA - 3 (15)	9 (48)

[a] Includes plants used as the external controls (EC) of pollen-limitation experiments conducted in 2017.

SM Table 3-4 Details of the number of plants which produced fruits, the number of fruits opened, and number of seeds examined in pollen-limitation experiments in 2017 and 2018, and for measures of natural levels of reproduction (open-pollination) in 2017 for the study species, *Hibbertia exutiacies*. Geographical coordinates for sample sites used in 2017 and 2018 are given. The area of the respective reserve in which the sample site was located is provided. Sample sizes for the number of tagged plants which produced fruits, and the subsequent number of fruits and seeds examined per sample site for pollen-limitation experiments conducted in 2017 and 2018 are provided. Specifically, sample sizes are given for these variables for the external controls (EC), internal controls (IC), and pollen addition treatments (PA). The number of tagged plants which produced fruits and the subsequent number of fruits and seeds examined per sample site are also given for measures of natural levels of reproduction (open-pollination) in 2017. S1 and S2 stand for sample site 1 and 2, respectively.

Reserve	Latitude	Longitude	Reserve area (ha)	Pollen-limitation experiments (2017)	Pollen-limitation experiments (2018)	Natural levels of reproduction (2017[a])
The Knoll	34° 55.92' S	138° 48.89' E	1.74	EC - 5 plants, 14 fruits (22 seeds) IC - 4 plants, 7 fruits (9 seeds) PA - 5 plants, 20 fruits (35 seeds)	EC - 5 plants, 18 fruits (30 seeds) IC - 5 plants, 15 fruits (28 seeds) PA - 5 plants, 19 fruits (37 seeds)	9 plants, 30 fruits (45 seeds)
Malcolm Wicks Reserve	34° 59.21' S	138° 46.59' E	7.90	NA	EC - 3, 7 (8) IC - 3, 8 (12) PA - 3, 7 (11)	NA
Wottons Scrub CP[b]	34° 59.48' S	138° 46.58' E	82.50	EC - 2, 4 (10) IC - 1, 2 (4) PA - 3, 13 (27)	EC - 5, 13 (19) IC - 5, 13 (25) PA - 5, 16 (26)	8, 24 (55)
Mount George CP	34° 55.92' S	138° 48.89' E	84.89	NA	EC - 2, 6 (8) IC - 3, 11 (14) PA - 3, 13 (19)	NA
Giles CP	34° 56.09' S	138° 43.74' E	108.92	NA	NA	5, 14 (28)

Filsell Hill CP	34° 57.70' S	138° 47.80' E	128.67	EC - 2,5 (14) IC - 3,7 (14) PA - 3,8 (18)	NA	7,17 (34)
Mark Oliphant CP - S1[b]	35° 1.53' S	138° 41.93' E	189.96	NA	NA	5, 11 (20)
Mark Oliphant CP - S2	35° 2.03' S	138° 41.97' E	189.96	EC - 3,12 (15) IC - 3,8 (13) PA - 3,11 (18)	EC - 5, 18 (25) IC - 5, 14 (23) PA - 5, 18 (26)	11,22 (41)
Belair NP	35° 1.46' S	138° 40.33' E	837.54	NA	NA	8, 28 (55)
Cleland CP	34° 59.21' S	138° 41.75' E	1027.47	EC - 5,13 (23) IC - 4,7 (11) PA - 5,17 (31)	EC - 3, 3 (3) IC - 3, 10 (11) PA - 3, 9 (11)	7,20 (36)

[a] Includes plants used as the external controls (EC) of pollen-limitation experiments conducted in 2017.

[b] Due to an initial mislabelling of seeds into the categories of viable, predated, and unfilled when first making and defining each of the three categories, seeds within fruits of four plant pairs of the pollen-limitation experiments were not considered for further statistical analysis. These plant pairs were from Wottons Scrub CP and Mark Oliphant CP - S1, respectively. For Wottons Scrub CP this included an additional: EC - 2 plants and 7 fruits, IC - 1 plant and 3 fruits, PA - 2 plants and 8 fruits. For Mark Oliphant CP - S1 this included: EC - 2 plants and 2 fruits, IC - 2 plants and 3 fruits, PA - 2 plants and 6 fruits.

SM File 3-1

An additional 1177 mature seeds were haphazardly sampled from the mature fruits (*n* = 712) of untagged open-pollinated plants (*n* = 91) across a number of sample sites in mid-December (10/12 to 21/12) 2018 (see SM File 3-1, Table 3-1 below). Seeds within these fruits were graded into the three categories of viable, predated, and unfilled as per the definitions of each seed category given in the section *Pollination and reproduction* of the Methods. This allowed for a general comparison between the percentages of each seed category within fruits collected earlier (i.e., from tagged flowers of the pollen-limitation experiment in 2018) versus later in development (i.e., from untagged flowers). However, it should be noted that unlike those fruits initially tagged as flowers, the flowering time(s) of those fruits haphazardly sampled from untagged open-pollinated plants later in the season is unknown. Consequently, whereas the earlier sampled fruits represent a seed cohort which have been developing for approximately the same length of time under the same relative environmental conditions, the length of time that seeds within the later sampled fruits have been developing is less clear (i.e., the length of time from when ovules were fertilized). This may potentially cause problems in comparing the two seed samples if the relative influences of abiotic and biotic factors on seed production shift within a single reproductive season. Thus, we simply examined whether the percentage of each seed category within fruits collected earlier (i.e., from tagged flowers) versus later in development (i.e., from untagged flowers) broadly corresponded to one another, rather than use statistical analyses.

Of the 1177 mature seeds examined in 2018, 710 (60.32 %) were unfilled, 432 (36.70 %) showed evidence of predation (although in many cases the amount of frass was noticeably lower than seeds in 2017 (Pers. Obs.)), and 35 (2.97 %) were graded as viable (although these seeds still appeared somewhat underdeveloped compared to viable seeds from mature fruits sampled around the same time in 2017 (Pers. Obs.)). Thus, compared to fruits derived from the tagged flowers of plants in the pollen-limitation experiment in 2018, there was a greater percentage of viable seeds within fruits sampled from untagged flowers later in the season (2.97 % versus 0.00 %), although sample sizes differed considerably (712 fruits and 1177 seeds from untagged flowers versus 218 fruits and 336 seeds from the pollen addition and control treatments). Similarly, the percentage of seeds suffering pre-dispersal seed predation was greater in fruits sampled later in the season from untagged flowers versus fruits sampled earlier from tagged flowers (36.70 % versus 11.90 %). Nevertheless, for fruits sampled earlier (i.e., from tagged flowers) and later in development (i.e., from untagged flowers), the majority of seeds were graded as inviable, and this inviability was predominately a result of seeds being unfilled (i.e., abortion). Ultimately, the high level of seed abortion across sample sites in 2018 strongly implies an abiotic constraint limited viable seed production of *H. exutiacies* throughout the study region.

SM File 3-1, Table 3-1 Details of the number untagged open-pollinated plants, mature fruits and seeds haphazardly sampled of *Hibbertia exutiacies* in 2018. Geographical coordinates for sample sites are given. The area of the respective reserve in which the sample site was located is provided. S1 and S2 stand for sample site 1 and 2, respectively.

Reserve	Latitude	Longitude	Reserve area (ha)	No. plants, fruits and seeds sampled in 2018
The Knoll	34° 55.92' S	138° 48.89' E	1.74	10 plants, 84 fruits, 139 seeds
Malcolm Wicks Reserve	34° 59.21' S	138° 46.59' E	7.90	9 plants, 74 fruits, 117 seeds
Wottons Scrub CP - S1	34° 59.48' S	138° 46.58' E	82.50	12 plants, 94 fruits, 187 seeds
Mount George CP - S2[a]	34° 59.76' S	138° 45.29' E	84.89	10 plants, 71 fruits, 111 seeds
Giles CP	34° 56.09' S	138° 43.74' E	108.92	10 plants, 84 fruits, 158 seeds
Filsell Hill CP	34° 57.70' S	138° 47.80' E	128.67	10 plants, 70 fruits, 105 seeds
Mark Oliphant CP - S2	35° 2.03' S	138° 41.97' E	189.96	10 plants, 75 fruits, 116 seeds
Belair NP - S2[a]	35° 1.45' S	138° 40.25' E	837.54	10 plants, 74 fruits, 116 seeds
Cleland CP	34° 59.21' S	138° 41.75' E	1027.47	10 plants, 86 fruits, 128 seeds

[a] Additional sample sites not used as part of the manipulative experiments (2017 and 2018) and mensurative census (2017 only) of *H. exutiacies*. For Mount George CP, an alternative sample site to the one used for pollen-limitation experiments in 2018 was required to collect a larger sample size of mature fruits later in the season. For Belair NP, the sample site used in 2017 could not be reused due to poor flowering and fruiting in 2018.

CHAPTER FOUR

4. SOIL MOISTURE LIMITS FRUIT-SET OF *HIBBERTIA EXUTIACIES* N.A.WAKEF. (DILLENIACEAE), A POLLEN-LIMITED SCLEROPHYLLOUS SHRUB THAT SUFFERS EXTENSIVE PRE-DISPERSAL SEED PREDATION.

Alex G. Blackall[1], Duncan A. Mackay[1], Molly A. Whalen[1]

[1]College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

4.1 ABSTRACT

Premise: The beginning of spring 2018 saw historically low rainfall in the Adelaide Hills, a peri-urbanised region of southern Australia. Possibly in response to this low rainfall, *Hibbertia exutiacies*, a common shrub, mostly aborted its reproduction, producing large numbers of unfilled seeds. In contrast, few seeds in 2017 were unfilled. However, most of these seeds were ultimately predated by the larvae of pre-dispersal seed predators in 2017. Thus, in 2019, we investigated if water availability limited reproduction of *H. exutiacies* in the field. Moreover, we predicted that pre-dispersal seed predation would be lower in 2019 than in 2017 due to the fewer seeds that appeared available to support populations of pre-dispersal seed predators in 2018.

Methods: Two sample sites with populations of *H. exutiacies* known to have flowered adequately the previous two years were chosen in 2019. Twenty plants were selected per sample site. Ten plants received additional water, once per week, across their flowering period (mid-September to early November). We recorded flower number weekly for each plant. We also tagged flowers on watered and unwatered plants to measure fruit-set, seed number per fruit, and levels of seed predation.

Results: Watering impacted the flowering phenology of plants in both sample sites, resulting in watered plants having more flowers later into the season. However, on average, flower number per plant did not differ between watered and unwatered plants over the course of the flowering period. Watering increased fruit-set per plant in one of the sample sites only. Seed number per fruit was not affected by watering in either sample site, with relatively few unfilled seeds produced. In contrast to our prediction, pre-dispersal seed predation in 2019 was qualitatively comparable to the high rates observed in 2017.

Conclusion: Although water limited fruit production in one of our sample sites, pre-dispersal seed predation was likely the most significant constraint on the output of viable seeds for *H. exutiacies* in 2019. Thus, populations of seed predators did not evidently decline after 2018, and *H. exutiacies* was unable to temporally escape high levels of pre-dispersal seed predation. The extent to which declines in spring rainfall under climate change will interact with pre-dispersal seed predators to impact reproduction of *H. exutiacies* requires further long-term study.

KEYWORDS: water-limitation; abiotic resources; rainfall; climate change; chalcid wasps

4.2 INTRODUCTION

Plant reproduction is limited by access to both biotic resources, such as pollinators (Johnson and Bond 1997; Pauw 2007) and compatible mates (de Waal et al. 2015; Delnevo et al. 2020), as well as abiotic resources, such as soil nutrients (McCall and Primack 1987; Burkle and Irwin 2009) and water availability (de Jong and Klinkhamer 1989; Gallagher and Campbell 2017). Reproduction may be limited predominantly by a single resource or simultaneously by multiple resources (Campbell and Halama 1993). In the latter case, an increased availability of one resource may only increase reproduction if other required resources are likewise increased (Brookes et al. 2008; Recart and Campbell 2021). The degree to which any resource constrains reproduction may vary both spatially and temporally (e.g., see studies by Gross 1996; Dudash and Fenster 1997; Hampe 2005; Hove et al. 2016). Consequently, plants display a myriad of adaptations that favour continued survival and reproduction under variable conditions. However, within natural communities, reproductive output may not remain adequate for long-term population persistence when large changes to resource availability occur (e.g., via anthropogenic impacts). Thus, from a conservation perspective it is of value to understand how plant reproduction responds to both current and potentially altered environmental conditions (Hobbs and Yates 2003).

Hibbertia exutiacies is a common, perennial, sclerophyllous shrub that grows within the dry sclerophyll forests and woodlands of the Adelaide Hills, a peri-urbanised region of southern Australia (Armstrong et al. 2003). In a prior study, across two consecutive years (2017 and 2018), spatio-temporal variability in the pollination and reproduction of *H. exutiacies* between fragmented populations in the Adelaide Hills was assessed (Chapter Three). Marked temporal variability in the efficiency of the pollination process was found, with pollen-limitation of fruit-set (here defined as the proportion of flowers producing swollen carpel(s) containing developing seed(s)) for plants in 2017 but not 2018. However, although fertilized flowers of *H. exutiacies* may develop fruits (i.e., presence of swollen carpel(s) containing developing seed(s)), large numbers of seeds within these fruits may contain shrivelled or more often no endosperm. Indeed, the production of seeds with shrivelled or no endosperm occurs regularly across species of *Hibbertia* (Schatral and Fox 1994; Schatral et al. 1994; Cochrane 2002; Erickson et al. 2016 p. 85). Furthermore, for those seeds provisioned with healthy endosperm, the majority of these seeds may be attacked by the larvae of pre-dispersal seed predators. Consequently, there were significant differences between 2017 and 2018 in the number of viable, predated, and unfilled seeds within fruits of *H. exutiacies* (Chapter Three).

For open-pollinated plants in 2017, over half the seeds examined were predated prior to dispersal by insect larvae, the remainder predominantly viable with a small number of unfilled seeds. In contrast, for open-pollinated plants in 2018, around one fifth of seeds were predated, with all remaining seeds unfilled. This almost complete abortion of seeds (i.e., not filled with endosperm) by plants of *H. exutiacies* in 2018 suggests an abiotic constraint limited viable seed-set across the whole of the study region. In support, the beginning of spring in 2018 saw historically low rainfall throughout the Adelaide Hills, with South Australia experiencing its third driest September on record (e.g., September rainfall at the Mount Lofty weather station (Station number: 23842) was 31.4 mm in 2018 versus 140.0 mm in 2017, against a longer-term average (2009-2021) of 111.2 mm; see Fig 4-1 for weather station location). Moreover, seed abortion was also high in 2018 for the co-flowering common legume, *Pultenaea daphnoides* (Chapter Two), as well as other co-flowering species (Pers. Obs.), suggesting historically low rainfall in September of 2018 negatively impacted the reproduction of a range of plant species in the Adelaide Hills. Indeed, rainfall is a strong determinant of reproductive output for plants within sclerophyll communities across Australia (e.g., Hansen et al. 1991; Hansen et al. 1992), and lower rainfall may be an added pressure on plants already negatively impacted by current landscape disturbance (Morgan 1999). Moreover, given average spring rainfall in the Adelaide Hills is modelled to decline by 17.0-25.2 % by the year 2070 (Charles and Fu 2015), increasing spring rainfall deficits may considerably reduce the reproduction of local plant species.

We hypothesized that water availability during the reproductive period of *H. exutiacies* significantly limits reproduction via reduced flowering, fruit, and seed production. Since populations of *H. exutiacies* begin to flower in the Adelaide Hills during September, we considered that lower rainfall during flowering, rather than in the later months during fruit and seed maturation, has a stronger impact on plant reproduction. The timing of water deficit is known to be of critical importance to the reproduction of other plant species growing in similar Mediterranean-type climates (Aragón et al. 2008). Moreover, rainfall returned to normal to above-average levels in the following months of October and November in 2018 (long-term averages of 65.6 mm and 43.0 mm, respectively (Mount Lofty weather station)). Thus, the historically low rainfall in September of 2018 stands out as a critical period of rainfall deficit. To test our hypothesis that water was a limiting abiotic resource, we watered plants of *H. exutiacies in situ* within two populations across their flowering period and compared flower, fruit, and seed production to unwatered controls. Five predictions were made:

Prediction 1: Flower number per plant would increase with additional watering. This was hypothesized as the overall flowering of *H. exutiacies* populations was less in the drier spring of 2018 (i.e., specifically in September) versus the wetter spring of 2017. It was also observed that flowering of *H. exutiacies* finished earlier in 2018 (Pers. Obs.); therefore, we also predicted that watering would extend the flowering period of *H. exutiacies*.

Prediction 2: Fruit-set (here defined as the proportion of flowers producing swollen carpel(s) containing developing seed(s)) of watered plants would be greater than that of their unwatered controls. This was predicted because the fruit-set of open-pollinated plants did not differ between 2017 and 2018; however, pollen addition to flowers in 2017 increased fruit-set, whereas pollen addition had no detectable effect in 2018. Thus, it is hypothesised that the lack of rainfall at the start of spring in 2018 did not allow for fruit-set to increase with supplementary pollination, compared to plants in the wetter spring of 2017. It is also of note that flowers of *H. exutiacies* in 2018 were supplemented with pollen in mid to late October and not during September itself. Thus, low rainfall in September 2018 may have resulted in reduced fruit and seed development throughout the whole of the flowering period, and not just for those flowers present on plants in September.

Prediction 3: The number of developing seeds (a combination of viable, predated, and unfilled seeds) per fruit would increase with watering. This was hypothesized as the mean number of developing seeds per fruit per plant was lower (1.37 ± 0.42) in the drier spring of 2018 than in the wetter spring of 2017 (1.75 ± 0.63).

Prediction 4: We also predicted the number of viable (i.e., filled) seeds per fruit would increase with watering. However, due to potentially high rates of seed predation (e.g., as observed in 2017), we considered both viable and predated seeds combined as the appropriate response variable under the assumption that predated seeds would have otherwise been viable. Thus, we predicted the number of viable + predated seeds per fruit would increase with watering.

Prediction 5: Rates of pre-dispersal seed predation of plant species often show considerable temporal variability among years (Kolb et al. 2007), and for long-lived perennials, variable weather conditions that result in occasional years of reproductive failure can allow seeds to escape constant rates of pre-dispersal predation by reducing seed predator abundance relative to the number of seeds produced the following year (Solbreck and Sillén-Tullberg 1986; Solbreck and Knape 2017). Thus, we hypothesized that *H. exutiacies* may temporally escape high rates of pre-dispersal seed predation by having occasional years of reproductive failure and, as a result, the rate of pre-dispersal seed predation would be expected to be lower in 2019 than in 2017, due to fewer seeds being available to support the reproduction and development of pre-dispersal seed predators in 2018.

4.3 METHODS

4.3.1 Study species

Hibbertia exutiacies N.A.Wakef. (Dilleniaceae) is a common sclerophyllous shrub that grows within the dry sclerophyll forests and woodlands of the Adelaide Hills (Armstrong et al. 2003). It is low growing to a height of 30-50 cm, and nectarless yellow flowers (~10-15 mm diameter) are produced along the length of branches in spring (Jessop 1986; supplementary material (SM) Fig. 4-1a). Each flower typically contains 3-8 stamens with anthers opening by terminal pores and lateral slits (Jessop 1986). The anthers are functionally poricidal (Tucker and Bernhardt 2000), and flowers are visited by native bees (species of *Lasioglossum* and *Exoneura*) capable of buzz-pollination (Chapter Three). The two lateral carpels typically each contain four ovules (Jessop 1986). Following fertilisation, each carpel may form a follicle, therefore, when both carpels produce seeds, the fruit may be considered to be a pair of follicles (Clarke and Lee 2019). Seeds contain abundant endosperm, while the embryo is minute (Horn 2007). The breeding system of *H. exutiacies* is predominantly outcrossing, with low fruit-set produced via selfing (Chapter Three). No seed is produced via autonomous self-pollination (Chapter Three). Although the root system of *H. exutiacies* has not been studied, *Hibbertia* species inhabiting similar Mediterranean-type regions of South Australia possess shallow taproots, penetrating less than 30 cm into the soil, with prominent lateral root development (Specht and Rayson 1957).

4.3.2 Sample sites

Two sample sites were chosen in the Adelaide Hills (Fig. 4-1). One in Mark Oliphant Conservation Park (CP) (MOCP - Lat: 35° 2.03', Long: 138° 41.97') and one in Wottons Scrub CP (WSCP - Lat: 34° 59.21', Long: 138° 46.59'). Locations for the sample sites were selected based on both accessibility, and prior knowledge that the populations of *H. exutiacies* in these reserves had flowered adequately the previous two years (Chapter Three). The three nearest weather stations to each sample site (with available data) were used to estimate local rainfall (Fig. 4-1). This was done by averaging the recorded rainfall of the three nearest weather stations for each sample site, respectively (see SM Fig. 4-2 and 4-4). The chosen weather stations had continuous data from at least the year 2001 (i.e., 19 years). Thus, averaged rainfall data from 2001-2019 was used as an estimate of mean rainfall for each sample site over the recent past. Prior to our experiment, long-term forecasts predicted below-average rainfall for the spring of 2019 (Bureau of Meteorology).

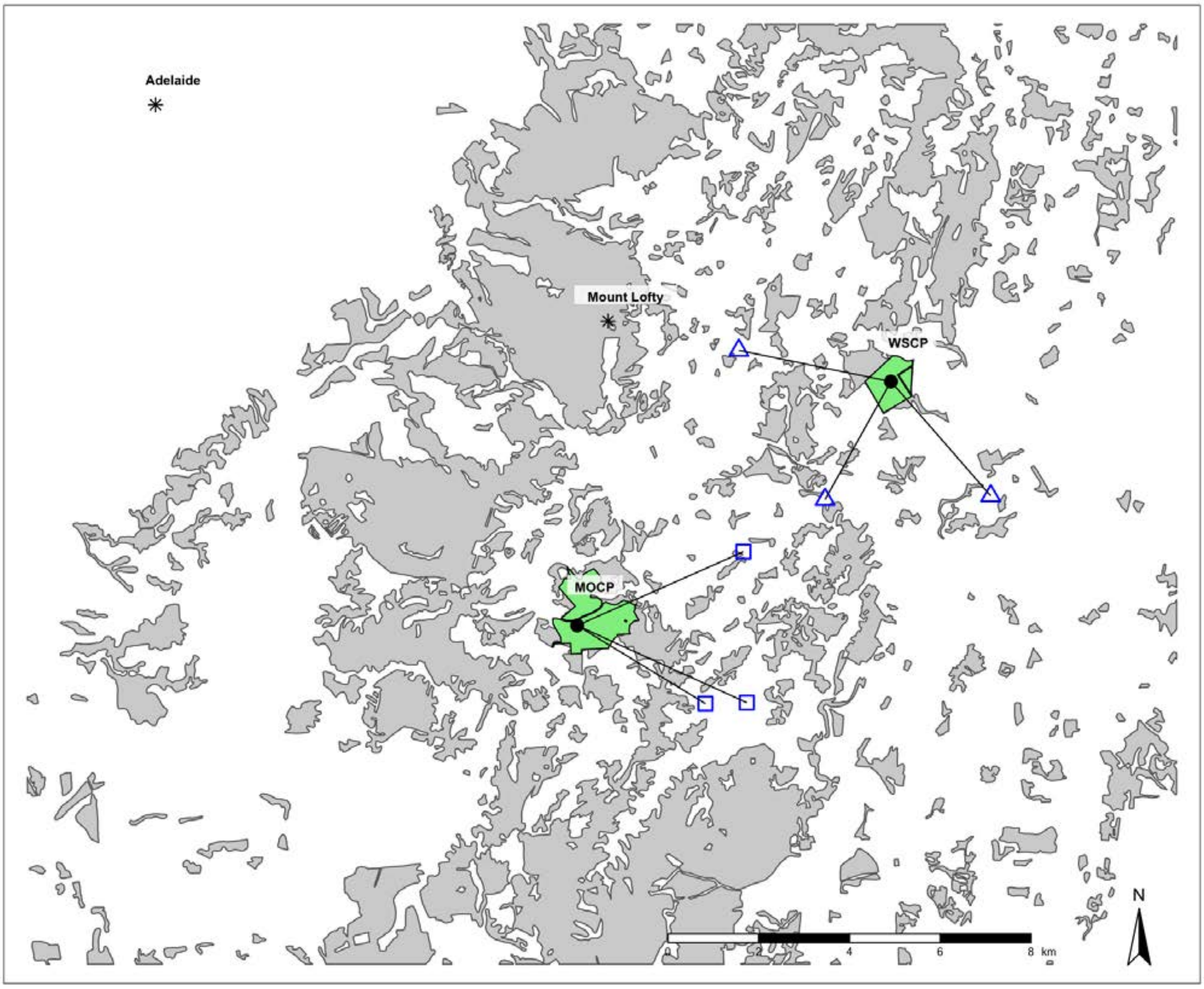


Fig. 4-1 (above) Location of sample sites (black circles) within the reserves of Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP), highlighted in green. The distribution of remaining native vegetation is highlighted in grey. Also shown are the three nearest weather stations (Bureau of Meteorology) with available data to the sample site in MOCP (squares) and WSCP (triangles), respectively. Weather station numbers are **MOCP**: 023817, 023727, 023108; **WSCP**: 023707, 023866, 023891. Adelaide, the capital city of South Australia, and Mount Lofty, the highest point within the study region (720 m), are both marked by an asterisk. The scale bar represents a total distance of 8 km. Native vegetation layer assembled by Department for Environment and Water (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

4.3.3 Experimental design

Ten pairs of *H. exutiacies* individuals were chosen within each sample site. Each plant pair consisted of two similar sized individuals, with comparable numbers of flower buds, situated within 5 m of each other. Thus, plant pairings were chosen to limit both inherent differences between control and treatment plants in size and initial reproductive input (i.e., numbers of flower buds) and spatial variability within sample sites which cause differences in water infiltration (e.g., soil density, slope, etc.). One individual of each pair was randomly assigned to the treatment group, which received 1 L of additional water per week above that received naturally through rainfall, while the other was otherwise unmanipulated and served as a control. This quantity of water was chosen based on both a need to carry water to plants in each sample site and visual confirmation that 1 L of water wetted the soil evenly across the chosen individuals of *H. exutiacies*, presumably providing greater access of water in the soil to treatment plants. Each plant was located within a ~0.5 × 0.5 m plot, and 1 L of water per week equated to approximately an additional 4 mm of rainfall extra per week. Water was applied with a watering can, which was held in proximity above the plant, with an even distribution of water applied slowly to avoid excessive run-off. Watering typically occurred after 16:00 hours when conditions were cooler to avoid excessive evapotranspiration. Treatment application ran for nine weeks from 12/09/2019 - 08/11/2019 for MOCP and 13/09/2019 - 09/11/2019 for WSCP. This period of nine weeks is termed the experimental period (i.e., the date of first watering to the date of last watering within each sample site).

Prior to watering, volumetric soil moisture content was measured for each plant using a soil moisture probe to a depth of 6 cm (Thetaprobe ML2X, Delta T, Cambridge, UK). Following the methods of Aragón et al. (2008), measurements were taken within 20 cm of each plant on both their northern and southern sides and then averaged. Soil moisture measurements were taken between midday and 15:00 hours. One two occasions, the 26/09/2019 and 08/11/2019 for MOCP, and the 27/09/2019 and 09/11/2019 for WSCP, additional soil moisture measurements were conducted 30 minutes after watering for five control plants and five watered plants, each within the same plant pairing (i.e., five plant pairings used per sample site). This was done to confirm, at least in the short term, that watering was having a measurable effect on the soil moisture of watered plants. Ultimately, it is assumed that measurements of volumetric soil moisture content reflect access of water in the soil for both control and treatment plants.

4.3.4 Flower production

At each date of watering, the number of open flowers on each focal plant was counted. When many flowers were present per plant, flower number was extrapolated from counting flowers on only a portion of the plant. Older, wilted-looking flowers where petals had begun to abscise were excluded. Most plants across treatments had not started to flower on the first day of watering, while most plants had finished flowering by the final day of watering. Specifically, prior to watering, one control plant in MOCP had already started flowering with two flowers open, while four watered plants and two control plants were still flowering on the final day of watering in MOCP. Thus, there was an overall mean of 0.45 ± 0.83 flowers remaining per plant across treatments on the final day of watering in MOCP. For WSCP, no plants were flowering prior to the first watering treatment. However, five watered plants and one control plant were still flowering on the final day of watering, with an overall mean of 2.05 ± 6.28 flowers remaining per plant across treatments. Thus, the experimental period captured the flowering period of most individuals across treatments (see Fig. 4-6 in the Results).

4.3.5 Fruit production

On the 17/10/2019 for MOCP, and 10/10/2019 and 12/10/2019 for WSCP, a set number of flowers were tagged per plant. The dates for the tagging of flowers within each sample site approximately coincided with peak flowering for both control and watered plants. For MOCP, this meant treatment plants had been watered on five occasions prior to the tagging of flowers and were watered on four occasions afterwards. For WSCP, this meant treatment plants had been watered on four occasions prior to the tagging of flowers and were watered on another five occasions afterwards. In WSCP, due to generally better flowering, ten flowers were tagged per plant. In MOCP, five flowers were tagged per plant excluding two control plants for which only three flowers could be tagged. One plant pairing was excluded due to the low number of flowers produced. Tagged flowers were monitored for fruit development.

We considered a flower to have developed into a fruit based on the presence of swollen carpel(s) containing developing seed(s), which demonstrated initial seed development, without regard to the viability of seeds within fruits. Fruits were separable from persistent calyxes by the obviously enlarged ovaries containing developing seeds. Fruits were removed on 30/11/2019 and 25/11/2019 for MOCP and WSCP, respectively. Fruits were retrieved earlier than would have been done if collecting for mature seed because *Hibbertia* are known to dehisce and drop their seeds quickly, making the timing of collection for mature seeds difficult to estimate (Fox et al. 1987 p. 86; Cochrane 2002; Pers. Obs.). On retrieval one of the tagged branches on a treatment plant in MOCP contained both a remaining calyx and fruit. Thus, this plant was considered to have had 6 flowers tagged, as it could not be determined which of the flowers had been tagged initially. Fruits were kept under refrigeration in paper envelopes until examination of their seeds (~4℃).

4.3.6 Seed production

Although fruits were retrieved prior to full maturity (occurring from mid-December onwards), developing seeds within could be graded into three categories: **1.)** Viable seeds which contained firm white endosperm filling most of the seed (Fig. 4-2a-b). **2.)** Predated seeds which contained insect larvae (or their remains), frass (excrement of insect larvae), entry or exit holes within the seed coat, and the majority of endosperm consumed or not present (Fig. 4-2c-d; SM Fig. 4-1b-h). **3.)** Unfilled seeds, which were either empty or contained a small amount of poorly formed often shrivelled endosperm not filling the seed (SM Fig. 4-1i). Unfilled seeds were considered to have aborted during development.

Two larval types were distinguishable within predated seeds, here termed Larvae-1 (Fig. 4-2c), and Larvae-2 (Fig. 4-2d), respectively. Larvae-2 was observed consuming the endosperm of developing and fully mature seeds, moving between the separate ovaries of a single fruit to consume seeds (SM Fig. 4-1c-d). In contrast, Larvae-1 was found enclosed within developing seeds, suspended within a white coloured fluid when smaller than the size of developing seeds (SM Fig. 4-1e), before growing to fill the mature seed entirely (Fig. 4-2c; SM Fig. 4-1f-h). A single individual of Larvae-1 appeared to develop within a single seed only, and was not found present with large amounts of frass (Fig. 4-2c; SM Fig. 4-1f-h). This compares with Larvae-2 which leaves predated seeds overloaded with frass (SM Fig. 4-1b). Thus, the predation of seeds with entry/exit holes and abundant frass was attributed to Larvae-2. Seeds which contained small individuals of Larvae-1 or Larvae-2 but contained no other contents (either endosperm or large amounts of frass) were considered to have aborted during maturation, with the larvae within having died due to starvation (SM Fig. 4-1j).

To measure differences between treatments in the number of developing (viable + predated + unfilled) seeds per fruit (Prediction 3), and the number of viable + predated seeds per fruit (Prediction 4), we divided the total number of developing seeds or viable + predated seeds sampled per plant by the number of fruits sampled per plant. Thus, we obtained the mean number of developing seeds per fruit, and the mean number of viable + predated seeds per fruit for each plant. To avoid confusion, note that the mean number of developing seeds per fruit per plant refers to the mean number of developing seeds per fruit (a measure calculated for each individual plant) averaged across plants. Similarly, the mean number of viable + predated seeds per fruit per plant refers to the mean number of viable + predated seeds per fruit averaged across plants. We also calculate and present the mean seed number per fruit per plant for viable, predated, and unfilled seeds separately, for qualitative comparison.

Fig. 4-2 (**a**) Developing seed of *H. exutiacies* graded as viable. (**b**) Endosperm contained within the prior seed. (**c**) Developing seed with an individual of Larvae-1 (an arbitrary title given to this morphologically distinct larvae). (**d**) Individual of Larvae-2 (an arbitrary title given to this morphologically distinct larvae).

4.3.7 Fruits and seeds from untagged flowers

Due to the low number of fruits produced from tagged flowers (i.e., due to low fruit-set), a random sample of fruits which had developed from untagged flowers was also sampled from watered and unwatered plants on the 11/12/2019 for MOCP, and 03/12/2019 for WSCP, respectively. Seeds within these fruits were likewise graded as viable, predated, and unfilled. This sample was used as an additional dataset to analyse the impact of watering on seed production per fruit. However, in contrast to fruits derived from tagged flowers, the length of time for which seeds within fruits from untagged flowers had been developing is less clear. This may cause problems for interpretation. For example, some fruits from untagged flowers may have been derived from flowers produced after the watering treatment had finished. Although, given flowering was mostly finished by the last day of watering, it is assumed that fruits from untagged flowers are likely derived from a period in which some level of watering treatment was occurring. However, the length of time each of these fruits experienced this increased watering cannot be determined. Thus, each of the two samples of fruits were analysed separately. Seed numbers were averaged per plant as described above.

4.3.8 Statistical analysis

All statistical models were run within the program R (R Core Team 2020). Linear and generalised linear mixed-effect models (LMM and GLMM, respectively) were constructed within the packages ‘lme4’ (Bates et al. 2015) and ‘glmmTMB’ (Brooks et al. 2017), respectively. Diagnostic checks of mixed-effect models were performed within the package ‘DHARMa’ (Hartig 2021). Statistical significance of predictors in (G)LMM’s were tested via an Analysis of Deviance, using a Type II F-test with Kenward-Roger corrected degrees of freedom for LMM’s, and a Type II Wald Chi-square test for GLMM’s (implemented in package ‘car’; Fox and Weisberg 2019). Multiple comparisons were performed via the package ‘multcomp’ (Hothorn et al. 2008a), with a Bonferroni correction used to account for inflated Type I error. Fisher-Pitman permutation tests were implemented via the package ‘coin’ (Hothorn et al. 2008b). Figures were constructed via the packages ‘ggplot2’ (Wickham 2016) and ‘tmap’ (Tennekes 2018). Lastly, due to the considerable differences between MOCP and WSCP in rainfall received and the number of flowers produced, we analysed each sample site individually.

4.3.9 Soil moisture

To assess if watering altered soil moisture across the experimental period, an LMM, constructed for each sample site, was used to test if soil moisture was explained by the predictors of treatment, the number of days since the first water treatment was applied, and their interaction. The number of days since the first water treatment was applied was treated as a categorical variable, with nine levels for each date on which soil moisture was measured (12/09/2019 - 08/11/2019 for MOCP, and 13/09/2019 - 09/11/2019 for WSCP, respectively). Plant pairing was considered a blocking factor within the experimental design and treated as a random effect. Plants were nested within each plant pairing to account for repeated measures of individual plants over time. Thus, this model design was equivalent to that of a repeated measure ANOVA. However, no variance was attributed to plant pairing in WSCP (i.e., variance of the specified random effect was singular). Thus, plant pairing was removed from the random effect structure, with only plants implicitly nested in each plant pairing included in the random effect structure. The response variable of soil moisture was also square root transformed for plants in WSCP to improve fit of model residuals and meet model assumptions.

We next analysed the subset of plant pairings, separately for each sample site, for which soil moisture was measured 30 minutes after watering of their respective treatment plants. To construct an appropriate response variable, soil moisture was measured at the beginning and end of a 30-minute period for both control and treatment plants. Plants in the watering treatment group were watered at the beginning of the 30-minute period. The soil moisture value at the beginning of the period was subtracted from the value at the end of the period (i.e., after 30 minutes) for each plant. For each sample site, the response variable was analysed by an LMM with the predictors of treatment, the number of days since the first water treatment was applied, and their interaction. The number of days since the first water treatment was applied was treated as a categorical variable, with two levels for the two dates on which additional soil moisture measurements were recorded (26/09/2019 and 08/11/2019 for MOCP, 27/09/2019 and 09/11/2019 for WSCP, respectively). Again, no variance was attributed to plant pairing in WSCP and only plant was included within the random effect structure.

4.3.10 Number of flowers and flowering phenology (Prediction 1)

The number of flowers per plant over the experimental period was modelled via a Poisson GLMM. The predictors included treatment, the number of days since the first water treatment was applied, and their interaction. Here, the number of days since the first water treatment was applied was treated as a continuous variable, with a second-order polynomial term included, due to the obvious quadratic nature of the response (see Fig. 4-6 in the Results). Plant pairing and plants nested within each plant pair were included as random effects. A significant interaction between the number of days since the first water treatment was applied and treatment would suggest the impact of watering on flower number is dependent on date (e.g., as would be the case if there was a shift in flowering phenology between watered and control plants). For both sample sites, hurdle models were implemented to account for zero-inflation (Martin et al. 2005; Zeileis et al. 2008), with predictors and random effects from the conditional model also included in the zero component of the model. Due to overdispersion, a negative binomial distribution (Zeileis et al. 2008; Lindén and Mäntyniemi 2011), rather than a Poisson distribution, was ultimately used to model flower number in WSCP.

4.3.11 Fruit-set (Prediction 2)

For each sample site, the fruit-set of control (unwatered) and treatment (watered) plants was compared by a Binomial GLMM. Both models included treatment as the predictor and plant pairing as the sole random effect. We also compared the fruit-set of control plants between MOCP and WSCP with a Binomial GLM. Thus, a statistically significant difference would suggest natural levels of fruit-set also differed for unwatered plants between sample sites.

4.3.12 Developing seeds per fruit (Prediction 3)

The mean number of developing seeds per fruit was compared between treatments within each sample site by a Fisher-Pitman permutation test. For each sample site, this test was done twice, once for those fruits sampled from tagged flowers, and again for those fruits sampled from untagged flowers. Fisher-Pitman permutation tests were used due to the non-normal distribution of the response variable.

4.3.13 Viable + predated seeds per fruit (Prediction 4)

Fisher-Pitman permutation tests were used to compare the mean number of viable + predated seeds per fruit between treatments. However, permutational tests can also be sensitive to differences in variance, resulting in possible rejection of the null hypothesis, even though the overall mean of the response variable between groups does not differ (Quinn and Keough 2002 p. 46). Thus, a square root transformation was used on the mean number of viable + predated seeds per fruit, as calculated from those fruits sampled from tagged flowers, to produce similar distributions between control and watered plants. This was done as we were specifically interested in a change in the of average of the mean number of seeds per fruit (i.e., mean seed number per fruit per plant) between treatments rather than a change in their variance.

4.3.14 Pre-dispersal seed predation (Prediction 5)

We used data from open-pollinated plants tagged in 2017, a year of high pre-dispersal seed predation, to qualitatively compare levels of predation between 2017 and 2019. Data were available for a total of 176 fruits sampled from 60 open-pollinated plants in 2017 (Chapter Three). These fruits were produced by flowers tagged between mid-October to early November, from eight sample sites located across seven reserves including plants in the sample sites of MOCP and WSCP used in the present study. We compared these data from 2017 with unwatered control plants in 2019.

4.4 RESULTS

4.4.1 Rainfall and soil moisture

MOCP received more rainfall from the first day of watering until retrieval of tagged flowers than WSCP (~107.00 mm versus ~60.00 mm, respectively), suggesting plants in MOCP experienced wetter conditions than plants in WSCP. Thus, relative to their unwatered controls, the additional 1 L of water per week over nine weeks resulted in ~34 % higher total water input (i.e., watering + rainfall) per treatment plant in MOCP, and ~60 % higher total water input per treatment plant in WSCP, respectively. Temporal variation in mean soil moisture per plant per treatment within each sample site appeared to reflect the pattern of rainfall received in the week prior to the reading of soil moisture of plants, suggesting soil moisture of both watered plants and control plants was predominantly controlled by natural rainfall (SM Fig. 4-2). In support, there was no significant interaction between treatment and the number of days since the first water treatment was applied or a main effect of treatment on mean soil moisture per plant in both MOCP and WSCP, respectively (Table 4-1). Thus, watering did not result in measurable differences in soil moisture between watered and control plants averaged over the course of the experimental period (i.e., the nine weeks of watering). However, mean soil moisture per plant, pooled across treatments, differed significantly between the days on which soil moisture was measured in each sample site (Table 4-1 and Fig. 4-3). Thus, soil moisture significantly fluctuated similarly for both watered and control plants across the experimental period in each sample site. Lastly, pooled across treatments, mean soil moisture per plant across the experimental period was significantly higher for plants in MOCP versus WSCP (*t*-test: t = 3.946, df = 38, $p = 3.316 \times 10^{-4}$; Fig. 4-4a). Thus, plants in MOCP experienced consistently wetter conditions than plants in WSCP. This also held true when the final soil moisture measurements for MOCP, which were taken during active rainfall (see last soil moisture measure in Fig. 4-3a), were not used in calculating mean soil moisture per plant (*t*-test: t = 2.155, df = 38, p = 0.038).

In contrast, the percentage soil moisture of treatment plants clearly increased following watering relative to readings taken 30 minutes earlier, whereas no difference was observed for control plants across both sample sites (Fig. 4-5). Thus, there was a statistically clear treatment effect of watering in the short-term, with soil moisture increasing for watered plants in MOCP and WSCP, respectively (Table 4-1). There was also a significant main effect of day on the mean percentage difference in soil moisture in MOCP (Table 4-1). The mean percentage difference in soil moisture, pooled across treatments, was lower the second date measurements were taken in MOCP (Fig. 4-5). This can be explained by the already high values of soil moisture for plants on this particular day, which was the last day of watering for MOCP (see Fig. 4-3a).

Table 4-1 Models of the effect of treatment (watering versus unwatered) and the number of days since the first water treatment was applied (Days) on plants of *Hibbertia exutiacies* in the samples sites of Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP). Significant results highlighted in bold.

Sample Site	Model	Response	Predictors	Test Statistic	*p-value*
MOCP	Gaussian	Soil moisture per plant	Days[a]	$F_{(8,\,144)} = 86.149$	**$< 2 \times 10^{-16}$**
			Treatment	$F_{(1,\,9)} = 1.499$	0.2519
			Days × Treatment	$F_{(8,\,144)} = 0.436$	0.8979
	Gaussian	Difference in soil moisture per plant before and 30 min. after watering	Days[b]	$F_{(1,\,8)} = 7.255$	**0.027**
			Treatment	$F_{(1,\,4)} = 23.372$	**0.008**
			Days × Treatment	$F_{(1,\,8)} = 4.508$	0.066
	Truncated Poisson (Hurdle Model)	Flowers per plant	Poly(Days)[c]	$\chi^2_2 = 220.39$	**$< 2 \times 10^{-16}$**
			Treatment	$\chi^2_1 = 0.004$	0.950
			Poly(Days) × Treatment	$\chi^2_2 = 34.696$	**2.924×10^{-08}**
		Probability of zero flowers per plant	Poly(Days)[c]	$\chi^2_2 = 31.098$	**1.767×10^{-07}**
			Treatment	$\chi^2_1 = 0.855$	0.355
			Poly(Days) × Treatment	$\chi^2_2 = 7.727$	**0.021**
WSCP	Gaussian	(Soil moisture per plant)^0.5	Days[a]	$F_{(8,\,144)} = 72.440$	**$< 2 \times 10^{-16}$**
			Treatment	$F_{(1,\,18)} = 2.187$	0.157
			Days × Treatment	$F_{(8,\,144)} = 0.153$	0.996
	Gaussian	Difference in soil moisture per plant before and 30 min. after watering	Days[b]	$F_{(1,\,8)} = 0.142$	0.716
			Treatment	$F_{(1,\,8)} = 22.034$	**0.002**
			Days × Treatment	$F_{(1,\,8)} = 1.220$	0.302
	Truncated Negative Binomial (Hurdle Model)	Flowers per plant	Poly(Days)[c]	$\chi^2_2 = 109.383$	**$< 2 \times 10^{-16}$**
			Treatment	$\chi^2_1 = 0.059$	0.808
			Poly(Days) × Treatment	$\chi^2_2 = 26.970$	**1.392×10^{-06}**
		Probability of zero flowers per plant	Poly(Days)[c]	$\chi^2_2 = 37.630$	**6.742×10^{-09}**
			Treatment	$\chi^2_1 = 0.002$	0.963
			Poly(Days) × Treatment	$\chi^2_2 = 5.173$	0.075

[a] Categorical predictor with nine levels for each date on which soil moisture was measured (12/09/2019 - 08/11/2019 for MOCP and 13/09/2019 - 09/11/2019 for WSCP).

[b] Categorical predictor with two levels for the two dates on which additional soil moisture measurements were recorded (26/09/2019 and 08/11/2019 for MOCP and 27/09/2019 and 09/11/2019 for WSCP).

[c] Continuous predictor modelled with a second-order polynomial.

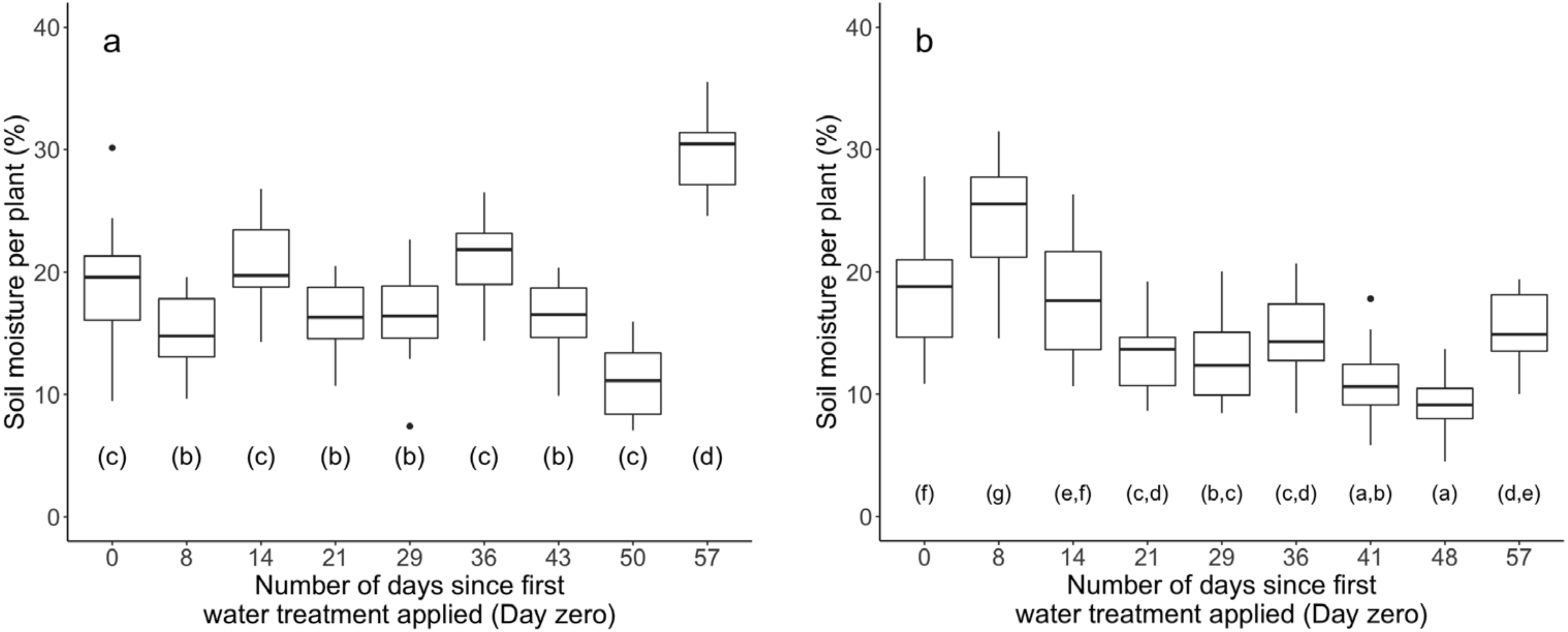


Fig. 4-3 Soil moisture per plant, pooled across treatments, for each day of watering in sample sites located within (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. Boxplots of soil moisture sharing the same letter indicate plants on each of these days were not statistically different from one another in mean soil moisture. Soil moisture was measured for all plants prior to the watering treatment being applied for each day of measurement. The first watering treatment was applied on day zero.

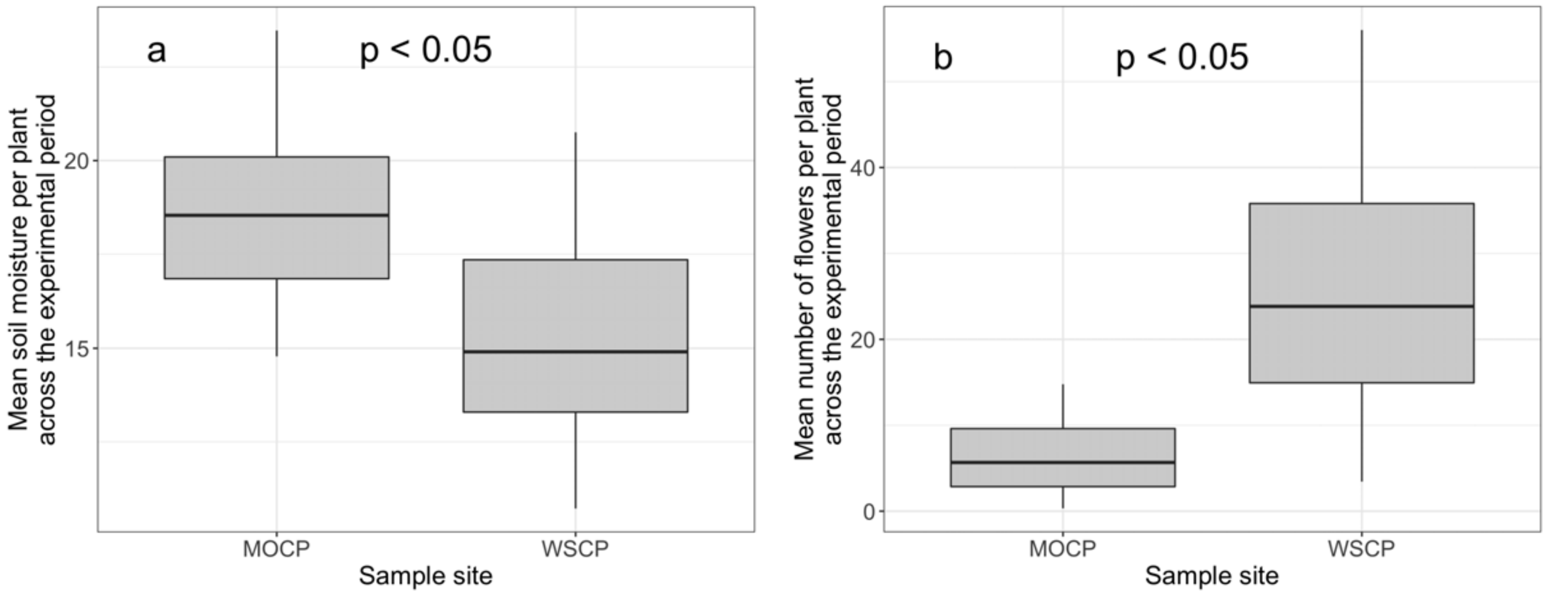


Fig. 4-4 (**a**) Mean soil moisture per plant across the experimental period (i.e., averaged across the nine weeks of watering for each plant) and pooled across treatments for sample sites within Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP), respectively. (**b**) Mean flower number per plant across the experimental period and pooled across treatments for sample sites located within MOCP and WSCP, respectively.

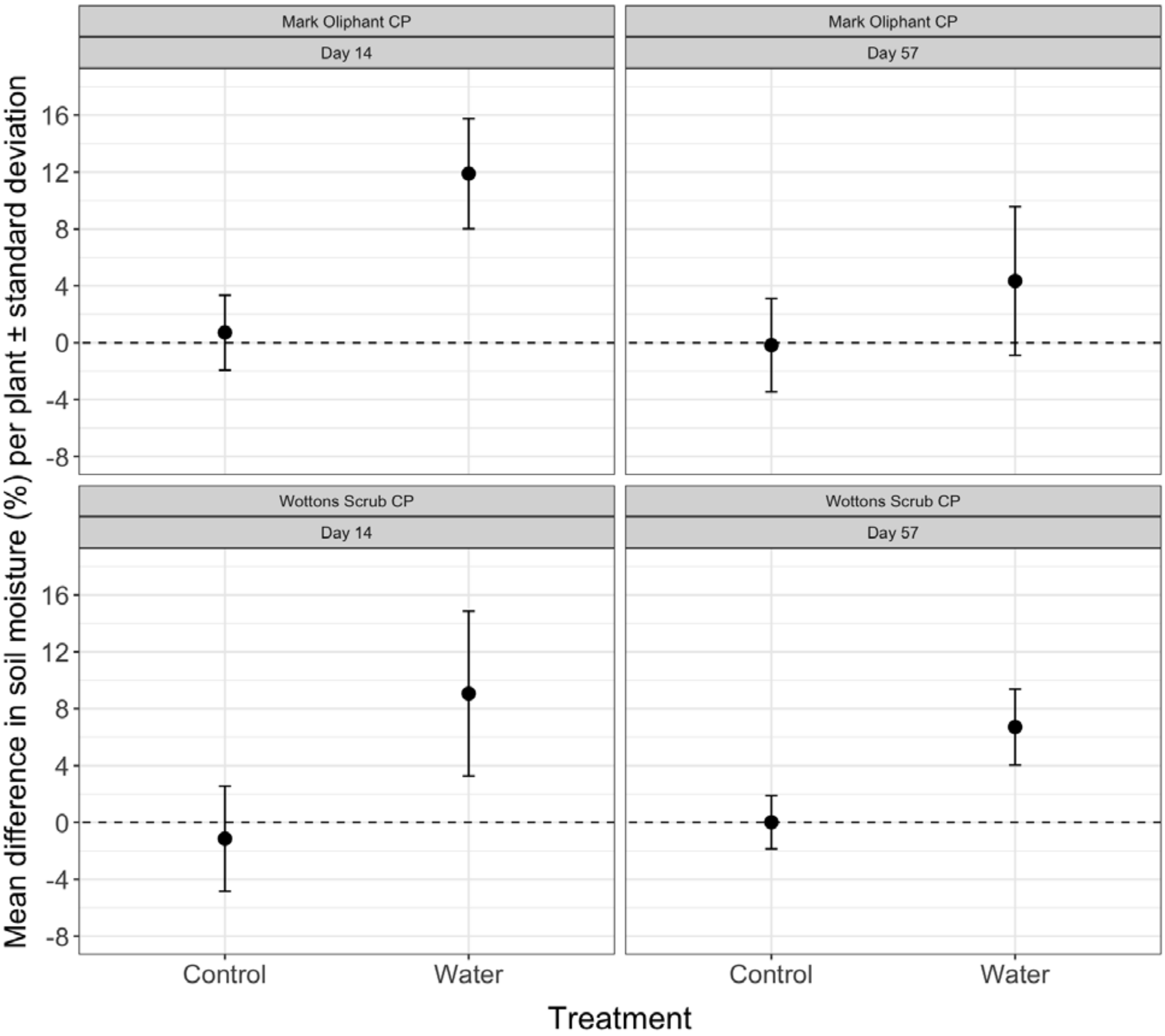


Fig. 4-5 Mean difference in soil moisture per plant relative to soil moisture recorded 30 minutes earlier, ± standard deviation. The difference in soil moisture was recorded for the same 5 plants in each treatment, across each sample site by day combination. Watered plants received 1 L of water immediately following their first measure of soil moisture, whereas control plants remained unwatered.

4.4.2 Number of flowers and flowering phenology (Prediction 1)

Peak flowering was earlier for WSCP (04/10/2019 - 1530 flowers) than for MOCP (11/10/2019 - 397 flowers). There was also a conspicuous difference in the number of flowers produced between sample sites (Fig. 4-4b and Fig. 4-6), with a mean ± standard deviation over the experimental period of 6.33 ± 4.37 flowers per plant in MOCP, versus 26.9 ± 15.20 flowers in WSCP, for plants pooled across treatments. This difference was statistically significant (Welch *t*-test: $t = 3.946$, df = 37.01, $p = 3.418 \times 10^{-4}$). Moreover, in both MOCP and WSCP, there was a statistically significant interaction between the number of days since the first water treatment was applied and treatment on the number of flowers per plant (Table 4-1). Thus, the difference in the average number of flowers per plant between watered and unwatered plants varied across the experimental period in both MOCP (Fig. 4-6a) and WSCP (Fig. 4-6b). Likewise, there was an interaction between these two predictors on the probability of plants having zero flowers in MOCP (Table 4-1). Thus, the probability of plants having zero flowers across the experimental period was different between control and watered plants in MOCP. In contrast, there was only a main effect of the number of days since the first water treatment was applied on plants having zero flowers in WSCP (Table 4-1). Thus, the probability of plants having zero flowers across the experimental period was similar between control and watered plants in WSCP.

These statistical interactions suggested there was a detectable shift in flowering phenology between watered and control plants. This was also visually evident in plots of the data. In MOCP, control plants had a higher number of flowers earlier in the flowering season compared to watered plants (Fig. 4-6a). Specifically, few watered plants had begun to flower until the fourth round of watering was applied in MOCP (i.e., day 21), compared to control plants (Fig. 4-6a). In contrast, control and treatment plants began flowering at roughly the same time in WSCP (Fig. 4-6b). Watered and control plants both reached peak flowering at the same time in MOCP (Fig. 4-6a) and WSCP (Fig. 4-6b). However, later in the season, watered plants typically had a greater number of flowers than control plants in both MOCP (Fig. 4-6a) and WSCP (Fig. 4-6b). Compared to MOCP, control plants in WSCP also had a considerably higher average number of flowers at peak flowering, and immediately after, versus watered plants (Fig. 4-6b). However, ultimately, there was no statistically clear difference between treatments in the mean number of flowers per plant across the experimental period in both MOCP (*t*-test: $t = 0.194$, df = 18, $p = 0.848$) and WSCP (*t*-test: $t = 0.908$, df = 18, $p = 0.376$). Thus, watering did not increase the average number of flowers produced by plants over the experimental period, but only resulted in differences in the flowering phenology of watered versus unwatered control plants of *H. exutiacies* (Fig. 4-6; also see SM Fig. 4-3).

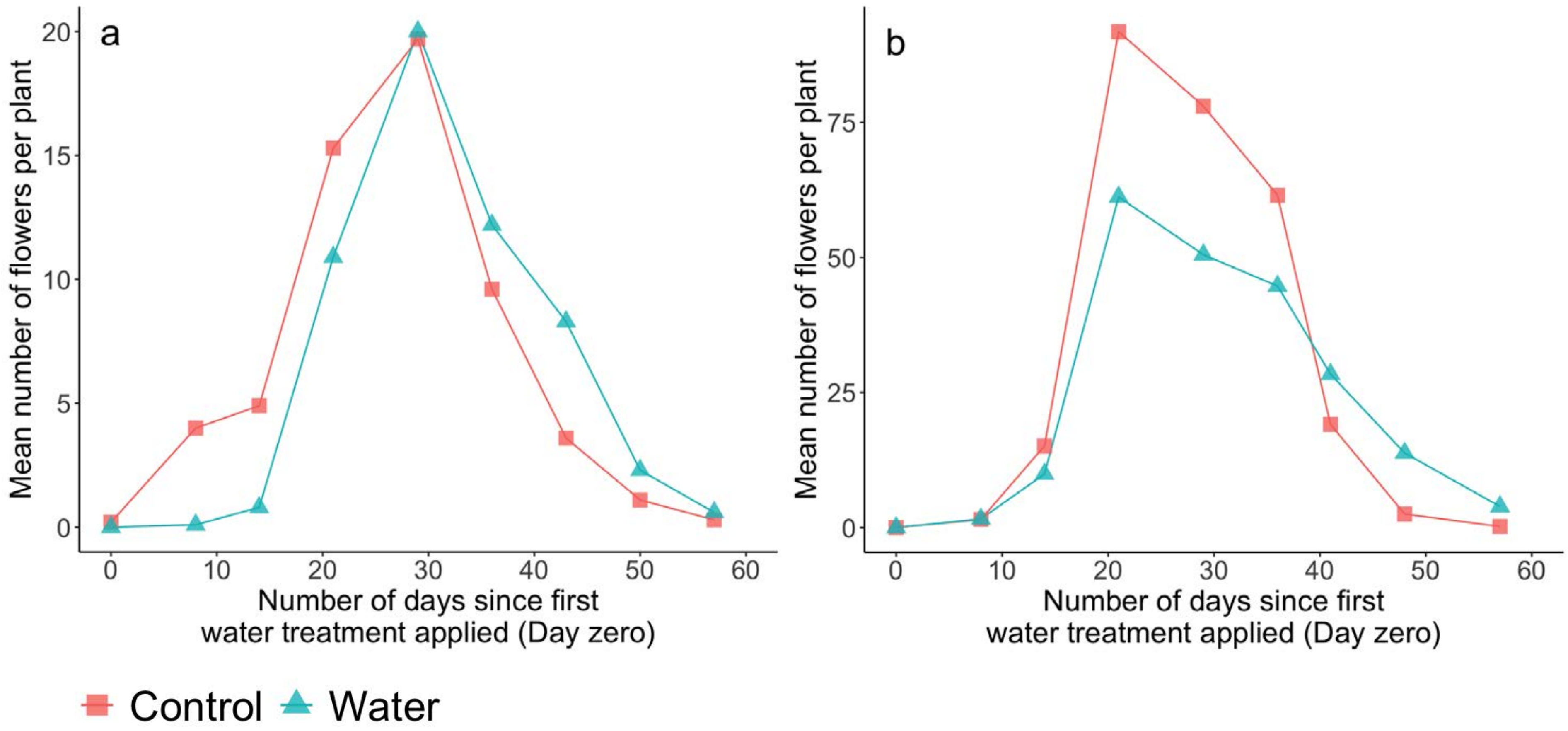


Fig. 4-6 The mean number of flowers per plant per treatment for each day of watering in the sample sites located in (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. The first watering treatment was applied on day zero. Note that different scales are used for each y-axis.

4.4.3 Fruit-set (Prediction 2)

The overall proportion of flowers which produced fruits was greater for watered plants versus control plants in both MOCP and WSCP (Fig. 4-7). However, the magnitude of this effect was clearly larger in WSCP (0.24 in watered versus 0.11 in control) than MOCP (0.28 in watered versus 0.24 in control). As such, watering only significantly increased fruit-set in WSCP (Table 4-2). Moreover, comparing the fruit-set of control plants between MOCP and WSCP, fruit-set was significantly higher for control plants in MOCP (Binomial GLM: χ^2_1 = 6.578, *p* = 0.020). Thus, natural levels of fruit-set were higher in the wetter sample site of MOCP.

The overall fruit-set of treatment plants in WSCP was also similar to that of control plants in MOCP (Fig. 4-7), and thus watering of plants in the drier sample site of WSCP appeared to result in a similar overall fruit-set to that of unwatered plants in the wetter sample site of MOCP.

4.4.4 Developing seeds per fruit (Prediction 3)

For fruits derived from tagged flowers, there was no effect of treatment on the mean number of developing seeds per fruit per plant in MOCP (*Z* = 0.324, *p* = 0.785) or WSCP (*Z* = 0.6158, *p* = 0.638). Likewise, for fruits derived from untagged flowers, there was no effect of treatment on the mean number of developing seeds per fruit per plant in MOCP (*Z* = 0.538, *p* = 0.604) or WSCP (*Z* = 1.134, *p* = 0.289). Thus, there was no evidence that watered plants had a higher number of developing seeds per fruit compared to unwatered controls (Table 4-3).

4.4.5 Viable + predated seeds per fruit (Prediction 4)

Similarly, for fruits derived from tagged flowers, there was no effect of treatment on the mean number of viable + predated seeds per fruit per plant in MOCP (*Z* = -0.469, *p* = 0.722) or WSCP (*Z* = -0.591, *p* = 0.658). Likewise, for fruits derived from untagged flowers, there was no effect of treatment on the mean number of viable + predated seeds per fruit per plant in MOCP (*Z* = 1.376, *p* = 0.172) or WSCP (*Z* = 0.275, *p* = 0.816). Thus, there was no evidence that watered plants had a higher number of viable + predated seeds per fruit compared to unwatered controls (Table 4-3). This suggests watering had no impact on the provisioning of seeds within fruits. In support, the mean number of unfilled seeds per fruit per plant was low for both watered and unwatered controls (Table 4-3).

4.4.6 Pre-dispersal seed predation (Prediction 5)

Pooled across MOCP and WSCP, 112 fruits (derived from both tagged and untagged flowers) from 20 control plants were opened and their seeds examined in 2019 (Table 4-3). Of these 112 fruits, 85 (75.89 %) contained at least one predated seed. Thus, the majority of fruits suffered attack from pre-dispersal seed predators in 2019. Likewise, out of 281 developing seeds examined for predation, 184 (65.48 %) were predated. Thus, the majority of seeds within fruits suffered attack from pre-dispersal seed predators. Of these 184 predated seeds, 138 (75.00 %) were predated by individuals of Larvae-1 (Fig. 4-2c), while 46 (25.00 %) were predated by Larvae-2 (Fig. 4-2d). Both larval types also appeared to avoid each other. Only three out of a 112 fruits (2.68 %) contained individuals (or evidence) of both larval types. Thus, fruits were mostly predated by one larval type or the other. Moreover, there was a significant negative relationship between the proportion of fruits with seeds predated by Larvae-1 per plant and the proportion of fruits with seeds predated by Larvae-2 per plant (Spearman's rho (r_s) = -0.698, $p = 6.254 \times 10^{-4}$, $n = 20$). This negative relationship also held when watered plants were included ($r_s = -0.557$, $p = 1.862 \times 10^{-4}$, $n = 40$; Fig. 4-8a).

The results observed in 2019 were qualitatively comparable to those previously observed in 2017. Thus, in 2017, pooled across plants and reserves, 67.05 % (118/176) of fruits had at least one predated seed. Similarly, 60.51 % (190/314) of seeds suffered predation, with 80.00 % (152/190) of predated seeds attacked by individuals of Larvae-1 versus 20.00 % (38/190) attacked by Larvae-2. Again, both larval types appeared to avoid each other, with only 6.25 % (11/176) of fruits with individuals (or evidence) of both larval types. For those plants with a minimum one fruit suffering seed predation, there was a significant negative relationship between the proportion of fruits with seeds predated by Larvae-1 per plant and the proportion of fruits with seeds predated by Larvae-2 per plant ($r_s = -0.444$, $p = 0.001$, $n = 50$; Fig. 4-8b). Using data only for the sample sites of MOCP and WSCP in 2017, WSCP had a greater percentage of seeds predated at 76.36 % versus 65.85 % in MOCP, respectively. This pattern was also similar in 2019, with 70.76 % seeds predated in WSCP versus 57.27 % of seeds in MOCP, respectively.

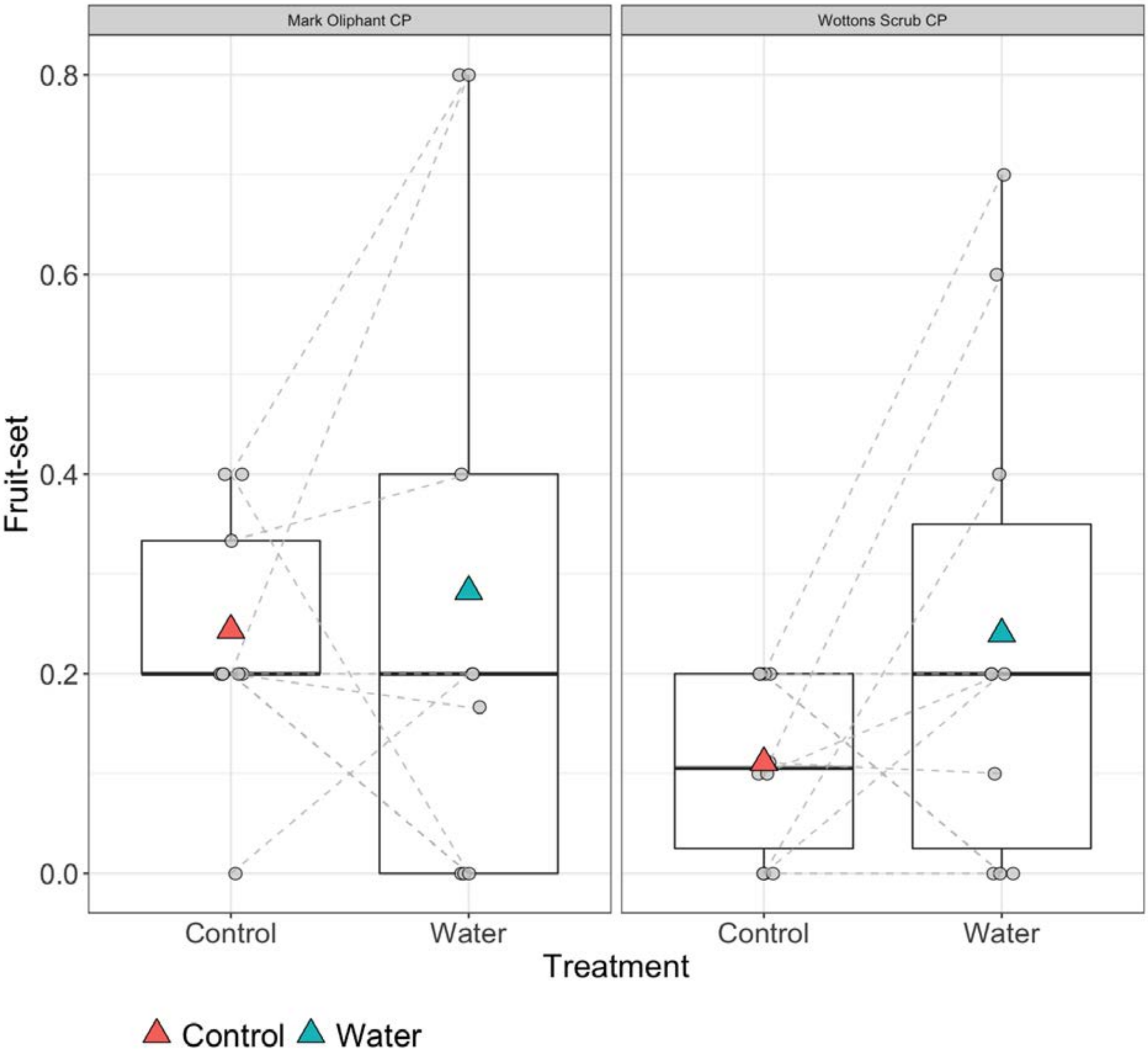


Fig. 4-7 Fruit-set of tagged plants of *Hibbertia exutiacies* which were either provided with an additional 1 L of water, one day a week, for nine weeks (Water), or were unwatered (Control), in sample sites located within (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. Coloured triangles represent the proportional number of flowers which set fruit, pooled across plants within each sample site by treatment combination (i.e., population proportion). The fruit-set of each individual plant is also plotted (grey datapoints). Dashed lines connect plants within the same plant pairing (i.e., similar sized control and watered plants growing within 5 m of one another).

Table 4-2 The effect of watering on the fruit-set of control and treatment plants of *Hibbertia exutiacies* in the sample sites of Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP). The random intercept term for each model is presented along with their standard deviation. The intercept and slope values of the fixed effect, on the scale of the link function, are provided. The intercept represents the estimated response of the control, with the slope the estimated difference between the control and watering treatment. 95 % confidence intervals (Wald) are provided in brackets. Significant results highlighted in bold.

Sample Site	Response	Predictor	Random effect	Model	Intercept	Slope	Test statistic	*p-value*
MOCP	Fruit-set	Watering Treatment	Plant Pair: 0.545	Binomial	-1.210 (-2.05, -0.37)	0.219 (-0.77, 1.21)	χ_1^2 = 0.189	0.664
WSCP	Fruit-set	Watering Treatment	Plant Pair: 0.700	Binomial	-2.269 (-3.08, -1.45)	0.996 (0.19, 1.80)	χ_1^2 = 5.843	**0.016**

Table 4-3 The number of plants and fruits sampled per sample site in Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP) for control (unwatered) and treatment (water) plants of *Hibbertia exutiacies*. The mean number, ± standard deviation, of developing, viable + predated, viable, predated, and unfilled seeds per fruit per plant for each treatment is provided. The total number of seeds within each of these categories is provided in brackets. Values of the mean number of developing and viable + predated seeds per fruit per plant sharing the same superscript are not statistically different from one another within the same sample by sample site combination.

Sample	Sample Site	Treatment	No. of plants sampled[1]	No. of fruits opened	Developing	Viable + Predated	Viable	Predated	Unfilled
Fruit-set Experiment (i.e., tagged flowers)	MOCP	Control	8	10	2.38 ± 1.09^{a} (23)	1.75 ± 1.16^{b} (15)	0.50 ± 1.41 (4)	1.25 ± 0.89 (11)	0.63 ± 1.03 (8)
		Water	6	13	2.21 ± 0.81^{a} (31)	1.83 ± 0.83^{b} (28)	0.21 ± 0.40 (2)	1.62 ± 0.89 (26)	0.38 ± 0.49 (3)
	WSCP	Control	7	11	1.86 ± 1.07^{a} (21)	1.57 ± 1.13^{b} (19)	0.50 ± 0.58 (6)	1.07 ± 0.98 (13)	0.29 ± 0.49 (2)
		Water	7	24	1.60 ± 0.35^{a} (40)	1.60 ± 0.35^{b} (40)	0.26 ± 0.25 (7)	1.35 ± 0.37 (33)	0.00 ± 0.00 (0)
Additional Sample (i.e., untagged flowers)	MOCP	Control	10	43	2.16 ± 0.52^{a} (88)	1.92 ± 0.65^{b} (70)	0.43 ± 0.49 (18)	1.50 ± 0.76 (52)	0.23 ± 0.28 (17)
		Water	10	70	2.02 ± 0.67^{a} (144)	1.49 ± 0.72^{b} (94)	0.20 ± 0.21 (13)	1.29 ± 0.80 (81)	0.53 ± 0.63 (50)
	WSCP	Control	10	48	3.10 ± 0.61^{a} (151)	2.62 ± 0.75^{b} (127)	0.38 ± 0.47 (19)	2.24 ± 0.52 (108)	0.46 ± 0.52 (23)
		Water	10	47	2.75 ± 0.75^{a} (130)	2.53 ± 0.75^{b} (119)	0.36 ± 0.47 (15)	2.17 ± 0.60 (104)	0.18 ± 0.31 (9)

[1] For the fruit-set experiment includes only those tagged plants which produced fruits.

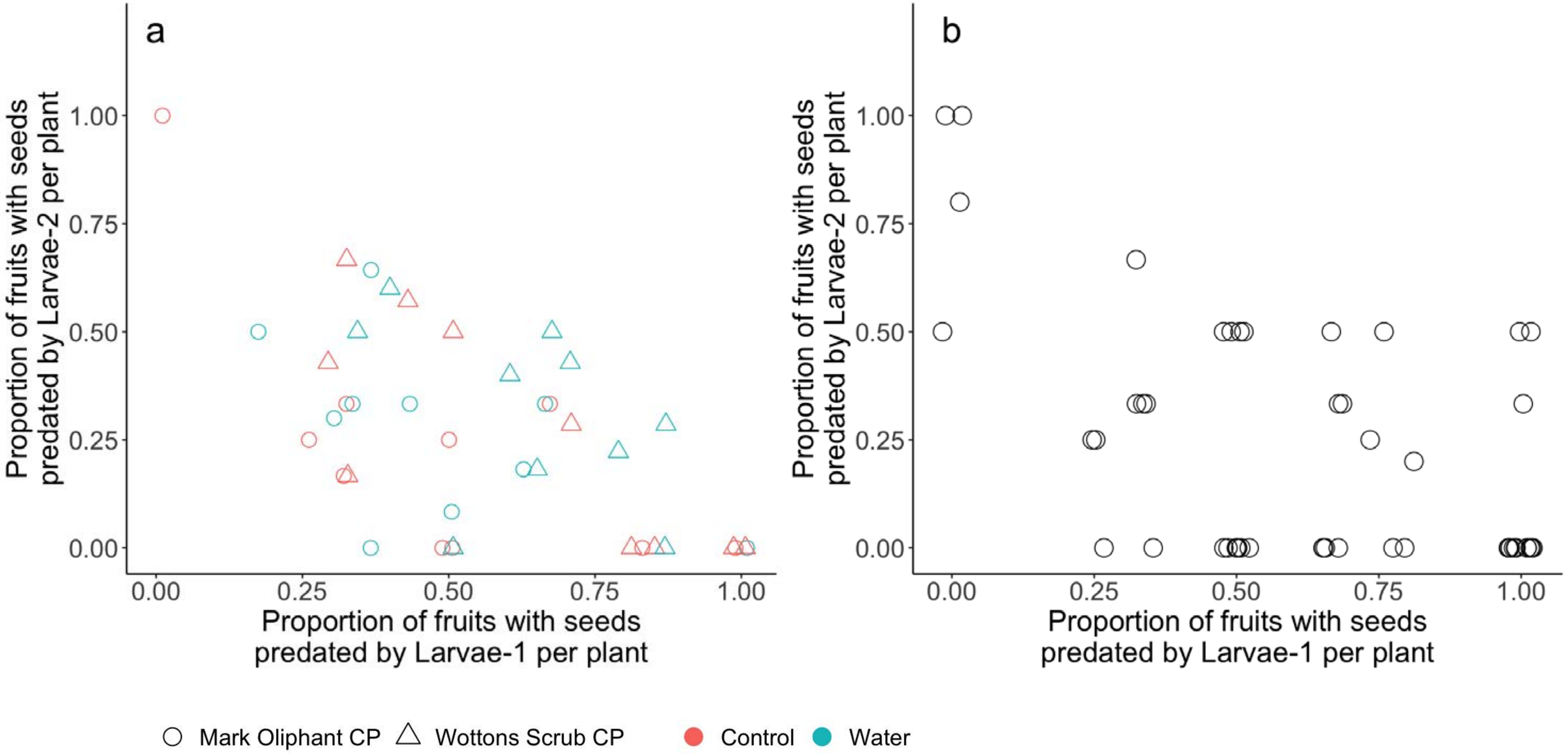


Fig. 4-8 The proportion of fruits sampled per plant which contained at least one seed predated by either Larvae-1 or Larvae-2 (arbitrary titles given to morphologically distinct larvae). Each plotted point represents a single plant. (**a**) Results for plants sampled in two sample sites within the reserves of Mark Oliphant CP (MOCP) and Wottons Scrub CP (WSCP) for both watered and unwatered control plants in 2019. (**b**) Results for unmanipulated plants sampled across seven different reserves in 2017. Ten plants with no predated seeds were excluded from plot (**b**).

4.5 DISCUSSION

Although watering had a measurable short-term impact on soil moisture (Fig. 4-5), only the fruit-set of treatment plants in the drier sample site of WSCP increased after experimental watering (Fig. 4-7). Thus, the prediction that watering would increase fruit-set was supported in only one of two sample sites. However, seed abortion, although prevalent the previous year (2018), was not particularly frequent in 2019, with the majority of seeds within fruits of both watered and unwatered plants suffering predation. Consequently, even though fruit-set was greater for watered plants in WSCP, these plants did not produce an appreciably higher total number of viable seeds from their tagged flowers, due to high rates of seed predation (Table 4-3).

4.5.1 Rainfall

A measurable impact of increased water availability on the reproductive variables of flower number, fruit-set, and production of developing and viable + predated seeds is likely dependent on the extent to which plants are already limited by water availability. Thus, we would expect our watering treatment to have a stronger effect when water availability is already low. In comparison to 2017 and 2018, both sample sites experienced less rainfall from the first day of soil moisture measurements until retrieval of tagged flowers. Specifically, MOCP received an estimated 153.3 mm of rainfall in 2018 (although 41.7 mm was recorded for a single day in late November and much of this may have been run-off) and 141.7 mm in 2017, versus 106.6 mm in 2019 (SM Fig. 4-4a). Similarly, WSCP received an estimated 153.2 mm of rainfall in 2018 (likewise 45.3 mm was recorded for a single day in late November) and 121.6 mm in 2017, versus only 60.0 mm in 2019 (SM Fig. 4-4b). However, regarding the month of September alone, which was suggested as a critical period in terms of the effect of water stress on the reproduction of *H. exutiacies*, September 2018 experienced the lowest recorded rainfall (36.5 mm and 33.8 mm for MOCP and WSCP, respectively) versus 2019 (55.2 mm and 59.8 mm) and 2017 (137.3 mm and 122.2 mm; SM Fig. 4-4). However, September rainfall in 2019 was still below the average of September rainfall since 2001 in both MOCP (mean: 97.9 mm) and WSCP (mean: 102 mm), respectively. Thus, reduced water availability may be expected to impact the reproduction of *H. exutiacies* under these relatively drier conditions.

4.5.2 Soil moisture

The applied watering treatment did not result in an overall higher soil moisture of treatment plants versus their unwatered controls averaged across the nine weeks of watering in MOCP and WSCP. Rather, soil moisture per plant within each sample site appeared to reflect levels of recent rainfall (SM Fig. 4-2). However, although watering did not increase soil moisture of plants over the course of the experiment, the percentage soil moisture of treatment plants was significantly higher when measured 30 minutes after watering (Fig. 4-5). Thus, watered individuals were at least gaining access to periods of relatively wetter soil moisture periodically throughout the flowering season.

4.5.3 Number of flowers and flowering phenology (Prediction 1)

Decreases in the number of flowers produced per plant (see review by Descamps et al. 2021) and altered flowering phenology (e.g., de Jong and Klinkhamer 1989) have been recorded for other wild plants under water-stress. However, regardless of the relatively dry conditions experienced by plants of *H. exutiacies* in 2019, no effect of watering was found on the average number of flowers produced by plants in either sample site across the experimental period. Thus, the prediction that watering would increase the number of flowers produced per plant was not supported. However, it should be noted that when watering treatments were first applied, plants already had a large number of flower buds present. Thus, it is possible watered plants had already invested a certain level of resources into flower production, and the subsequent level of watering applied was not enough to alter this investment. For the summer-flowering Mediterranean sub-shrub, *Helianthemum squamatum* (Cistaceae) only soil moisture prior to flowering was significantly related to flower production (Aragón et al. 2008). Hence, providing larger quantities of water to plants of *H. exutiacies*, or applying the watering treatment earlier, may have resulted in increased flower production.

In contrast to overall flower number, watering impacted the flowering phenology of *H. exutiacies*, with watered plants tending to begin to flower after their respective controls in MOCP, and have more flowers later into the season in both sample sites (Fig. 4-6). Specifically, we predicted that watering would extend the flowering period of *H. exutiacies*. This was supported in WSCP, where watered and control plants began to flower at the same time, but watered plants had more flowers later into the season (Fig. 4-6b). Moreover, five watered plants versus only one unwatered plant were still flowering on the final day of watering. Watered plants in MOCP also had more flowers later into the season. However, most watered plants also began flowering after their respective controls in MOCP. Hence, although watered plants had more flowers later into the season in MOCP, this was due to a shift in flowering phenology to also begin to flower later rather than an extension of flowering per se (Fig. 4-6a). Thus, our prediction was not completely supported in MOCP. A similar shift in flowering phenology was observed for watered plants of the monocarpic *Cynoglossum officinale* (Boraginaceae) in a sand dune habitat within the Netherlands, where watered plants flowered a few days after control plants and, subsequently, a few days later into the flowering season (de Jong and Klinkhamer 1989). In the present study, irrespective of delayed flowering in watered individuals of *H. exutiacies*, peak flowering was the same for both treatment and control plants in MOCP and WSCP, respectively. This is an observation also seen for *C. officinale* (de Jong and Klinkhamer 1989).

4.5.4 Fruit-set (Prediction 2)

Fruit-set (here defined as the proportion of flowers producing swollen carpel(s) containing developing seed(s)) was significantly higher for watered plants in the sample site of WSCP (Fig. 4-7). In contrast, because watering did not increase the fruit-set of plants in MOCP, fruit-set was not limited by soil moisture, at least for the amount of water we provided. Thus, the prediction that watering would increase fruit-set relative to unwatered plants was supported in only one of the two sample sites, indicating spatial variability in the degree of water stress between populations of *H. exutiacies* in the Adelaide Hills. The lack of a detectable effect of watering in MOCP matches the higher rainfall and soil moisture of plants in MOCP versus the drier sample site of WSCP. The mechanistic causes underlying the detrimental impacts of water stress on plant reproduction are complex and involve multiple processes from the cellular to the whole-plant level (see review by Farooq et al. 2009). However, there are at least two broad reasons why water stress may have limited fruit-set of *H. exutiacies* in WSCP.

First, assuming unwatered and watered plants received the same rate of visitation by pollinators, increased fruit-set with watering of plants in WSCP suggests that otherwise visited flowers of *H. exutiacies* were not producing fruits due to water-limitation. Certainly, water deficits have wide-ranging detrimental impacts on the processes of photosynthesis (Pinheiro and Chaves 2011), which limits the availability of resources for reproduction (i.e., photosynthetic carbon assimilates; Lemoine et al. 2013). Reduced transpiration rates under water stress limits nutrient uptake from the soil (Farooq et al. 2009), and water stress may impair active nutrient absorption and mechanisms of nutrient transport in the roots of plants (Tanguilig et al. 1987). In particular, the impacts of water stress on plant reproduction have been well studied for numerous cultivated crops. For example, Fang et al. (2010) observed that the fruit-set of two cultivars of the widely grown legume, *Cicer arietinum* (chickpea), declined under water stress. An important factor limiting fruit-set was the impaired ability of pollen tubes to reach the ovary in water stressed plants (Fang et al. 2010). Similarly, Hu et al. (2019) found that drought stress in *Gossypium hirsutum* (cotton) limited energy supply to the style, which limited pollen tube elongation, which ultimately results in reduced ovule fertilization. Even when ovules are fertilized, fruit development may still be aborted. For example, competition under water stress for limited resources between fertilized ovules results in greater fruit abortion in maize (Shen et al. 2019). Wild plants are likely better equipped against water stress than many cultivated crops (Johnson et al. 2000). Nevertheless, water stress is still a significant abiotic pressure on the growth, reproduction, and survival of wild plants (Chaves et al. 2002). Whether water stress impacts pollen-style interactions or increases competition between fertilized ovules for resources in *H. exutiacies* is unknown, and the exact physiological mechanism(s) behind increased fruit-set in watered plants of *H. exutiacies* requires further study. However, a mechanistic understanding of the impacts of water stress on *H. exutiacies* would certainly be of benefit in understanding how declining rainfall under climate change may impact plant reproduction. It should also be recognized that the water stress of paternal plants (i.e., pollen donors) may likewise impact pollen performance, reducing fruit-set of maternal plants (i.e., pollen receivers) regardless of their water status (Recart et al. 2019). However, we had no control over the water stress of pollen donors in our natural study system.

Second, water stress can also result in changes to floral morphology, such as flower size (Gallagher and Campbell 2017) and lower quantities of nectar and pollen (Waser and Price 2016). This may result in reduced floral visitation by pollinators (Höfer et al. 2021). The fertilization of *H. exutiacies* flowers requires pollinator visitation (Chapter Three), therefore, fruit-set is entirely dependent on flowers first being visited by pollinators. Hence, watered plants in WSCP may have been more attractive to the pollinators of *H. exutiacies*, resulting in higher flower visitation, allowing for higher fruit-set. This scenario does not necessarily imply the fruit-set of unwatered plants was pollen-limited, as watered plants may have had resources simultaneously increased for both floral attraction and the provisioning of subsequently visited flowers. Flowers of *H. exutiacies* are nectarless, providing pollen as the sole reward to their native bee buzz-pollinators (Chapter Three). Thus, if pollen quantity per flower declined under water stress, this could theoretically result in flowers of reduced attractiveness to pollinators. Sonicating bees of the North American buzz-pollinated perennial *Solanum elaeagnifolium* (Solanaceae) are able to assess pollen returns from that species' poricidal anthers (Buchmann and Cane 1989). If the pollinators of *H. exutiacies* can likewise assess pollen returns, they may preferentially visit individuals of *H. exutiacies* offering greater pollen quantity per flower (e.g., possibly those plants with access to greater soil moisture). The impact(s) of water deficits on plant-pollinator interactions are not particularly well understood but will be particularly relevant under future climate scenarios (Descamps et al. 2021).

4.5.5 Developing seeds per fruit (Prediction 3) and viable + predated seeds per fruit (Prediction 4)

We predicted that the number of developing seeds per fruit, a combination of viable + predated + unfilled seeds, would increase in watered plants. This is because of an observed decrease in developing seed number per fruit for plants in 2018 versus 2017 (Chapter Three), although the size of the difference was not particularly large (mean per fruit per plant of 1.37 versus 1.75). However, we found no effect of watering on developing seed number per fruit (Table 4-3), suggesting watering had no impact on the number of fertilized ovules which at least initiated seed development per fruit.

However, from the plant's perspective, it is likely more important how many fertilized ovules per fruit can be provisioned with required resources (e.g., endosperm) to produce viable seeds. Thus, under the assumption that predated seeds would have otherwise been viable, we predicted that viable + predated seed number per fruit would be higher in watered plants. This is because we observed an almost complete abortion of seeds of *H. exutiacies* in 2018, when rainfall was exceptionally low in the month of September (SM Fig. 4-4). Indeed, a number of the biochemical processes used to provision seeds may breakdown under water deficits, resulting in higher levels of seed abortion (e.g., see Awasthi et al. 2014). Furthermore, in contrast to annuals, perennial plants are able to make trade-offs between the benefit(s) of current reproduction, versus survival and future opportunities for reproduction (Aragón et al. 2009). Thus, in response to stressful conditions, perennial plants may actively divert limited resources away from sexual reproduction and into other plant structures, such as their root system (e.g., perennial versus annual cultivars of cotton; De Souza and Da Silva 1987). Overall, an increased allocation of biomass to the root system is a common response of both cultivated and wild plants under drought stress (Eziz et al. 2017). *Hibbertia exutiacies* can also resprout after fire (Pers. Obs.); consequently, annual reproductive output may be less critical for population persistence than it is for obligate seeding species unable to resprout after fire (Hansen et al. 1991). This perennial nature combined with a capability to resprout after fire likely results in a flexible strategy of resource allocation between different plant organs and the production of offspring.

However, in contrast to our prediction, viable + predated seed number per fruit did not increase with watering in either MOCP or WSCP, respectively (Table 4-3). Nonetheless, although the number of viable + predated seeds per fruit was not increased by watering, watering did increase the fruit-set of plants in WSCP, which resulted in an overall greater number of viable + predated seeds produced, simply due to the increased number of fruits developed from tagged flowers (Table 4-3). However, because of the high rate of pre-dispersal seed predation in 2019, these watered plants still did not produce a substantially higher number of viable seeds overall (Table 4-3).

4.5.6 Pre-dispersal seed predation (Prediction 5)

The prediction that pre-dispersal seed predation of *H. exutiacies* would be lower in 2019, in comparison to 2017, due to the low number of seeds that appeared available to pre-dispersal seed predators in the intervening year of 2018 was not supported. In both 2017 and 2019 overall rates of pre-dispersal seed predation were high at 60.51 % and 65.48 %, respectively. This level of pre-dispersal seed predation is noteworthy, particularly considering the results of Moles et al. (2003), who observed an average of only 7.50 ± 16.87 % pre-dispersal seed predation for 41 plant species inhabiting sclerophyll communities at Ku-ring-gai Chase National Park, Sydney, Australia. Moreover, the percentage of seeds that were predated was higher in WSCP than in MOCP in both 2017 and 2019, suggesting certain populations may be more prone to seed predator attack.

Two morphologically distinct types of seed predators were found in the seeds of *H. exutiacies*. The adult form of Larvae-2 is unknown, although the larvae were observed exiting the fruits of *H. exutiacies* and constructing a cocoon, presumably for pupation (SM Fig. 4-1k). Unfortunately, no adults were successfully reared. The morphology of Larvae-1 is comparable to that of seed feeding members of the large and diverse wasp superfamily of Chalcidoidea (Fig. 4-2c; SM Fig. 4-1e-h). These seed-feeding chalcids share a similar larval life-history to that of Larvae-1, whereby a single larva develops at the expense of an individual seed, consuming the seeds contents as it matures within the developing seed (Jansen-González et al. 2020). In support, several chalcid wasps, including adults of the predominantly seed-feeding genus *Megastigmus* Dalman (Janšta et al. 2018) emerged from mature seeds of *H. exutiacies*. Individuals of Larvae-1 and Larvae-2 also appeared to avoid each other, both at the level of individual fruits, and at the plant level, with plants with a high proportion of fruits with seeds being predated by Larvae-1 tending to have a lower proportion of fruits with seeds being predated by Larvae-2 and vice versa (Fig. 4-8). This suggests that these two types of pre-dispersal seed predators may compete with each other, but this remains to be tested.

Individuals of Larvae-1 were also found alive in seeds kept at room temperature for approximately three months, and an individual of Larvae-1 was found alive within a seed kept under refrigeration for approximately eight months. This suggests individuals of Larvae-1 may be capable of entering a period of diapause within the seed between the reproductive episodes of *H. exutiacies*. Some species of seed-feeding chalcids can even enter a prolonged diapause across multiple years, avoiding adverse conditions (e.g., Douglas Fir seed chalcid, *Megastigmus spermotrophus*; Roux et al. 1997). Prolonged diapause of individuals of Larvae-1 across multiple years in response to adverse conditions (e.g., 2017-2019), may explain why the pre-dispersal predation rate of developing seeds in 2019 was unimpacted by the apparent lack of successful pre-dispersal seed predation in 2018. However, it is unknown if Larvae-1 is capable of prolonged diapause, and high seed predation in 2019 could also have been due to effective dispersal of seed predators from unassessed areas of lower seed abortion in 2018. Life-history studies of the pre-dispersal seed predators of *H. exutiacies* are required to further understand these seed predation dynamics both spatially and temporally.

4.5.7 Future research recommendations

The results of this study, combined with the findings of Chapter Three, demonstrate that over a three-year period reproduction of the common plant, *H. exutiacies*, was limited by a mixture of pollen- and water-limitation of fruit-set, high rates of pre-dispersal seed predation, and occasional extensive seed abortion, all of which showed considerable temporal variability between years. Here, in light of these results, we offer three interrelated directions for future research. First, studies should combine watering treatments with supplementary pollination. For plants requiring obligate pollinator visitation, the extent to which increased access to water will increase reproduction will be moderated by the combined availability of soil moisture and pollinator visitation (Recart and Campbell 2021). In addition, future studies of the effect(s) of experimental watering on plant reproduction should also simultaneously measure aspects of associated plant physiology (e.g., leaf water potential, measures of photosynthesis, etc.) to better understand the biological significance of the quantity of water provided. Second, fruit-set is a relative measure of reproduction and may not necessarily be associated with the total number of fruits produced by a plant (Herrera 1991). For example, although fruit-set of control plants was lower in WSCP versus MOCP, plants in WSCP produced a greater number of flowers, such that lower fruit-set may still have resulted in higher total fruit production per plant. While it is tempting to simply multiply our measure of fruit-set per plant by the number of flowers produced to estimate total fruit production, this may be unreliable. In particular, fruit-set was measured near the peak of flowering and is unlikely to be representative of the probability a flower produces fruit throughout the entire flowering period. Indeed, the extent to which particular biotic and abiotic resources limit plant reproduction may change over the course of a single flowering season (Hampe 2005; Hove et al. 2016). Thus, future studies should attempt to measure total fruit production per plant. Third, the high level of pre-dispersal seed predation in *H. exutiacies*, and that of *Hibbertia* generally (Sweedman and Brand 2006 p. 187), deserves further investigation (as also advocated by Schatral 1996). Populations of *H. exutiacies* flower over an extended period, potentially from the beginning of spring in early September into November and longer in suitable years. This is considerably longer than some other co-flowering species, such as *P. daphnoides*, which suffer lower rates of pre-dispersal seed predation (Chapter Two). The extent to which levels of pre-dispersal seed predation change during individual flowering seasons within populations of *H. exutiacies*, and whether plants can reduce predation by altering their flowering phenology (Sercu et al. 2020), is worthy of further investigation.

4.5.8 Summary

The watering treatment did not significantly increase the number of viable and predated seeds per fruit in either sample site, suggesting watering had no impact on the provisioning of seeds within fruits in 2019. However, fruit-set of treatment plants in one of the two sample sites significantly increased with watering, indicating some spatial variability in the degree of water stress, and the watering treatment similarly altered the flowering phenology of plants in the two sample sites studied, although the possible influence of this phenological shift on reproductive output remains unclear. Pre-dispersal seed predation however was likely the most significant constraint on the output of viable seeds in 2019 and, ultimately, few viable seeds were produced by both watered plants and their unwatered controls across both sample sites.

4.6 REFERENCES:

Aragón CF, Escudero A, Valladares F (2008) Stress-induced dynamic adjustments of reproduction differentially affect fitness components of a semi-arid plant. Journal of Ecology 96:222-229.

Aragón CF, Méndez M, Escudero A (2009) Survival costs of reproduction in a short-lived perennial plant: live hard, die young. American Journal of Botany 96:904-911.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Awasthi R, Kaushal N, Vadez V, Turner NC, Berger J, Siddique KH, Nayyar H (2014) Individual and combined effects of transient drought and heat stress on carbon assimilation and seed filling in chickpea. Functional Plant Biology 41:1148-1167.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Brookes RH, Jesson LK, Burd M (2008) A test of simultaneous resource and pollen limitation in *Stylidium armeria*. New Phytologist, 179:557-565.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378-400.

Buchmann SL, Cane JH (1989) Bees assess pollen returns while sonicating *Solanum* flowers. Oecologia 81:289-294.

Burkle LA, Irwin RE (2009) The effects of nutrient addition on floral characters and pollination in two subalpine plants, *Ipomopsis aggregata* and *Linum lewisii*. Plant Ecology 203:83-98.

Campbell DR, Halama KJ (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. Ecology 74:1043-1051.

Charles SP, Fu G (2015) Statistically downscaled projections for South Australia. Goyder Institute for Water Research Technical Report Series No. 15/1, Adelaide, South Australia.

Chaves MM, Pereira JS, Maroco J, Rodrigues ML, Ricardo CPP, Osório ML, Carvalho I, Faria T, Pinheiro C (2002) How plants cope with water stress in the field? Photosynthesis and growth. Annals of Botany 89:907-916.

Clarke I, Lee H (2019) Name that flower. Melbourne University Press, Melbourne, Australia.

Cochrane A (2002) Seed notes for Western Australia. No. 17 *Hibbertia*. Wildflower Society of Western Australia.

Delnevo N, van Etten EJ, Byrne M, Petraglia A, Carbognani M, Stock WD (2020) Habitat fragmentation restricts insect pollinators and pollen quality in a threatened Proteaceae species. Biological Conservation 252:108824.

Descamps C, Quinet M, Jacquemart AL (2021) The effects of drought on plant-pollinator interactions: what to expect? Environmental and Experimental Botany 182:104297.

De Souza JG, Da Silva JV (1987) Partitioning of carbohydrates in annual and perennial cotton (*Gossypium hirsutum* L.). Journal of Experimental Botany 38:1211-1218.

Dudash MR, Fenster CB (1997) Multiyear study of pollen limitation and cost of reproduction in the iteroparous *Silene virginica*. Ecology 78:484-493.

Erickson TE, Barrett RL, Symons DR, Turner SR, Merritt DJ (2016) An atlas to the plants and seeds of the Pilbara region. In: Erickson TE, Barrett RL, Merritt DJ, Dixon KW (eds) Pilbara seed atlas and field guide: plant restoration in Australia's arid northwest. CSIRO Publishing, Clayton South, Victoria, pp 43-256.

Eziz A, Yan Z, Tian D, Han W, Tang Z, Fang J (2017) Drought effect on plant biomass allocation: a meta-analysis. Ecology and Evolution 7:11002-11010.

Fang X, Turner NC, Yan G, Li F, Siddique KH (2010) Flower numbers, pod production, pollen viability, and pistil function are reduced and flower and pod abortion increased in chickpea (*Cicer arietinum* L.) under terminal drought. Journal of Experimental Botany 61:335-345.

Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects, mechanisms and management. Agronomy for Sustainable Development 29:185-212

Fox J, Dixon B, Monk D (1987) Germination in other plant families. In: Langkamp P (ed) Germination of Australian native plant seed. Inkata Press, Melbourne and Sydney, pp 83-97.

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks, California.

Gallagher MK, Campbell DR (2017) Shifts in water availability mediate plant-pollinator interactions. New Phytologist 215:792-802.

Gross CL (1996) Is resource overlap disadvantageous to three sympatric legumes? Australian Journal of Ecology 21:133-143.

Hampe A (2005) Fecundity limits in *Frangula alnus* (Rhamnaceae) relict populations at the species' southern range margin. Oecologia 143:377-386.

Hansen A, Pate JS, Hansen AP (1991) Growth and reproductive performance of a seeder and a resprouter species of *Bossiaea* as a function of plant age after fire. Annals of Botany 67:497-509.

Hansen A, Pate JS, Hansen AP (1992) Growth, reproductive performance and resource allocation of the herbaceous obligate seeder *Gompholobium marginatum* R. Br. (Fabaceae). Oecologia 90:158-166.

Hartig F (2021) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1.

Herrera CM (1991) Dissecting factors responsible for individual variation in plant fecundity. Ecology 72:1436-1448.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Höfer RJ, Ayasse M, Kuppler J (2021) Bumblebee behavior on flowers, but not initial attraction, is altered by short-term drought stress. Frontiers in Plant Science 11:564802.

Horn JW (2007) Dilleniaceae. In: Kubitzki K (ed) The families and genera of vascular plants, vol 9, flowering plants, eudicots, 1st edn. Springer, Berlin, Heidelberg, pp. 132-154.

Hothorn T, Bretz F, Westfall P (2008a) Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Hothorn T, Hornik K, van de Wiel MA, Zeileis A (2008b) Implementing a class of permutation tests: the coin package. Journal of Statistical Software 28:1-23.

Hove AA, Mazer SJ, Ivey CT (2016) Seed set variation in wild *Clarkia* populations: teasing apart the effects of seasonal resource depletion, pollen quality, and pollen quantity. Ecology and Evolution 6:6524-6536.

Hu W, Liu Y, Loka DA, Zahoor R, Wang S, Zhou Z (2019) Drought limits pollen tube growth rate by altering carbohydrate metabolism in cotton (*Gossypium hirsutum*) pistils. Plant Science 286:108-117.

Jansen-González S, Teixeira SDP, Pereira RA (2020) Larval strategy of two species of seed-feeding Chalcidoidea parallels that of parasitoid koinobionts. Oecologia Australis 24:903-916

Janšta P, Cruaud A, Delvare G, Genson G, Heraty J, Křížková B, Rasplus JY (2018) Torymidae (Hymenoptera, Chalcidoidea) revised: molecular phylogeny, circumscription and reclassification of the family with discussion of its biogeography and evolution of life-history traits. Cladistics 34:627-651.

Jessop JP (1986) Dilleniaceae. In: Jessop JP, Toelken HR (eds) Flora of South Australia, part 1, Lycopodiaceae-Rosaceae, 4th edn. South Australian Government Printing Division, Adelaide, pp 354-358.

Johnson SD, Bond WJ (1997) Evidence for widespread pollen limitation of fruiting success in Cape wildflowers. Oecologia 109:530-534.

Johnson WC, Jackson LE, Ochoa O, Van Wijk R, Peleman J, Clair DS, Michelmore RW (2000) Lettuce, a shallow-rooted crop, and *Lactuca serriola*, its wild progenitor, differ at QTL determining root architecture and deep soil water exploitation. Theoretical and Applied Genetics 101:1066-1073.

de Jong TJ, Klinkhamer PG (1989) Limiting factors for seed production in *Cynoglossum officinale*. Oecologia 80:167-172.

Kolb A, Ehrlén J, Eriksson O (2007) Ecological and evolutionary consequences of spatial and temporal variation in pre-dispersal seed predation. Perspectives in Plant Ecology, Evolution and Systematics 9:79-100.

Lemoine R, La Camera S, Atanassova R, Dédaldéchamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Coutos-Thévenot P, Maurousset L, Faucher M (2013) Source-to-sink transport of sugar and regulation by environmental factors. Frontiers in Plant Science 4:272.

Lindén A, Mäntyniemi S (2011) Using the negative binomial distribution to model overdispersion in ecological count data. Ecology 92:1414-1421.

Martin TG, Wintle BA, Rhodes JR, Kuhnert PM, Field SA, Low-Choy SJ, Tyre AJ, Possingham HP (2005) Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecology Letters 8:1235-1246.

McCall C, Primack RB (1987) Resources limit the fecundity of three woodland herbs. Oecologia 71:431-435.

Moles AT, Warton DI, Westoby M (2003) Do small-seeded species have higher survival through seed predation than large-seeded species? Ecology 84:3148-3161.

Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology 13:266-273.

Pauw A (2007) Collapse of a pollination web in small conservation areas. Ecology 88:1759-1769.

Pinheiro C, Chaves MM (2011) Photosynthesis and drought: can we make metabolic connections from available data? Journal of Experimental Botany 62:869-882.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, United Kingdom.

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Recart W, Campbell DR (2021) Water availability affects the relationship between pollen intensity and seed production. AoB Plants 13:plab074.

Recart W, Ottoson B, Campbell DR (2019) Water influences how seed production responds to conspecific and heterospecific pollen. American Journal of Botany 106:713-721.

Roux G, Roques A, Menu F (1997) Effect of temperature and photoperiod on diapause development in a Douglas fir seed chalcid, *Megastigmus spermotrophus*. Oecologia 111:172-177.

Schatral A (1996) Floral predators, pollinators and seed set in Western Australian species of the genus *Hibbertia* (Dilleniaceae). In: Hopper SD, Chappill JA, Harvey MS, George AS (eds) Gondwanan heritage: past, present and future of the Western Australian biota. Surrey Beatty & Sons, Sydney, NSW, pp 203-211.

Schatral A, Fox JED (1994) Quality and viability of seeds in the genus *Hibbertia*. Seed Science and Technology 22:273-284.

Schatral A, Kailis SG, Fox JED (1994) Seed dispersal of *Hibbertia hypericoides* (Dilleniaceae) by ants. Journal of the Royal Society of Western Australia 77:81-85.

Sercu BK, Moeneclaey I, Bonte D, Baeten L (2020) Induced phenological avoidance: a neglected defense mechanism against seed predation in plants. Journal of Ecology 108:1115-1124.

Shen S, Liang XG, Zhang L, Zhao X, Liu YP, Lin S, Gao Z, Wang P, Wang ZM, Zhou SL (2020) Intervening in sibling competition for assimilates by controlled pollination prevents seed abortion under postpollination drought in maize. Plant, Cell and Environment 43:903-919.

Solbreck C, Sillén-Tullberg B (1986) The role of variable weather for the dynamics of a seed-seed predator system. Oecologia 71:59-62.

Solbreck C, Knape J (2017) Seed production and predation in a changing climate: new roles for resource and seed predator feedback? Ecology 98:2301-2311.

Specht RL, Rayson P (1957) Dark Island heath (Ninety-mile Plain, South Australia). III. The root systems. Australian Journal of Botany 5:103-114.

Sweedman L, Brand G (2006) Collection guidelines for common Australian families and genera. In: Sweedman L, Merritt D (eds) Australian seeds a guide to their collection, identification and biology. CSIRO Publishing, Collingwood, Victoria, pp 173-198.

Tanguilig VC, Yambao EB, O'Toole JC, De Datta SK (1987) Water stress effects on leaf elongation, leaf water potential, transpiration, and nutrient uptake of rice, maize, and soybean. Plant and Soil 103:155-168.

Tennekes M (2018) tmap: thematic maps in R. Journal of Statistical Software 84:1-39.

Tucker SC, Bernhardt P (2000) Floral ontogeny, pattern formation, and evolution in *Hibbertia* and *Adrastaea* (Dilleniaceae). American Journal of Botany 87:1915-1936.

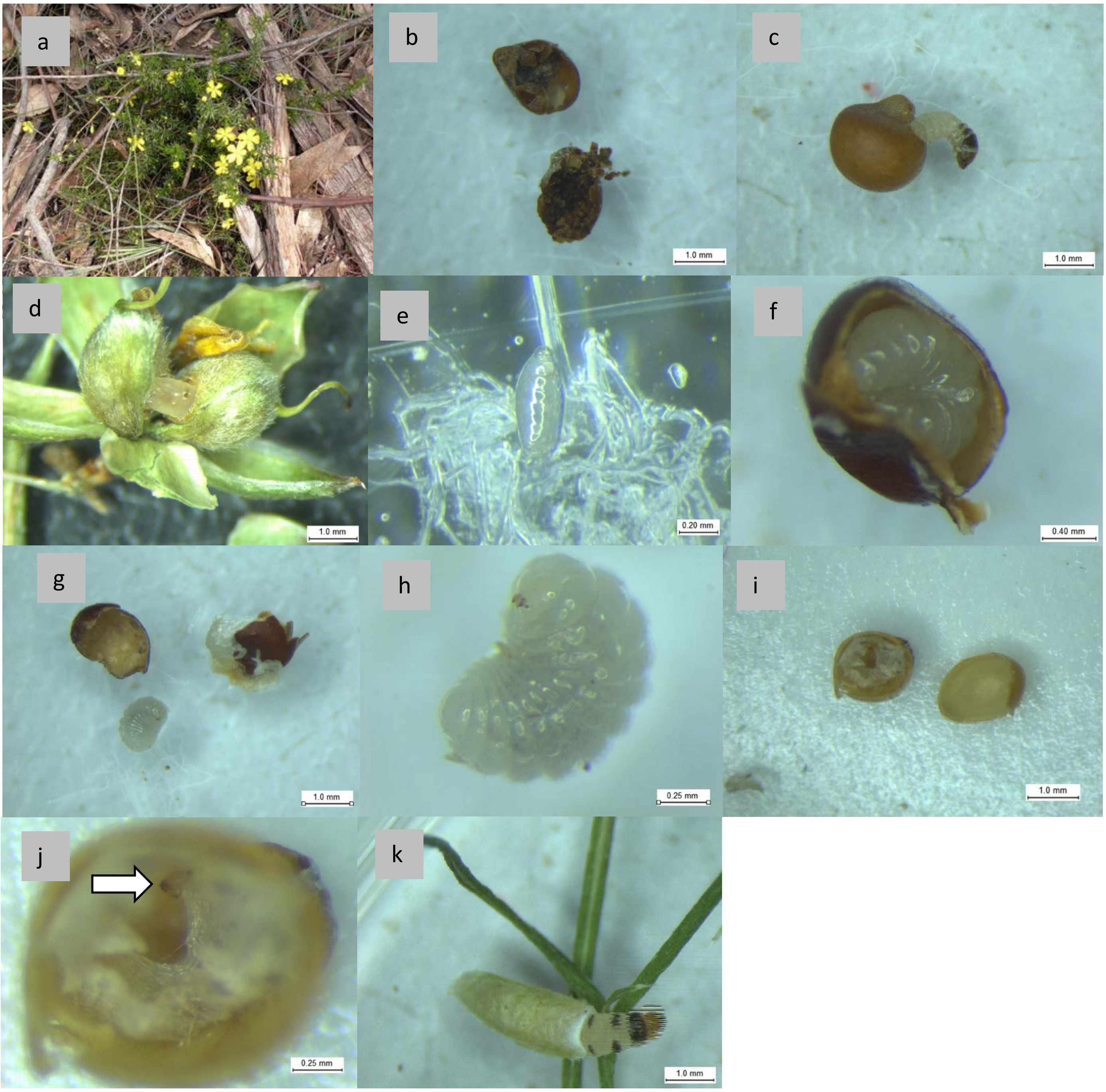
de Waal C, Anderson B, Ellis AG (2015) Relative density and dispersion pattern of two southern African Asteraceae affect fecundity through heterospecific interference and mate availability, not pollinator visitation rate. Journal of Ecology 103:513-525.

Waser NM, Price MV (2016) Drought, pollen and nectar availability, and pollination success. Ecology 97:1400-1409.

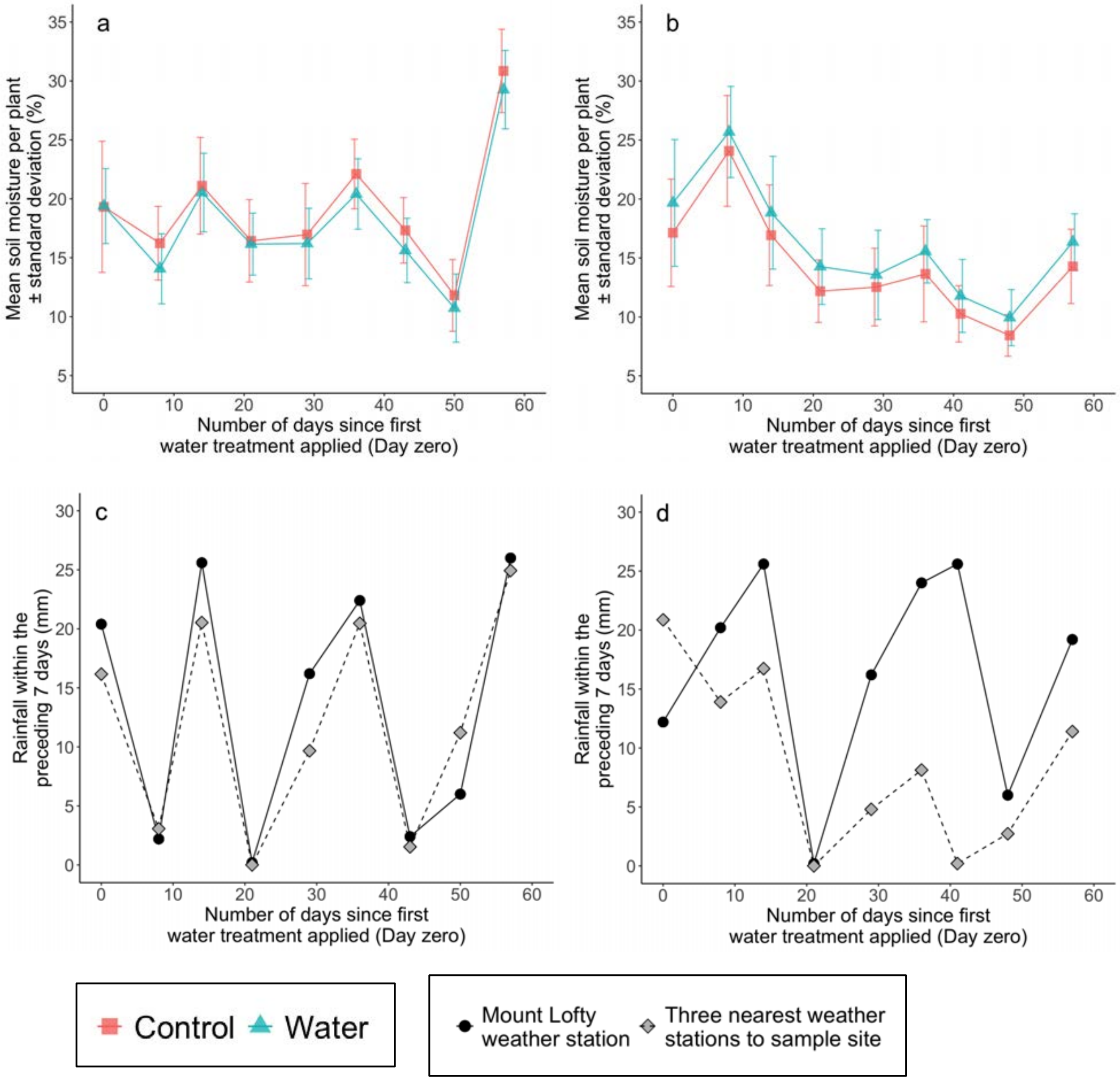
Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Zeileis A, Kleiber C, Jackman S (2008) Regression models for count data in R. Journal of Statistical Software 27:1-25.

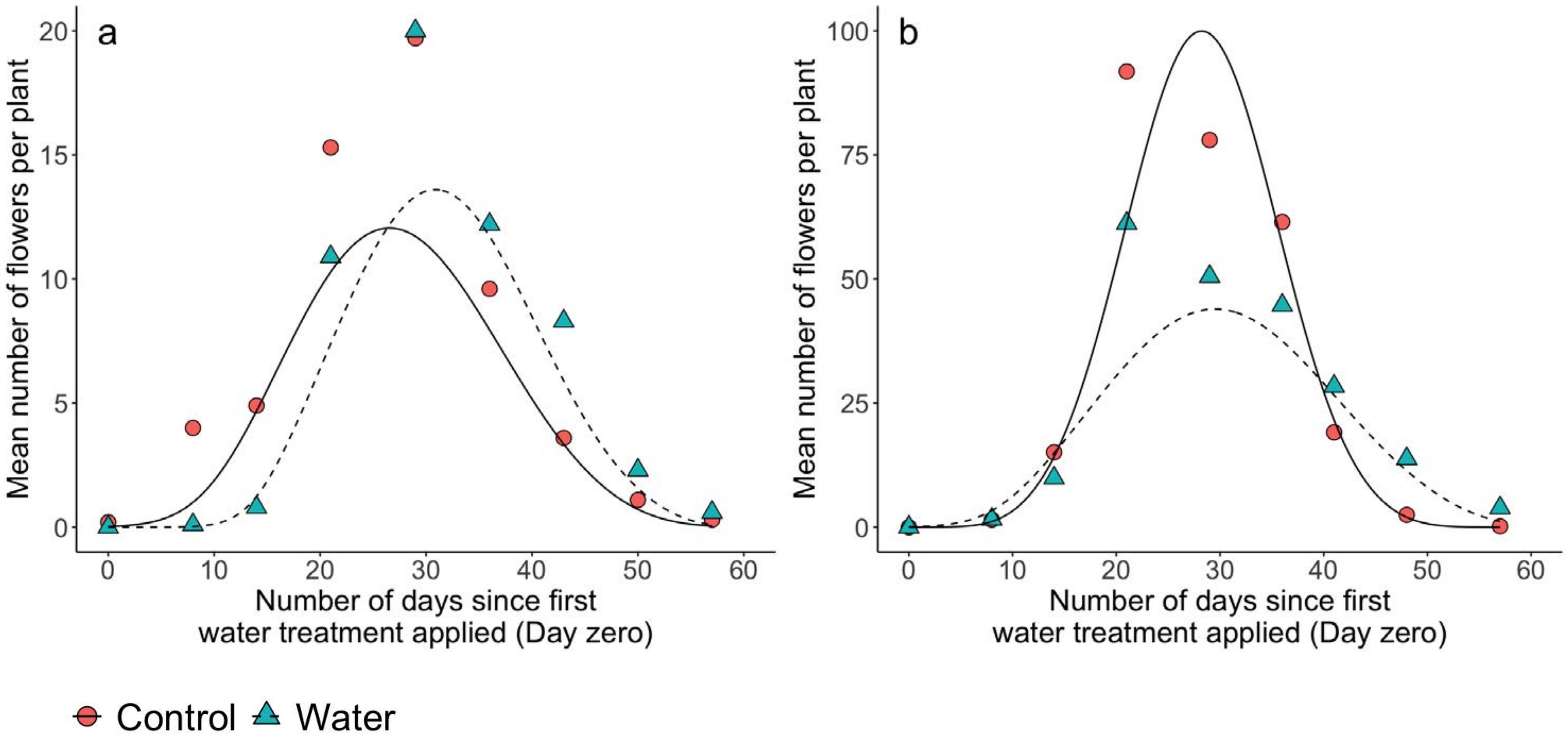
4.7 SUPPLEMENTARY MATERIAL



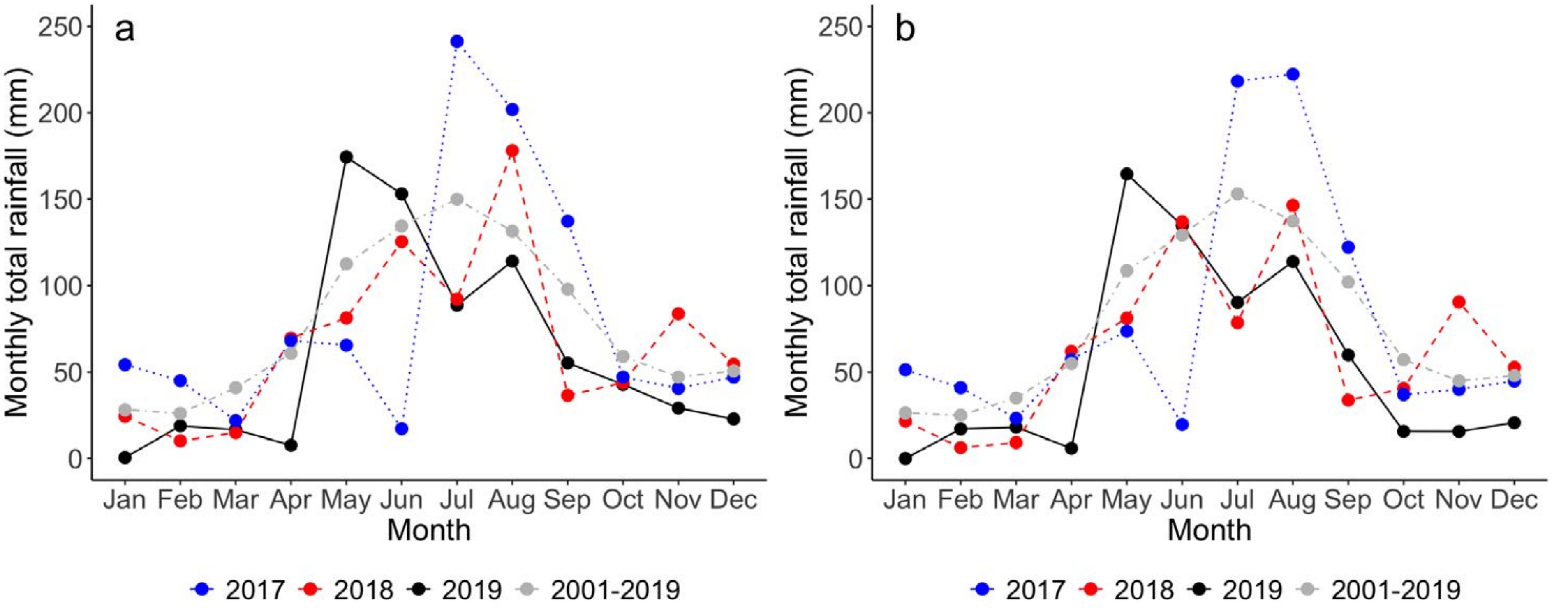
SM Fig. 4-1 (**a**) Flowering plant of *Hibbertia exutiacies*. (**b**) Predated seed of *H. exutiacies* containing insect frass. (**c**) Predated seed of *H. exutiacies* containing an individual of Larvae-2 (an arbitrary title given to this morphologically distinct larvae). (**d**) Fruit of *H. exutiacies* with an individual of Larvae-2 moving between follicles. (**e**) A small individual of Larvae-1 (an arbitrary title given to this morphologically distinct larvae) found within a developing seed of *H. exutiacies*. (**f-h**) Larger individuals of Larvae-1 found within developing seeds of *H. exutiacies*. (**i**) Two halves of an unfilled seed of *H. exutiacies*. The papery inner part of the seed coat can be seen in the left half of the cut unfilled seed. (**j**) Unfilled seed of *H. exutiacies* with a minute individual of Larvae-2 (arrow). (**k**) Individual of Larvae-2 within a cocoon.



SM Fig. 4-2 Mean soil moisture per plant, ± standard deviation, measured for 10 control (unwatered) and 10 watered plants in sample sites located in the reserves of (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. Soil moisture was measured prior to watering treatments being applied for each day of measurement (nine measurements in total). The first watering treatment was applied on day zero. Bottom plots show rainfall (mm) recorded within the preceding seven days prior to soil moisture measurements being taken at each sample site for both the Mount Lofty weather station (Station number: 23842), as well as estimated for the sample site itself by calculating average rainfall recorded at the three nearest weather stations (with available data) to each sample site (see Fig. 4-1 for details) in (**c**) MOCP and (**d**) WSCP, respectively.



SM Fig. 4-3 The mean number of flowers per plant per treatment for each day of watering in the sample sites located in (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. Trend lines were estimated from statistical models (see Methods and Results). The first watering treatment was applied on day zero. Note that different scales are used for each y-axis.



SM Fig. 4-4 Monthly total rainfall (mm) in the years of 2017, 2018, 2019, and averaged for the years 2001-2019, estimated for sample sites within the reserves of (**a**) Mark Oliphant CP (MOCP) and (**b**) Wottons Scrub CP (WSCP), respectively. Rainfall was estimated for the sample site itself by calculating the average rainfall recorded at the three nearest weather stations (with available data) to each sample site, respectively (see Fig. 4-1 for details).

CHAPTER FIVE

5. IMPACT OF RESERVE AREA ON REPRODUCTION OF A MOTH-POLLINATED *STACKHOUSIA* SM. (CELASTRACEAE) SPECIES IN A FRAGMENTED LANDSCAPE.

Alex G. Blackall[1], Duncan A. Mackay[1], Molly A. Whalen[1]

[1]College of Science and Engineering, Flinders University, GPO Box 2100, Adelaide, South Australia 5001, Australia

5.1 ABSTRACT

Premise: Landscape disturbance frequently results in reduced pollination and reproduction of animal-pollinated plants. However, biases remain in our understanding of the impact(s) of landscape disturbance. In particular, most previous studies have focused on impacts on rare and threatened plants and plants with diurnal rather than nocturnal pollinators.

Methods: We used the common, putatively nocturnally moth-pollinated plant, *Stackhousia aspericocca* ssp. *Cylindrical inflorescence*, to examine the impact(s) of landscape disturbance on plant reproduction. First, we sought to confirm that night-flying moths visit flowers and carry pollen of *S. aspericocca* ssp. *Cylindrical inflorescence*. Subsequently, in two consecutive years, we compared natural levels of fruit and seed production across a range of reserves of varying sizes that hosted populations in our study region within southern Australia. We hypothesized that plant reproduction would show a positive relationship with reserve area.

Results: We confirmed floral visitation by night-flying settling moths (Geometridae and Noctuidae), which carried large pollen loads of the study species (8/14 moths $>$ 100 pollen grains). Two individuals of the moth species *Oenochroma vinaria* (Geometridae) each carried over 3000 pollen grains. Plant reproduction, measured as proportional fruit-set and mean seed number per fruit, showed no relation to reserve area in either year. Thus, the hypothesis that plant reproduction would increase with reserve area was not supported. Furthermore, plant reproduction showed limited variability among reserves more generally, suggesting mean levels of plant reproduction are spatially similar throughout the fragmented landscape of the study region.

Conclusion: This is one of a few studies directly observing nocturnal moth pollination within Australia, and the first examining the impact of landscape disturbance on a moth-pollinated plant. In contrast to most studies, reproduction of *S. aspericocca* ssp. *Cylindrical inflorescence* was not significantly affected by landscape disturbance, with limited detectable spatial variation in plant reproduction among sites. It is hypothesized that this lack of spatial variability in reproduction is due to effective pollination by common, night-flying moths, which may utilise both the reserves and their surrounding matrix to complete their life cycle(s).

KEYWORDS: Celastraceae; *Stackhousia*; phalenophilous; *Pimelea*; habitat loss; fragmentation; Lepidoptera

5.2 INTRODUCTION

Animal-mediated pollination and plant reproduction, two interrelated processes, often decline under the combined effects of habitat loss and fragmentation (reviewed by Aizen et al. 2002; Hobbs and Yates 2003; Harris and Johnson 2004; Aguilar et al. 2006). As such, the negative consequences of landscape disturbance (here used to describe the combined effects of habitat loss and fragmentation and other interrelated processes (e.g., edge effects)) on pollination and plant reproduction are often greater as the areas of vegetation fragments, or sizes of plant populations, decline (see studies by Jennersten 1988; Lamont et al. 1993; Aizen and Feinsinger 1994; Morgan 1999; Cunningham 2000; Donaldson et al. 2002; Lennartsson 2002; Johnson et al. 2004). However, biases remain in our understanding of the impact(s) of landscape disturbance. For example, rare and threatened plants which are *a priori* likely vulnerable to landscape disturbance, are over-represented in the literature (as recognised by Hobbs and Yates 2003 and Aguilar et al. 2006; see Chapter One). Moreover, studies are typically conducted over a single flowering season (Aguilar et al. 2006; see Chapter One), although the impact of landscape disturbance may fluctuate between years (e.g., see Morgan 1999). Many studies also measure effects on pollination and plant reproduction at the most severe scale(s) of disturbance, for instance, in small vegetation patches under 5 ha in area (Donaldson et al. 2002) or in roadside verges of native vegetation (Lamont et al. 1993). Although these may be important to current conservation efforts (New et al. 2021), it is somewhat less clear how the negative effects of landscape disturbance manifest themselves in larger areas that are often designated as conservation reserves (e.g., see Cunningham 2000 and Pauw 2007). In addition, most studies have focused on diurnal pollinators, ignoring the importance of nocturnal pollinators such as night-flying moths to flowering plant communities (Macgregor et al. 2015).

Moths represent the most diverse group of flower visiting insects globally (Ollerton 2017), and plants pollinated by night-flying moths have floral adaptations unlike those of butterfly (and diurnal insect taxa more broadly) pollinated plants, representing a unique plant-pollinator system (Faegri and van der Pijl 1979; Willmer 2011). However, pollination by night-flying moths has been understudied relative to that by less diverse groups of diurnal pollinators (e.g., Hymenoptera), although moths are likely important pollinators across many plant communities (see reviews by Macgregor et al. 2015; Hahn and Brühl 2016; Buxton et al. 2018). Moreover, research on moth pollination is itself biased towards hawkmoths (Sphingidae), which are important pollinators of many plants globally (Johnson et al. 2017). In comparison, pollination by settling moths (a broad term encompassing the diverse nectar-feeding macro-moths commonly of the families Noctuidae and Geometridae) has received relatively less attention (Buxton et al. 2018), although these insects can be high-quality pollinators (Zhang and Gao 2021). Nevertheless, the potential role of night-flying settling moths as pollinators within natural plant communities (Oliveira et al. 2004; Atwater et al. 2013), as well as in agricultural landscapes (Walton et al. 2020; Robertson et al. 2021), is increasingly being recognized and studied. Even for plant communities in which animal-mediated pollination is typically seen to be rare (e.g., New Zealand Subantarctic Islands), recent work suggests nocturnal settling moth pollination may be important (Buxton et al. 2019). Thus, given evidence of declines in moth abundance and diversity throughout many areas of world (Sánchez-Bayo and Wyckhuys 2019; but see Wagner et al. 2021), and a general lack of understanding of the importance of these nocturnal flower visitors to pollination (Van Zandt et al. 2020), it is of increasing importance to study this nocturnal plant-pollinator interaction.

Unsurprisingly, few studies have focused on moth pollination within Australia (Buxton et al. 2018). Even pollination by hawkmoths has been comparatively understudied relative to the rest of the world (Johnson et al. 2017). Likewise, little work on pollination by settling moths has been undertaken, with published observations of settling moth flower visitation providing few details and limited actual evidence of pollination (e.g., reviewed by Armstrong 1979; but see Finch et al. (2019) for recent work on specialised moth pollination in the Australian plant, *Breynia oblongifolia* (Phyllanthaceae)). Here, we study a putatively moth-pollinated species of the genus *Stackhousia* Sm. (Celastraceae). This small genus of flowering plants is predominantly confined to Australia (Barker 1986), and species of *Stackhousia* are often assumed to be pollinated by night-flying moths (e.g., Clarke and Lee 2019 p. 186). However, although diurnal visitation by flies (Diptera) to the alpine species *Stackhousia pulvinaris* has been recorded (Inouye and Pyke 1988; Milla and Encinas-Viso 2020), and pollen of an unidentified species of *Stackhousia* was found on a native bee in eastern Australia by

Bernhardt and Burns-Balogh (1986), there appears to be no published evidence of moth pollination in any species of *Stackhousia*. Nevertheless, nocturnal visitation by moths to flowers of some *Stackhousia* species has been irregularly observed (W.R. Barker Pers. Comms.), which is suggestive of moth pollination. Likewise, although studies have examined the general ecology (e.g., response to fire) of some species of *Stackhousia* (e.g., *S. tryonii* (Batianoff et al. 1990) and *S. subterranea* (Gilfedder and Kirkpatrick 1998)), there appear to be no published studies regarding their levels of fruit and seed production.

We studied nocturnal floral visitation and reproduction of the common plant, *Stackhousia aspericocca* Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker, within the fragmented landscape of the Adelaide Hills, a peri-urbanised section of the Southern Mount Lofty Ranges, South Australia, Australia. First, we investigated the likelihood of pollination by night-flying settling moths via direct nocturnal observations of flowering plants and analysis of moth pollen loads in 2017. Second, in 2018 and 2019, natural levels of plant reproduction were measured across eight reserves of increasing area (1.40 to 1027.47 ha) to examine impacts of landscape disturbance on reproductive output. It was hypothesized that plant reproduction, measured as the proportion of flowers forming fruits (fruit-set) and mean seed number per fruit, would show a positive relationship with reserve area. This is one of few studies directly observing nocturnal moth pollination within Australia and, to our knowledge, the first study to examine the impact of variation in landscape characteristics on a nocturnally moth-pollinated plant in Australia.

5.3 METHODS

5.3.1 Study species

The herbaceous perennial *Stackhousia aspericocca* Schuch. occurs throughout sclerophyll forests and drier mallee communities of South Australia and western Victoria, Australia (Barker 1977). Within the study region it is a common plant of dry sclerophyll forests and woodlands (Armstrong et al. 2003). Two subspecies of *S. aspericocca* are recognised within the study region (Barker et al. 2005). Initially, Barker (1977) recognised two distinct races of *S. aspericocca*, *Stackhousia aspericocca* Schuch. race *1* W.R.Barker (1977), with flowers arranged cylindrically around the floral spike (Fig. 5-1a), and *Stackhousia aspericocca* Schuch. race *2* W.R.Barker (1977), with flowers directed unilaterally. Subsequently, in Barker (1986), each race was considered a subspecies, referred to as *Stackhousia aspericocca* Schuch. ssp. *A* W.R.Barker, and *Stackhousia aspericocca* Schuch. ssp. *B* W.R.Barker, respectively. Currently, the accepted names for these subspecies are

Stackhousia aspericocca Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker, and *Stackhousia aspericocca* Schuch. ssp. *One-sided inflorescence* (W.R.Barker 697) W.R.Barker, respectively (Australian Plant Census, accessed 04/05/2022, https://chah.gov.au/council-of-heads-of-australasian-herbaria/). While both subspecies may be found growing near one another (Pers. Obs.), *S. aspericocca* ssp. *Cylindrical inflorescence* preferentially inhabits relatively wetter areas within the study region (Barker 1977). No intergradation between the two subspecies has been observed in the study region (Barker 1977). For this study, *S. aspericocca* ssp. *Cylindrical inflorescence* was the most abundant of the two subspecies within the chosen sample sites (Pers. Obs.). For example, for those plants of *S. aspericocca* ssp. *Cylindrical inflorescence* tagged to measure reproduction across 2018 and 2019, only 4.88 % had one or more plants of *S. aspericocca* ssp. *One-sided inflorescence* within 2 m, versus 78.05 % of plants with one or more individuals of *S. aspericocca* ssp. *Cylindrical inflorescence* within 2 m.

Flowers of both subspecies have a corolla consisting of five petals united centrally to form a tubular flower (Barker 1986; Fig. 5-1a). Across the two subspecies, flower length, from the base of the flower to the opening of the corolla, is ~6-8mm, while the distance between the stigma and the opening of the corolla tube is ~4-6 mm (Pers. Obs.). Flowers are typically white (ssp. *Cylindrical inflorescence*) to deep yellow in colouration (ssp. *One-sided inflorescence*; Barker 1977). Stamens are of unequal length, with three long and two short stamens (Barker 1986). The 3-(rarely 5)-partite gynoecium (Barker 1986) does not elongate up the corolla tube, remaining near the base of the flower (Pers. Obs.). A small amount of viscous fluid is present at the base of the gynoecium, presumably representing a floral reward in the form of nectar (Pers. Obs.). Flowers are borne along a floral spike that can grow over 50 cm in height, and multiple flowering stems may be present per plant (Pers. Obs.). The inflorescence persists throughout the flowering season (spring to early summer), so that basally developing fruits may be present while flowering continues higher up the inflorescence (Pers. Obs.). Flowers produce a "musky" scent at night, which is absent during the day, and cut inflorescences kept in water will likewise produce a nightly scent (Pers. Obs.). Such floral traits are suggestive of nocturnal pollination by night-flying moths, particularly by settling moths (so-called phalenophilous pollination syndrome; Faegri and van der Pijl (1979); Willmer 2011; see examples in Johnson et al. (1993), Oliveira et al. (2004), Okamoto et al. (2008)). Reproduction via autonomous self-pollination does not occur for plants in the study region (0 fruits/238 flowers, n = 3 plants).

Following successful fertilization, flowers form a typically 1-3 (up to 5) seeded non-fleshy schizocarp (Barker 1986; i.e., fruit "splitting at maturity into one-seeded segments" Clarke and Lee (2019) p. 343; Fig. 5-1b). Each of these one-seeded segments is termed a coccus, containing a single seed within a carpel (Clarke and Lee 2019; Fig. 5-1e-f). In contrast, flowers that do not produce fruits leave behind the floral bract and two bracteoles which subtended the flower (Fig. 5-1c). Due to the fruit splitting into one-seeded segments prior to dispersal from the plant, the number of seeds formed per fruit can be counted without dissection of the fruit itself (Fig. 5-1b). Dispersal appears to be passive (Pers Obs.), and seeds likely possess physiological dormancy (Vening et al. 2017). Following seed dispersal, the hypanthium remains persistent on the floral stem (Barker 1986; Fig. 5-1d).

5.3.2 Sample sites

Selected reserves were managed by the Department of Environment and Water, excluding two private reserves (Nurrutti and Malcolm Wicks) owned by the National Trust of South Australia. Reserve area ranged from 1.40 ha up to 1027.47 ha (Table 5-1). In all but the smallest reserve (Nurrutti), plants within sample sites were located a minimum of 40 m from the edge of the reserve (a delimitation used by Cunningham 2000). However, due to factors such as terrain, prescribed burns, and the location of plant populations, it was not possible to hold constant the distance from each sample site to the edge of their respective reserve. Furthermore, there was also native vegetation outside reserve boundaries (Fig. 5-2). Nonetheless, reserve area is positively correlated with the amount of native vegetation surrounding sample sites (e.g., the correlation between reserve area and the amount of native vegetation within 1000 m of sample sites is Spearman's rho (r_s) = 0.976). Thus, sample sites within smaller reserves are generally surrounded by less native vegetation (Fig. 5-2).

5.3.3 Plant community

The Adelaide Hills possesses a temperate climate (hot, dry summers and cool, wet winters) with a mean annual rainfall of ~700-1000 mm (Bureau of Meteorology), suitable for *Eucalyptus* L'Hér. (Myrtaceae) forests and woodlands (Armstrong et al. 2003). Indeed, most remnant vegetation within the study region is composed of dry sclerophyll forest and woodlands of *Eucalyptus* (Armstrong et al. 2003). The dominant vegetation type(s) at the chosen sample sites were dry sclerophyll forests and woodlands of messmate stringybark (*Eucalyptus obliqua* L'Hér.) and brown stringybark (*E. baxteri* (Benth.) Maiden & Blakely ex J.M.Black), with an understorey dominated by sclerophyllous legumes and *Hibbertia* Andrews (Dilleniaceae) species.

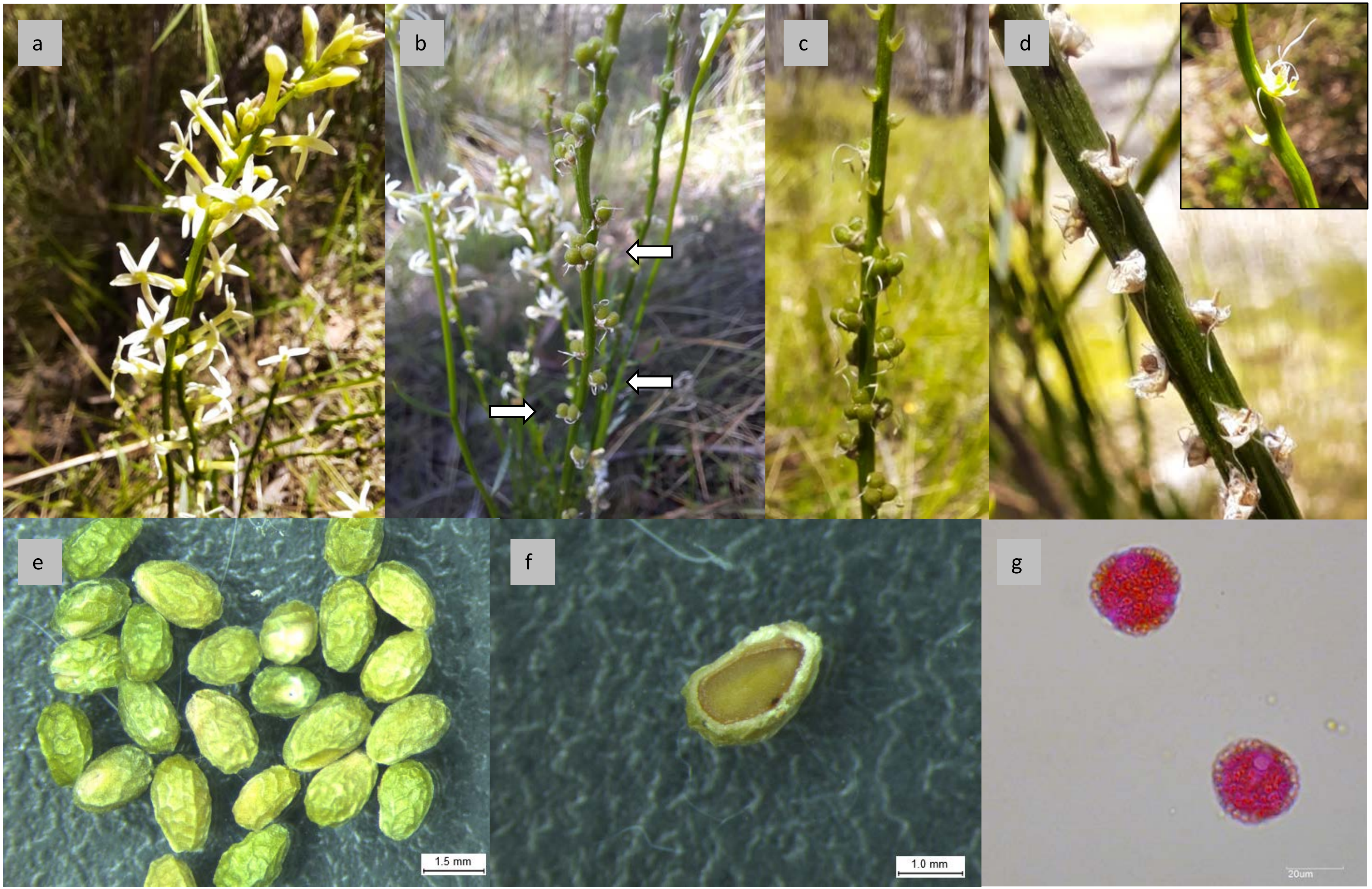

a
b
c
d
e
f
g
1.5 mm
1.0 mm
20um

Fig. 5-1 (above) Flowers, fruits, and pollen of *Stackhousia aspericocca* ssp. *Cylindrical inflorescence*. (**a**) Inflorescence showing both open flowers and closed flower buds. (**b**) Plant with both flowers and fruits present across different flowering stems. Fruits are schizocarps splitting into cocci. Arrows highlight, from the bottom upwards, fruits with two, one, and three cocci per fruit, respectively. (**c**) Post flowering inflorescence showing basally developing fruits and remaining bracts and bracteoles from flowers which have not developed fruits. (**d**) Stem showing the persistent remains of the hypanthium following fruit development and dispersal. Inset shows the hypanthium which held a fruit containing three cocci (cocci were removed for photo). (**e**) Cocci. (**f**) Cut coccus showing single seed. (**g**) Reticulate pollen grains (400× magnification, scalebar = 20 μm).

Table 5-1 Coordinates for sample sites within reserves, reserve area (ha), and sample size (minus the loss of tagged plants and inflorescences) for the number of focal plants and flowers tagged within sample sites (2018 and 2019), and additional plants and flowers tagged (2019 only), used to measure reproduction of *Stackhousia aspericocca* ssp. *Cylindrical inflorescence*. Sample sites in some reserves were also used to observe nocturnal flower visitation to *S. aspericocca* ssp. *Cylindrical inflorescence* and *S. aspericocca* ssp. *One-sided inflorescence*. CP stands for Conservation Park.

Reserve	Latitude	Longitude	Reserve area (ha)	Focal plants and flowers tagged (2018)	Focal plants and flowers tagged (2019)	Additional plants and flowers tagged (2019)[b]
Nurrutti Reserve	35° 1.90' S	138° 44.53' E	1.40	5 plants, 127 flowers	5 plants, 92 flowers	2 plants, 40 flowers
Malcolm Wicks Reserve	34° 55.92' S	138° 48.89' E	7.90	5, 140	5, 94	3, 40
Mylor CP	35° 2.30' S	138° 46.17' E	45.10	4, 107	6, 136	4, 110
Wottons Scrub CP	34° 59.00' S	138° 46.70' E	82.50	4, 100	5, 128	5, 76
Giles CP	34° 55.90' S	138° 43.35' E	108.92	5, 124	6, 90	3, 43
Filsell Hill CP	34° 57.59' S	138° 47.83' E	128.67	4, 72	4, 74	NA[c]
Mark Oliphant CP	35° 1.63' S	138° 41.85' E	189.96	5, 98	5, 139	5, 114
Cleland CP	34° 58.16' S	138° 42.66' E	1027.47	5, 70	5, 88	1, 7
Scott Creek CP[a]	35° 05.31' S	138° 41.07' E	713.95	NA	NA	NA

[a] Scott Creek CP was only used for observations of nocturnal visitation to flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* and *S. aspericocca* ssp. *One-sided inflorescence*.

[b] Additional plants were located within 2 m of focal plants in 2019.

[c] All additional plants lost due to plant death (at least of above ground tissues) and herbivory of stems with tagged inflorescences.

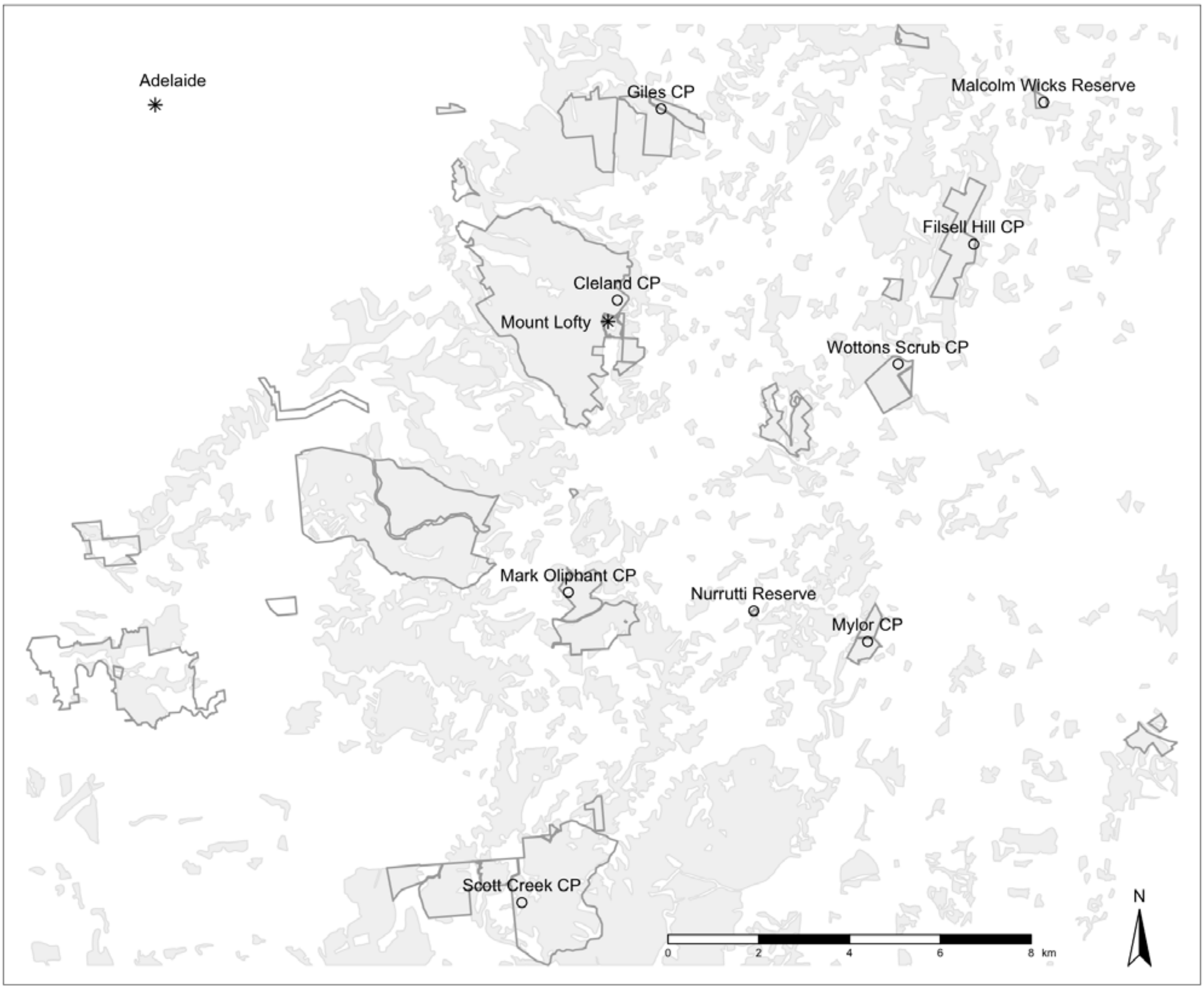


Fig. 5-2 Location of sample sites. All reserves under protection by the Department for Environment and Water (DEW) and two private reserves (Nurrutti and Malcolm Wicks) owned by the National Trust of South Australia are outlined. Adelaide, the capital city of South Australia, and Mount Lofty (720 m), the highest point within the study region, are both marked by an asterisk. The scale bar represents a total distance of 8 km. Native vegetation layer assembled by DEW (Native Vegetation Floristic Areas - NVIS - Statewide (Incomplete Version); Dataset Number: 898).

5.3.4 Pollinator observations - 2017

In the spring of 2017, nocturnal surveys were conducted to observe if night-flying moths foraged at flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* and, when present, *S. aspericocca* ssp. *One-sided inflorescence*. Because we were primarily interested in confirming whether night-flying moths are attracted to this taxon's apparently phalenophilous floral traits, no systematic diurnal observations were performed. Observations of floral visitors were undertaken by slowly walking along trails within reserves that were bordered by an abundance of flowering plants (500-1700 inflorescences of *S. aspericocca* ssp. *Cylindrical inflorescence*). There was a mean ± SD of 14.30 ± 4.47 open flowers per inflorescence across the surveys. This method was used to cover as many flowering plants as possible per survey, given that other studies of phalenophilous plants have observed infrequent floral visitation (e.g., Pettersson 1991; Oliveira et al. 2004). To enable observations of floral visitors after dark, a red-light source was used, generated by covering a hand-held torch with red cellophane (Funamoto and Ohashi 2017). A red-light source is thought to lessen the potential disturbance of flower-visiting moths and is a commonly used technique in studies of moth pollination (Kearns and Inouye 1993 p. 389). Video cameras capable of recording in infra-red (Panasonic HC-VX980M) were also used to film floral visitation to inflorescences of *S. aspericocca* ssp. *Cylindrical inflorescence*. Cameras were positioned ~1-2 m from several inflorescences of *S. aspericocca* ssp. *Cylindrical inflorescence*, without regard to individual plants, with filming lasting 1-3 hours (mean: 1 hour, 48 minutes). Personal observations and video recordings started at or shortly after sunset, finishing before 23:00 hours. Surveys took place over 11 nights, from 06/10/17-13/11/17, across several reserves (see Table 5-2 in Results).

A sweep net was used during observation periods to capture flower-visiting moths, which were subsequently placed in kill jars containing ethyl acetate and then placed in individual specimen tubes, before being frozen. Following the methods of Devoto et al. (2011) and Banza et al. (2015), sampled moths were later swabbed for pollen with a small cube of fuchsin jelly (Beattie 1971). Pollen was sampled from the area between the base of the antennae and the labium, each compound eye, and the proboscis (uncoiled when possible). The cube of fuchsin jelly was subsequently melted onto a microscope slide and preserved with a coverslip sealed with colourless nail varnish. Pollen grains were identified under a compound microscope (100-400 × magnification), using a pollen library constructed from co-flowering plants. The reticulate pollen grains of *S. aspericocca* (Fig. 5-1g) were distinctive from those of other co-flowering plant species (Pers. Obs.), although pollen of the two subspecies could not be separated. It is possible that some pollen grains become attached to moths via pollen contamination during the sampling process. Thus, following Devoto et al. (2011), moths carrying fewer than five pollen grains of the same plant taxon were considered not to have visited the respective plant. Netted moths were identified where possible using the guides of McQuillan and Forrest (1985), Common (1990), Marriott (2011, 2012), Marriott et al. (2017), and McQuillan et al. (2019).

It should be noted that floral visitation and pollen load analysis alone do not definitively demonstrate pollination. However, the combination of direct observation of flower visitation to flowers of *S. aspericocca* ssp. *Cylindrical inflorescence*, and finding pollen of *S. aspericocca* adhering to moths, provides stronger evidence of moth pollination than simply observing flower visitation alone (Van Zandt et al. 2020). However, ultimately, experimental studies are required to directly confirm pollination by night-flying moths.

5.3.5 Plant reproduction - 2018 and 2019

In each year, a minimum of five focal plants were haphazardly selected within each sample site to measure natural levels of plant reproduction. On each of these plants, when possible, two inflorescences were selected, and a haphazardly chosen number of newly and near opened flowers were tagged on each inflorescence. Flowers were tagged on each inflorescence by knotting twine between two sections of the inflorescences and counting the number of flowers encompassed. Across both years, a mean ± SD of 10.98 ± 3.83 flowers were tagged per inflorescences. All 40 plants in 2018 had two inflorescences tagged, while two of 42 plants had only a single inflorescence tagged in 2019.

Differences in plant reproduction may be related to aspects of the plant itself, such as plant size (Bruna and Kress 2001), or to local variables, such as the density of flowering conspecifics (Kunin 1997). Thus, for each focal plant in 2018 and 2019, several variables considered potentially important to their reproduction were measured. This included the number of stems per plant, both flowering and non-flowering, used as a measure of plant size; the number of open flowers per plant at the time of tagging; the number of flowering conspecifics (both subspecies) within 2 m; the number of inflorescences (min. one flower open) of conspecifics within 2 m; and the number of open flowers on conspecifics within 2 m at the time of tagging. The latter three measures were used as measures of conspecific floral density. Focal plants were selected so that there was little to no overlap of the 2 m radius between plants. Because stems branch above ground level in *S. aspericocca* ssp. *Cylindrical inflorescence* (Baker 1977), a stem was counted separately from a conjoined stem when greater than 10 cm in length.

In 2019, additional plants within the 2 m radius of focal plants were also tagged. This was done to increase replication for measures of plant reproduction. For these additional plants, measures of conspecific floral density were not undertaken. However, plant size, and the number of open flowers per plant at the time of tagging were recorded. Thus, the total number of plants tagged for measures of reproduction in 2019 was increased from 42 to 68, relative to 40 plants tagged in 2018. However, final sample size (number of plants, inflorescences, flowers) was reduced due to factors such as plant death (at least of above ground tissues) and herbivory of stems with tagged inflorescences (Table 5-1).

In both years, plants were tagged from mid-October to early November. Following flowering, the number of fruits and seeds produced per fruit were recorded in late November to early December (~1-1.5 months later). Fruit-set was calculated by summing the number of fruits produced within the tagged segment of each inflorescence (one or two per plant) and dividing by the original number of flowers tagged. Thus, a proportional measure of fruit-set was obtained. Similarly, mean seed number per fruit was calculated by summing the number of seeds produced across fruits within the tagged segment of each inflorescence and dividing by the number of fruits produced. To avoid confusion, note that the mean number of seeds per fruit per plant refers to the mean number of seeds per fruit (a measure calculated for each individual tagged plant) averaged across tagged plants. Fruit-set and mean seed number per fruit were significantly correlated with one another in 2018 (Pearson's $r = 0.487$, $p = 0.002$, $n = 37$) and 2019 ($r = 0.598$, $p = 2.297 \times 10^{-7}$, $n = 63$; supplementary material (SM) Fig. 5-1).

5.3.6 Statistical analysis

Here, models used to analyse fruit-set and seed production are detailed. All statistical models were run within the program R (R Core Team 2020). Figures were constructed via the packages 'ggplot2' (Wickham 2016) and 'tmap' (Tennekes 2018). Linear mixed-effect models (LMM) were constructed via the package 'lme4' (Bates et al. 2015). Statistical significance was tested via an Analysis of Deviance, using a Type II Wald F test with Kenward-Roger corrected degrees of freedom (implemented in package 'car'; Fox and Weisberg 2019). Generalised linear mixed-effect models (GLMM) were constructed via the package 'glmmTMB' (Brooks et al. 2017). Statistical significance of predictors in GLMM's were tested via an Analysis of Deviance, using a Type II Wald Chi-square test (implemented in package 'car'). Multiple comparisons were performed via the package 'multcomp' (Hothorn et al. 2008), with a Bonferroni correction used to account for inflated Type I error.

Bonferroni-corrected *p*-values are presented (*p*-value × no. of comparisons). Diagnostic checks of mixed-effect models were performed within the package 'DHARMa' (Hartig 2021).

5.3.7 Plant reproduction, plant size, and conspecific floral density

We used Spearman's rank correlations to explore relationships between fruit-set, or mean seed number per fruit, and variables considered potentially important to the reproduction of individual plants in 2018 and 2019 (i.e., plant size, number of flowering conspecifics within 2 m, etc.). However, both plant size (i.e., number of stems per plant) and the number of open flowers per plant were positively correlated within one another in 2018 ($r_s = 0.859$, $p = 9.774 \times 10^{-12}$, $n = 37$) and 2019 ($r_s = 0.790$, $p = 1.351 \times 10^{-14}$, $n = 63$). Likewise, measures of conspecific floral density were positively correlated with one another in 2018 ($r_s \geq 0.934$, $p < 2.2 \times 10^{-16}$, $n = 37$) and 2019 ($r_s = \geq 0.923$, $p < 2.2 \times 10^{-16}$, $n = 41$). Hence, conclusions were qualitatively comparable among the correlated variables. Thus, we present correlations of plant reproduction with plant size and the number of conspecific flowers within 2 m of tagged plants (for correlations with additional variables see SM Fig. 5-2, 5-3, 5-4, and 5-5). Correlations were performed by pooling data for plants across reserves. Thus, the inherent assumption is that if a correlation among variables exists, it is largely consistent across sample sites. However, it is possible that sample sites differ in the relationships exhibited (e.g., see Costin et al. 2001). Thus, any significant correlation exhibited for plants across reserves was further explored for plants within sample sites via visual inspection of the plotted data.

5.3.8 Fruit-set and reserve area

To test the hypothesis that fruit-set would show a positive relationship with reserve area, a GLMM with a Beta-Binomial error structure to account for overdispersion (Harrison 2015), was used to analyse the relationship between fruit-set and reserve area in 2018 and 2019. Reserve was treated as a random effect to account for the inherent pseudo-replication between individual plants within sample sites. The independent predictor of reserve area was log transformed prior to analysis to reduce right skewness. To see if mean fruit-set differed between the reserves chosen (i.e., treating the effect of sample site within each reserve as fixed and independent), a generalised linear model (GLM), with a quasibinomial distribution to account for overdispersion, was used to analyse fruit-set between sample sites within each year. Significance was tested via an Analysis of Deviance, using a Type II F-test.

5.3.9 Mean seed number per fruit and reserve area

To test the hypothesis that mean seed number per fruit would show a positive relationship with reserve area, a LMM was used to analyse the relationship between mean seed number per fruit and reserve area in 2018 and 2019. Reserve was again treated as a random effect, and the independent predictor of reserve area was log transformed prior to analysis. In 2018, there was no variance attributed to reserve (i.e., variance of the specified random effect was singular) and a partially Bayesian method was used to prevent a singular fit via the 'blmer' function in the package 'blme' (Chung et al. 2013). To see if mean seed number per fruit differed on average for plants between the reserves chosen, a one-way ANOVA was used to analyse mean seed number per fruit between sample sites for each year. Assumptions of normality and equality of variance were checked via plots of model residuals (i.e., Q-Q plots, S-L plots). Following checks of residuals, a James Second Order test was used to account for heterogeneity of variance in 2018 (Quinn and Keough 2002 pg. 195). The James Second Order test was conducted via the R package 'onewaytests' (Dag et al. 2018).

5.3.10 Plant-to-plant variability and reserve area

Some prior studies have found plants within smaller fragments (Aguilar and Galetto 2014) or populations (Jacquemyn et al. 2002) exhibit greater plant-to-plant variability in successful reproduction. Consequently, for both years, the coefficient of variation (CV = (SD/mean) × 100) for fruit-set, and mean seed number per fruit, among plants within sample sites was calculated. These standardized measures can be interpreted as reflecting the degree of uncertainty in reproduction for plants within sample sites (Oostermeijer et al. 1998). Pearson's correlations were used to examine the relationship between sample site CVs, for both fruit-set and mean seed number per fruit, and reserve area (log transformed). A negative correlation would suggest plant-to-plant variance in reproduction is greater for sample sites within reserves of smaller area.

5.3.11 Fruit-set and mean seed number per fruit between 2018 and 2019

A Beta-Binomial GLMM was used to analyse if fruit-set differed for plants between 2018 and 2019. Reserve was treated as a random effect to account for correlations between plants within reserves across years. Similarly, a nested reserve by year random factor was used to account for correlations between plants within reserves in each year. A LMM was used to analyse if mean seed number per fruit differed for plants between years, with the same random effect structure as the previous model.

5.4 RESULTS

5.4.1 Flower visitation - 2017

- ***Video recordings***

Across eight nights (Table 5-2), over a total of 27 hours, 182 inflorescences of *S. aspericocca* ssp. *Cylindrical inflorescence* were recorded. An average of 12.13 ± 8.13 inflorescences were filmed per video (*n* = 15 videos). Sixteen moths were recorded visiting at least one inflorescence of *S. aspericocca* ssp. *Cylindrical inflorescence* (Fig. 5-3a-c), and 9/15 recordings had at least one moth visitation. Moth visits per inflorescence lasted from a few seconds to more than two minutes (mean ± SD of 27.33 ± 37.85 seconds per inflorescence). Some visits may not have resulted in pollination (e.g., moths resting on an inflorescence); nevertheless, moths typically appeared to be actively nectaring at flowers (i.e., head directed towards opening of flowers; deliberate movement between flowers on inflorescence(s)). For an individual plant filmed in Mark Oliphant CP (16/10/2017), a visiting moth spent a total of 5 minutes 51 seconds visiting flowers between three of the plant's inflorescences (Fig. 5-3a). There was a tendency for moths to visit flowers closer to the top of the inflorescence (Fig. 5-3b-c), and in some cases, moths moved acropetally up the inflorescence when visiting flowers (Fig. 5-3a). Floral visitation was only observed after-dark, and not under crepuscular (twilight) conditions.

- ***Personal observations***

A total of 25 moths were observed visiting flowers of *S. aspericocca* ssp. *Cylindrical inflorescence*, with an additional two moths observed visiting flowers of *S. aspericocca* ssp. *One-sided inflorescence* (Table 5-2). Floral visitation was again only observed after dark (mean: 21:13). Moth visitation appeared more active on relatively warmer nights with little wind (Pers. Obs.).

Table 5-2 Details related to the personal observations of nocturnal visitors to the flowers of *Stackhousia aspericocca* ssp. *Cylindrical inflorescence* and *S. aspericocca* ssp. *One-sided inflorescence*. Given are the reserves in which surveys occurred, the date of each survey, the start time and end time (24-hour clock) of each survey, times when moths were caught or otherwise seen but not caught on the flowers of either subspecies, whether a video camera was used during the survey, and the number of observers per survey. Notes refer to additional moths caught or otherwise seen but not caught visiting the inflorescences of co-flowering *Pimelea linifolia* ssp. *linifolia* during surveys. Note that the time given for when moths were caught or otherwise seen but not caught on flowers of *S. aspericocca* (either subspecies) refers to personal observations of floral visitors in the field. Each of these times refers to either the capture or observation without capture of a single moth (except Scott Creek CP - 9/11/17). Values in parentheses under camera used give the total time (hours:minutes) of video recordings per night (i.e., pooled across the number of cameras used per night), followed by the number of moths recorded visiting flowers. CI = *S. aspericocca* ssp. *Cylindrical inflorescence*, OSI = *S. aspericocca* ssp. *One-sided inflorescence*. NA = no moths personally observed visiting the flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* or *S. aspericocca* ssp. *One-sided inflorescence* during the survey.

Reserve	Date	Start time	End time	Moth(s) caught (Time)	Moth(s) observed (Time)	*Stackhousia aspericocca* ssp.	Camera used	Number of observers	Notes
Mark Oliphant CP	6/10/17	19:44	20:44	19:56		CI	Yes (1:21, 0)	1	
Nurrutti Reserve	8/10/17	19:48	21:40	21:05		CI	Yes (3:43, 2)	1	
Mylor CP	10/10/17	19:38	22:14		20:19	CI	No	1	
				20:24		CI			
					21:29	OSI			
				21:46		CI			
				22:00		CI			
Mylor CP	15/10/17	20:07	21:31	20:36		CI	No	1	
					21:01	CI			
					21:15	OSI			

Mark Oliphant CP	16/10/17	20:40	22:50		21:12	CI	Yes (4:04, 2)	3	Two additional moths caught visiting the inflorescences of *Pimelea linifolia* ssp. *linifolia*.
				21:22		CI			
				21:41		CI			
				21:54		CI			
				22:10		CI			
				22:27		CI			
Scott Creek CP	18/10/17	20:22	22:44			NA	Yes (1:41, 0)	1	
Scott Creek CP	23/10/17	20:08	21:57			NA	Yes (4:05, 1)	1	Additional moth caught visiting the inflorescence of *Pimelea linifolia* ssp. *linifolia*.
Giles CP	26/10/17	20:14	22:42		20:20	CI	Yes (6:02, 4)	2	Additional moth observed visiting the inflorescence of *Pimelea linifolia* ssp. *linifolia*.
					20:21	CI			
					21:15	CI			
Giles CP	8/11/17	20:41	21:26			NA	Yes (2:21, 2)	2	
Scott Creek CP	9/11/17	20:19	22:02		20:32[a]	CI	Yes (3:48, 5)	2	
				21:14		CI			
				21:19		CI			
				21:25		CI			
				21:49		CI			
Giles CP	13/11/17	20:22	22:22	20:53		CI	No	1	
					22:09	CI			

[a] Two moths observed visiting an inflorescence of the same plant of *S. aspericocca* ssp. *Cylindrical inflorescence*.

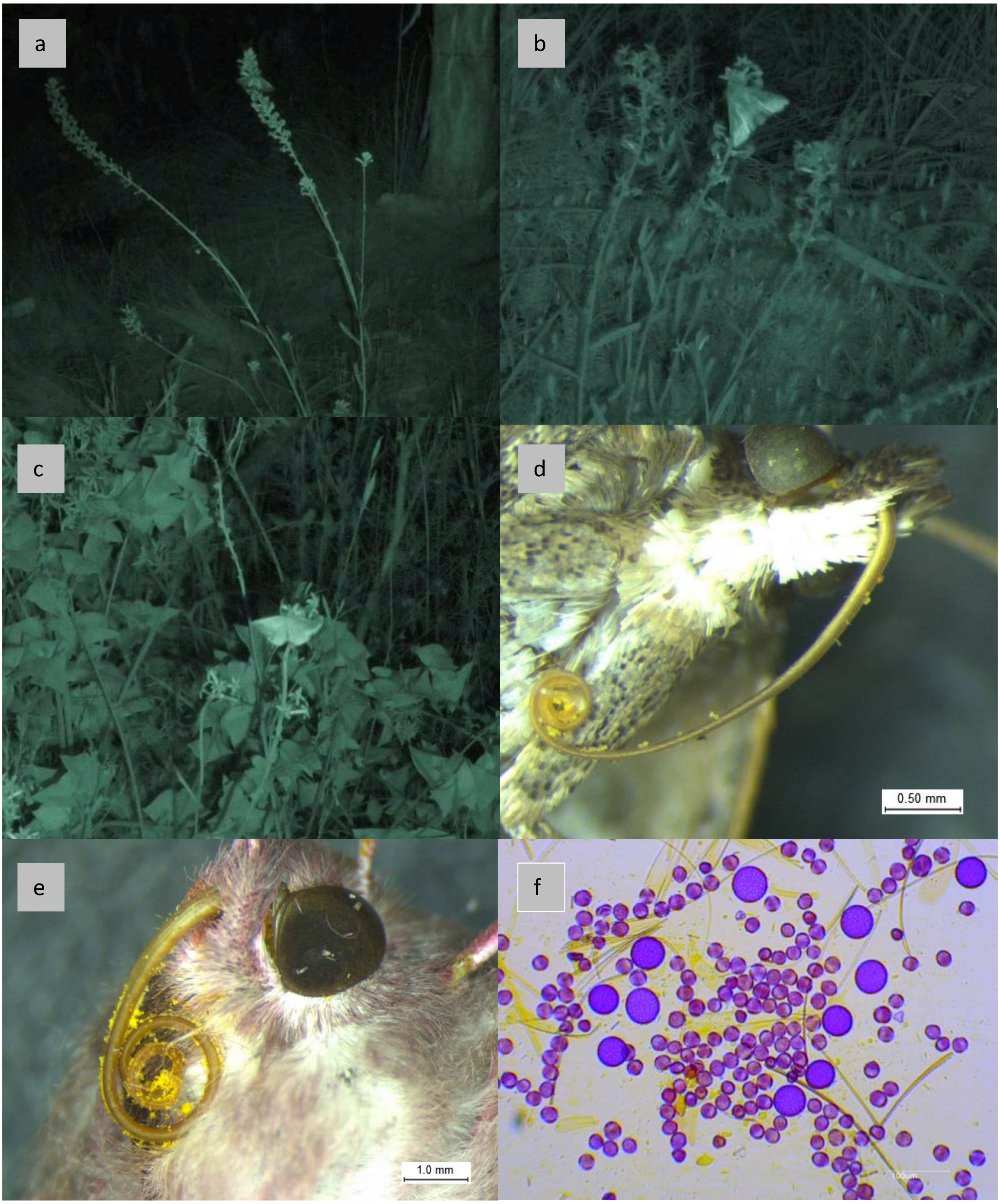


Fig. 5-3 Moths recorded visiting inflorescences of *Stackhousia aspericocca* ssp. *Cylindrical inflorescence*. (**a**) Mark Oliphant CP 16/10/2017. (**b**-**c**) Scott Creek CP 09/11/2017. (**d**) *Taxeotis* sp. (Geometridae) captured visiting an inflorescence of *S. aspericocca* ssp. *Cylindrical inflorescence*. (**e**) *Oenochroma vinaria* (Geometridae) captured visiting an inflorescence of *S. aspericocca* ssp. *Cylindrical inflorescence*. (**f**) Pollen grains sampled from the specimen of *O. vinaria* pictured in (**e**). Smaller pollen grains of *S. aspericocca* (either subspecies) are abundant, with some larger pollen grains belonging to the genus *Pimelea* (likely that of *Pimelea linifolia* ssp. *linifolia*) also present (100× magnification, scalebar = 100 μm).

5.4.2 Pollen load analysis - 2017

Of the 25 moths observed visiting flowers of *S. aspericocca* ssp. *Cylindrical inflorescence*, 16 were captured for pollen load analysis (Table 5-2). Given the pollen of each subspecies could not be separated, the phrase "*S. aspericocca* pollen" refers to pollen of either subspecies, given some moths could have visited flowers of *S. aspericocca* ssp. *One-sided inflorescence* prior to being captured on *S. aspericocca* ssp. *Cylindrical inflorescence*. Two moths carried fewer than five pollen grains overall and are not considered further (Table 5-3). The remaining 14 moths each carried more than five pollen grains of *S. aspericocca*, ranging from 11 to 3537 pollen grains (Table 5-3). These moths belonged to the large and diverse families of Geometridae and Noctuidae (Table 5-3). Overall, the sampled moths mostly carried *S. aspericocca* pollen versus pollen of other co-flowering plants (Table 5-3). The bright yellow pollen grains of *S. aspericocca* were visible on the proboscis of captured moths (Fig. 5-3d-e) and were also found in abundance between the labial palps and labium in which the proboscis recoils. Notably, the two *Hakea* moths, *Oenochroma vinaria* Guenée (Geometridae), carried 3442 and 3537 *S. aspericocca* pollen grains, respectively (Fig. 5-3e-f).

Most co-flowering plants were not expected to be pollinated by night-flying moths. However, both specimens of *O. vinaria* carried 56 and 224 pollen grains, respectively, belonging to plants of the genus *Pimelea* (likely co-flowering *Pimelea linifolia* Sm. ssp. *linifolia* (Thymelaeaceae); Table 5-3). In addition, an individual of *Persectania ewingii* (Westwood) (Noctuidae) carried 114 pollen grains of the genus *Pimelea* (Table 5-3). *Pimelea* blossoms are often visited by butterflies (Armstrong 1979) and have a floral morphology typical of butterfly pollination (so-called psychophilous pollination syndrome; Faegri and van der Pijl 1979; Willmer 2011). Nevertheless, this pollen load analysis suggests some night-flying moths that visit *S. aspericocca* may also visit flowers of co-flowering *Pimelea* species (Fig. 5-3f). This was further supported by observations of moths visiting flowers of *P. linifolia* ssp. *linifolia* while conducting surveys on flowering *S. aspericocca* (notes in Table 5-2). Three of these moths were captured and found to carry pollen belonging to both *Pimelea* and *S. aspericocca*. The sampled moths included another individual of *P. ewingii*, the Bogong moth, *Agrotis infusa* (Boisduval) (Noctuidae), and the Southern Whistling moth, *Hecatesia thyridion* Feisthamel (Noctuidae). An additional individual of the Tree Lucerne moth, *Uresiphita ornithopteralis* (Guenée) (Crambidae), opportunistically sampled from LED light-traps (Green et al. 2012), was also found to carry *S. aspericocca* pollen.

Table 5-3 Moths caught visiting the flowers of *Stackhousia aspericocca* ssp. *Cylindrical inflorescence* in 2017. For each specimen, the reserve where the moth was captured, the date, and the identity of the captured moth (ID) are provided. Moths were identified to either family (Fam.), subfamily (Subfam.), genus, or species level. The wingspan (twice the length from the centre of the thorax to the tip of the forewing), the number of grains of *Stackhousia aspericocca* pollen (potentially from either subspecies), *Pimelia* spp. pollen, unknown plant pollen, and the total number of pollen grains on each moth is provided. The mean ± standard deviation (SD) of each of these pollen categories is given below their respective columns. Moths carrying fewer than 5 pollen grains of a particular plant taxon were considered not to have visited the respective plant, and these pollen grains were not included in counts of total pollen grains carried for each moth, or the mean number of pollen grains carried across moths.

Reserve	Date	ID - (wingspan)	Pollen			
			Stackhousia aspericocca	*Pimelia* spp.	Unknown[a]	Total
Mark Oliphant CP	06/10/2017	Fam. Noctuidae - (~40 mm)	287	0	104	391
Nurrutti Reserve	08/10/2017	Fam. Geometridae - (~34 mm)	38	0	0	38
Mylor CP	10/10/2017	*Scopula* sp. (Geometridae, Subfam. Sterrhinae) - (~28 mm)	135	0	0	135
		Oenochroma vinaria (Geometridae, Subfam. Oenochrominae) - (~50 mm)	3442	56	49	3547
		Geometridae - (~24 mm)	40	0	3	40
Mylor CP	15/10/2017	Unidentified[b]	0	0	0	0
Mark Oliphant CP	16/10/2017	Noctuidae (Subfam. *Heliothinae*) - (~40 mm)	687	0	148	835
		Persectania ewingii (Noctuidae, Subfam. Hadeninae) - (~40 mm)	15	114	114	243
		O. vinaria - (~50 mm)	3537	224	1	3761
		Noctuidae - (~34 mm)	1010	2	182	1192
		Unidentified[b]	0	0	2	0

Scott Creek CP	09/11/2017	*Taxeotis* sp. (Geometridae, Oenochrominae) - (~24 mm)	136	0	18	154
		Geometridae - (~24 mm)	11	0	7	18
		P. ewingii - (~40 mm)	366	0	131	497
		Taxeotis sp. - (~24 mm)	82	0	22	104
Giles CP	13/11/2017	*Taxeotis* sp. - (~24 mm)	81	0	0	81
			Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
			616.69 ± 1155.70	24.63 ± 61.43	48.44 ± 64.15	689.75 ± 1204.27

[a] Although not sampled within the study sites most unknown pollen was of the same general morphology as pollen of the genus *Eucalyptus* (e.g., *E. obliqua*).

[b] Unidentified moths carried fewer than five pollen grains in total.

5.4.3 Fruit-set and mean seed number per fruit - 2018

Excluding plants suffering from mortality or herbivory, all plants produced fruits, with a single plant producing zero fruits on one of its two tagged inflorescences. Pooled across plants and sample sites, overall fruit-set was 62.89 % in 2018 (527 fruits/838 flowers), with overall fruit-set ranging from 51.40 % to 87.90 % between sample sites (Fig. 5-4a). Similarly, pooled across plants and sample sites, mean seed number per fruit per plant was 2.10 ± 0.42, with mean seed number per fruit per plant ranging from 1.85 ± 0.75 to 2.31 ± 0.12 between sample sites (Fig. 5-5a). There was evident variability between individual plants within sample sites (see individual data points in Fig. 5-4a and Fig. 5-5a). Nevertheless, there was no clear correlation between plant size (r_s = -0.083; p = 0.621 , n = 37), or the number of conspecific flowers within 2 m (r_s = 0.284; p = 0.088, n = 37), and the fruit-set of plants across sample sites. Likewise, there was no clear correlation between plant size (r_s = 0.053; p = 0.754, n = 37), or the number of conspecific flowers within 2 m (r_s = 0.216; p = 0.198, n = 37), and mean seed number per fruit for plants across sample sites.

There was no significant effect of reserve area, and thus there was no statistically significant trend for increased fruit-set with increasing reserve area in 2018 (Table 5-4). This was also true of mean seed number per fruit per plant (Table 5-4). Thus, there was no support for the hypothesis that levels of plant reproduction would show a positive relationship with reserve area. There was also no evidence of significant spatial variation generally, with no statistically clear difference between sample sites for plants in both fruit-set ($F_{7,29}$ = 1.214, p = 0.327; Fig. 5-4a) and mean seed number per fruit (James Second Order test: J = 12.595, $h(\alpha)$ = 29.437, $p > 0.05$; Fig. 5-5a).

In addition, there was no statistically significant relationship between plant-to-plant variation (CV) within sample sites and reserve area for both fruit-set (r = -0.438; p = 0.278, n = 8) and mean seed number per fruit (r = 0.097; p = 0.820, n = 8) in 2018 (SM Fig. 5-6a-b). Thus, reserve area is not related to local among-plant variability or uncertainty in plant reproduction.

5.4.4 Fruit-set and mean seed number per fruit - 2019

Excluding plants suffering from mortality or herbivory, all tagged plants except one produced fruits. However, five plants produced zero fruits on one of their two tagged inflorescences. Pooled across plants and sample sites, overall fruit-set was 58.46 % in 2019 (743/1271), with fruit-set ranging from 37.70 % to 74.60 % between sample sites (Fig. 5-4b). Overall, mean seed number per fruit per plant was 1.90 ± 0.49 in 2019, with mean seed number per fruit per plant ranging from 1.47 ± 0.55 to 2.28 ± 0.46 between sample sites (Fig. 5-5b). Again, there was evident variability between individual plants within sample sites (see individual data points in Fig. 5-4b and Fig. 5-5b). Nevertheless, there was no clear correlation between plant size (r_s = -0.025; p = 0.846, n = 64), or the number of conspecific flowers within 2 m (r_s = 0.152; p = 0.343, n = 41), and the fruit-set of plants across sample sites. Similarly, mean seed number per fruit was not correlated with plant size (r_s = -0.187; p = 0.143, n = 63). However, mean seed number per fruit was significantly positively correlated with the number of conspecific flowers within 2 m (r_s = 0.405; p = 0.009, n = 40). Thus, there was a tendency in 2019 for plants surrounded by a greater floral density of conspecifics to have a higher mean seed number per fruit (Fig. 5-6). However, this positive relationship was not particularly clear for all plants within sample sites (SM Fig. 5-7).

As in 2018, there was no significant effect of reserve area on fruit set in 2019, and thus there was no statistically significant trend for increased fruit-set with increasing reserve area in 2019 (Table 5-4). This was also true of mean seed number per fruit per plant (Table 5-4). Thus, there was no support for the hypothesis that levels of plant reproduction would show a positive relationship with reserve area. There was also no statistically clear difference between sample sites in mean seed number per fruit ($F_{7,55}$ = 1.914, p = 0.085; Fig. 5-5b). However, fruit-set was significantly different between sample sites ($F_{7,56}$ = 2.414, p = 0.031), although pairwise comparisons suggested plants in Mark Oliphant only approached significance in being of higher fruit-set than plants sampled in Wottons Scrub (z = 2.948, p = 0.090; Fig. 5-4b).

There was also no statistically clear relationship between plant-to-plant variation (CV) within sample sites and reserve area for both fruit-set (r = -0.323; p = 0.435, n = 8) and mean seed number per fruit (r = -0.539; p = 0.168, n = 8) in 2019 (SM Fig. 5-6c-d). Thus, reserve area was not significantly related to uncertainty in plant reproduction.

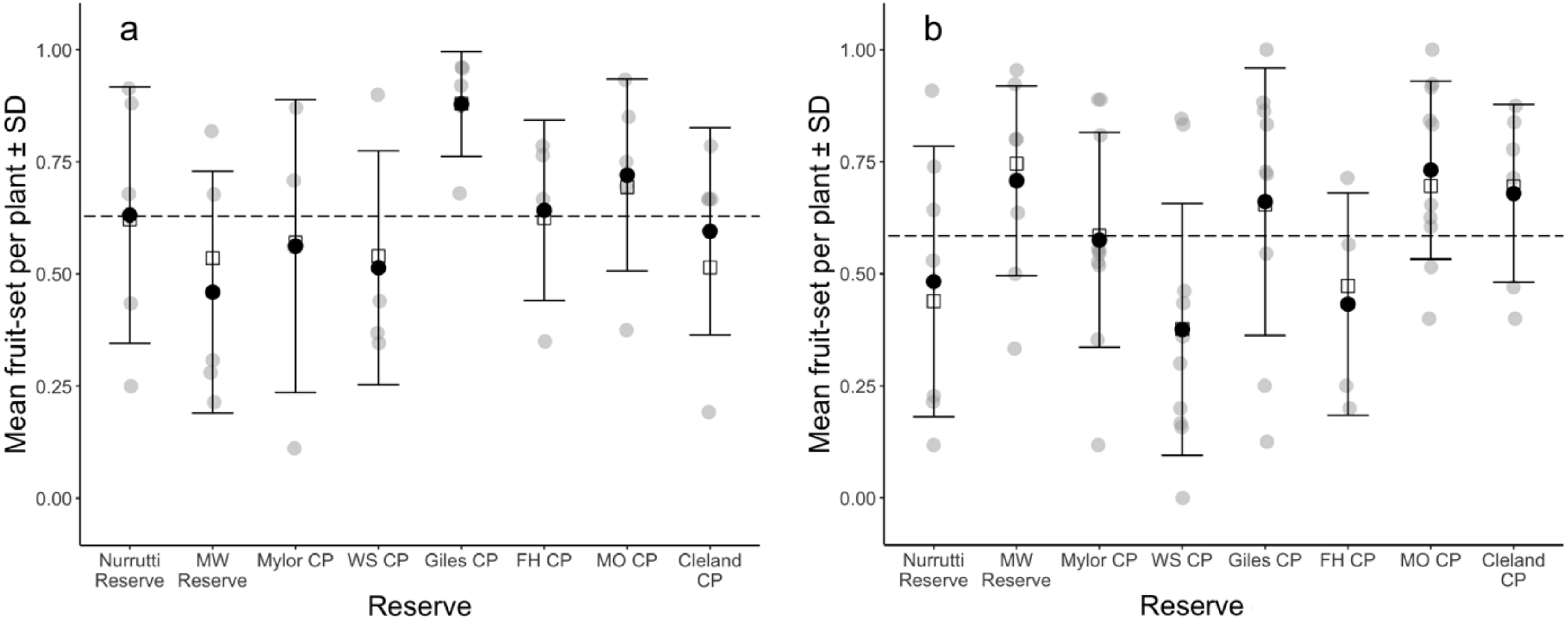


Fig. 5-4 Mean fruit-set per plant (black points) ± standard deviation (SD) of tagged plants for sample sites within reserves in (**a**) 2018 and (**b**) 2019. Square datapoints represent the proportion of the total number of fruits produced from the total number of flowers tagged, pooled across plants per sample site (i.e., population proportion). Fruit-set for each of the individual tagged plants within sample sites is also plotted (grey data points). The dotted line in each plot represents the overall fruit-set of tagged plants pooled across sample sites (i.e., population proportion). Reserves are ordered in ascending order of reserve area. MW Reserve = Malcolm Wicks Reserve, WS CP = Wottons Scrub CP, FH CP = Filsell Hill CP, MO CP = Mark Oliphant CP.

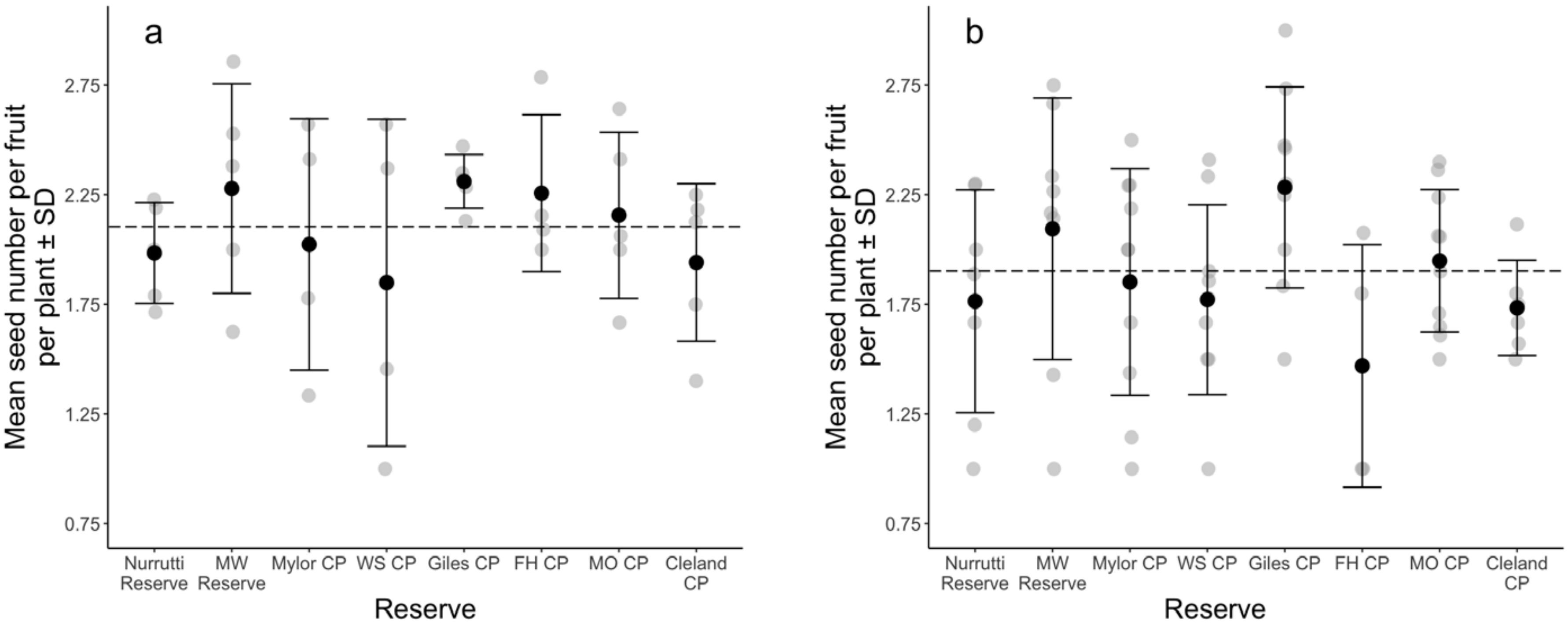


Fig. 5-5 Mean seed number per fruit per plant (black points) ± standard deviation (SD) of tagged plants for sample sites within reserves in (**a**) 2018 and (**b**) 2019. Mean seed number per fruit for each of the individual tagged plants within sample sites is also plotted (grey data points). The dotted line in each plot represents the mean seed number per fruit per plant, for plants pooled across sample sites. Reserves are ordered in ascending order of reserve area. MW Reserve = Malcolm Wicks Reserve, WS CP = Wottons Scrub CP, FH CP = Filsell Hill CP, MO CP = Mark Oliphant CP.

Table 5-4 Models of fruit-set and mean seed number per fruit for 2018, 2019, and 2018 vs. 2019. The random intercept terms (nested from top to bottom) for each model are presented along with their standard deviations. The intercept and slope on the scale of the link function for each model are provided along with their 95 % confidence intervals (Wald) in brackets.

Year	Response	Predictor	Random Effect(s)	Intercept	Slope	Model	Test Statistic	*p-value*
2018	Fruit-set	Reserve area (log transformed)	Reserve: 0.222	0.306 (-0.50, 1.12)	0.053 (-0.13, 0.24)	Beta-Binomial	$\chi^2_1 = 0.320$	0.572
	Mean number of seeds per fruit	Reserve area (log transformed)	Reserve: 0.188[1]	2.121 (1.68, 2.57)	-0.005 (-0.10, 0.09)	Gaussian	$F_{(1,\ 5.491)} = 0.009$	0.930
2019	Fruit-set	Reserve area (log transformed)	Reserve: 0.350	0.080 (-0.77, 0.92)	0.064 (-0.13, 0.26)	Beta-Binomial	$\chi^2_1 = 0.425$	0.515
	Mean number of seeds per fruit	Reserve area (log transformed)	Reserve: 0.182	1.929 (1.51, 2.35)	-0.011 (-0.11, 0.08)	Gaussian	$F_{(1,\ 6.648)} = 0.051$	0.828
2018 vs. 2019	Fruit-set	Year	Reserve: 0.272 Reserve by Year: 0.178	0.503[2] (0.11, 0.90)	-0.165 (-0.61, 0.28)	Beta-Binomial	$\chi^2_1 = 0.523$	0.469
	Mean number of seeds per fruit	Year	Reserve: 0.176 Reserve by Year[1]: 0.115	2.100[2] (1.89, 2.30)	-0.214 (-0.43, 0.00)	Gaussian	$F_{(1,\ 7.078)} = 3.926$	0.088

[1] Partially Bayesian method used to prevent a singular fit via the ‘blmer’ function in the package ‘blme’ (Chung et al. 2013).

[2] Intercept for the categorical predictor year represents the estimated response of plants in the first year (2018).

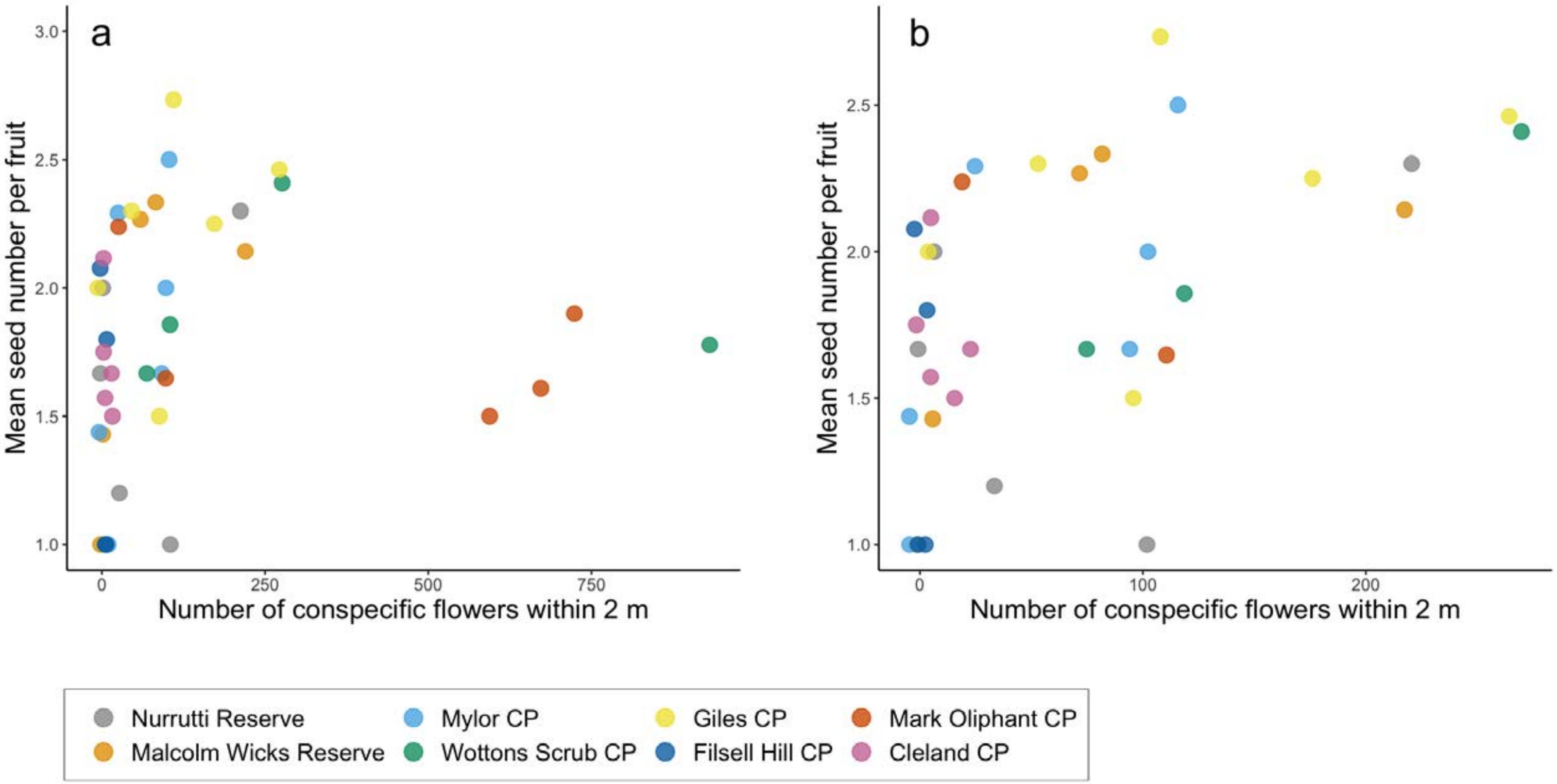


Fig. 5-6 (**a**) Relationship between the mean seed number per fruit of tagged plants and the number of conspecific flowers (open at the time of tagging) within 2 m of tagged plants in 2019, pooled across sample sites. Points are randomly jittered at a width of 10 to aid visualisation. (**b**) Plot excluding plants surrounded by greater than 300 conspecific flowers. Points are randomly jittered at a width of 5 to aid visualisation.

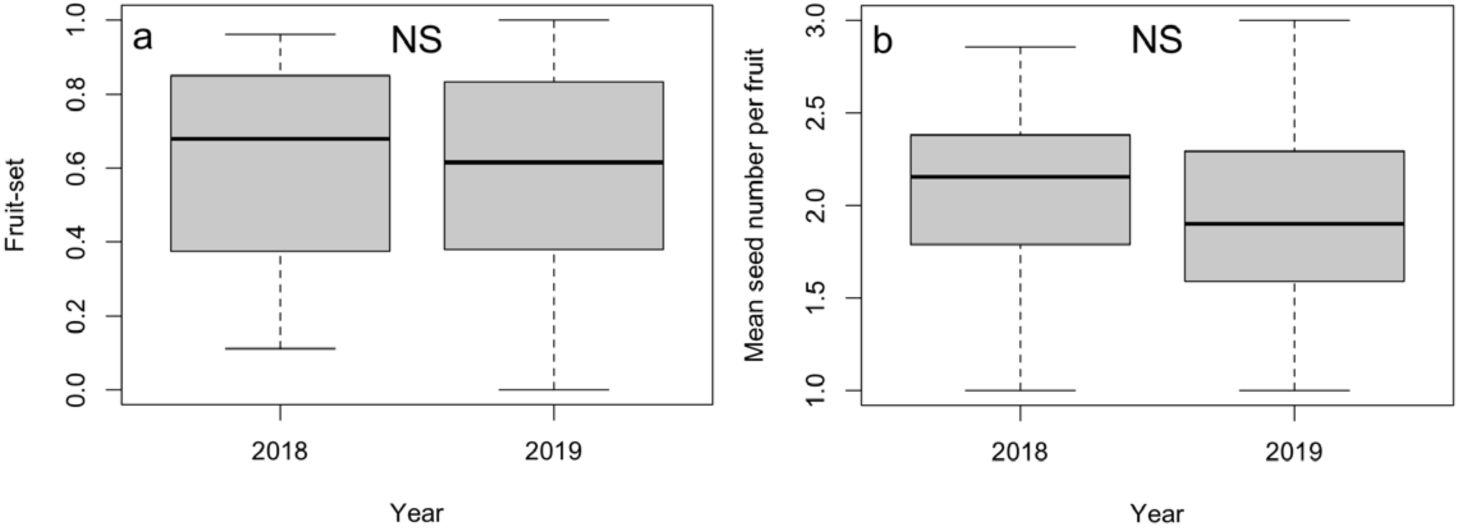


Fig. 5-7 (**a**) Fruit-set of tagged plants, pooled across reserves, in 2018 and 2019. (**b**) Mean seed number per fruit of tagged plants, pooled across reserves, in 2018 and 2019. NS stands for non-significant.

5.4.5 Fruit-set and mean seed number per fruit - 2018 versus 2019

Although fruit-set was typically lower for plants in 2019 (Fig. 5-7a), there was no statistically significant difference in the fruit-set of tagged plants between 2018 and 2019 (Table 5-4). Similarly, there was no statistically clear difference in mean seed number per fruit for tagged plants between 2018 and 2019 (Fig. 5-7b), although lower mean seed number per fruit in 2019 approached statistical significance (i.e., $p < 0.10 \geq 0.05$; Table 5-4). This was reflected in the relative numbers of one-seeded fruits (i.e., fruits with one coccus) between years, with 22.01 % (116/527) of fruits with one seed in 2018, versus 32.30 % (240/743) of fruits with one seed in 2019.

5.5 DISCUSSION

Floral visitation by night-flying settling moths to flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* was confirmed in this study; with visiting moths carrying large pollen loads of the study species. This supports *S. aspericocca* ssp. *Cylindrical inflorescence* being assigned a phalenophilous pollination syndrome. However, plant reproduction, measured as fruit-set and mean seed number per fruit, showed no relation to reserve area across two years. Thus, the hypothesis that plant reproduction would show a positive relationship with reserve area was not supported. Ultimately, plant reproduction showed limited variability among reserves more generally, suggesting mean levels of plant reproduction are spatially similar throughout the fragmented landscape of the study region. Thus, plant reproduction within the smallest reserves is comparable to that of plants in the largest reserves.

5.5.1 Moth pollination

We confirmed visitation by night-flying settling moths to flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* (n = 25 moths) and *S. aspericocca* ssp. *One-sided inflorescence* (n = 2). Floral visitation was always observed after dark (Table 5-2), suggesting pollination is nocturnal rather than crepuscular. Thus, pollination is not restricted to a limited period after sunset (e.g., hawkmoth pollinators in East Africa; Martins and Johnson 2013).

Moths captured visiting the flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* carried between 11 to 3537 pollen grains of *S. aspericocca*, with 8/14 moths carrying over 100 pollen grains (Table 5-3). Studies examining the pollen loads of settling moths typically find individuals to carry fewer than 100 pollen grains and rarely more than 1000 (e.g., Pettersson

1991; Devoto et al. 2011; Atwater 2013; LeCroy et al. 2013; Banza et al. 2015). However, direct comparisons between studies are difficult, given differences between plant floral ecology (e.g., flower shape, differences in pollenkitt), methods used to sample moths (i.e., light-trap versus direct collection from flowers), and recent evidence that much of the pollen acquired by moths may become lost after floral visitation (Smith et al. 2021). Nevertheless, for this study system, moths remove, and at least initially carry, large amounts of *S. aspericocca* pollen.

The largest pollen-loads (> 3000 pollen grains) were found on two individuals of the *Hakea* moth, *O. vinaria* (Fig. 5-3e-f), a relatively large moth noted as a “powerful-flying nocturnal species” (McQuillan and Forrest 1985 p. 16). These pollen loads are comparable to those found on some hawkmoth pollinators (e.g., Alarcón et al. 2008). A recent meta-analysis found that larger bodied bees and flies deposit greater amounts of pollen to the stigmas of visited flowers than do smaller bodied counterparts (Földesi et al. 2020). If this trend holds true for flower-visiting moths, then larger sized moths may be more effective pollinators of *S. aspericocca*. Certainly, larger moths typically carried higher pollen loads than smaller moths in this study (Table 5-3), putatively allowing for the deposition of greater amounts of pollen to the stigmas of *S. aspericocca* flower(s). In support, for the common, European moth-pollinated plant, *Silene vulgaris* (Caryophyllaceae), there was a positive correlation between the number of pollen grains deposited by visiting moths on the stigma of a flower and the amount of conspecific pollen remaining on the visitors’ body (Pettersson 1991). However, high pollen loads on flower visitors do not always translate to effective pollen deposition on the stigma(s) of visited flowers (Adler and Irwin 2006). Furthermore, the quality of pollen carried and delivered to flowers may not be closely related to the quantity of pollen carried by floral visitors, but may also have important implications for seed production (Ramsey and Vaughton 2000). Thus, care should be taken linking pollinator effectiveness to the number of pollen grains carried by flower visitors without further study.

During nectaring, *O. vinaria* was observed to continue to beat its wings while perched on an inflorescence, rather than completely settling. Similar “fluttering” behaviour by two species of flower-visiting geometrid moths was reported by Atwater (2013) within a sandhill ecosystem in Florida, North America. In the study by Atwater (2013), the two moths which exhibited fluttering behaviour tended to have increased pollen collection compared with those moth species not observed fluttering when nectaring. Here, specimens of the fluttering *O. vinaria* also carried the highest pollen-loads (Table 5-3). Smaller geometrids that settled and did not flutter on the inflorescences to nectar (e.g., the captured *Scopula* Schrank sp. and *Taxeotis* Guest spp.), carried fewer grains of *S. aspericocca* pollen (Table 5-3). Nevertheless, it is not clear if moths exhibiting such fluttering behaviour are necessarily more effective pollinators of the plants they visit (Pettersson 1991).

Behavioural differences, combined with large variability in pollen grains carried between moths, suggest moth species may vary in their pollination effectiveness (Herrera 1987), perhaps presenting an opportunity for specialization by plants on pollinators (Schemske and Horvitz 1984). However, given the relative variety of moths found carrying *S. aspericocca* pollen, and the stability of plant reproduction, both spatially and temporally, pollination may be rather unspecialised and provided by a guild of moth species, functionally equivalent in pollination effectiveness (Pettersson 1991). Additionally, while no diurnal observations of floral visitors were undertaken, day-flying Lepidoptera were haphazardly observed on two occasions visiting flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* (Pers. Obs.). Thus, day-time visitors may contribute to pollination and plant reproduction. Certainly, in this regard, a more in-depth study of the pollination ecology of *S. aspericocca* ssp. *Cylindrical inflorescence* would prove enlightening. Particularly, experimental bagging of plants could be used to directly measure the relative contributions of nocturnal versus diurnal visitors to reproduction (e.g., see studies by Wolff et al. 2002; Ortega-Baes et al. 2010; Funamoto and Ohashi 2017; Doubleday and Eckert 2018; Hattori et al. 2020).

Finally, although not a focus of the study, it is of interest that some of the moths captured visiting flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* carried pollen grains of a co-flowering *Pimelea* species, and vice versa (Table 5-3). Species of *Pimelea* are generally considered to have flowers adapted toward butterfly pollination. Nevertheless, it is known that long tongued bombyliid flies may also visit flowers of *Pimelea* species (Armstrong 1979; Hingston and McQuillan 2000; Pers. Obs.). From the results presented here, it appears night-flying moths may likewise be relatively frequent floral visitors. To our knowledge this is the first recorded observation of floral visitation by night-flying moths to flowers of *Pimelea* within Australia, although other genera within the family globally exhibit settling moth pollination (e.g., *Struthiola ciliata* in southern Africa (Makholela and Manning 2006), species of *Diplomorpha* in temperate East Asia (Okamoto et al. 2008)). In addition, pollen grains belonging to *Pimelea* species have been found on the probosces of the noctuid moths *Helicoverpa punctigera* and *H. armigera* collected from traps in eastern Australia (Gregg 1993).

Given *S. aspericocca* ssp. *Cylindrical inflorescence* and co-flowering species of *Pimelea* possibly share pollinators, and exhibit both similar (e.g., elongated corolla tube) and different (e.g., nocturnal production of scent) floral adaptations, a comparative study of their pollination ecology would be a productive avenue in further teasing apart their floral adaptations (phalenophilous versus psychophilous pollination syndromes).

5.5.2 Reserve area and plant reproduction

It is frequently expected that the negative consequences of habitat loss and fragmentation to pollination and plant reproduction are greater as the areas of vegetation fragments decline and the isolation of vegetation fragments from other such habitat increases (Hobbs and Yates 2003). However, it is somewhat unclear how often the negative effects of habitat loss and fragmentation commonly manifest themselves in those larger reserves that are often designated as areas of conservation. For instance, Cunningham (2000) found plant fecundity declined for two of four common plant species within linear strips of mallee woodland (e.g., roadside verges) versus larger woodland reserves of 26 to 800 ha in New South Wales, Australia, suggesting strong negative effects of landscape disturbance on reproductive output at the severest scale(s) of disturbance. However, only one plant species, the generalist pollinated *Acacia brachybotrya* (Fabaceae), showed an additional increase in reproduction within a fragment area of over 140 000 ha (Cunningham 2000). Thus, even for detrimentally affected plant species within this system, reproduction may be relatively robust to landscape disturbance within moderately sized reserves.

Similarly, reproductive success of the widely distributed, hawkmoth-pollinated plant *Cestrum parqui* (Solanaceae) within the fragmented landscape of the Chaco Serrano Forest of central Argentina declined substantially in forest fragments of 5.30 ha or less (Aguilar and Galetto 2004). However, reproduction of *C. parqui* considerably increased within a 13.60 ha forest fragment, with little further increase in reproduction in fragment sizes of 92.10 ha and greater than 300 ha, highlighting the importance to conservation of remaining medium to large forest fragments in this system (Aguilar and Galetto 2004). A similar conclusion regarding the importance of larger fragment area was reached by Hadley et al. (2014), the authors of which observed a 40.00 % increase in seed production for the common, hummingbird pollinated herb, *Heliconia tortuosa* (Heliconiaceae), across a log transformed 0.64 to 1300 ha fragment area gradient in fragmented premontane tropical forest within Costa Rica. In particular, larger forest fragment area was important due to the behaviour of this plant species traplining hummingbird pollinators, which avoid crossing gaps in forest cover as small as 30 m (Hadley et al. 2014).

In comparison, when no apparent effects of habitat loss and fragmentation occur, plant reproduction may be linked to habitat scale variables unrelated to fragment area. For example, local habitat scale variables (e.g., amount of leaf litter) were of greater importance than landscape scale effects in maintaining plant pollination and reproduction for three species of insect-pollinated orchids across urban bushland remnants surrounding Perth, Western Australia (Newman et al. 2013). This result suggests that these orchid species would benefit most from targeted conservation efforts improving the habitability of individual reserves. Certainly, knowing the scale at which plants and their pollinators respond to disturbance is critical for effective conservation (Paton et al. 2004), and Paton (2000) suggested insect-plant pollination systems may typically operate at spatial scales smaller than individual fragments of vegetation within southern Australia.

Even at the most severe scale(s) of fragmentation, within-site factors may be most important. For example, reproduction of the endangered composite, *Leucochrysum albicans* subsp. *albicans* var. *tricolor*, located within roadside verges of remaining grasslands in western Victoria, showed no relation to population size (Costin et al. 2001). However, there was clear variability between sites, with evidence suggesting site-specific factors unrelated to population size significantly moderated reproduction (Costin et al. 2001). In contrast, however, reproduction of the sympatric endangered composite, *Rutidosis leptorrhynchoides*, was significantly associated with population size (Morgan 1999), further demonstrating the individual response(s) of plant species to landscape disturbance (e.g., Cunningham 2000) and validating the need for continued empirical studies of species responses to landscape disturbance (Broadhurst and Young 2007).

Here, reproduction of the common, moth-pollinated plant, *S. aspericocca* ssp. *Cylindrical inflorescence*, showed no clear relationship between reserve area and reproduction, as measured by fruit-set and mean seed number per fruit. As such, the hypothesis that plant reproduction would increase with reserve area was not supported. There was also little variation between reserves in mean levels of plant reproduction. Thus, local site-specific factors influencing reproduction did not result in clear, consistent, differences in plant reproduction between sample sites among reserves. In contrast, plants within sample sites clearly differed in reproduction, and there was evidence that plants surrounded by a greater conspecific floral density produced higher mean seed number per fruit in 2019. However, this did not translate into marked differences in mean seed number per fruit between reserves. Moreover, levels of plant-to-plant variation (CV) in fruit-set and mean seed number per fruit were not related to reserve area. Lastly, reproduction of *S. aspericocca* ssp. *Cylindrical inflorescence* showed no relationship to reserve area across the two years studied, and there was limited between-year variability in levels of reproduction. Ultimately, taken together, the results suggest reproduction of *S. aspericocca* ssp. *Cylindrical* *inflorescence* is spatially similar throughout the fragmented landscape of the Adelaide Hills. Consequently, as far as the measured variables of reproduction reflect population persistence, reserve area alone cannot be used as a reliable indicator of conservation value, and small and large reserves both potentially represent valuable contributions to the conservation of *S. aspericocca* ssp. *Cylindrical inflorescence*.

Although the breeding system of the study species is undetermined, reproduction does not occur via autonomous self-pollination. Thus, floral visitation is required for reproduction, whether reproduction occurs via self-pollination (facilitated autogamy and geitonogamy) or outcrossing. The limited variation among reserves in measures of reproductive output suggests that differential access to pollinator(s) among sample sites does not differentially limit fruit and seed production, and pollination may be relatively robust to current landscape fragmentation. Indeed, it must be recognized that the matrix surrounding reserves is not “ecologically irrelevant” (Jules and Shahani 2003 p. 459) and represents available habitat for a variety of pollinators. For example, several cereal grasses may be used as food-plants by the larvae of *P. ewingii*, while the larvae of *O. vinaria* feed on native species of *Hakea* and *Grevillea* (Proteaceae) (McQuillan and Forrest 1985), common throughout the study region, including alongside vegetated roadside corridors (Pers. Obs.). Indeed, the species *O. vinaria* and *P. ewingii* are common to southern Australia generally (McQuillan et al. 2019). Similarly, species of the endemic genus *Taxeotis* are mainly found within eucalypt forests and mallee of southern Australia (Common 1990) and are common small moths of remnant bushland fragments in Victoria (Marriot 2012). Thus, although sample sites within smaller reserves were surrounded by less remnant native vegetation than those situated in larger reserves, pollinator availability throughout the study region may be relatively similar among reserves, due to continued reproduction and survival of pollinators throughout the surrounding matrix. This was also suggested by Donaldson et al. (2002), who observed no difference in reproduction of the moth-pollinated herbaceous perennial, *Gladiolus liliaceus* (Iridaceae), between small (< 2 ha) and large (> 30 ha) renosterveld shrubland fragments in South Africa. These authors hypothesized the broad larval host range of the generalist noctuid moth pollinator allowed for its continued reproduction and survival throughout the surrounding agricultural matrix, buffering the reproduction of *G. liliaceus*. Similarly, Skogen et al. (2016) found no impact of landscape disturbance on the pollination of the hawkmoth-pollinated plant, *Oenothera harringtonii* (Onagraceae), an herbaceous annual of semi-disturbed areas of arid shortgrass prairies within the United States. This lack of impact of landscape disturbance on pollination was due, in part, to both the dietary generalist nature of the plant’s hawkmoth pollinators, as well as the highly mobile adults, which allowed for successful gene flow between the fragmented populations (Skogen et al. 2019). Indeed, high pollinator mobility can effectively connect fragmented populations, such that measures of fragment area and isolation may not be particularly informative for certain plants (Ritchie et al. 2019). Given species of Lepidoptera often disperse pollen further than other insect pollinators (Herrera 1987; Miyake and Yahara 1998; Young 2002), the lack of an apparent impact of reserve area on the reproduction of *S. aspericocca* ssp. *Cylindrical inflorescence* may also result from long-distance pollen dispersal between spatially separated populations.

Importantly, it should be said the above arguments do not imply that there have not been declines in the abundance and diversity of moths within the study region. The local abundance and diversity of Lepidoptera have certainly declined due to landscape disturbance and related anthropogenic impacts within southern Australia (Braby et al. 2021), and smaller reserves may be less diverse in their Lepidopteran assemblages than larger ones (Williams 2009, 2011). However, any potential differences in the abundance and diversity of floral visitors among reserves is not resulting in detectable differences in the reproduction of this moth-pollinated plant. Plant reproduction may be lower across the study region generally, relative to the past, but to our knowledge data do not exist to test this hypothesis.

Lastly, this study measured quantitative changes in reproduction. However, pollen quality may differ between reserves (e.g., Delnevo et al. 2020), resulting in offspring of lower fitness. Certainly, reductions in genetic diversity of offspring and offspring quality (as measured via offspring germination, growth, and survival) appear to be a common feature of plant populations within disturbed landscapes generally (reviewed by Aguilar et al. 2019). Thus, although the results of the current study are encouraging, with reproduction appearing relatively stable across the range of reserve areas currently conserving populations of *S. aspericocca* ssp. *Cylindrical inflorescence* within the study region, reduced genetic quality of offspring within smaller reserves remains to be assessed.

5.5.3 Summary

Floral visitation by night-flying settling moths to the flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* was confirmed, with visiting moths carrying large pollen loads of the study species. However fruit-set and mean seed number per fruit, showed no relationship to reserve area in the two years studied. Thus, the hypothesis that plant reproduction would increase with reserve area was not supported. Indeed, there was limited spatial variation in plant reproduction among reserves in both years, suggesting mean levels of plant reproduction are similar across reserves throughout the fragmented landscape of the study region. It is hypothesized that this lack of marked spatial variability in reproduction is due to effective pollination by common, night-flying moths, which can more or less utilise the reserves and surrounding matrix to complete their life cycle(s). Nevertheless, more recent declines in once common butterfly (Braby et al. 2021) and moth species (Green et al. 2021) in areas of southern Australia raise concerns about future pollinator abundance and diversity in the study region.

5.6 REFERENCES:

Adler LS, Irwin RE (2006) Comparison of pollen transfer dynamics by multiple floral visitors: experiments with pollen and fluorescent dye. Annals of Botany 97:141-150.

Aguilar R, Galetto L (2004) Effects of forest fragmentation on male and female reproductive success in *Cestrum parqui* (Solanaceae). Oecologia 138:513-520.

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980.

Aguilar R, Cristóbal-Pérez EJ, Balvino-Olvera FJ, de Jesús Aguilar-Aguilar M, Aguirre-Acosta N, Ashworth L, Lobo JA, Martén-Rodríguez S, Fuchs EJ, Sanchez-Montoya G, Bernardello G (2019) Habitat fragmentation reduces plant progeny quality: a global synthesis. Ecology Letters 22:1163-1173.

Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. Ecology 75:330-351.

Aizen MA, Ashworth L, Galetto L (2002) Reproductive success in fragmented habitats: do compatibility systems and pollination specialization matter? Journal of Vegetation Science 13:885-892.

Alarcón R, Davidowitz G, Bronstein JL (2008) Nectar usage in a southern Arizona hawkmoth community. Ecological Entomology 33:503-509.

Armstrong JA (1979) Biotic pollination mechanisms in the Australian flora - a review. New Zealand Journal of Botany 17:467-508.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Atwater MM (2013) Diversity and nectar hosts of flower-settling moths within a Florida sandhill ecosystem. Journal of Natural History 47:2719-2734.

Banza P, Belo AD, Evans DM (2015) The structure and robustness of nocturnal Lepidopteran pollen-transfer networks in a Biodiversity Hotspot. Insect Conservation and Diversity 8:538-546.

Barker WR (1977) Taxonomic studies in *Stackhousia* Sm. (Stackhousiaceae) in South Australia. Journal of the Adelaide Botanic Garden 1:69-82.

Barker WR (1986) Stackhousiaceae. In: Jessop JP, Toelken HR (eds) Flora of South Australia, part 2, Leguminosae-Rubiaceae, 4th edn. South Australian Government Printing Division, Adelaide, pp 803-807.

Barker WR, Barker RM, Jessop JP, Vonow HP (2005). Census of South Australian vascular plants. Journal of the Adelaide Botanic Gardens Supplement 1:1-396.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67:1-48.

Batianoff GN, Reeves RD, Specht RL (1990) *Stackhousia tryonii* Bailey: a nickel-accumulating serpentine-endemic species of central Queensland. Australian Journal of Botany 38:121-130.

Beattie AJ (1971) A technique for the study of insect-borne pollen. The Pan-Pacific Entomologist 47:82.

Bernhardt P, Burns-Balogh P (1986) Observations of the floral biology of *Prasophyllum odoratum* (Orchidaceae, Spiranthoideae). Plant Systematics and Evolution 153:65-76.

Braby MF, Williams MR, Douglas F, Beardsell C, Crosby DF (2021) Changes in a peri-urban butterfly assemblage over 80 years near Melbourne, Australia. Austral Entomology 60:27-51.

Broadhurst L, Young A (2007) Seeing the wood and the trees - predicting the future for fragmented plant populations in Australian landscapes. Australian Journal of Botany 55:250-260.

Brooks ME, Kristensen K, van Benthem KJ, Magnusson A, Berg CW, Nielsen A, Skaug HJ, Maechler M, Bolker BM (2017) glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. The R Journal 9:378-400.

Bruna EM, Kress WJ (2002) Habitat fragmentation and the demographic structure of an Amazonian understory herb (*Heliconia acuminata*). Conservation Biology 16:1256-1266.

Buxton MN, Anderson BJ, Lord JM (2018) The secret service - analysis of the available knowledge on moths as pollinators in New Zealand. New Zealand Journal of Ecology 42:1-9.

Buxton MN, Anderson BJ, Hoare RJ, Lord JM (2019) Are moths the missing pollinators in subantarctic New Zealand? Polar Research 38:3545.

Chung Y, Rabe-Hesketh S, Dorie V, Gelman A, Liu J (2013) A nondegenerate penalized likelihood estimator for variance parameters in multilevel models. Psychometrika 78:685-709.

Clarke I, Lee H (2019) Name that flower. Melbourne University Press, Melbourne, Australia.

Common IFB (1990) Moths of Australia. Melbourne University Press, Melbourne, Australia.

Costin BJ, Morgan JW, Young AG (2001) Reproductive success does not decline in fragmented populations of *Leucochrysum albicans* subsp. *albicans* var. *tricolor* (Asteraceae). Biological Conservation 98:273-284.

Cunningham SA (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Dag O, Dolgun A, Konar NM (2018). onewaytests: an R package for one-way tests in independent groups designs. The R Journal 10:175-199.

Delnevo N, van Etten EJ, Byrne M, Petraglia A, Carbognani M, Stock WD (2020) Habitat fragmentation restricts insect pollinators and pollen quality in a threatened Proteaceae species. Biological Conservation 252:108824.

Devoto M, Bailey S, Memmott J (2011) The ‘night shift’: nocturnal pollen-transport networks in a boreal pine forest. Ecological Entomology 36:25-35.

Donaldson J, Nänni I, Zachariades C, Kemper J (2002) Effects of habitat fragmentation on pollinator diversity and plant reproductive success in renosterveld shrublands of South Africa. Conservation Biology 16:1267-1276.

Doubleday LA, Eckert CG (2018) Experimental evidence for predominant nocturnal pollination despite more frequent diurnal visitation in *Abronia umbellata* (Nyctaginaceae). Journal of Pollination Ecology 22:67-74.

Faegri K, van der Pijl L (1979) The principles of pollination ecology. Pergamon Press, New York.

Finch JT, Power SA, Welbergen JA, Cook JM (2019) A non-pollinating moth inflicts higher seed predation than two co-pollinators in an obligate pollination mutualism. Ecological Entomology 44:780-791.

Földesi R, Howlett BG, Grass I, Batáry P (2021) Larger pollinators deposit more pollen on stigmas across multiple plant species - a meta-analysis. Journal of Applied Ecology 58:699-707.

Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage, Thousand Oaks, California.

Funamoto D, Ohashi K (2017) Hidden floral adaptation to nocturnal moths in an apparently bee-pollinated flower, *Adenophora triphylla* var. *japonica* (Campanulaceae). Plant Biology 19:767-774.

Gilfedder L, Kirkpatrick JB (1998) Distribution, disturbance tolerance and conservation of *Stackhousia gunnii* in Tasmania. Australian Journal of Botany, 46:1-13.

Green D, MacKay D, Whalen M (2012) Next generation insect light traps: the use of LED light technology in sampling emerging aquatic macroinvertebrates. Australian Entomologist 39:189-194.

Green K, Caley P, Baker M, Dreyer D, Wallace J, Warrant E (2021) Australian Bogong moths *Agrotis infusa* (Lepidoptera: Noctuidae), 1951-2020: decline and crash. Austral Entomology 60:66-81.

Gregg PC (1993) Pollen as a marker for migration of *Helicoverpa armigera* and *H. punctigera* (Lepidoptera: Noctuidae) from western Queensland. Australian Journal of Ecology 18:209-219.

Hadley AS, Frey SJ, Robinson WD, Kress WJ, Betts MG (2014) Tropical forest fragmentation limits pollination of a keystone understory herb. Ecology 95:2202-2212.

Hahn M, Brühl CA (2016) The secret pollinators: an overview of moth pollination with a focus on Europe and North America. Arthropod-Plant Interactions 10:21-28.

Harris LF, Johnson SD (2004) The consequences of habitat fragmentation for plant-pollinator mutualisms. International Journal of Tropical Insect Science 24:29-43.

Harrison XA (2015) A comparison of observation-level random effect and Beta-Binomial models for modelling overdispersion in Binomial data in ecology and evolution. PeerJ 3:e1114.

Hartig F (2021) DHARMa: residual diagnostics for hierarchical (multi-level/mixed) regression models. R package version 0.4.1.

Hattori M, Tamada Y, Itino T (2020) The relative importance of diurnal and nocturnal pollinators of *Platanthera hologlottis* Maxim. (Orchidaceae). Journal of Plant Interactions 15:106-110.

Herrera CM (1987) Components of pollinator “quality”: comparative analysis of a diverse insect assemblage. Oikos 50:79-90.

Hingston AB, McQuillan PB (2000) Are pollination syndromes useful predictors of floral visitors in Tasmania? Austral Ecology 25:600-609.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Hothorn T, Bretz F, Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50:346-363.

Inouye DW, Pyke GH (1988) Pollination biology in the Snowy Mountains of Australia: comparisons with montane Colorado, USA. Australian Journal of Ecology 13:191-205.

Jacquemyn H, Brys R, Hermy M (2002) Patch occupancy, population size and reproductive success of a forest herb (*Primula elatior*) in a fragmented landscape. Oecologia 130:617-625.

Jennersten OLA (1988) Pollination in *Dianthus deltoides* (Caryophyllaceae): effects of habitat fragmentation on visitation and seed set. Conservation Biology 2:359-366.

Johnson SD, Ellis A, Carrick P, Swift A, Horner N, Van Rensburg SJ, WJ Bond (1993) Moth pollination and rhythms of advertisement and reward in *Crassula fascicularis* (Crassulaceae). South African Journal of Botany 59:511-513.

Johnson SD, Collin CL, Wissman HJ, Halvarsson E, Ågren J (2004) Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. Biotropica 36:148-155.

Johnson SD, Moré M, Amorim FW, Haber WA, Frankie GW, Stanley DA, Cocucci AA, Raguso RA (2017) The long and the short of it: a global analysis of hawkmoth pollination niches and interaction networks. Functional Ecology 31:101-115.

Jules ES, Shahani P (2003) A broader ecological context to habitat fragmentation: why matrix habitat is more important than we thought. Journal of Vegetation Science 14:459-464.

Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado.

Kunin WE (1997) Population size and density effects in pollination: pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. Journal of Ecology 85:225-234.

Lamont BB, Klinkhamer PG, Witkowski ETF (1993) Population fragmentation may reduce fertility to zero in *Banksia goodii* - a demonstration of the Allee effect. Oecologia 94:446-450.

LeCroy KA, Shew HW, van Zandt PA (2013) Pollen presence on nocturnal moths in the Ketona Dolomite glades of Bibb County, Alabama. Southern Lepidopterists' News 35:136-142.

Lennartsson T (2002) Extinction thresholds and disrupted plant-pollinator interactions in fragmented plant populations. Ecology 83:3060-3072.

Macgregor CJ, Pocock MJ, Fox R, Evans DM (2015) Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. Ecological Entomology 40:187-198.

Makholela T, Manning JC (2006) First report of moth pollination in *Struthiola ciliata* (Thymelaeaceae) in southern Africa. South African Journal of Botany 72:597-603.

Marriot P (2011) Part 3 - waves and carpets - Geometridae (C). Entomological Society of Victoria.

Marriot P (2012) Part 4 - emeralds and allies - Geometridae (B). Entomological Society of Victoria.

Marriot P, Edwards T, Kallies A, Hewish M, Williams S (2017) Part 8 - night moths and allies - Noctuoidea (B). Entomological Society of Victoria.

Martins DJ, Johnson SD (2013) Interactions between hawkmoths and flowering plants in East Africa: polyphagy and evolutionary specialization in an ecological context. Biological Journal of the Linnean Society 110:199-213.

McQuillan PB, Forrest JA (1985) A guide to common moths of the Adelaide region (No. 5). South Australian Museum, Adelaide, South Australia.

McQuillan P, Forrest J, Keane D, Grund R (2019) Caterpillars, moths and their plants of southern Australia. Butterfly Conservation South Australia Incorporated, Adelaide, South Australia.

Milla L, Encinas-Viso F (2020) Plant-pollinator communities in the Australian Alps. Australasian Plant Conservation 28:13-16.

Miyake T, Yahara T (1998) Why does the flower of *Lonicera japonica* open at dusk? Canadian Journal of Botany 76:1806-1811.

Morgan JW (1999) Effects of population size on seed production and germinability in an endangered, fragmented grassland plant. Conservation Biology 13:266-273.

New TR, Sands DP, Taylor GS (2021) Roles of roadside vegetation in insect conservation in Australia. Austral Entomology 60:128-137.

Newman BJ, Ladd P, Brundrett M, Dixon KW (2013) Effects of habitat fragmentation on plant reproductive success and population viability at the landscape and habitat scale. Biological Conservation 159:16-23.

Okamoto T, Kawakita A, Kato M (2008) Floral adaptations to nocturnal moth pollination in *Diplomorpha* (Thymelaeaceae). Plant Species Biology 23:192-201.

Oliveira PE, Gibbs PE, Barbosa AA (2004) Moth pollination of woody species in the Cerrados of Central Brazil: a case of so much owed to so few? Plant Systematics and Evolution 245:41-54.

Ollerton J (2017) Pollinator diversity: distribution, ecological function, and conservation. Annual Review of Ecology, Evolution, and Systematics 48:353-376.

Oostermeijer JGB, Luijten SH, Křenová ZV, Den Nijs HC (1998) Relationships between population and habitat characteristics and reproduction of the rare *Gentiana pneumonanthe* L. Conservation Biology 12:1042-1053.

Ortega-Baes P, Saravia M, Sühring S, Godínez-Alvarez H, Zamar M (2011) Reproductive biology of *Echinopsis terscheckii* (Cactaceae): the role of nocturnal and diurnal pollinators. Plant Biology 13:33-40.

Paton DC (2000) Disruption of bird-plant pollination systems in southern Australia. Conservation Biology 14:1232-1234.

Paton DC, Rogers D, Harris W (2004) Birdscaping the environment: restoring the woodland system of the Mt Lofty region. In Lunney D (ed) Conservation of Australia’s forest fauna, 2nd edn. Royal Zoological Society of New South Wales, Mosman, NSW, Australia, pp 331-358.

Pauw A (2007) Collapse of a pollination web in small conservation areas. Ecology 88:1759-1769.

Pettersson MW (1991) Pollination by a guild of fluctuating moth populations: option for unspecialization in *Silene vulgaris*. The Journal of Ecology 79:591-604.

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press, Cambridge, United Kingdom.

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsey M, Vaughton G (2000) Pollen quality limits seed set in *Burchardia umbellata* (Colchicaceae). American Journal of Botany 87:845-852.

Ritchie AL, Dyer RJ, Nevill PG, Sinclair EA, Krauss SL (2019) Wide outcrossing provides functional connectivity for new and old *Banksia* populations within a fragmented landscape. Oecologia 190:255-268.

Robertson SM, Dowling AP, Wiedenmann RN, Joshi NK, Westerman EL (2021) Nocturnal pollinators significantly contribute to apple production. Journal of Economic Entomology 114:2155-2161.

Sánchez-Bayo F, Wyckhuys KA (2019) Worldwide decline of the entomofauna: a review of its drivers. Biological Conservation 232:8-27.

Schemske DW, Horvitz CC (1984) Variation among floral visitors in pollination ability: a precondition for mutualism specialization. Science 225:519-521.

Skogen KA, Jogesh T, Hilpman ET, Todd SL, Rhodes MK, Still SM, Fant JB (2016) Land-use change has no detectable effect on reproduction of a disturbance-adapted, hawkmoth-pollinated plant species. American Journal of Botany 103:1950-1963.

Skogen KA, Overson RP, Hilpman ET, Fant JB (2019) Hawkmoth pollination facilitates long-distance pollen dispersal and reduces isolation across a gradient of land-use change. Annals of the Missouri Botanical Garden 104:495-511.

Smith GP, Davidowitz G, Raguso RA, Bronstein JL (2021) Proboscis curling in a pollinator causes extensive pollen movement and loss. Ecological Entomology 47:234-241.

Tennekes M (2018) tmap: thematic maps in R. Journal of Statistical Software 84:1-39.

Van Zandt PA, Johnson DD, Hartley C, LeCroy KA, Shew HW, Davis BT, Lehnert MS (2020) Which moths might be pollinators? approaches in the search for the flower-visiting needles in the Lepidopteran haystack. Ecological Entomology 45:13-25.

Vening GS, Guja LK, Spooner PG, Price JN (2017) Seed dormancy and germination of three grassy woodland forbs required for diverse restoration. Australian Journal of Botany 65:625-637.

Wagner DL, Fox R, Salcido DM, Dyer LA (2021) A window to the world of global insect declines: moth biodiversity trends are complex and heterogeneous. Proceedings of the National Academy of Sciences 118:e2002549117.

Walton RE, Sayer CD, Bennion H, Axmacher JC (2020) Nocturnal pollinators strongly contribute to pollen transport of wild flowers in an agricultural landscape. Biology Letters 16:20190877.

Wickham H (2016) ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.

Williams MR (2009) Butterflies and day-flying moths in a fragmented urban landscape, south-west Western Australia: patterns of species richness. Pacific Conservation Biology 15:32-46.

Williams MR (2011) Habitat resources, remnant vegetation condition and area determine distribution patterns and abundance of butterflies and day-flying moths in a fragmented urban landscape, south-west Western Australia. Journal of Insect Conservation 15:37-54.

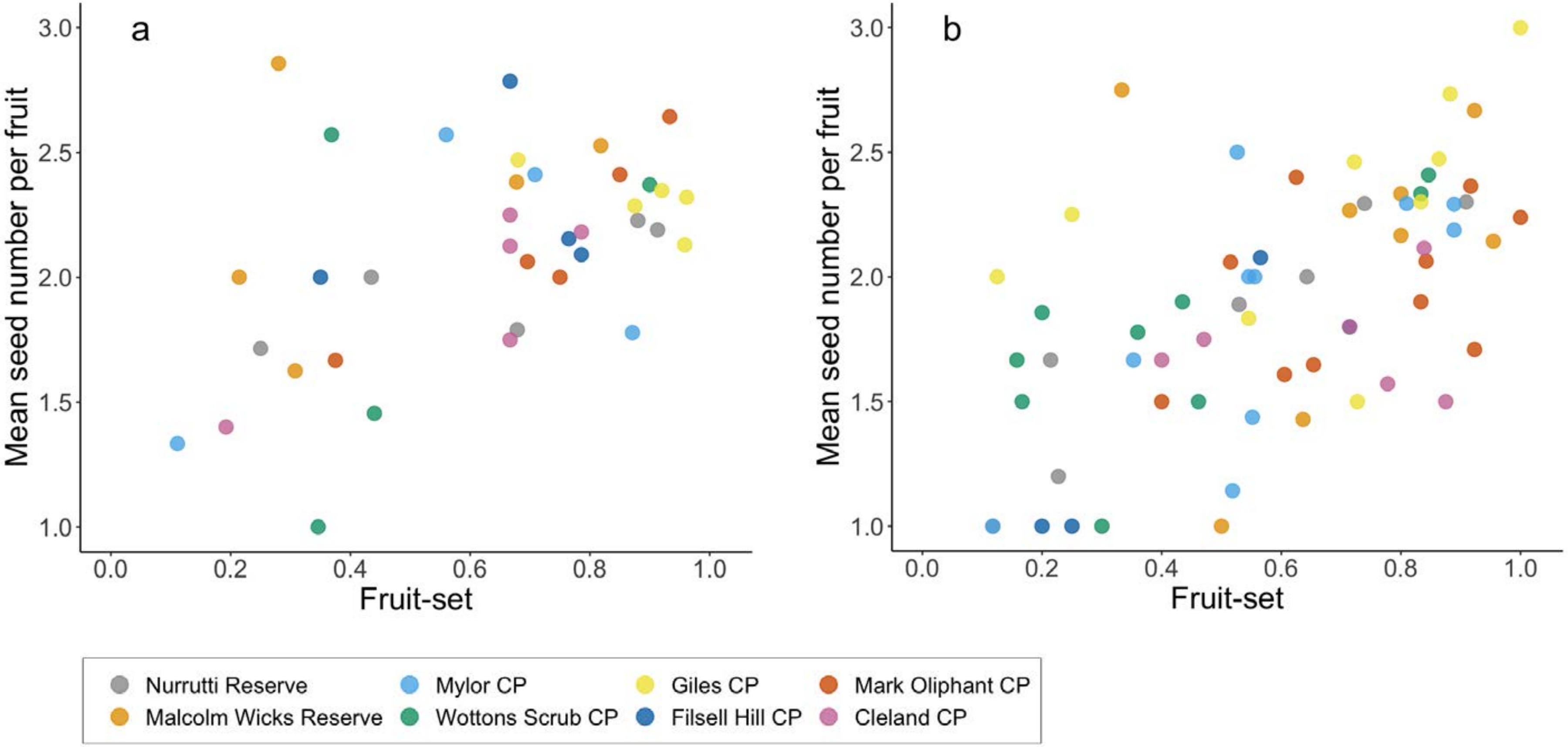
Willmer P (2011) Pollination and floral ecology. Princeton University Press, Princeton, New Jersey.

Wolff D, Braun M, Liede S (2003) Nocturnal versus diurnal pollination success in *Isertia laevis* (Rubiaceae): a sphingophilous plant visited by hummingbirds. Plant Biology 5:71-78.

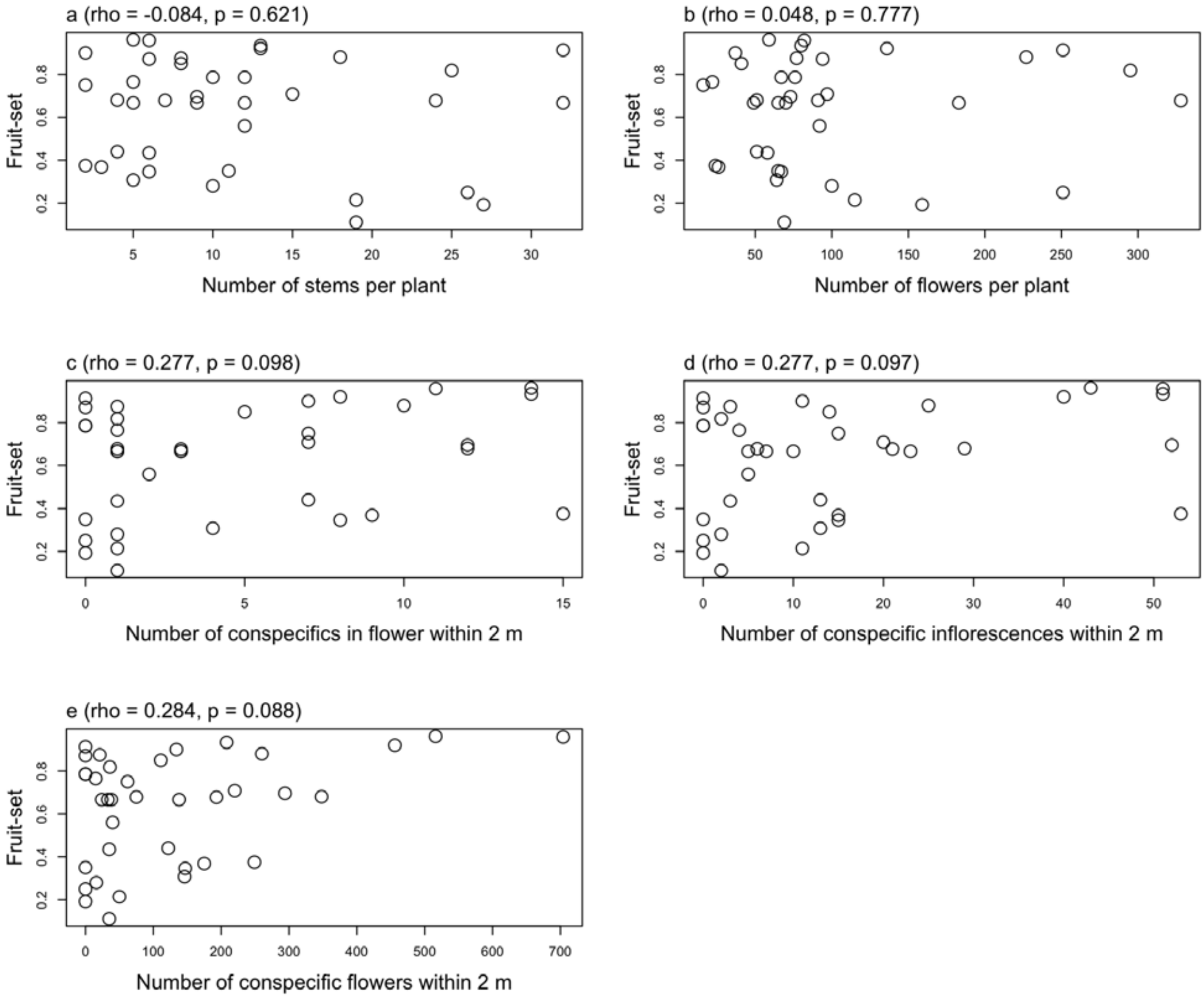
Young HJ (2002) Diurnal and nocturnal pollination of *Silene alba* (Caryophyllaceae). American Journal of Botany 89:433-440.

Zhang W, Gao J (2021) A comparative study on the reproductive success of two rewarding *Habenaria* species (Orchidaceae) occurring in roadside verge habitats. BMC Plant Biology 21:1-12.

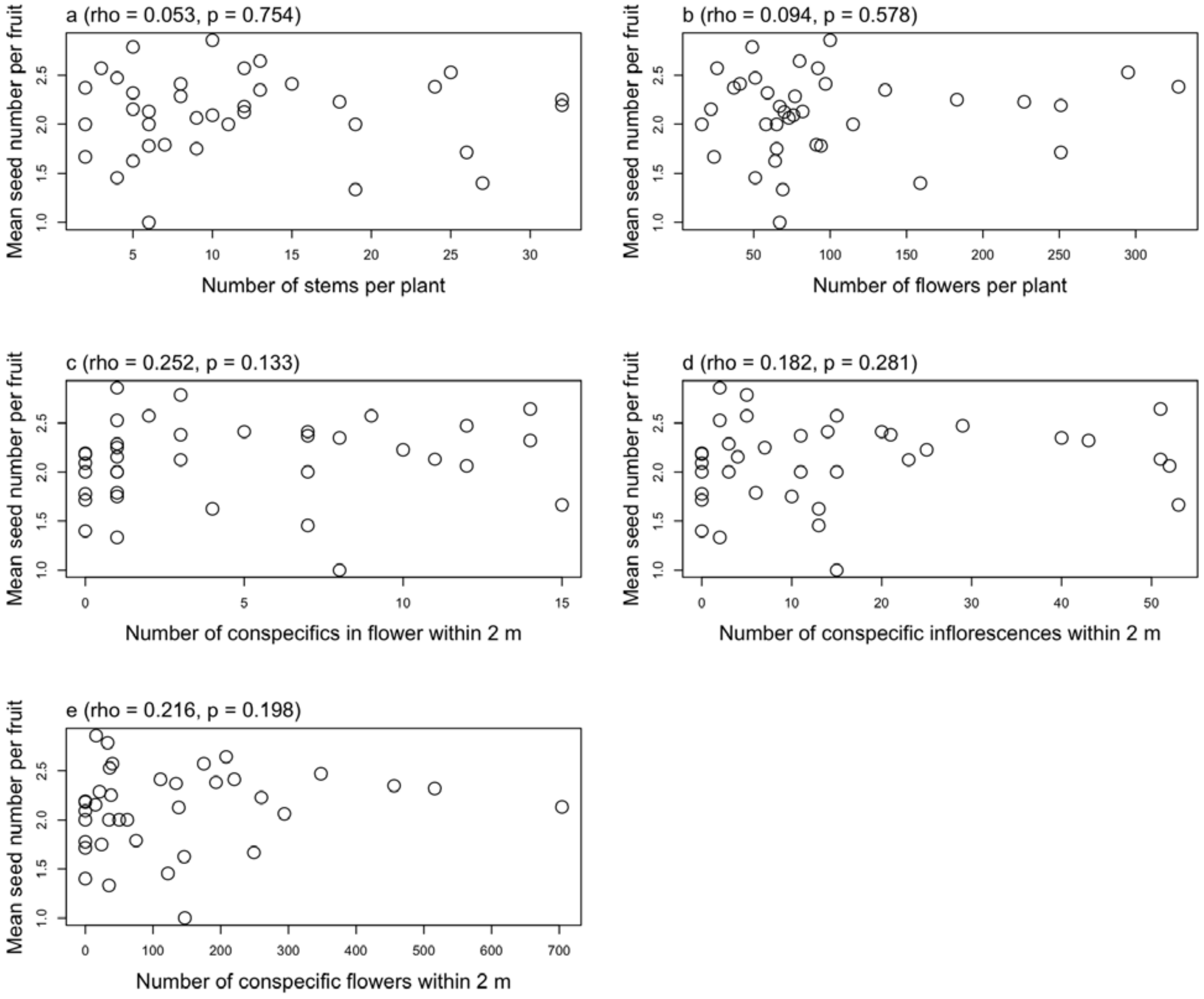
5.7 SUPPLEMENTARY MATERIAL



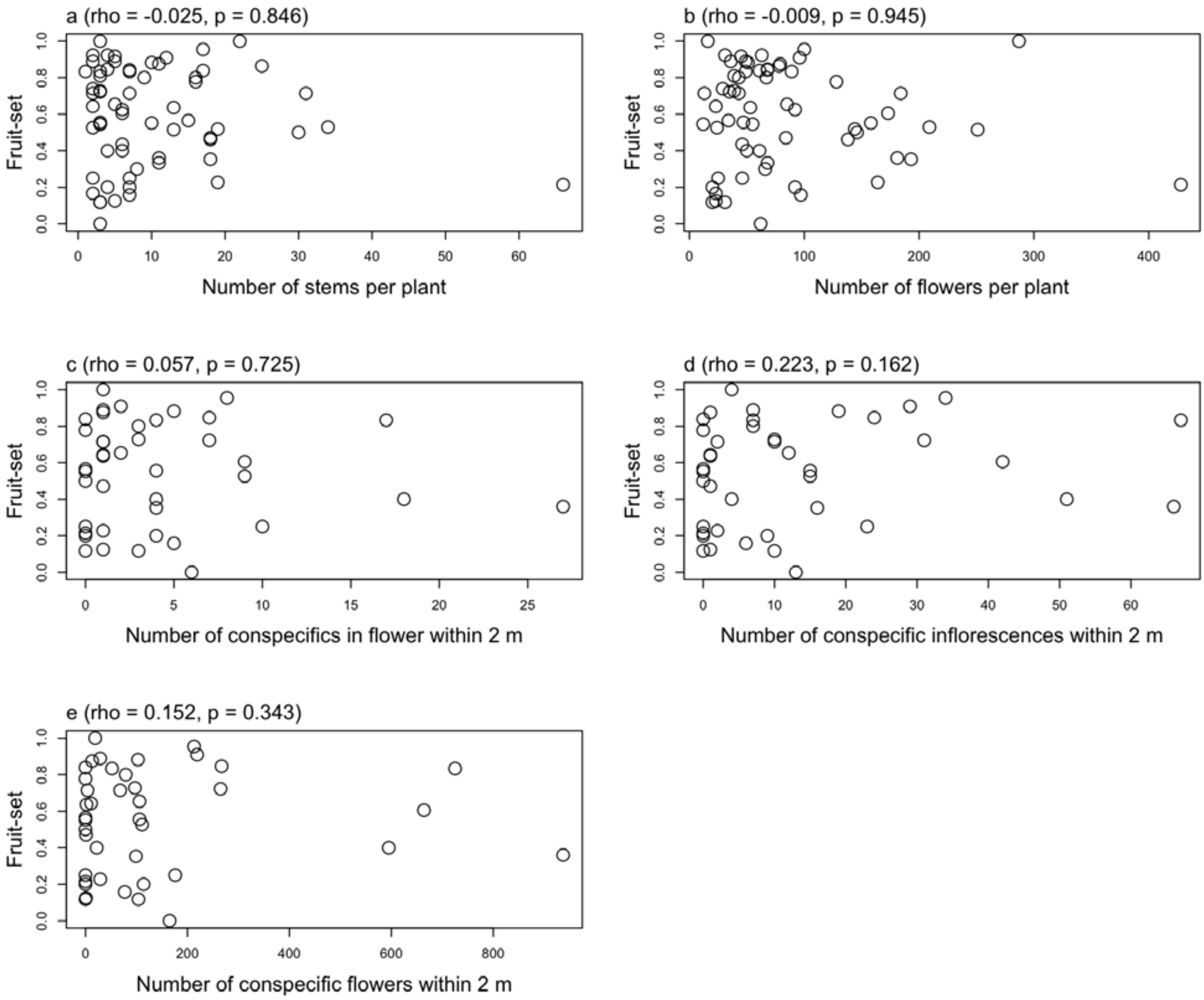
SM Fig. 5-1 Relationship between the mean seed number per fruit and fruit-set of tagged plants, pooled across sample sites, in (**a**) 2018 and (**b**) 2019.



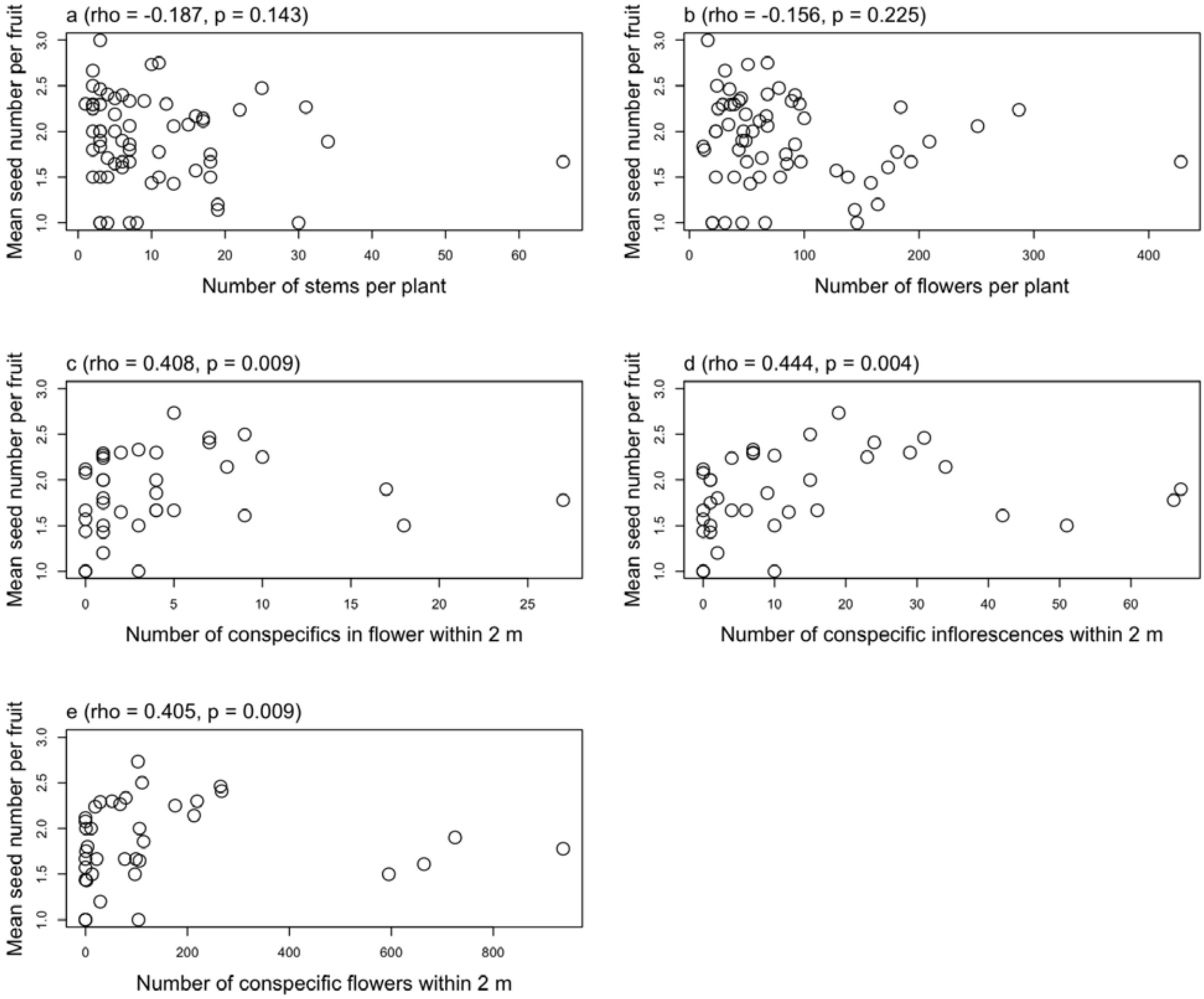
SM Fig. 5-2 Relationships in 2018 between the fruit-set of tagged plants, pooled across sample sites, and (**a**) the numbers of stems per plant (i.e., a measure of plant size), (**b**) the number of flowers per plant (open flowers at the time of tagging), (**c**) the number of conspecifics in flower within 2 m of the tagged plant, (**d**) the number of conspecific inflorescences (min. one flower open) within 2 m of the tagged plants, and (**e**) the number of conspecific flowers (open at the time of tagging) within 2 m of the tagged plants. The results of Spearman's correlations between each of the variables is provided above each plot, respectively.



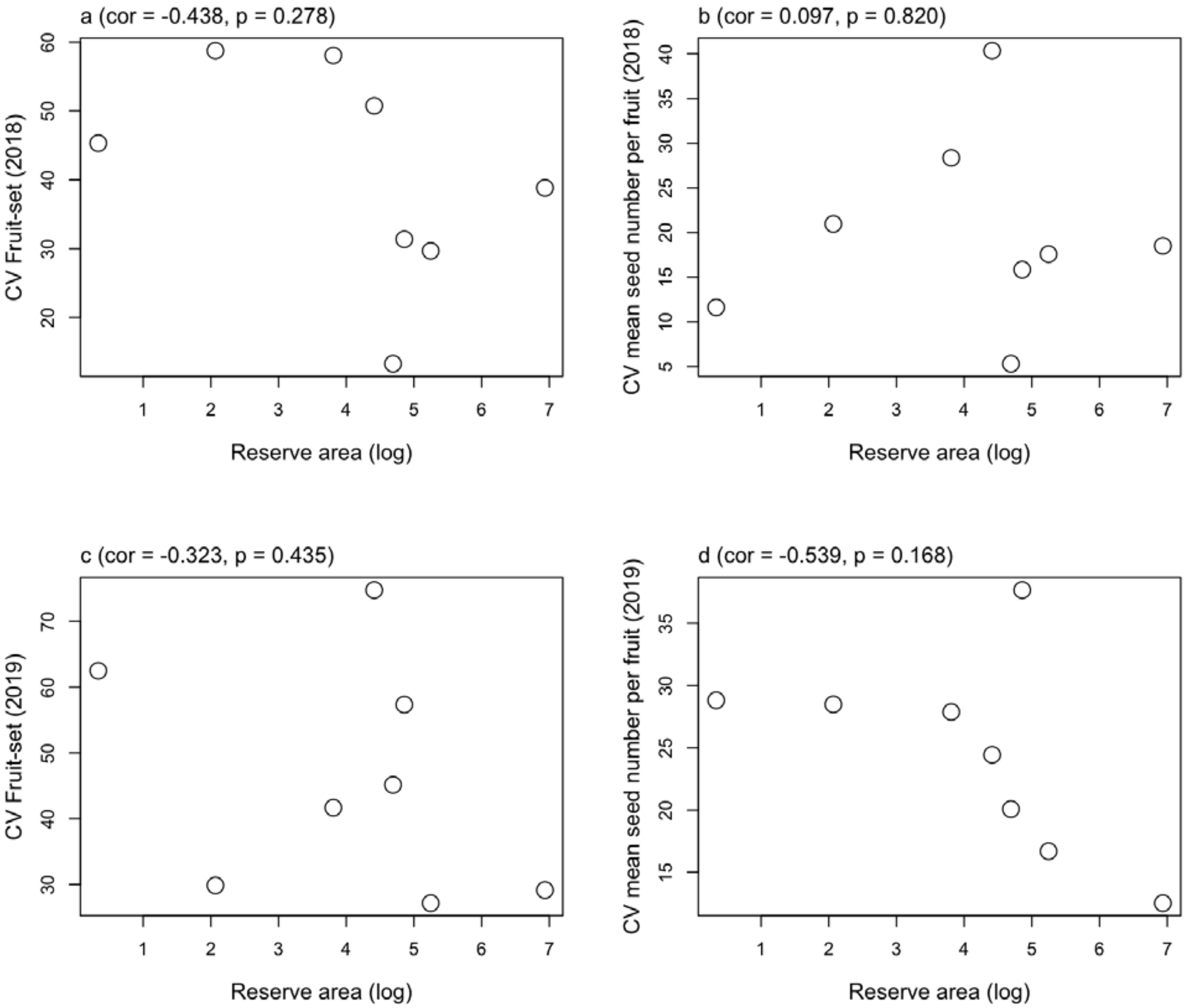
SM Fig. 5-3 Relationships in 2018 between the mean seed number per fruit of tagged plants, pooled across sample sites, and (**a**) the numbers of stems per plant (i.e., a measure of plant size), (**b**) the number of flowers per plant (open flowers at the time of tagging), (**c**) the number of conspecifics in flower within 2 m of the tagged plant, (**d**) the number of conspecific inflorescences (min. one flower open) within 2 m of the tagged plants, and (**e**) the number of conspecific flowers (open at the time of tagging) within 2 m of the tagged plants. The results of Spearman's correlations between each of the variables is provided above each plot, respectively.



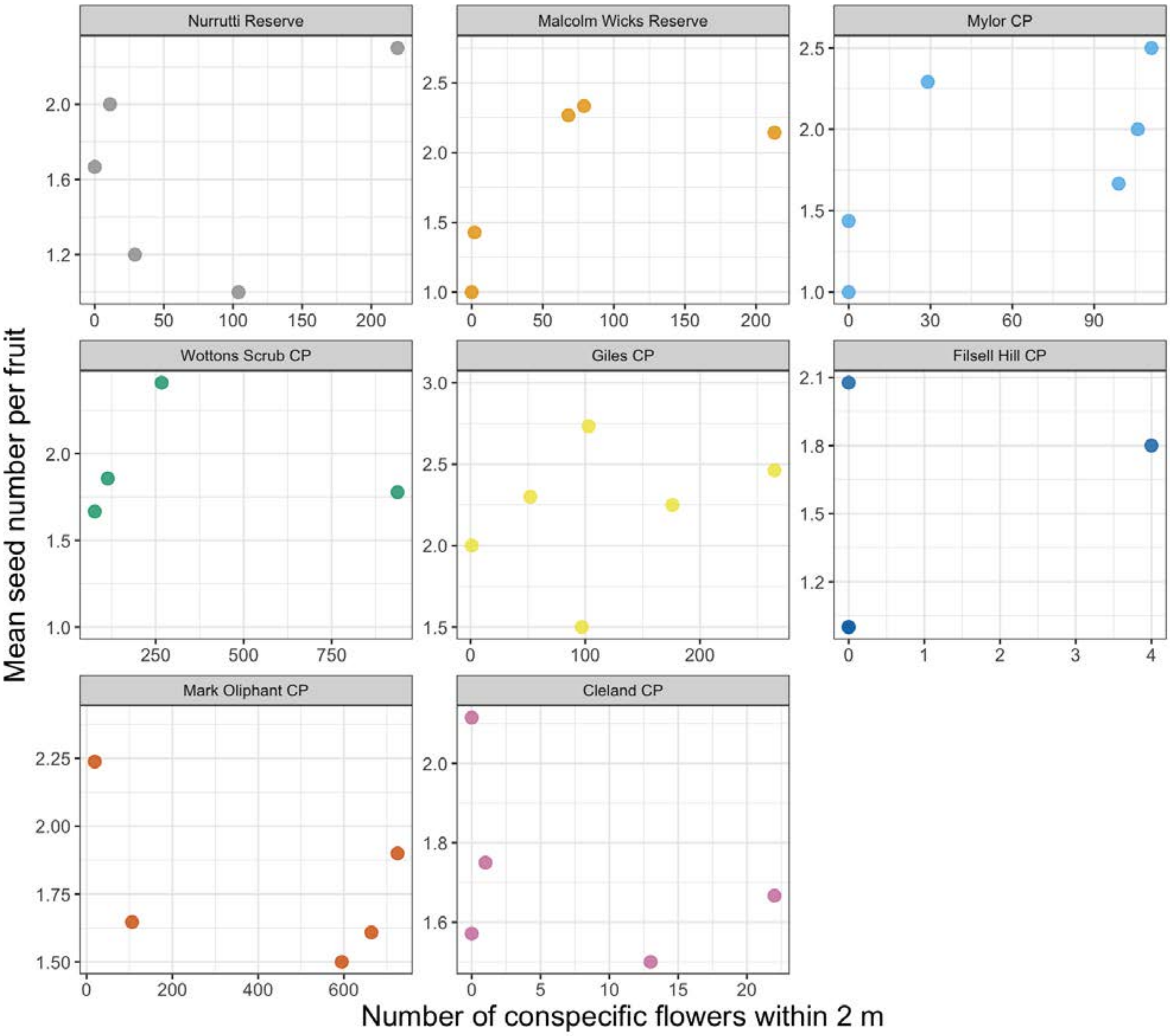
SM Fig. 5-4 Relationships in 2019 between the fruit-set of tagged plants, pooled across sample sites, and (**a**) the numbers of stems per plant (i.e., a measure of plant size), (**b**) the number of flowers per plant (open flowers at the time of tagging), (**c**) the number of conspecifics in flower within 2 m of the tagged plant, (**d**) the number of conspecific inflorescences (min. one flower open) within 2 m of the tagged plants, and (**e**) the number of conspecific flowers (open at the time of tagging) within 2 m of the tagged plants. The results of Spearman's correlations between each of the variables is provided above each plot, respectively.



SM Fig. 5-5 Relationships in 2019 between the mean seed number per fruit of tagged plants, pooled across sample sites, and (**a**) the numbers of stems per plant (i.e., a measure of plant size), (**b**) the number of flowers per plant (open flowers at the time of tagging), (**c**) the number of conspecifics in flower within 2 m of the tagged plant, (**d**) the number of conspecific inflorescences (min. one flower open) within 2 m of the tagged plants, and (**e**) the number of conspecific flowers (open at the time of tagging) within 2 m of the tagged plants. The results of Spearman's correlations between each of the variables is provided above each plot, respectively.



SM Fig. 5-6 Relationships between log-transformed reserve areas and coefficients of variation (CV) of measures of reproductive output of plants tagged in sample sites. (**a**) Relationship with the CV of fruit-set in 2018. (**b**) Relationship with the CV of mean seeds per fruit in 2018. (**c**) Relationship with the CV of fruit-set in 2019. (**d**) Relationship with the CV of mean seeds per fruit in 2019. The results of Pearson's correlations between each of the variables is provided above each plot, respectively.



SM Fig. 5-7 Relationship between the mean seed number per fruit of tagged plants within sample sites and the number of conspecific flowers (open at the time of tagging) within 2 m of tagged plants in 2019. Note the scale of the y- and x-axis varies between individual plots. Also note that two plants in Filsell Hill CP both had a mean seed number per fruit of one seed and zero conspecific flowers within 2 m.

CHAPTER SIX

6. GENERAL DISCUSSION

The overarching aim of this research was to examine spatio-temporal variability in pollination and reproduction of some common plant species across a range of reserve areas within a fragmented landscape and to test whether reserve area is a significant predictor of reproductive success. Common plant species were chosen because of a pre-existing bias toward the study of landscape effects on rare and threatened species (Hobbs and Yates 2003; Aguilar et al. 2006). Specifically, in this study, three common plant species with contrasting pollination systems were chosen to examine and compare effects of landscape disturbance (here used to describe the combined effects of habitat loss and fragmentation and other interrelated processes (e.g., edge effects)).

Research was undertaken in the Adelaide Hills, a region representative of a typically fragmented landscape (a landscape where remaining native vegetation cover is between 10-60 %, as defined by McIntyre and Hobbs (1999)). The three plant species selected for study were *Hibbertia exutiacies* N.A.Wakef.*, Pultenaea daphnoides* J.C.Wendl., and *Stackhousia aspericocca* Schuch. ssp. *Cylindrical inflorescence* (W.R.Barker 1418) W.R.Barker.

Hibbertia exutiacies was selected because it is buzz-pollinated by native bees and because of its high level of insect pre-dispersal seed predation, a relatively understudied biotic interaction involving plants in fragmentated landscapes (Hobbs and Yates 2003). *Stackhousia aspericocca* ssp. *Cylindrical inflorescence* was selected because its floral syndrome was suggestive of nocturnal moth-pollination, a relatively understudied plant-pollinator interaction (Macgregor et al. 2015; Hahn and Brühl 2016; Buxton et al. 2018). *Pultenaea daphnoides* was selected because its papilionaceous floral morphology likely allows for a relatively wider range of bee floral visitors to act as pollinators, including the pervasive introduced honeybee, which may buffer reproduction against possible declines in native pollinators due to landscape disturbance (e.g., see Gross 2001). This contrasts with the relatively more specialized pollination system of the buzz-pollinated *H. exutiacies* in which fewer native bees are able to act as efficient pollinators and honeybees are unable to buzz-pollinate (Buchmann 1983).

The following hypotheses were initially tested:-

1.) That the likelihood of successful pollination increased in reserves of greater area.
2.) That the relationship between reserve area and successful pollination was temporally consistent across years.

These hypotheses were tested for *P. daphnoides* (Chapter Two) and *H. exutiacies* (Chapter Three). There do not appear to be any other studies of "egg and bacon" peas (a broad term for members of the tribes Mirbelieae (which includes *Pultenaea*) and Bossiaeeae) or species of *Hibbertia* which measure the magnitude of pollen-limitation in relation to reserve or fragment area.

Pultenaea daphnoides and *H. exutiacies* are both pollinated by native bees in the Adelaide Hills and may possibly share some pollinators (e.g., species of *Lasioglossum* such as *L.* (*Chilalictus*) *erythrurum*). However, only reproduction of the buzz-pollinated *H. exutiacies* was significantly pollen-limited, although the magnitude of this pollen-limitation was unrelated to reserve area (Chapter Three). The current level of landscape disturbance in the Adelaide Hills does not appear to have resulted in significant spatial variability among reserves in the efficiency of the pollination process of *H. exutiacies*.

A relatively wide range of generalist foraging bees including honeybees visit and likely pollinate the flowers of *P. daphnoides*, and this diversity of floral visitors may reduce the risk of reproductive failure from a scarcity of pollinators. Interestingly, similar species of "egg and bacon" peas visited by native bees and honeybees in other fragmented landscapes of Australia do not appear to be limited by a lack of pollinators (Gross 2001; Lomov et al. 2010). In contrast, although *P. daphnoides* and *H. exutiacies* may possibly share some pollinators, pollination of the buzz-pollinated *H. exutiacies* is necessarily restricted to a subset of native bees which are able to buzz the anthers of *H. exutiacies* and honeybees are unable to buzz-pollinate (Buchmann 1983). Thus, the significant pollen-limitation of *H. exutiacies* reproduction may at least in part result from this species' relatively more specialized plant-pollinator interaction, supporting the view that pollinator specialization results in a greater likelihood of plant reproductive failure (Bond 1994; Wolowski et al. 2014; Bennett et al. 2020). Nevertheless, it should be noted that reproduction of *H. exutiacies* was not pollen-limited in all years of this study and thus pollen-limitation of reproduction is temporally variable.

Further consideration of this temporal variation suggested that abiotic factors such as rainfall may have been involved in mediating the degree of pollen-limitation. This hypothesis was addressed in Chapter Four (see below). Future studies should seek to understand the degree to which the specialized buzz-pollination syndrome of *H. exutiacies* contributes to the risk of pollen-limitation and assess the relative influences of pollen quantity versus quality in mediating this pollen-limitation. It would also be of interest to study the extent to which the pollination of *P. daphnoides* is now mediated by introduced honeybees.

We next tested the following hypotheses:-

3.) That the level of plant reproduction was higher in reserves of greater area.
4.) That the relationship between reserve area and plant reproduction was temporally consistent across years.

These hypotheses were tested for *P. daphnoides* (Chapter Two), *H. exutiacies* (Chapter Three), and *S. aspericocca* ssp. *Cylindrical inflorescence* (Chapter Five).

Of the three species examined, only the reproduction of *H. exutiacies* was significantly positively related to reserve area and then only in one of the years studied and only following removal of the smallest reserve assessed (Chapter Three). Reproduction in the smallest reserve appeared to be greater than would be expected based on reserve area alone and further analysis using a “scale-of-effect” approach suggested that the reproduction of plants in smaller reserves embedded in a landscape with a larger amount of native vegetation may be comparable to that of plants in larger reserves. Consequently, small reserve area alone is not negatively related to the reproduction of *H. exutiacies*.

Similar “scale-of-effect” approaches have found that the composition of the landscape surrounding plants may mediate levels of pollination and plant reproduction. For example, Steffan-Dewenter et al. (2001) found that visitation by bees and levels of insect pre-dispersal seed predation significantly increased in experimental arrays of the daisy, *Centaurea jacea* within an agricultural landscape of Germany when surrounded by a greater amount of semi-natural habitat within 250 m, but this positive relationship weakened as the amount of semi-natural habitat was calculated at ever greater distances up to 3000 m. Taki et al. (2007) measured both bee abundance and richness and seed production of the bee-pollinated woodland herb, *Erythronium americanum* (Liliaceae) in relation to forest cover at 250 m up to 1500 m in fragmented Carolinian forest, Canada. It was found that both bee abundance and richness and seed production of *E. americanum* was significantly positively related with forest cover at 750 m (Taki et al. 2007). In comparison, the scale-of-effect analysis conducted here on the bee-pollinated *H. exutiacies* found viable seed production was positively related to the amount of native vegetation at a much greater distance of ~2400-3400 m.

Assessing the composition of the landscape surrounding sample sites may also confirm the importance of fragment area alone. For example, seed-set of the hummingbird pollinated herb *Heliconia tortuosa* (Heliconiaceae) in Costa Rica was not related to the amount of forest cover sounding sample sites within fragments, but positively related to the area of the fragment itself (Hadley et al. 2014). This contrasts with the findings of *H. exutiacies*, in which reserve area alone did not appear to be of particular importance, although further research is needed to partition the independent effects of reserve area and surrounding native vegetation in this system (e.g., see Fahrig 2013).

Pre-dispersal seed predation by insects has been understudied relative to pollination in terms of the possible effect(s) of landscape disturbance on plant reproduction (Hobbs and Yates 2003) and yet such seed predation may be one of the most significant constraints on population growth for particular plant species (Louda 1982). Moreover, studies have shown that levels of insect pre-dispersal predation of fruits and seeds may respond to landscape characteristics such as fragment area and isolation (Cunningham 2000; Steffan-Dewenter et al. 2001; Rabasa et al. 2009; Matesanz et al. 2015). For example, Matesanz et al. (2015) observed increased insect pre-dispersal seed predation of *Centaurea hyssopifolia* (Asteraceae) growing on more isolated gypsum outcrops in central Spain. High levels of insect pre-dispersal seed predation may also interact with the pollination dynamics of plants, such that possible increases of seed production in larger fragments or populations are not realised due to simultaneously high levels of seed predation (e.g., see Steffan-Dewenter et al. 2001 and Johnson et al. 2004). Thus, levels of pre-dispersal seed predation may well need to be studied in order to properly understand the influence of landscape disturbance on pollination and net plant reproductive output of particular plant species.

The buzz-pollinated *H. exutiacies* suffers high levels of pre-dispersal seed predation and the significant spatial variability in reproduction found in Chapter Three appeared to be largely due to this predation which directly lowered the output of viable seeds, rather than pollination. Thus, the current level of landscape disturbance in the Adelaide Hills appears to significantly influence the spatial variability of pre-dispersal seed predation but not pollination of *H. exutiacies*. The results here further emphasise the importance of considering biotic interactions other than animal-pollination when examining factors determining plant reproduction in fragmented landscapes. A similar conclusion was also reached by Faast (2009) and Faast and Facelli (2009) who studied spatio-temporal variability of florivory on the reproductive output of the orchids *Caladenia rigida* and *C. tentaculata* in the Mount Lofty Ranges. It is interesting that the production of viable seed is low for many *Hibbertia* species (Fox et al. 1987 p. 86; Schatral et al. 1994; Cochrane 2002) and that insect pre-dispersal seed predation appears to be common (Sweedman and Brand 2006 p. 187). Thus, *Hibbertia* appears to be a good candidate genus to further study the possible impact(s) of landscape disturbance on the spatio-temporal dynamics of insect pre-dispersal seed predation.

No previous published study appears to have examined the impact(s) of variation in landscape characteristics on a nocturnally moth-pollinated plant in Australia, and thus the results of Chapter Five are novel. In comparison to *H. exutiacies*, reproduction of *S. aspericocca* ssp. *Cylindrical inflorescence* was comparable between smaller and larger reserves in each of the years studied, and mean reproduction was similar between the two years. Thus, reproduction of this plant showed the least spatio-temporal variability of the three species. Some of the moth species caught visiting flowers of *S. aspericocca* ssp. *Cylindrical inflorescence* are known to be common to the study region and the relatively short floral tube of *S. aspericocca* ssp. *Cylindrical inflorescence* (e.g., shorter than a number of co-flowering species of *Pimelea* (Pers. Obs.)) may allow for a variety of moth species to function as pollinators. Thus, the pollination system of *S. aspericocca* ssp. *Cylindrical inflorescence* may be rather generalised and similar in this way to that of *P. daphnoides*, and for both these species there may be a relatively low risk of reproductive failure from a scarcity of pollinators. However, given the lack of research of nocturnal moth-pollination in Australia, similar studies on other species of *Stackhousia* which are likely nocturnally moth-pollinated (e.g., *S. monogyna* (Clarke and Lee 2019 p. 186) and *S. subterranea* (Pers. Obs.)) would prove enlightening.

Reserve area alone was not positively related to the reproduction of *P. daphnoides* (Chapter Two). Indeed, in 2018, viable seed production was almost limited to the two smallest reserves which resulted in a significant negative relationship between reserve area and viable seed production. However in 2017, there was no significant relationship between reserve area and viable seed production. Thus, unlike for *S. aspericocca* ssp. *Cylindrical inflorescence*, but similar to *H. exutiacies*, the relationship between reserve area and plant reproduction was not temporally consistent for *P. daphnoides*. The significant negative relationship in 2018 was due to the almost complete abortion of developing seeds in larger reserves which may have been related to historically low early spring rainfall (Chapter Four, see below).

6.1 Implications for Conservation

6.1.1 Reserve area

The principle that a larger fragment of continuous habitat is of greater conservation value than a collection of smaller habitat fragments (e.g., Diamond 1975) has influenced reserve designs globally (Armsworth et al. 2018; Fahrig et al. 2022), including in South Australia (Bryan 2002). However, small patches of habitat may contribute significantly to biodiversity conservation, particularly within heavily modified landscapes (Lindenmayer 2019; Wintle et al. 2019). Recent reviews have highlighted that sets of smaller fragments typically hold a greater number of species than equivalent larger fragments within a landscape (i.e., single large or several small (SLOSS) debate; Fahrig 2020; Riva and Fahrig 2022). For example, the individual studies of Rösch et al. (2015) and Honnay et al. (1999) both found that the species richness of plants was greater when pooled across many small fragments versus a few larger fragments of equal area in their study systems of calcareous grasslands in Germany and forest fragments across Belgium and France, respectively.

These findings do not imply that a single smaller reserve is of greater conservation value than a single larger reserve (Fahrig 2020; Riva and Fahrig 2022). However, they highlight that sets of small habitat fragments across a landscape may contribute significantly to the conservation of current biodiversity and should not be viewed, collectively, as of lesser conservation value than a similarly sized larger patch of habitat (Fahrig 2020; Riva and Fahrig 2022). This is particularly important given over half of reserves globally are < 100 ha, and the median protected area of new reserves has declined over the past decades to < 50 ha (Volenec and Dobson 2020). Indeed, the protection and restoration of small native vegetation fragments will be particularly important in the Adelaide Hills and the Southern Mount Lofty Ranges overall, where 55.00 % of native vegetation occurs in fragments of ≤ 500 ha, and 69.00 % of remaining native vegetation patches are 1-10 ha in size (Armstrong et al. 2003).

The research conducted here did not measure biodiversity per se (i.e., measures of species richness, diversity indices, etc.). However, the reduced reproductive output of plants within smaller reserves is one mechanism through which plant populations may decline and become locally extinct in small reserves. Thus, a negative effect of smaller reserve area on plant reproduction would be of conservation concern. However, the findings here do not suggest the studied plant species are at greater risk of reproductive failure within smaller reserves versus larger reserves. Thus, as far as successful reproduction reflects population persistence, reserve area alone cannot be used as a reliable indicator of conservation value, and small and large reserves both potentially represent valuable contributions to the conservation of these three studied plant species.

However, some caution is warranted. While the overall results are positive, populations within both smaller and more isolated fragments may still be more vulnerable to threatening processes (Saunders et al. 1991; Laurance 2002; Stenhouse 2004; Laurance et al. 2011; Haddad et al. 2015), and extinction debts in smaller patches may remain for longer-lived species, particularly plants (Ramalho et al. 2014, 2018; Helm et al. 2006; Krauss et al. 2010; Aguilar et al. 2018). Thus, the future viability of plant populations within small reserves and fragments cannot be assumed and ongoing conservation efforts will be required to successfully manage the flora and fauna of native vegetation fragments in the Adelaide Hills (e.g., see Hills and Fleurieu Landscape Plan 2021 - 2026).

In this regard, the role of non-government and community volunteer organisations in managing small private reserves and other patches of native vegetation will be important (Stephens 2001). This was effectively highlighted here by Nurrutti Reserve, a small 1.40 ha private nature reserve managed by the National Trust of SA (i.e., open to the public but not managed by any level of government; https://www.nationaltrust.org.au/places/nurrutti-reserve/, last accessed 25/05/2022). This small reserve has an active volunteer bushcare group mostly focused on weed removal. It was found that mean reproduction of *P. daphnoides* and *S. aspericocca* ssp. *Cylindrical inflorescence* in Nurrutti was equal to or greater than plants of both species in larger reserves. Thus, successful reproduction was maintained in this small reserve for two common plants with different pollinators (bee- versus nocturnally moth-pollinated) and growth forms (woody versus herbaceous). Nurrutti also harbours 16 plant species of state or regional significance and is part of the "Valley of the Bandicoots", a community project initiated and managed by the Aldgate Valley Landcare Group (another volunteer organisation) to conserve and restore a corridor of native vegetation as habitat for local flora and fauna. Thus, Nurrutti emphasises the conservation value of small reserves alone, and their possible role in conjunction with other small and large patches of habitat across the landscape (e.g., by increasing landscape connectivity).

Ultimately, while large reserve area will be important to the conservation of some species, the effective protection and management of the many smaller patches of native vegetation across fragmented landscapes should be a principal conservation priority globally (e.g., see similar sentiments in Lindenmayer 2019; Arroyo-Rodríguez et al. 2020; Volenec and Dobson 2020; Riva and Fahrig 2022). In particular, non-governmental organisations and private citizens will play a significant role in the protection and restoration of small remaining habitat patches in the Southern Mount Lofty Ranges and elsewhere in Australia (Stephens 2001). The finding that small reserve area alone does not negatively impact the reproductive output of these three plant species may also suggest the chosen plants would be important in restoration projects of small public or private reserves and landholdings, although this requires further study.

6.1.2 Lower rainfall and future climate change

Seed abortion for *P. daphnoides* and *H. exutiacies* was widespread in 2018, a year of historically low early spring rainfall. Specifically, viable seed production of *P. daphnoides* was almost limited to the two smallest reserves assessed in 2018 (Chapter Two). This was a particularly striking result, and emphasises the role smaller reserves may play in maintaining at least some viable seed production across fragmented landscapes in particular years. Moreover, although no pollen-limitation experiments were conducted in 2018, the termination of many pods and their developing seeds is inconsistent with the widespread pollen-limitation of reproduction, at least from a pollen quantity perspective (Burd 2004). The high level of seed abortion across reserves may have resulted from lower water availability (i.e., water-limitation), however, it remains unclear why viable seed production was maintained in the two smallest reserves studied in 2018 (e.g., no evidence of localised rainfall events).

In comparison, almost no viable seeds were produced by plants of *H. exutiacies* across the sampled reserves in 2018 (Chapter Three). Thus, plant reproduction was equally limited across the studied populations. Additionally, pollen-limitation experiments in 2018 clearly demonstrated that the reproductive failure of *H. exutiacies* was unrelated to pollination efficiency and seed abortion was likely a direct result of water-limitation. This contrasts with the results of Faast (2009), who examined spatio-temporal variability in the pollination and reproduction of the orchids of *C. rigida* and *C. tentaculata* in the Mount Lofty Ranges. In particular, Faast (2009) observed that reproductive failure of the orchid *C. rigida* in the drought year of 2006 was most likely due to a lack of pollinators, rather than a cause of lower rainfall per se. Thus, lower rainfall may also indirectly influence plant reproduction via its effect on pollinators (e.g., lower abundance, altered diversity, etc.). Importantly, neither effect is mutually exclusive, and lower rainfall may simultaneously negatively impact plant reproduction both directly, via resource-limitation, and indirectly via negative effects on a plants pollinator(s).

An experimental field study of two populations of *H. exutiacies* in 2019 assessed the influence of water availability on reproduction (Chapter Four). However, the findings were mixed, with increased watering of plants in the field similarly altering the flowering phenology of plants in both populations but significantly increasing fruit production in only one of the populations, and having no significant effect on seed abortion in either population. Indeed, pre-dispersal seed predation of *H. exutiacies* plants in 2019 was qualitatively comparable to the high rates observed in 2017. Thus, the abundance of seed predators was not obviously impacted by high seed abortion in the intervening year of 2018 and at least within the two studied populations plants of *H. exutiacies* were unable to temporally escape high levels of pre-dispersal predation. Further study is required to better understand this complex system, in which over three years plant reproduction was limited by a mixture of pollen- and water-limitation, high levels of pre-dispersal seed predation, and occasional extensive seed abortion, all of which showed either significant spatial variability, temporal variability, or both. *Hibbertia exutiacies* like other perennial plant species may be able to use stored reserves for reproduction (e.g., see Ida et al. 2013) and this may be a productive avenue of future research. How populations of *H. exutiacies* successfully cope with such high levels of pre-dispersal seed predation remains an unresolved question.

It is expected that there will be significant variation between plant species in the susceptibility of their reproduction to lower rainfall and some plant species will fare better than others under drier conditions. In support, and in direct contrast to *P. daphnoides* and *H. exutiacies*, both the mean fruit-set and mean seed number per fruit of *S. aspericocca* ssp. *Cylindrical inflorescence* (Chapter Five) was relatively stable between years (2018 versus 2019), and reproduction of this plant may be relatively robust to both landscape disturbance and among-year variability in spring rainfall (at least over the short-term).

Nevertheless, although some plant species may perform relatively better than others under drier conditions, average spring rainfall in the Adelaide Hills is projected to decline by 17.0-25.2 % by the year 2070 (Charles and Fu 2015), and this overall decline in spring rainfall may result in reduced reproduction of many common plant species relative to historic levels, irrespective of or in combination with any impact(s) of landscape disturbance. Thus, future studies examining the impact(s) of landscape disturbance on pollination and plant reproduction should also consider the possible influence(s) of climate change in the study region. Indeed, building-up the climate resilience of ecosystems is a strong focus of the current "Hills and Fleurieu Landscape Plan 2021 - 2026". In particular, although the research presented here did not explicitly assess the impact(s) of future climatic conditions on plant reproduction, the results of *P. daphnoides* suggest that the conservation of small native vegetation fragments throughout the landscape may be critical to the climate resilience of this species within the Adelaide Hills.

6.2 Concluding Statement

The relationship between pollination, reproduction, and reserve area varied between the plant species studied and among years for *P. daphnoides* and *H. exutiacies*. There was no evidence however that small reserve area alone negatively impacts the pollination or reproduction of the three studied understorey plants. Because the plant species studied vary considerably in their ecology (e.g., pollinator specialization, levels of insect pre-dispersal seed predation, woody versus herbaceous), it is possible that many other common plants in the Adelaide Hills are robust to the present levels of landscape disturbance. However, the idiosyncratic response of the three species studied and the significant temporal variability observed between years for *P. daphnoides* and *H. exutiacies*, emphasises the importance of continued empirical studies across multiple years in further understanding pollination and plant reproduction within fragmented landscapes, particularly under possible future drier climate scenarios. Indeed, although detailed empirical studies of the impact(s) of landscape disturbance on single plant species, as presented here, may not by themselves allow for the generalisation of common effects across plant species, they remain exceedingly important for at least two reasons. Firstly, empirical studies represent the raw data used by both qualitive and quantitate reviews (e.g., meta-analyses), and although multi-species studies in the same location are valuable, they often necessitate a less comprehensive study per plant species. Thus, detailed empirical studies of single plant species are clearly required to understand the possible impact(s) of landscape disturbance on species with complex spatio-temporal interactions among a number of abiotic and biotic resources, as exemplified here by *H. exutiacies*. Secondly, although both qualitive and quantitate reviews which have examined the impact(s) of landscape disturbance on pollination and plant reproduction have found an overall negative influence of landscape disturbance, such reviews do not reliably assess the replicability of the findings of individual studies. This requires within-study replication, either spatially or temporally, or as close to possible direct replication of previous studies, although direct replications are difficult in field ecology. Thus, there remain calls for greater within-study replication of ecological studies to better assess the replicability of results (Filazzola and Cahill 2021), which in turn will strengthen confidence in the findings of future cross-species and/or cross-system syntheses (Nakagawa and Parker 2015). Indeed, the significant temporal variability observed here between consecutive years for *P. daphnoides* and *H. exutiacies* highlights the potential limitation(s) of studies conducted across single flowering seasons. In particular, given the majority of prior studies have been conducted across a single flowering season, many current studies may fail to capture the true complexity of their studied system(s). Ultimately, future studies of the impact(s) of landscape disturbance on pollination and plant reproduction should at a minimum be replicated across two flowering seasons, which will allow for future systematic reviews to begin to assess the frequency and significance of temporal variability in the presence and magnitude of landscape disturbance effects in fragmented landscapes, and whether such temporal variability is related to particular ecological traits.

6.3 REFERENCES:

Aguilar R, Ashworth L, Galetto L, Aizen MA (2006) Plant reproductive susceptibility to habitat fragmentation: review and synthesis through a meta-analysis. Ecology Letters 9:968-980.

Aguilar R, Calviño A, Ashworth L, Aguirre-Acosta N, Carbone LM, Albrieu-Llinás G, Nolasco M, Ghilardi A, Cagnolo L (2018) Unprecedented plant species loss after a decade in fragmented subtropical Chaco Serrano forests. PLoS One 13:e0206738.

Armstrong DM, Croft SJ, Foulkes JN (2003) A biological survey of the Southern Mount Lofty Ranges, South Australia, 2000-2001. Department for Environment and Heritage, South Australia.

Armsworth PR, Jackson HB, Cho SH, Clark M, Fargione JE, Iacona GD, Kim T, Larson ER, Minney T, Sutton NA (2018) Is conservation right to go big? Protected area size and conservation return-on-investment. Biological Conservation 225:229-236.

Arroyo-Rodríguez V, Fahrig L, Tabarelli M, Watling JI, Tischendorf L, Benchimol M, Cazetta E, Faria D, Leal IR, Melo FP, Morante-Filho JC (2020) Designing optimal human-modified landscapes for forest biodiversity conservation. Ecology Letters 23:1404-1420.

Bennett JM, Steets JA, Burns JH, Burkle LA, Vamosi JC, Wolowski M, Arceo-Gómez G, Burd M, Durka W, Ellis AG, Freitas L (2020) Land use and pollinator dependency drives global patterns of pollen limitation in the Anthropocene. Nature Communications 11:3999.

Bond WJ (1994) Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London B 344:83-90.

Bryan BA (2002) Reserve selection for nature conservation in South Australia: past, present and future. Australian Geographical Studies 40:196-209.

Buchmann SL (1983) Buzz pollination in angiosperms. In: Jones CE, Little RJ (eds) Handbook of experimental pollination biology. Van Nostrand Reinhold, New York, pp 73-133.

Burd M (2004) Offspring quality in relation to excess flowers in *Pultenaea gunnii* (Fabaceae). Evolution 58:2371-2376.

Buxton MN, Anderson BJ, Lord JM (2018) The secret service - analysis of the available knowledge on moths as pollinators in New Zealand. New Zealand Journal of Ecology 42:1-9.

Charles SP, Fu G (2015) Statistically downscaled projections for South Australia. Goyder Institute for Water Research Technical Report Series No. 15/1, Adelaide, South Australia.

Clarke I, Lee H (2019) Name that flower. Melbourne University Press, Melbourne, Australia.

Cochrane A (2002) Seed notes for Western Australia. No. 17 *Hibbertia*. Wildflower Society of Western Australia.

Cunningham SA (2000) Effects of habitat fragmentation on the reproductive ecology of four plant species in mallee woodland. Conservation Biology 14:758-768.

Diamond JM (1975) The island dilemma: lessons of modern biogeographic studies for the design of natural reserves. Biological Conservation 7:129-146.

Faast R (2009) The reproductive ecology of two terrestrial orchids, *Caladenia rigida* and *Caladenia tentaculata*. Dissertation, University of Adelaide.

Faast R, Facelli JM (2009) Grazing orchids: impact of florivory on two species of *Caladenia* (Orchidaceae). Australian Journal of Botany 57:361-372.

Fahrig L (2013) Rethinking patch size and isolation effects: the habitat amount hypothesis. Journal of Biogeography 40:1649-1663.

Fahrig L (2020) Why do several small patches hold more species than few large patches? Global Ecology and Biogeography 29:615-628.

Fahrig L, Watling JI, Arnillas CA, Arroyo-Rodríguez V, Jörger-Hickfang T, Müller J, Pereira HM, Riva F, Rösch V, Seibold S, Tscharntke T (2022) Resolving the SLOSS dilemma for biodiversity conservation: a research agenda. Biological Reviews 97:99-114.

Filazzola A, Cahill Jr JF (2021) Replication in field ecology: identifying challenges and proposing solutions. Methods in Ecology and Evolution 12:1780-1792.

Fox J, Dixon B, Monk D (1987) Germination in other plant families. In: Langkamp P (ed) Germination of Australian native plant seed. Inkata Press, Melbourne and Sydney, pp 83-97.

Gross CL (2001) The effect of introduced honeybees on native bee visitation and fruit-set in *Dillwynia juniperina* (Fabaceae) in a fragmented ecosystem. Biological Conservation 102:89-95.

Haddad NM, Brudvig LA, Clobert J, Davies KF, Gonzalez A, Holt RD, Lovejoy TE, Sexton JO, Austin MP, Collins CD, Cook WM (2015) Habitat fragmentation and its lasting impact on Earth's ecosystems. Science Advances 1:e150005.

Hadley AS, Frey SJ, Robinson WD, Kress WJ, Betts MG (2014) Tropical forest fragmentation limits pollination of a keystone understory herb. Ecology 95:2202-2212.

Hahn M, Brühl CA (2016) The secret pollinators: an overview of moth pollination with a focus on Europe and North America. Arthropod-Plant Interactions 10:21-28.

Helm A, Hanski I, Pärtel M (2006) Slow response of plant species richness to habitat loss and fragmentation. Ecology Letters 9:72-77.

Hills and Fleurieu Landscape Plan 2021 - 2026. https://www.landscape.sa.gov.au/hf/about-us/our-plans/our-regions-plan. Last accessed: 25/05/2022.

Hobbs RJ, Yates CJ (2003) Impacts of ecosystem fragmentation on plant populations: generalising the idiosyncratic. Australian Journal of Botany 51:471-488.

Honnay O, Hermy M, Coppin AP (1999) Effects of area, age and diversity of forest patches in Belgium on plant species richness, and implications for conservation and reforestation. Biological Conservation 87:73-84.

Ida TY, Harder LD, Kudo G (2013) Demand-driven resource investment in annual seed production by a perennial angiosperm precludes resource limitation. Ecology 94:51-61.

Johnson SD, Collin CL, Wissman HJ, Halvarsson E, Ågren J (2004) Factors contributing to variation in seed production among remnant populations of the endangered daisy *Gerbera aurantiaca*. Biotropica 36:148-155.

Krauss J, Bommarco R, Guardiola M, Heikkinen RK, Helm A, Kuussaari M, Lindborg R, Öckinger E, Pärtel M, Pino J, Pöyry J (2010) Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. Ecology Letters 13:597-605.

Laurance WF (2002) Hyperdynamism in fragmented habitats. Journal of Vegetation Science 13:595-602.

Laurance WF, Camargo JL, Luizão RC, Laurance SG, Pimm SL, Bruna EM, Stouffer PC, Williamson GB, Benítez-Malvido J, Vasconcelos HL, Van Houtan KS (2011) The fate of Amazonian forest fragments: a 32-year investigation. Biological Conservation 144:56-67.

Lindenmayer D (2019) Small patches make critical contributions to biodiversity conservation. Proceedings of the National Academy of Sciences 116:717-719.

Lomov B, Keith DA, Hochuli DF (2010) Pollination and plant reproductive success in restored urban landscapes dominated by a pervasive exotic pollinator. Landscape and Urban Planning 96:232-239.

Louda SM (1982) Limitation of the recruitment of the shrub *Haplopappus squarrosus* (Asteraceae) by flower- and seed-feeding insects. The Journal of Ecology 70:43-53.

Macgregor CJ, Pocock MJ, Fox R, Evans DM (2015) Pollination by nocturnal Lepidoptera, and the effects of light pollution: a review. Ecological Entomology 40:187-198.

Matesanz S, Gómez-Fernández A, Alcocer I, Escudero A (2015) Fragment size does not matter when you are well connected: effects of fragmentation on fitness of coexisting gypsophiles. Plant Biology 17:1047-1056.

McIntyre S, Hobbs R (1999) A framework for conceptualizing human effects on landscapes and its relevance to management and research models. Conservation Biology 13:1282-1292.

Nakagawa S, Parker TH (2015) Replicating research in ecology and evolution: feasibility, incentives, and the cost-benefit conundrum. BMC Biology 13:88.

Rabasa SG, Gutiérrez D, Escudero A (2009) Temporal variation in the effects of habitat fragmentation on reproduction of the Mediterranean shrub *Colutea hispanica*. Plant Ecology 200:241-254.

Ramalho CE, Laliberté E, Poot P, Hobbs RJ (2014) Complex effects of fragmentation on remnant woodland plant communities of a rapidly urbanizing biodiversity hotspot. Ecology 95:2466-2478.

Ramalho CE, Laliberté E, Poot P, Hobbs R (2018) Effects of fragmentation on the plant functional composition and diversity of remnant woodlands in a young and rapidly expanding city. Journal of Vegetation Science 29:285-296.

Riva F, Fahrig L (2022) The disproportionately high value of small patches for biodiversity conservation. Conservation Letters e12881.

Rösch V, Tscharntke T, Scherber C, Batáry P (2015) Biodiversity conservation across taxa and landscapes requires many small as well as single large habitat fragments. Oecologia 179:209-222.

Saunders DA, Hobbs RJ, Margules CR (1991) Biological consequences of ecosystem fragmentation: a review. Conservation Biology 5:18-32.

Schatral A, Kailis SG, Fox JED (1994) Seed dispersal of *Hibbertia hypericoides* (Dilleniaceae) by ants. Journal of the Royal Society of Western Australia 77:81-85.

Steffan-Dewenter I, Münzenberg U, Tscharntke T (2001) Pollination, seed set and seed predation on a landscape scale. Proceedings of the Royal Society of London. Series B: Biological Sciences 268:1685-1690.

Stenhouse RN (2004) Fragmentation and internal disturbance of native vegetation reserves in the Perth metropolitan area, Western Australia. Landscape and Urban Planning 68:389-401.

Stephens S (2001) Visions and viability: how achievable is landscape conservation in Australia? Ecological Management and Restoration 2:189-195.

Sweedman L, Brand G (2006) Collection guidelines for common Australian families and genera. In: Sweedman L, Merritt D (eds) Australian seeds a guide to their collection, identification and biology. CSIRO Publishing, Collingwood, Victoria, pp 173-198.

Taki H, Kevan PG, Ascher JS (2007) Landscape effects of forest loss in a pollination system. Landscape Ecology 22:1575-1587.

Volenec ZM, Dobson AP (2020) Conservation value of small reserves. Conservation Biology 34:66-79.

Wintle BA, Kujala H, Whitehead A, Cameron A, Veloz S, Kukkala A, Moilanen A, Gordon A, Lentini PE, Cadenhead NCR, Bekessy SA (2019) Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. Proceedings of the National Academy of Sciences 116:909-914.

Wolowski M, Ashman TL, Freitas L (2014) Meta-analysis of pollen limitation reveals the relevance of pollination generalization in the Atlantic forest of Brazil. PLoS One 9:e89498.