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The pollination ecology and reproductive  
success of the Australian shrub *Grevillea*  
*macleayana*

Samantha M. Lloyd  
University of Wollongong

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**The Pollination Ecology and Reproductive Success of the  
Australian shrub *Grevillea macleayana***

**A thesis submitted in fulfillment of the requirements for the award of the  
degree**

**DOCTOR OF PHILOSOPHY**

**from the**

**UNIVERSITY OF WOLLONGONG**

**by**

**Samantha M. Lloyd**

**SCHOOL OF BIOLOGICAL SCIENCES**

**2006**

## **Certification**

I, Samantha M. Lloyd, declare that this thesis, submitted in fulfillment of the requirements for the award of Doctor of Philosophy, in the School of Biological Sciences, University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. The document has not been submitted for qualifications at any other academic institution.

Samantha Lloyd

6<sup>th</sup> April 2006

"To describe different groups of plant, Linnaeus had used extraordinary terms like 'bridal chamber' and 'nuptials'. For prudish Britons, this sexualized version of nature verged on the pornographic, and battles over botanical textbooks resembled current debates about allowing children to watch violent videos. Self-appointed moral guardians of society declared that they wanted to protect young women from the corrupting influences of botanical education" (Fara, 2003)....

Fortunately they failed!

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## Abstract

Theory predicts that plants that are more attractive to pollinators (via greater floral rewards) should have greater reproductive success, produce higher quality seed, and hence have greater fitness. Within a species, we assume that competition for effective pollinators is more intense because plants look the same, and thus, attracting pollinators may be more difficult. Moreover, plant-pollinator systems are highly variable and, in Australia, they have been subject to disruption by habitat fragmentation and the introduction of the European Honeybee. Ultimately, some individuals within a population will be more fit than others, however, there is little empirical evidence on the relationships between floral traits and plant fitness. This study examines the links between floral rewards, pollinator foraging behaviour, reproductive success, plant mating system parameters, and some non-reproductive plant traits and environmental variables, in an Australian woody shrub.

Variation may be evident in five primary components of plant-pollinator systems: (1) floral traits (e.g. flower, nectar, and pollen production); (2) pollinator foraging behaviour (e.g. insects, honeyeaters, and mammals); (3) reproductive success (e.g. pollen transfer, seed production and viability); (4) plant mating system and genetics (e.g. self-compatible species with low outcrossing rates) and (5) non-reproductive plant traits and environmental variables (e.g. plant size and density, climatic conditions). Our current understanding of the extent of intraspecific variation within these variables and how these variables interact within pollination systems is poor. This study quantifies intraspecific variation among *Grevillea macleayana* plants in each of these five components of the plant-pollination system, using three sites studied over three years. The broad aims are to: (1) quantify variation among plants in characteristics conferring attractiveness to pollinators (floral traits), pollinator foraging behaviour, reproductive success, and mating system variables and (2) determine how these components are related, and identify the interactions most important in explaining variation among plants.

*Grevillea macleayana* is a rare, hermaphroditic, bird-pollinated, medium to large shrub, with a large floral display. It has a fragmented distribution on the south-east coast of NSW, Australia. *Grevillea macleayana* is self-compatible and has low genetic diversity. It is visited by a suite of potential pollinators including honeybees,

honeyeaters, and the Eastern Pygmy Possum. However, evidence suggests that honeybees do not facilitate pollen transfer.

I quantified variation among *G. macleayana* plants in three floral traits: monthly inflorescence number; nectar production (i.e. volume per inflorescence and sugar concentration); and pollen production. I found substantial variation among plants in inflorescence production at every site. At each site, a small number of plants (three to five) produced over half the inflorescences for the study plants (19 in total), over the survey period. I also found significant variation among plants in nectar volume, but less variation in nectar sugar concentration. I did not detect significant variation among plants in pollen production. These results were consistent with previous studies on other Proteaceae species and provide evidence that floral display and nectar production are the most important floral rewards.

I quantified variation among plants in four aspects of honeybee and honeyeater foraging behaviour: the number of honeybees and honeyeaters; the number of inflorescences visited per plant; the foraging time per inflorescence; and the foraging time per plant. I found significant variation among plants in at least one feature of honeybee and honeyeater foraging behaviour, for one or two survey seasons per site. Contrary to the expectation that all pollinators will respond positively to similar floral traits, there were very few similarities between honeybees and honeyeaters in how they responded to variation in floral characteristics. These results provide some evidence that honeybees and honeyeaters may be responding differently to variation in floral cues and rewards.

I quantified variation among plants in two aspects of female reproductive success: monthly seed number, and nocturnal and diurnal pollen deposition. Plants varied substantially in seed numbers over the study period. Moreover, at each site, a small number of plants contributed to more than half the seed production of the survey population. I detected very low seed-to-inflorescence ratios, and these varied substantially among plants. However, plants with greater inflorescence numbers also had greater reproductive success (maternal seed numbers). Interestingly, there were no significant differences in pollen deposition between diurnal and nocturnal surveys, at two of the three sites. This result indicates that nocturnal pollinators may have an important role in pollinating *G. macleayana* plants.

I quantified variation among plants in two aspects of the *G. macleayana* plant mating system, using six microsatellite loci: family outcrossing rates (i.e. calculated for individual adults and their seed); and levels of biparental inbreeding for outcrossed seed. I found very low outcrossing rates across all families, and some plants were significantly different from zero and from each other. I also found very low biparental inbreeding rates across all families. The very low family outcrossing rates detected in this study indicates that whilst this is a mixed mating system, individuals are predominantly selfed.

I quantified variation among *G. macleayana* plants in six other non-reproductive plant traits and environmental variables that are likely to be related to plant vigour and hence, reproductive success: plant height, plant area, distance to nearest conspecific, canopy cover, leaf moisture content, and leaf photosynthetic yield. I found substantial variation among plants in height, area, and distance to nearest conspecific. I also found significant variation among plants in mean canopy cover, and slight, but significant variation among plants in leaf photosynthetic yield and leaf moisture.

Having detected significant variation among plants (in three populations) in the previously described five key components of pollination ecology, I then explored the strongest relationships among these variables. I used correlation and regression analyses to test for significant or consistent trends between dependent and independent variables. The most important trends in this system were:

- Significant positive regressions between inflorescence production (size) and nectar production (volume) and (non-significant) positive trends between inflorescence production and nectar production, suggesting no immediate trade-offs between resource allocation for inflorescence and nectar production.
- Numerous significant regressions between floral rewards (inflorescence and/or nectar production) and both honeybee and honeyeater foraging behaviour. These results support previous studies that have found greater numbers of pollinators or greater foraging activity associated with greater floral rewards.
- Significant positive correlations between seed production and both inflorescence and nectar production, suggesting: (1) no immediate trade-offs between resource allocation for floral traits and seed production, and (2) plants with greater floral rewards have greater reproductive success.



- Significant negative relationship between outcrossing rates and inflorescence numbers per plant. Plants with more inflorescences may be receiving more honeyeater visits (and within-plant activity), resulting in increased geitonogamous pollen movement and decreased outcrossing rates.
- Significant positive relationships between plant size (area or height) and both inflorescence and seed number, suggesting that larger plants may have greater carbon stores and resource availability.
- Significant negative regressions and (non-significant) negative trends between both inflorescence and seed number and canopy cover, suggesting that increased shade may reduce photosynthetic yield and resource availability for inflorescence and seed production.

The holistic approach used in this study has contributed to our understanding of intraspecific variation in plant-pollination systems and how this variation is related to plant reproductive success. Furthermore, my study has challenged some of the widely held beliefs about plant attraction to pollinators and added to our limited knowledge of some important plant processes (e.g. outcrossing rates) and their role in this pollination system. In trying to determine the most important relationships among the numerous components of the *G. macleayana* system, I have revealed a very complex plant-pollinator system. Whilst some of the relationships I found were as predicted, trends were not always consistent and it is clear that patterns of floral attraction, pollinator behaviour and reproductive success are not always intuitive.

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## Chapter 1 - General Introduction

### 1.1 Variation in Plant Pollination Systems

In *The Origin of Species*, Darwin (1859) described the “*struggle for existence*” as the competition (for often limited resources) that all life must engage in, to survive and reproduce successfully. Importantly, Darwin (1859) recognised that competition would be most intense between individuals of the same species (i.e. intraspecific competition), because conspecifics are morphologically more similar and are more likely to share the same resources, and be exposed to the same dangers and environmental stress than more distantly related organisms. Due to intense intraspecific competition, variation in some advantageous morphological and physiological traits may increase an individual’s chance of reproduction and survival. Provided this variation is heritable, individuals in a population with these heritable traits should have greater reproductive success and potentially greater fitness<sup>1</sup> than conspecifics (Pyke, 1981; Stearns, 1992). This process of intraspecific competition and variation in heritable traits will ultimately shape the mating systems and life history traits of the species involved. However, variation in reproductive success is not only a reflection of mating system traits and heritable variation, but also phenotypic plasticity in response to external influences, such as the environmental conditions present within a population.

Pollination ecology provides an opportunity to study the selection and evolution of floral traits, by quantifying: intraspecific variation in floral traits, the response of pollinators, and the consequences for plant reproductive success and potential fitness (Real and Rathcke, 1991; Kearns and Inouye, 1993; Mitchell and Marshall, 1998; Herrera, 2005). Unfortunately, despite approximately 245 years of published works on pollination (beginning with Kölreuter’s 1761 report, *Vorläufige Nachricht*; cited in Waser, 2006), our understanding of plant-pollinator interactions is still relatively rudimentary and specific knowledge of plant-pollinator interactions is lacking (Buchmann and Nabham, 1996; Kearns and Inouye, 1997; Kearns *et al.*, 1998). Furthermore, few pollination studies have taken a broad systems approach to investigating plant-pollinator systems. Many only address one or two components of plant-pollinator systems (e.g. flower production and pollinator activity) and are

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<sup>1</sup> Plant fitness is defined as the ability of an individual plant to contribute its genes to subsequent generations, relative to other plants in the population (Lincoln *et al.*, 1982).

therefore unable to explore how variation is linked to reproductive success, which ultimately determines fitness and drives the evolution of floral traits.

## 1.2 Plant Attraction and Pollination Ecology

Pollination is one of the most important ecological processes on earth, ensuring the reproduction of angiosperm plants. Moreover, most flowering plant species worldwide are entirely or partly pollinated by animals, as opposed to abiotic processes, such as wind or water (Buchmann and Nabham, 1996; Waser, 2006). Pollination by animals is largely considered a mutualistic relationship, rewarding a pollinator with energy (from nectar and pollen) and providing a plant with reproductive success through both male and female function (i.e. pollen transfer and seed production) (Faegri & van der Pijl, 1979; Heinrich, 1983; Waser, 2006). Whilst this relationship may appear to be mutualistic, conflicts may develop between plants and pollinators in specific interactions. Specifically, the pollinator behaviour that provides the most effective pollen transfer for the plant may not provide the pollinator with the greatest energy reward (Zimmerman, 1988; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994; Gegear and Lavery, 2001). Pollinator infidelity, intra-plant movements and low visit frequencies could all be features of a pollinator's foraging behaviour that compromise effective pollen transfer and reduce reproductive success.

One of the fundamental expectations of pollination ecology is that the most attractive plants will have greater potential male and/or female reproductive success (via increased pollinator visits) and hence fitness (Darwin, 1859; Zimmerman, 1988; Eckhart, 1991; Oldroyd *et al.*, 1997). Moreover, selection should favour plant and floral traits that attract the most efficient pollinators, thus maximising the deposition of viable pollen and reducing competition for pollination (Darwin, 1859; Ramsey, 1988; Conner and Rush, 1996; Gómez and Zamora, 2006). For instance, Darwin (1859) predicted that insects would visit flowers with greater nectar production more frequently, consequently these flowers would produce more outcrossed seed and therefore have increased fitness (*"Those individual flowers which had the largest glands or nectaries, and which excreted most nectar, would be oftenest visited by insects, and would be oftenest crossed; and so in the long-run would gain the upper hand"* pg 140; Darwin, 1859). However, attracting pollinators is not the result of a single plant characteristic, but rather a combination of many characteristics (e.g. floral display, plant size) (Scogin,

1983). Evidence suggests that the plant characteristics most effective at increasing plant attractiveness, and therefore, competitive ability are floral display and nectar production, however, pollen production is also recognised as an important floral reward (Scogin, 1983; Klinkhamer and de Jong, 1993; Brody and Mitchell, 1997; Salguero-Faria and Ackerman, 1999). Many studies have found that large floral displays (e.g. Eckhart, 1991; Mitchell *et al.*, 2004) and greater nectar production (e.g. Dreisig, 1995; Klinkhamer *et al.*, 2001) are most attractive to pollinators (Table 1.1 – at the end of this Chapter). Plants with these floral characteristics may, in many instances, also have greater reproductive success (Table 1.2 - at the end of this Chapter).

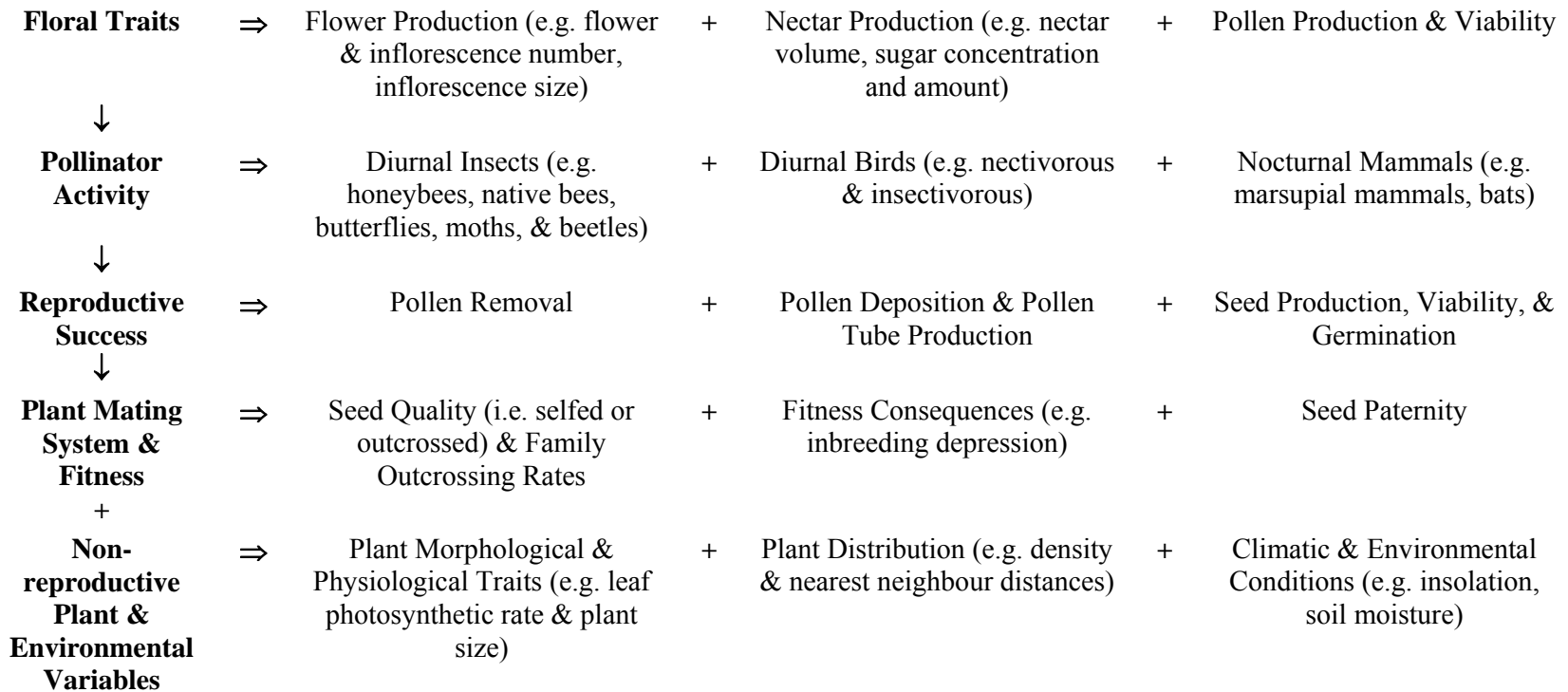
Robertson (1895) proposed the idea that competition may arise between plants due to a limited number of effective pollinators and that this competition may drive variation between plant species in flowering phenology and other traits. Pollinator behaviour, has therefore, been interpreted as a selective force acting on a wide variety of floral traits (Whelan and Goldingay, 1986; Caruso, 2000). However, appearing more attractive to pollinators may create a dilemma for plants (Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994). For example, greater nectar production may encourage more pollinator visits, but it may also lead to longer foraging bouts on the same plant, increasing self-pollination and inbreeding (in self-compatible species), and potentially decreasing plant reproductive success (Heinrich, 1983; Rathcke, 1992). Furthermore, investment in floral traits involves a cost to a plant's limited resource supply (Pyke, 1981; Heinrich, 1983; Zimmerman, 1988; Klinkhamer and de Jong, 1993). Plants must find a balance between attracting a pollinator (i.e. providing a reward that meets the energy needs of a pollinator) and ensuring its own reproductive requirements (i.e. effective pollen transfer) (Heinrich and Raven, 1972; Paton, 1986a).

The various studies referred to above, and in Tables 1.1 and 1.2, have established that increased floral rewards may increase pollinator visits, and that increased pollinator visits or fidelity, may lead to increased reproductive success and the prediction of greater fitness. However, evolution within plant-pollination systems depends on the existence of inter-plant variation in a broad range of traits, on which selection can act. In fact, little is known about the scale of variation in traits such as nectar production and floral display (Conner and Rush, 1996; Kearns *et al.*, 1998; Biernaskie *et al.*, 2002) and how pollinator behaviour and reproductive success respond to this variation (Heinrich,

1983; Shmida and Kadmon, 1991; Rathcke, 1992; Hegland and Totland, 2005). There is also a lack of detailed information on pollinator foraging behaviour, with respect to patterns of movement within and between plants, and variation in pollination service to single plant species (Klinkhamer *et al.*, 1994; Price *et al.*, 2005). Rectifying this situation requires system-wide studies that explore the relationships between each component of the plant-pollinator system (Figure 1.1).

Much of the existing work on pollination has been focussed on annual and other short-lived plant species, due to their short reproductive episodes and life cycles. However, many of the plant species we want to understand and manage are long-lived perennial species that require longer and more complex studies. If we are to understand the evolutionary implications of previous studies and current theories on variation in pollination, we need studies that address the relationships between floral traits, pollinators and reproductive success (Rathcke, 1992). Furthermore, given the potential ecological crisis in global pollination systems, we now more than ever need research on the most important and basic aspects of pollination systems (Kearns *et al.*, 1998).

For the remainder of this literature review I have divided information on variation within plant-pollinator systems into five components: (1) floral traits (i.e. flower/inflorescence production, nectar production and pollen production); (2) pollinator activity (e.g. pollinator foraging behaviour, Australian native and exotic pollinators); (3) reproductive success (i.e. pollen removal, pollen deposition, and seed production); (4) plant mating systems and reproductive fitness (e.g. seed quality, outcrossing rates, seed paternity, inbreeding depression); and (5) non-reproductive plant traits and environmental variables (e.g. plant size and distribution, resource availability, and climatic conditions) (Figure 1.1). Within each of these components there are substantial gaps in our knowledge, especially with respect to how different plant-pollinator processes are related to each other, to plant reproductive success, and to plant fitness. Each of these five components also relates to a major chapter of my thesis.



**Figure 1.1 - The primary characteristics of floral traits, pollinator activity, reproductive success, mating systems and fitness, and non-reproductive plant traits and environmental variables, as relevant to plant-pollinator systems.**

For the purpose of my literature review I have divided variation in plant-pollinator systems into five broad components, relating to the floral traits, pollinator activity, reproductive success, and plant mating system and fitness sequence of events in plant-pollinator systems. I have also addressed non-reproductive plant traits and environmental variables that may be important to plants and their pollinators. Each component includes (but is not theoretically limited to) three main topics of literature that I address in detail.



### 1.3 Floral Traits

#### 1.3.1 Floral Display

##### 1.3.1.1 Floral Display, Pollinators, and Reproductive Success

Floral display includes the colour, scent, number, size, density of flowers, quality and quantity of floral rewards (e.g. pollen and nectar), and timing of flower opening (phenology). Floral display is vital to attracting adequate pollinator visits (Kunin, 1997). Not surprisingly, theory predicts that large floral displays are typically more attractive to pollinators (Darwin, 1859; Faegri and van der Pijl, 1979; Zimmerman 1988; Rathcke, 1992). Furthermore, Waser (1983) proposed that floral traits may affect a pollinator's behaviour in three ways: in approaching the plant; whilst it forages within a plant; and interplant movements. In fact, it has been proposed that pollinator foraging behaviour is one of the three key ecological processes to have brought about the observed variation among angiosperms in flower size and shape, the other two being resource constraints associated with floral display, and plant interactions with “enemies”, such as herbivores, seed/fruit predators and disease (Galen, 1999).

Many studies have reported that plants with a greater number or density of flowers receive more frequent pollinator visits, often with a larger number of flowers probed (e.g. Paton, 1982a; Conner and Rush, 1996; reviewed by Ohashi and Yahara, 2001; Leiss and Klinkhamer, 2005; Table 1.1). Moreover, large floral displays may also increase the likelihood of successful pollination in plant species with limited pollen transfer, low plant densities or short blooming period (Koptur, 1984). Provided pollinator visits result in effective pollen transfer, plant reproductive success and fitness may be increased (Wyatt, 1982; Morgan, 2000; Ohashi and Yahara, 2001). For example, Salguero-Faria and Ackerman (1999) found a significant positive correlation between the number of open *Comparettia falcata* flowers and effective pollination visits by the Puerto Rican Emerald Hummingbird. Other studies have found that whilst plants with more flowers may receive more pollinator visits, the proportion of flowers visited per plant may be equal to, or even less than, plants with smaller floral displays (e.g. Klinkhamer *et al.*, 1989; Robertson and Macnair, 1995). Comprehensive reviews by Galen (1999) and Zimmerman (1988) address: (1) variation in flower size and form and (2) the relationship between variation in floral display, the response of pollinators and subsequent reproductive success, respectively. Table 1.1 presents several studies,

conducted after the Zimmerman review (1988), which have found substantial relationships between floral display and pollinator visits.

A range of studies have found male and female reproductive success (e.g. pollen transfer and seed production) increased as floral display increased (e.g. Campbell, 1989; Broyles and Wyatt, 1990; Devlin *et al.*, 1992). For example, Devlin *et al.* (1992) found a positive relationship between floral display (flower production) and male reproductive success (seed siring) in wild radish populations. Campbell (1989) also found a significant positive relationship between flower number and female reproductive success (seed production) in *Ipomopsis aggregata* plants. In a comprehensive study, Broyles & Wyatt (1990) found significant positive correlations between flower number per plant and both male reproductive success (seeds sired) and female reproductive success (seed production) in *Asclepias exaltata* plants. Furthermore, plants with the greatest total reproductive success contributed equally to male and female success, and it was proposed that female success is at least as important as male success in selecting for flower number (Broyles and Wyatt, 1990). Fewer studies have found positive relationships between each of floral display, pollinator foraging behaviour and plant reproductive success (e.g. Goldingay and Whelan, 1990; Brody and Mitchell, 1997; Table 1.2). For example, Vaughton and Ramsey (1998) found that both male and female plants of the herb *Wurmbea dioica* with more flowers received more honeybee and butterfly visits. They also found that pollen transfer (both removal and deposition) was faster in plants with larger flowers (but not with greater flower number) and the number of seed per plant increased with flower number. Table 1.2 outlines studies that have examined and found relationships between floral traits, pollinator behaviour and reproductive success.

Several authors have recognised that, within self-compatible species, plants with greater floral displays may have an increased proportion of self-fertilised seed (hereafter referred to as ‘selfing’) and lower outcrossing rates due to geitonogamous pollen transfer (Darwin, 1859; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994; Harder and Barrett, 1995). These results were attributed to pollinators visiting more flowers in succession, as flower number increased per plant. Not surprisingly, many studies have reported increased selfing in plants with larger floral displays (e.g. Klinkhamer and de Jong, 1990; de Jong *et al.*, 1993; Klinkhamer and de Jong, 1993; de Jong *et al.*, 1999;

Franceschinelli & Bawa, 2000). In self-compatible species, increased transport of self-pollen within plants may decrease reproductive success, due to increased genetic load and inbreeding depression, and in self-incompatible species may decrease seed set. The relationship between selfed and outcrossed pollinations, plant mating systems and the consequences for reproductive success and fitness are further explored in Section 1.6 and Chapter 5.

#### 1.3.1.2 Inflorescence and Flower Size

Many studies have found increasing numbers of pollinator visits with increasing inflorescence and/or flower size (e.g. Cruzan *et al.*, 1988; Thomson, 1988; Young and Stanton, 1990; Conner and Rush, 1996). For example, Thomson *et al.* (1982) and Thomson (1988) found that bumblebee visits to *Aralia hispida* inflorescences increased with both umbel number and size. Fewer studies have also found increased reproductive success (i.e. seed production and pollen transfer) with increasing inflorescence and/or flower size (e.g. Willson and Rathcke, 1974; Schemske, 1980a; Firmage and Cole, 1988). For example, Pyke (1982) found that waratahs (*Telopea speciosissima*) with larger inflorescences had greater fruit production. Even fewer studies have found positive correlations between increasing inflorescence size, pollinator visits and subsequent reproductive success (e.g. Ohara and Higashi, 1994; Vaughton and Ramsey, 1998; Table 1.2). Ohara and Higashi (1994) found that *Corydalis ambigua* plants with larger inflorescences were visited more often than plants with smaller inflorescences, and these plants subsequently set more seed. Young and Stanton (1990) proposed that larger wild radish flowers may have greater male reproductive success than smaller flowers, given that larger flowers produced more pollen, received more pollinator visits, had a greater proportion of pollen removed and were preferentially visited before small flowers. It has been suggested that the popularity of larger inflorescences by pollinators may be due to the reduced flight cost and subsequent increased foraging time on a potentially greater floral reward (Thomson *et al.*, 1982; Cruzan *et al.*, 1988).

#### 1.3.1.3 Excess Flower Production & the Male Function Hypothesis

Bateman's Principle proposes that increased effective pollen transfer (via pollinator visits) will increase the male contribution to reproductive fitness more than the female contribution, especially when maternal seed production is resource limited (Bateman,

1948). Floral characteristics that result in increased pollinator visits, but no subsequent increase in maternal seed production, are suggested as adaptations to enhance male reproductive success, via pollen dispersal and seed siring (Stanton *et al.*, 1986; Broyles and Wyatt, 1990). This is described as the “*male function hypothesis*” (Willson, 1979; Sutherland & Delph, 1984) and the “*pollen donation hypothesis*” (Broyles and Wyatt, 1990). The “*male function hypothesis*” predicts that fitness gained through male function (i.e. pollen donation) should increase with increasing flower number. Conversely, fitness increases via female function should plateau or decline with increasing flower number, due to resource limits on seed production (Campbell, 1989). For example, Willson and Rathcke (1974) found that pod production increased with flowers per inflorescence, to a limit of approximately 30 flowers per inflorescence. They proposed that inflorescences larger than this might have evolved for pollen donation, supporting the “*male function hypothesis*”. However, tests of the “*male function hypothesis*” have been few, presumably due to difficulties in quantifying paternity.

The “*male function hypothesis*” hypothesis predicts that hermaphroditic plants should have a lower fruit-to-flower ratio than monoecious or dioecious plants, and that self-compatible hermaphrodites should have a greater proportion of fruit set than self-incompatible plants (Sutherland & Delph, 1984). Moreover, it has been proposed that if the male contribution to fitness gained by a plant (via excess flower production and subsequent pollen donation) is greater than the female fitness losses (from undeveloped seeds), then selection should favour the overproduction of flowers (Willson and Rathcke, 1974; Sutherland and Delph, 1984 and references therein). However, not all studies support the “*male function hypothesis*” (e.g. Campbell, 1989; Jersáková and Kindlmann, 2004). Whilst Campbell (1989) found that pollen donation and deposition increased with flower number per *Ipomopsis aggregata* plant, seed production also increased disproportionately in plants with larger floral displays. It was concluded that female function increased with flower number (Campbell, 1989).

Sutherland and Delph (1984) and Ayre and Whelan (1989) have reviewed other hypotheses for excess flower production and low seed/fruit production, including: (1) “*pollinator limitation*”, whereby low fruit set is limited by low rates of pollinator visits, low pollinator densities, or ineffective pollinator foraging behaviour (Schemske,

1980b); (2) “*pollinator attraction*”, whereby it is assumed that increased flower production will increase plant attraction to pollinators (via increased floral rewards) and ensure adequate pollinator visits for effective pollen transfer (Willson and Rathcke, 1974; Stephenson, 1980; Rathcke, 1992); (3) “*bet-hedging*”, whereby a plant produces excess flowers to compensate for variation in reproductive success due to variation in pollinator densities or visits, and resources required for fruit development (Stephenson, 1980); and (4) “*selective abortion*”, whereby if more fruits are initiated than available resources are able to develop to maturity, a plant can select to abort poor quality fruits based on fertilised ovules or the genetic composition of seeds (Stephenson, 1980; Stephenson, 1981). Sutherland and Delph (1984) proposed that if any of the four female-based hypotheses are valid, then there should be no difference in fruit-to-flower ratios between hermaphrodites, monoecious or dioecious plants. However, they found that hermaphrodites had significantly lower fruit-to-flower ratios than either monoecious or dioecious species (Sutherland and Delph, 1984). Therefore, they concluded that none of the female-based fitness hypotheses explained the observed lower fruit-to-flower ratios and that the “*male function hypothesis*” provides the best explanation of this phenomenon in hermaphrodite species (Sutherland and Delph, 1984).

### 1.3.2 Nectar Production

#### 1.3.2.1 Nectar Production, Pollinators, and Reproductive Success

Nectar has been recognised as the most important floral reward, because it is the primary energy source provided by plants for nectarivorous vertebrates and insect pollinators, and is simple for pollinators to metabolise and use (Simpson and Neff, 1983; Ford and Paton, 1986; Kearns and Inouye, 1993). Darwin (1859) predicted that individual flowers that produce more nectar would be more frequently visited by insect pollinators, produce more outcrossed seed and therefore have greater fitness. However, the “*functional influence*” of nectar production on both pollinators and plant reproductive success is very complex (Cresswell, 1999). The nectar reward a pollinator encounters at an individual flower has the potential to affect pollinator foraging patterns and thus pollen movement in a number of ways, including: whether subsequent flowers are probed on that plant, the number of flowers probed per plant, the time spent foraging at individual flowers and plants, and interplant foraging distances and movements (Pyke and Waser, 1981; Zimmerman, 1988; Thomson, 1988; Rathcke, 1992).

Some authors have predicted that (as for flower number) the fitness gains (via pollen transfer) for an individual plant should increase with increasing nectar production up to a certain point, and thereafter, further nectar increases may even decrease plant fitness (Pyke, 1981; Klinkhamer and de Jong, 1993). Klinkhamer *et al.* (1994) suggested that some plants with greater floral rewards may actually be “*too attractive*”, given that increased pollen removal does not necessarily result in increased pollen export, because of greater within-plant pollen movement. As with increased floral display, increased nectar production may result in a greater proportion of within-plant pollinator movements, increasing geitonogamy in self-compatible species and reducing effective pollen transfer and seed production in self-incompatible species (Rathcke, 1992; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994). Quantifying the variation in plant nectar production within a population is particularly important in understanding how floral display influences pollinator behaviour and reproductive success (Shmida and Kadmon, 1991; Kearns and Inouye, 1993). It is also worth noting that discrepancies between flower and pollinator morphology may allow for nectar removal whilst inhibiting effective pollen transfer (e.g. nectar thieving). This concept will be explored in Chapter 3.

Not surprisingly, different plant species vary widely in the quantity and composition of nectar they produce (Pleasants and Chaplin, 1983; Simpson and Neff, 1983). Moreover, previous studies have found that, within a species, nectar production may vary significantly both within and between plants (Waser, 1983; Rathcke, 1992; Boose, 1997; see also Tables 1.1 and 1.2). Numerous studies have found that bird and insect pollinators are more frequent visitors, forage for longer, and/or consume more nectar on individual plants within a species with greater nectar rewards (insects reviewed in Kevan and Baker, 1983; Mitchell and Paton, 1990; Cresswell, 1999; Klinkhamer and van der Lugt, 2004; Table 1.1). Thomson (1988) found that bumblebees visited nectar-rich *Aralia hispida* flowers significantly more than nectar-poor flowers. Paton (1982a) also found significant positive correlations between plant nectar production (per flower per day) and both the number of honeyeater visits and foraging time per flower. Interestingly, when plants are located in dense patches, poor nectar producers may benefit from the increased foraging activity of pollinators, due to their high nectar producing near neighbours (Klinkhamer *et al.*, 2001; Leiss and Klinkhamer, 2005).

As for floral display, if these pollinator visits result in effective pollen transfer, then reproductive success may be increased, thereby increasing plant fitness (reviewed in Zimmerman, 1988; Rathcke, 1992). Though limited in number, some comprehensive studies have found positive relationships between the nectar production, pollinator activity, and reproductive success of plants within species (e.g. Zimmerman, 1983; Eckhart, 1991; Oldroyd, *et al.*, 1997; Table 1.2). For example, Real and Rathcke (1991) found significant variation among *Kalmia latifolia* plants in mean 24 hr nectar production. This variation was positively correlated with the mean rate of pollinator visits and a positive correlation was also found between the rate of pollinator visits and percent fruit set (Real and Rathcke, 1991). Galen and Plowright (1985) found that bumblebees visited more flowers and spent longer foraging on *Epilobium angustifolium* inflorescences enriched with nectar, than on nectar-depleted inflorescences. Pollen deposition (an indication of potential female reproductive success) was greatest on the female flowers of nectar-enriched inflorescences (Galen and Plowright, 1985).

#### 1.3.2.2 Internal and External Influences on Nectar Production

Nectar production is influenced by many internal and external variables including the cost of production, resource limitation, season, time of day, flower age, size of floral display, plant size and location (Darwin, 1989; Cruden *et al.*, 1983; Zimmerman and Pyke, 1986; reviewed by Zimmerman, 1988 and Rathcke, 1992; Nicolson and Nepi, 2005). Numerous environmental factors and climatic conditions have also been correlated with nectar standing crop and the rate of nectar production, including air temperature, relative humidity, soil moisture, temperature, and amount of sunlight (Cruden *et al.*, 1983; Pleasants, 1983; Corbet, 1990; Rathcke, 1992 and references therein – further discussed in Section 1.7). It is expected that increased air temperature and humidity, via evaporative losses, will increase sugar concentration and decrease the volume of nectar rewards; and rain will dilute the sugar concentration of nectar rewards whilst increasing the volume. For example, many studies have found positive relationships between soil moisture and nectar production (e.g. Zimmerman, 1983; Zimmerman and Pyke, 1988a; Lee and Felker, 1992; Wyatt *et al.*, 1992). Cruden *et al.* (1983) also found the initiation of nectar secretion to be temperature dependent. Plants in a population of *Pedicularis canadensis* began nectar secretion one and a half hours later on mornings following very cold nights, than following warm nights (Cruden *et al.*, 1983).

Many studies have found that nectar production varies with time of day and may be a reflection of the foraging activity of pollinators (e.g. Collins *et al.*, 1984; McFarland, 1985; Wolff *et al.*, 2003; Saffer, 2004). In Australia, most mammal pollinators are nocturnal or crepuscular, and pollinating birds are diurnal (Carthew and Goldingay, 1997). Therefore, nocturnal nectar production and/or pollen presentation may indicate pollination by mammals (Saffer, 2004). However, in a study of six Australian plant species, Saffer (2004) failed to detect any clear pattern in the timing of nectar production, with respect to bird or mammal pollinated species (i.e. mammal pollinated plants did not necessarily produce more nectar at night).

#### 1.3.2.3 *The Cost of Nectar Production*

Little is known about the physiological or reproductive costs of nectar production to plants and few studies have examined any such costs (Rathcke, 1992; Klinkhamer *et al.*, 2001). However, energy and resources expended on nectar production cannot be used for other plant functions, such as growth and seed production (Zimmerman, 1983). It has been proposed that the costs and benefits of nectar production should be “*balanced*” between the energetic needs of pollinators (a cost to the plant) and plant reproductive success, to provide the best possible chance of maximising plant fitness (Pyke, 1981). Therefore, “*balanced*” nectar production may not necessarily be the maximum quantity a plant is capable of producing (Zimmerman, 1983).

The resources required for nectar production have been linked with various physiological and abiotic processes, including underground rhizome systems (Southwick, 1984), photosynthesis (Zimmerman and Pyke, 1988a) and moisture availability (e.g. Boose, 1997; Carroll *et al.*, 2001). For example, Zimmerman and Pyke (1988a) found no significant difference in nectar production between the flowers of control and 50% defoliated *Polemonium foliosissimum* plants. They concluded that nectar production in this species was well buffered against changes in carbon resources and suggested plants increased photosynthesis in remaining leaves to compensate for the loss of photosynthetic area.

#### 1.3.2.4 *Nectar Production, Heritability, and Selection*

Despite the importance of nectar characteristics with respect to plant attraction and reproduction, we know very little about their heritability and responsiveness to natural



selection (Mitchell, 2004). For selection to occur, pollinators must preferentially visit plants based on phenotypic variation in nectar production, or be more effective at pollen transfer, and at least some of this variation must be heritable, and therefore, expressed in subsequent generations (Boose, 1997). Such selection would also be subject to substantial influence from environmental variables and associations with other plant characteristics (Rathcke, 1992; Shuel, 1992; Mitchell, 2004). Intraspecific and interspecific variation in nectar production may itself be a heritable trait in response to natural selection (Rathcke, 1992; McDade and Weeks, 2004a, b). Therefore, the extent to which selection of nectar characteristics might result in evolutionary change would likely be determined by the genetic basis of the characteristics and the influence of environmental variables (Boose, 1997; Mitchell *et al.*, 1998).

A limited number of studies have demonstrated genetic variation or heritability in nectar production (e.g. Pedersen, 1953a; Boose, 1997; Leiss *et al.*, 2004; reviewed in Mitchell, 2004). Pedersen (1953a) found evidence of the heritability of nectar, whereby self-pollination of low and high nectar producing plants resulted in progeny with low and high nectar production, respectively. Moreover, cross-pollination of a high and low nectar producer resulted in progeny with intermediate nectar production. More recent studies have concentrated primarily on nectar volume, sugar concentration, and total sugar, and results indicate that there is substantial genetic variation in these nectar characteristics (e.g. Boose, 1997; Mitchell, 2004). In the first study to report heritable variation in nectar production under field conditions, Leiss *et al.* (2004) reported that the offspring of high nectar-producing plants produced comparable nectar (to their parents) in both controlled and field conditions. However, the offspring of low nectar-producing plants produced more nectar (than their parents) in controlled conditions, indicating a “*genotype by environment interaction*” (Leiss *et al.*, 2004). One of the difficulties in identifying a genetic basis for nectar characteristics is that substantial and highly variable environmental effects confound attempts to identify genetic variation (Mitchell, 2004).

The direction and strength of selection for nectar production will vary with plant density, pollinator foraging patterns, competing environmental variables, and plant reproductive success (Salguero-Faria and Ackerman, 1999; Klinkhamer and van der Lugt, 2004). Hodges (1995) presents a model that proposes different selection

pressures, depending on pollinator abundance and suggests (1) when pollinator abundance is low there would be directional selection for increased nectar production; and (2) when pollinator abundance is high there would be directional selection for decreased nectar production, to reduce within-plant pollen movement in a self-incompatible species. Other studies have predicted that nectar production is under directional selection for increased, stabilized, or low-nectar producing phenotypes depending on pollination and resource limitations and reproductive success (e.g. Thomson, 1986; reviewed in Rathcke, 1992; Mitchell *et al.*, 1998; Salguero-Faria and Ackerman, 1999). Harder and Cruzan (1990) proposed that selection to maximise reproductive success via pollen transfer has had a “*greater evolutionary influence*” on the nectar production of individual plants than interspecific competition for pollinators. However, as Cresswell (1999) argued, when increased nectar rewards do not increase pollen transfer, selection on nectar production could not arise, but selection may act on other mechanisms within the system (e.g. patterns of pollinator movements to minimise geitonogamy).

### 1.3.3 Pollen Production

#### 1.3.3.1 *The Function of Pollen*

Pollen in flowers has two main functions: (1) pollen grains contain the male gametophyte, therefore, successful donation to conspecific flowers can facilitate reproductive success through male function; and (2) pollen acts as a major attractant (i.e. food source rich in protein, lipids and starch) for many pollinators (Faegri and van der Pijl, 1979; Eckhart, 1991; Kearns and Inouye, 1993). The extent of the influence of pollen production and donation on reproductive success will depend on several factors, including: pollen presentation (e.g. temporal staggering of pollen), pollen germination and fertilisation capacity, environmental conditions (e.g. temperature and humidity may effect anther dehiscence), plant resource availability (i.e. a plant’s ability to mature fertilised egg cells, within the female gametophyte, using available resources), and the efficiency of a pollinator at removing or depositing pollen (Stephenson, 1980; Eckhart, 1991; Thomson and Thomson, 1992; Dafni and Firmage, 2000).

#### 1.3.3.2 *Variation in Pollen Production, Floral Traits, and Reproductive Success*

Environmental conditions that place a plant under resource stress (e.g. nutritional stress) may influence pollen quality and quantity, which may in turn adversely affect male

reproductive success, by reducing pollen available for donation (Devlin *et al.*, 1992; Cruden, 2000). Many studies have reported that environmental conditions such as herbivory, soil fertility, and mycorrhizal infection, influence pollen production via flower production and the number of pollen grains (Allison, 1990; Lau *et al.*, 1995). Lau *et al.* (1995) found that male function, including pollen production, increased with increasing soil fertility (nitrogen and phosphorus) and mycorrhizal infection. Furthermore, a significant trade-off was detected between pollen production and pollen grain size, in plants with increased phosphorus and mycorrhizal infection (Lau *et al.*, 1995).

If the reproductive success of a plant species is limited by inadequate pollen production, then plants producing more pollen may have a substantial reproductive advantage (via greater floral display or increased pollen grains per flower). Whilst pollen production may vary within or among plants in a population (Brunet and Eckert, 1998; Klinkhamer and van der Veen-van Wijk, 1999; reviewed in Cruden, 2000), relatively few studies have quantified such variation, compared with the countless studies on variation in nectar production and floral display (Kearns and Inouye, 1993). Moreover, pollen production is typically measured for use in estimating pollen-to-ovule ratios and plant mating systems (e.g. Cruden, 1977; Koptur, 1984; Routley *et al.*, 1999), rather than quantifying intraspecific variation (but see Brunet and Eckert, 1998; Klinkhamer and van der Veen-van Wijk, 1999). Klinkhamer and van der Veen-van Wijk (1999) found a 28-fold difference among clonal families of *Echium vulgare* in pollen grains per flower, indicating that pollen production can vary substantially within a species. Brunet and Eckert (1998) detected a 12% coefficient of variation among *Aquilegia caerulea* plants in pollen production per flower.

Despite the scarcity of studies, pollen production has been positively correlated with measures of floral display, such as petal and corolla size, style and stamen length, and stigma depth (e.g. Young and Stanton, 1990; Klinkhamer and van der Veen-van Wijk, 1999; reviewed in Cruden, 2000). For example, Galen (2000) found that pollen production and pollen germination per flower was positively correlated with corolla size in *Polemonium viscosum* control plants. Furthermore, numerous studies have reported a negative relationship between pollen grain number and size, described as a trade-off between these two functions (reviewed in Cruden, 1997 and Cruden, 2000; Yang and

Guo, 2004). However, Thomson *et al.* (1989) reported significant positive correlations between pollen production and pollen grain size in *Aralia hispida* plants.

Some studies have reported positive relationships between pollen production and plant reproductive success, indicating a positive relationship between floral rewards and reproductive output (e.g. Allison, 1990; Young and Stanton, 1990; Stanton *et al.*, 1991). Stanton *et al.* (1991) found that wild radish plants with greater pollen production sired more seeds. Allison (1990) also found significant correlations between pollen production and seed set among plants of the wind pollinated species *Taxus canadensis*. However, greater pollen production may not always have positive reproductive outcomes, due to stigma clogging and reduced pollen carryover in nearby conspecifics (Cruden, 2000), and increased geitonogamy in self-compatible plants (de Jong *et al.*, 1993; Klinkhamer and de Jong, 1993; de Jong *et al.*, 1999). In preferentially outcrossed species, an increase in geitonogamy may decrease outcrossing rates and increase inbreeding depression, eventually reducing reproductive success (Barrett and Kohn, 1991). As Cruden (2000) proposed, both “*minimal and excessive pollen production may have a detrimental effect on fitness*”.

Whilst it has been proposed that variation in pollen production is not genetically based (reviewed in Delph *et al.*, 1997), a few studies have detected some evidence of genetic variation in pollen production and performance (e.g. Snow and Spira, 1991; Campbell *et al.*, 1996). Campbell *et al.* (1996) reported that pollen production in *Ipomopsis aggregata* plants increased with flower width, which varied within populations and was subject to pollinator-mediated phenotypic selection. Delph *et al.* (1997) suggested that genotype-environment interactions (with respect to pollen performance) might maintain genetic variation, due to variation among different genotypes in response to environmental change.

### 1.3.3.3 Pollen Viability

Pollen viability<sup>2</sup> is a measure of potential male fertility and therefore influences plant reproductive success (Kearns and Inouye, 1993). Pollen viability may be used to test directly for seed production via germination tests, and indirectly, by comparing the

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<sup>2</sup> Viability is defined as “*having the capacity to live, grow, germinate or develop*” by Lincoln *et al.* (1982).

results of viability tests with actual measures of seed siring (Kearns and Inouye, 1993; Thomson *et al.*, 1994). Viability tests include: testing pollen for enzyme-induced fluorescence (Heslop-Harrison and Heslop-Harrison, 1970); enzyme activity related to oxidation and reduction reactions (e.g. triphenyl tetrazolium chloride - Cook and Stanley, 1960) and stainability of the vegetative parts of pollen grains (e.g. phloxin-methyl green stains cellulose - Stanley and Linsken, 1974). However, all viability tests have specific advantages and disadvantages (reviewed in Dafni and Firmage, 2000). Thomson *et al.* (1994) cautioned that a general lack of correlation between viability tests and measures of seed-siring ability indicate that viability tests are not a reliable measure of potential seed production. Thomson *et al.* (1994) used fluorochromatic reaction (FCR) tests to measure pollen viability in *Erythronium grandiflorum*, and found FCR scores declined with pollen age. However, seed-siring success was not related to pollen age, and no correlation was found between measures of FCR scores and seed-siring ability.

## **1.4 Pollinator Activity**

### **1.4.1 Pollinator Foraging Behaviour and Plant Reproduction**

Many plants that reproduce sexually rely on animals for cross-fertilisation. To achieve successful cross-fertilisation, a plant must receive an adequate number of pollinator visits from the available, and possibly limited supply of pollinators. Furthermore, due to the complex set of tradeoffs involved in plant-pollinator interactions the ideal pollinator for many plant species may be difficult to predict. Pollinator visits would ideally result in the removal and deposition of an adequate amount of high quality pollen during the period of stigmatic receptivity. In some cases the ideal pollinator for a plant might move quickly between single flowers but have a high fidelity for that plant species, thus minimising lost or wasted pollen and potentially maximising reproductive success (Darwin, 1989; Stiling, 1996; Klinkhamer and de Jong, 1993). In contrast, the most efficient foraging strategy for a pollinator may be to concentrate on the food source with the highest energy intake per unit foraging time (Heinrich, 1983). Many plant-pollinator interactions represent a conflict where one party may benefit more than the other (Gegear and Lavery, 2001; Section 1.2).

The deposition of non-viable and/or excess pollen, as a result of interspecific pollinator foraging, may clog the stigmatic surface of flowers (Waser and Fugate, 1986; Cruden,

2000). This may, in turn, prevent the successful deposition and germination of compatible pollen, reducing plant reproductive success (Waser and Fugate, 1986). Furthermore, many pollinators will visit several flowers on the same plant during a single foraging bout, potentially increasing the movement of self-pollen and decreasing the number of outcrossed seed and reproductive success in preferentially outcrossed species, or seed production in self-incompatible species (Darwin, 1989; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994). Klinkhamer and van der Jugt (2004) demonstrated that plant density, and whether pollinators are making decisions at the individual plant, population, or community level, will have important consequences for plant reproductive success. Franceschinelli and Bawa (2000) found that territorial hummingbirds visited fewer flowers per plant in areas of high plant density, therefore, promoting outcrossing. However, in areas of low plant density, hummingbirds were more likely to visit many flowers per plant, therefore, promoting self-fertilisation via geitonogamy.

#### **1.4.2 Variation in Pollination Effectiveness Among Pollinators**

Faegri and van der Pijl (1979) recognised that different pollinators are likely to differ in their ability to remove and/or deposit pollen, and therefore, differ in their ability to pollinate particular plant species. Lau and Galloway (2004) suggest that if ineffective pollinators are responsible for reducing fitness, then they may also be a selective force directing floral trait evolution. Effective pollinators, however, may be responsible for directing floral trait evolution towards specialist mutualisms (Schemske and Horvitz, 1984). In fact, the morphological forms of angiosperm flowers are believed to have evolved due to selection promoting efficient pollen transfer by effective pollinators and excluding ineffective pollinators (Müller, 1883; Stebbins, 1970).

The contribution that different pollinators make to the reproductive success of particular plant species will depend on: floral morphology, plant mating systems, the pollinator's ability to remove and deposit pollen effectively, body size, mode of locomotion, frequency of visits, and patterns of movement within the plant and habitat (Faegri and van der Pijl, 1979; Pyke, 1981; Carthew, 1994; Castellanos *et al.*, 2004). Therefore, the behaviour of some pollinators may contribute more to the reproductive success of plants than other pollinators, and less efficient pollinators may even reduce the reproductive success of plants (Pyke, 1981; Schemske and Horvitz, 1984; Wilson and Thomson,

1991; Gross and Mackay, 1998; Utelli and Roy, 2000; Celebrezze and Paton, 2004; Lau and Galloway, 2004). For example, honeybee visitors were found to contact the anther lobes of *Calothamnus quadrifidus* (Myrtaceae) only half as often as bird pollinators (Collins *et al.*, 1984), this behaviour by honeybees would presumably result in lower seed development for flowers visited by honeybees than for those visited by birds.

Ineffective pollinators may decrease the male component of plant reproductive success by reducing the amount of pollen available for transfer by effective pollinators.

Ineffective pollinators may also limit the female component of fitness by reducing plant attraction to more effective pollinators, via the removal of nectar (Schemske and Horvitz, 1984; Paton, 1993; Lau and Galloway, 2004). Lau and Galloway (2004) found a significant reduction in the siring success of *Campanula americana* plants as a consequence of increased visits from ineffective pollinators (halictid bee) and reduced visits by efficient bumblebee pollinators. However, ineffective pollinators did not affect reproductive success when plants received more visits from bumblebees.

### 1.4.3 Pollinator Foraging Behaviour, Floral Traits and the Environment

Pollinator foraging behaviour may vary significantly among plants of the same species and as previously described (Sections 1.3.1 and 1.3.2), and may be correlated with plant characteristics, such as nectar and floral display (Table 1.1). Franceschinelli and Bawa (2000) described variation in hummingbird foraging behaviour as the result of plant density and floral display. Lloyd (1998) also found significant variation among *Banksia ericifolia* plants in the rate of bird visits and the frequencies of bird visits per plant were positively correlated with inflorescence number and nectar concentration. Reviews by Zimmerman (1988) and Rathcke (1992) examine the response of pollinators to various floral traits and plant characteristics. Zimmerman (1988) concluded that plants have the potential to manipulate pollinator behaviour, but whether this behaviour is to a plant's advantage was inconclusive. Rathcke (1992) concluded that floral rewards (in this case nectar) might influence pollinator behaviour after an initial visit, thereby affecting pollen transfer and potentially reproductive success. Pollinator visitation rates and abundance may also fluctuate with environmental variables such as ambient temperature, light levels, wind speed, relative humidity and time of day (Primack and Inouye, 1993; Richardson *et al.*, 2000; Llorens, 2004). The relationship between pollinator foraging behaviour and environmental variables is discussed in Section 1.7.

#### 1.4.4 Native Australian Pollinators

The isolation of the Australian continent for over 40 million years resulted in a great diversity of endemic pollinators, including birds (Ford *et al.*, 1979; Paton, 1986b; Buchmann and Nabhan, 1996), mammals (Carthew and Goldingay, 1997; Goldingay, 2000), and thousands of species of insects (e.g. solitary bees, flies and butterflies and beetles) (Michener, 1965; Armstrong, 1979; Buchmann and Nabhan, 1996). Australia is host to a very high diversity of endemic bee species, with over 1500 known native species (Cardale, 1993), although this number would likely be substantially higher if we knew of all the species yet to be identified. This diversity has not only been attributed to the isolation of the Australian continent, but also the very limited presence of social pollinating insects (e.g. native bees of the genus *Trigona* found in tropical north Australia), prior to the introduction of the European Honeybee (*Apis mellifera*) (Michener, 1965; Paton, 1986a; Buchmann and Nabhan, 1996). The evolution of many plant traits and floral rewards in Australian pollination systems lends itself to attracting generalist pollinators, which now also includes the European honeybee. However, this diverse suite of pollinators often has an equally diverse response to plant traits and floral rewards, and therefore, pollination effectiveness (discussed further in Chapter 3).

It has been suggested that Australia has one of the most diverse and widespread distribution of pollinating bird species in the world (Buchmann and Nabhan, 1996). More than 110 species of birds have been observed foraging at some 250-plant species (Ford *et al.*, 1979). It is approximated that 1000 Australian plant species may be pollinated by birds (Buchmann and Nabhan, 1996). Reviews by Ford *et al.* (1979) and Ford and Paton (1986) thoroughly examine birds as pollinators of Australian plants. Moreover, in the past 15 years many more studies have examined the role of honeyeaters in Australian plant-pollination systems (e.g. Pyke, 1988; Ramsey, 1988; Armstrong, 1991; Vaughton, 1996; Lloyd, 1998; Evans and Bunce, 2000; Richardson *et al.*, 2000; Celebrezze, 2002; Saffer 2004). Most honeyeaters (Family Meliphagidae) are reported to be effective pollinators, frequently carrying pollen of the flowers they visit and depositing this pollen to the stigmas of conspecific flowers (Paton, 1986b). The majority of these plant species are from Proteaceae, Myrtaceae, Epacridaceae, Fabaceae and Loranthaceae. The flowers of these families are often clustered together in dense inflorescences, some producing substantial quantities of nectar and being of bright and various colours.



Plants that utilise vertebrates as pollinators must be able to meet the energy requirements of these organisms (via nectar and pollen production), which are much greater than that of insect pollinators (Paton, 1986b; van Tets and Whelan, 1997; van Tets and Hulbert, 1999). Furthermore, the benefits associated with vertebrate pollination, compared with insects, must outweigh the increased cost of producing a larger nectar reward (Hingston *et al.*, 2004). Not surprisingly, some studies have demonstrated that honeyeater abundance fluctuates with the availability of nectar resources (via inflorescence density) within an area (Collins and Briffa, 1982; Ford, 1983; McFarland, 1986). Pyke (1983, 1988), however, found no significant correlation between seasonal patterns of honeyeater abundance and nectar energy production in three Sydney based sites.

Australian research on non-flying mammals as pollinators has been focussed in Western Australia and the eastern Australian seaboard, beginning with key publications such as Rourke and Wiens (1977), Carpenter (1978), Holm (1978), Wiens *et al.* (1979), Hopper (1980), Recher (1981), and Turner (1982). One of the most important early studies was by Carpenter (1978), suggesting that some *Banksia* species are adapted for mammal pollination via: the direction of excess nectar to the ground, excessive and odorous nectar production, hooked styles that allow pollen transfer to fur, and crepuscular and nocturnal nectar and pollen presentation. In the past twenty years, there has been a greater concentration of research on non-flying mammals as pollinators of Australian plants, increasing the profile of mammals as pollinators of many plant species (Goldingay *et al.*, 1987; Cunningham, 1991; Goldingay *et al.*, 1991; Carthew, 1993, 1994; Saffer, 1998; Evans and Bunce, 2000; Goldingay, 2000).

Non-flying mammals are known to visit 83 plant species for nectar and/or pollen on the Australian, African and South American continents (Carthew and Goldingay, 1997). Moreover, 12 species of Australian marsupials have been recorded visiting 59 Australian plant species, and many of these are in Proteaceae and Myrtaceae (Carthew and Goldingay, 1997). Clearly, this is an important group of pollinators in Australian plant-pollinator systems and especially unique in diversity and abundance in Australia. However, there is still a substantial lack of information on non-flying mammals as pollinators, especially their role in facilitating pollen transfer (reviewed in Carthew and Goldingay, 1997 and Goldingay, 2000).

## 1.4.5 European Honeybees in Australia

### 1.4.5.1 Honeybees and Australian Pollination Systems

European honeybees (*Apis mellifera*) were introduced to Australia in the 1820s in managed hives, to provide honey and pollinate crops (Pyke and Balzer, 1985; Pyke, 1990; Paton, 1996). However, as in numerous other countries, they did not remain confined to these managed hives and their distribution and abundance has increased substantially in the past 60 years (Paton, 1996). Honeybees have now spread to all regions of Australia, except for parts of the Australian Alps and inland desert, where climatic conditions and resources are incompatible with their needs (e.g. insufficient water in some desert areas) (Pyke, 1990; Oldroyd *et al.*, 1994; Paton 1996). Honeybees have been recorded visiting more than 1,000 plant species from more than 200 endemic Australian plant genera (Paton, 1996). There are now more than 525,000 managed hives in Australia, and the total number of hives (including feral hives) is likely to be much greater than this figure (Buchmann and Nabhan, 1996) (Figure 1.2). Furthermore, Australia is the only country to set aside areas of native vegetation as beekeeping reserves (Buchmann and Nabhan, 1996).

Honeybee interactions with native Australian plants and pollinators are very complex (Celebrezze and Paton, 2004) and their impact on ecosystems is unclear and difficult to assess (Butz Huryn, 1997; Oldroyd, *et al.*, 1997; Paton, 1997). There is much debate among scientists, agricultural bodies, apiarists and government agencies on the potential affect of honeybees on native ecosystems (e.g. Pyke, 1990; Westerkamp, 1991; Butz Huryn, 1997; Manning, 1997; Schwarz and Hurst, 1997; Pyke, 1999). Despite the valuable pollination service European honeybees provide to many agricultural crops, they are ineffective pollinators of other crops and some native plant species (O'Toole, 1993; Paton, 1993; Goulson, 2003). Some of these concerns were expressed in 2002, when the NSW Scientific Committee made a determination to list “*competition from feral honeybees*” (for tree hollows and floral resources) as a ‘key threatening process’ on the NSW *Threatened Species Conservation Act 1995*.

**Figure 1.2 - Managed honeybee hives approximately 50 m from the border of Booderee National Park, Jervis Bay, N.S.W. Australia (Photo: R.J. Whelan).**

It has been recognised that the potential impacts of honeybees may include altered rates of pollination, displacement of native pollinators via competition for floral resources and subsequent disruption of native pollination systems (Taylor and Whelan, 1988; Pyke, 1990, 1999; Paton, 1993, 1996, 1997; Buchmann and Nabham, 1996; Oldroyd *et al.*, 1997; Horskins and Turner, 1999). However, the potential impacts of honeybees on Australian plant species will vary depending on floral morphology, plant mating systems, native pollinator foraging behaviour, and the abundance and foraging behaviour of honeybees (Celebrezze and Paton, 2004). Reviews by Pyke (1990, 1999), Paton (1993, 1996), Manning (1997), and Paine (2004) have addressed the potential effects of honeybees on Australian plant species and ecosystems.

Pyke (1990) and Paton (1997) proposed four negative impacts of honeybees on Australian ecosystems: (1) they may displace native pollinators, resulting in decreased reproductive success (i.e. reduced seed production), via ineffective honeybee pollination services or changes in the behaviour of native pollinators; (2) the removal of nectar and pollen resources by honeybees (thereby reducing plant attractiveness), may reduce native pollinator visits, effective pollen transfer, and thus plant reproductive success (i.e. seed production); (3) patterns of movement within and among plants by honeybees

may change the genetic composition of seeds and populations, by altering rates of outcrossing and selfing; and (4) differences in honeybee foraging behaviour may influence the evolution of plant floral traits. It is also recognised that the presence of honeybees alone, or together with native pollinators, may enhance plant reproductive success, or leave it unchanged (Pyke, 1990; Paton, 1997; Horskins and Turner, 1999). Pyke (1999) recommended that rather than directing research at obtaining absolute “proof” of honeybee impacts, energy be shifted to finding vegetated areas where the reduction of feral honeybees is feasible and the potential conservation gains are high.

#### 1.4.5.2 Honeybee Foraging Behaviour and Plant Reproductive Success

One of the primary concerns raised in numerous studies is that honeybees are less effective pollinators of some native Australian plants, due to ineffective pollen removal and deposition (e.g. Pyke and Balzer, 1985; Taylor and Whelan, 1988; Vaughton, 1992; Gross and Mackay, 1998; Celebrezze and Paton, 2004). It has been proposed (Paton, 1986b; Taylor and Whelan, 1988; Paton, 1993) that the relatively large distance between the nectary and stigma or pollen (compared with the size of a honeybee) in some flowers may be one reason why honeybees are ineffective pollinators of some Australian species. Indeed, many studies have found that reproductive success is significantly reduced when vertebrates (but not honeybees) are excluded from the flowers of Australian plants (e.g. Paton and Turner, 1985; Vaughton, 1996; Gross and Mackay, 1998; Celebrezze, 2002). For example, Gross and Mackay (1998) found that honeybees deposited significantly less pollen than native bees and seed set was significantly lower in *Melastoma affine* flowers that were last visited by honeybees. Furthermore, Ramsey (1988) found that, whilst honeybees visited inflorescences of *Banksia menziesii* ten times more frequently than birds, pollen deposition on stigmas by birds was four times greater than honeybees. This resulted in approximately ten times greater fruit set by birds and it was interpreted that birds were more effective pollinators.

Honeybees have been found to move less frequently between plants in populations of some Australian species (e.g. Paton, 1986b; Richardson *et al.*, 2000; Beynon *et al.*, *unpublished*), which may reduce plant reproductive success. Furthermore, in some bird-pollinated self-compatible plant species, honeybee foraging behaviour may increase inbreeding to such a level that the effects of bird foraging activity are negligible

(England *et al.*, 2001). Whether this threatens the long-term existence of some Australian plant species is unknown and requires further attention (Paton, 1997). Increased or continual inbreeding is of conservation concern because it may have negative fitness effects for preferentially outcrossed plant species (Charlesworth and Charlesworth, 1987; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994) or even occasionally outcrossed species (Shields, 1982). The negative impacts of increased self-pollen transfer include interference of cross-pollen with self-pollen in self-incompatible species, and reduced offspring fitness due to the expression of lethal recessive genes (inbreeding depression) in self-compatible species (Charlesworth and Charlesworth, 1987; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994).

Despite conflicting evidence, some studies have found that honeybees are able to pollinate a range of Australian insect- and vertebrate-pollinated plants (Paton and Turner, 1985; Vaughton, 1992), including species of *Banksia*, *Grevillea*, *Callistemon*, and *Correa* (reviewed in Paton, 1996), *Styphelia* (Celebrezze, 2002), and *Eucalyptus costata* (Horskins and Turner, 1999). Celebrezze (2002) found that in three Australian plant species, the fruit set of plants exposed to honeybees alone was approximately equivalent to that of plants exposed to all pollinator groups (i.e. native birds, insects, and honeybees). However, in self-incompatible, insect-pollinated species *Grevillea sphacelata*, hand pollination was found to produce four to 13 times more fruit set than open pollination, supporting observations that honeybees were ineffective at pollen deposition. Some Australian plants may now actually depend on honeybees for their pollination service because their native pollinators have declined or disappeared, due to habitat clearance and degradation (Paton, 1996).

Much more research is needed to better understand the potential impacts of honeybees on Australian pollination systems (Pyke, 1990; Manning, 1997; Paton, 1996; Schwarz and Hurst, 1997; Goulson, 2003; Paine, 2004). Future research needs to measure the influence of honeybees on a wide variety of native flora and pollinating fauna species, using both experimental and descriptive studies (Paton, 1996). Ultimately, the presence of honeybees in Australian native ecosystems may result in benefits to some plant species, and a loss of reproductive success or resources to other plant species or native fauna (Paton, 1996).

## 1.5 Reproductive Success

In hermaphroditic plants, sexual reproductive success can be achieved via seed production (female success) and seed siring (male success) (Zimmerman, 1987; Thomson and Thomson, 1989; Broyles and Wyatt, 1990). Reproductive success can be used as an indication of plant fitness, and may be inferred in a variety of ways including, but not limited to: (1) successful pollen transfer (e.g. pollen removal, deposition, and pollen tube growth); (2) the quality of seed production (i.e. outcrossed or selfed seed, addressed in Section 1.6); and (3) seed production and viability.

Measures of plant reproductive success should ideally include estimates of both male and female function. However, many studies have been limited in their assessment of plant reproductive success by only measuring seed production due to the difficulty in accurately quantifying male reproductive success (Rush *et al.*, 1995). Moreover, many authors have questioned the reliability of pollen removal as a measure of potential seed siring and male reproductive success (Broyles and Wyatt, 1990; Wilson and Thomson, 1991; Thomson and Thomson, 1992; Klinkhamer *et al.*, 1994). Pollen deposition, pollen tube growth, and paternity analysis are generally considered more reliable estimates of the male contribution to reproduction fitness. Furthermore, in the past 15 years, researchers have begun to use molecular markers more readily to assess paternity and thus get a direct measure of male reproductive success (e.g. Broyles and Wyatt, 1990; Burczyk and Prat, 1997; Gerber *et al.*, 2000; reviewed in Bernasconi, 2003).

### 1.5.1 Pollen Transfer

Successful pollen transfer is determined by numerous extrinsic (e.g. pollinator foraging behaviour, climatic conditions) and intrinsic (e.g. flower number, stigmatic structure) components of the plant-pollinator system (Waser, 1993a; Krauss, 1994; Price *et al.*, 2005). Moreover, the quantity and type of pollen deposited onto flower stigmas may affect the number, size, germination or quality (i.e. selfed or outcrossed) of seed produced (Zimmerman and Pyke, 1988b; Colling, *et al.*, 2004). For example, Robertson and Ulappa (2004) found that individual *Lepidium papilliferum* plants that were hand-pollinated with pollen from distant conspecifics (> 75 m away) had significantly greater fruit set (%) than plants pollinated with pollen from near-neighbours (< 1 m).

It is generally agreed that pollen transfer via animal pollinators is inefficient, with only a small percentage of pollen removed being successfully deposited onto the stigmas of conspecific flowers (Harder and Thomson, 1989; Thomson and Eisenhart, 2003). For example, Thomson and Thomson (1989) found that bumblebees removed a mean of 62% of *Erythronium grandiflorum* pollen grains from donor flowers, but deposited a mean of just 0.52% of the removed pollen to the stigmas of conspecific flowers. Moreover, in many plant-pollinator systems, much of the pollen that is removed is actually transferred among flowers on the same plant, or moved to close neighbours. Thomson and Thomson (1989) suggested that because of the often poor delivery of pollen to conspecific flowers by pollinators, plants that present pollen in sequentially opening subunits may reduce the amount of pollen wasted (e.g. the inflorescences of many *Grevillea* spp.).

The efficiency of pollen transfer, as a result of pollinator foraging behaviour, may vary among different species of pollinator (Lau and Galloway, 2004). Inefficient pollinators may decrease male reproductive success by limiting the amount of pollen available for export by effective pollinators (Lau and Galloway, 2004). Additionally, ineffective pollen transfer may adversely affect plant reproductive success because of pollen loss to the donor (without subsequent seed siring) and the deposition of foreign pollen onto stigmas (interspecific pollen transfer), potentially clogging the stigma and reducing seed production (Rathcke, 1983). Waser and Fugate (1986) propose that the negative effects of foreign pollen transfer include “*clogging and actively disrupting the stigma surface, blocking the stylar transmitting tissue, and eliciting flower abscission or stigma closure*”. Previous studies have found that the prior deposition of foreign pollen has the potential to reduce the reproductive success of plants, by suppressing or interfering with the germination of conspecific pollen (e.g. Galen and Gregory, 1989; Waser and Fugate, 1986). Galen and Gregory (1989) found that *Polemonium viscosum* plants that received foreign pollen also experienced significantly reduced pollen grain fertilisation and produced fewer seeds.

#### 1.5.1.1 Pollen Removal and Export

Whilst the reliability of pollen removal as an indicator of reproductive success is questionable, pollen removal has been associated with patterns of floral reward production and pollinator foraging behaviour. Several studies have reported pollinators

removing greater quantities of pollen from plants with greater nectar production or floral displays (e.g. Pleasants and Chaplin, 1983; Campbell, 1989; Hodges, 1995; Vaughton and Ramsey, 1998; Johnson *et al.*, 2004). Pleasants and Chaplin (1983) found that daily pollen removal per flower in the milkweed herb, *Asclepias quadrifolia*, increased significantly with increasing nectar production. Previous studies have also found that pollen removal may be a positive function of pollinator visits (e.g. Mitchell and Waser, 1992; Hodges, 1995; Rush *et al.*, 1995; Sahley, 2001). For example, Hodges (1995) found a significant positive relationship between Hawkmoth visits per *Mirabilis multiflora* flower & pollen removal per anther.

A limited number of studies have found that pollen removal is a reliable indicator of subsequent pollen deposition or seed production (e.g. Broyles and Wyatt, 1990; Galen, 1992; Ashman, 1998). For example, the amount of pollen removed from flowers significantly affected the number of pollen grains deposited on flowers of the first plant subsequently visited in *Polemonium viscosum* (Galen, 1992). Broyles and Wyatt (1990) also found a significant positive correlation between the number of pollinaria removed and both the number of seed sired and seed produced in *Asclepias exaltata* plants. However, the assumption that pollen removal is related to the export of pollen to conspecific plants (and therefore seed production) is not always valid (Wilson and Thomson, 1991; Thomson and Thomson, 1992; Klinkhamer *et al.*, 1994). There is much reason to be cautious about pollen removal as an indication of subsequent deposition considering the within-plant foraging patterns of some pollinators (i.e. geitonogamous pollen transfer), variation in foraging behaviour among different groups of pollinators (e.g. grooming behaviour), and competition for generalist pollinators from other ‘attractive’ simultaneously flowering species (Wilson and Thomson, 1991; Thomson and Thomson, 1992; Klinkhamer *et al.*, 1994).

#### 1.5.1.2 Pollen Deposition

Many previous studies have found that stigma pollen loads (i.e. the number of pollen grains) increased with increased nectar production or floral display size (e.g. Thomson and Plowright, 1980; Campbell, 1989; Hodges, 1995; Vaughton and Ramsey, 1998). For example, Thomson (1986) found that *Erythronium grandiflorum* flowers with greater nectar reward (volume) received more pollen grains from bumblebees per visit. It was proposed that this was a function of longer bumblebee visits to these flowers.



Vaughton and Ramsey (1998) also found that *Wurmbea dioica* pollen deposition increased significantly with flower size (but not number) on female plants. However, percent seed set decreased significantly with increasing flower size and number, which may indicate a potential trade-off between flower production and female fecundity, or potential negative affects of greater pollen deposition (e.g. pollen mate choice or stigma clogging).

Some studies have found that pollen deposition is a positive function of pollinator visits and may be directly related to increased seed production (e.g. Mitchell and Waser, 1992; Hodges, 1995; Engel and Irwin, 2003; Waite and Ågren, 2004). For example, Hodges (1995) found a significant positive relationship between hawkmoth visits per *Mirabilis multiflora* flower and stigma pollen loads. However, the proportion of self-pollen deposited per plant also increased significantly with successive flower visits, which may prevent deposited non-self pollen from fertilizing ovules. Engel and Irwin (2003) reported (using path analysis) that stigma pollen loads increased with pollinator visitation per *Ipomopsis aggregata* flower. Plants with greater stigma pollen loads also produced significantly more seeds.

Stigma pollen loads may directly effect seed production (Quesada *et al.*, 2001; Davis, 2004). Quesada *et al.* (2001) found that *Pachira quinata* flowers receiving more than 400 pollen grains always developed mature fruits, however, flowers with less than 200 pollen grains never developed mature fruit. Furthermore, stigma pollen loads explained 34% of the variation in seed number per fruit. In a review of the pollen-to-ovule ratio of 96 plant species, Cruden (1977) suggests that just two to seven pollen grains per ovule per stigma are needed to maximise seed set (further discussed in Cruden, 2000). For example, just four viable pollen grains deposited onto *Mirabilis jalapa* stigmas resulted in 87% seed set and six viable pollen grains resulted in 97% seed set of *Viola nephrophylla* flowers (Cruden, 1977).

#### 1.5.1.3 Pollen Carryover

Pollen carryover has been defined as “the extent to which pollen from one flower is picked up and transferred beyond the next flower visited” (Waser and Price, 1984). Pollen carryover, resulting from floral variability and the foraging patterns of pollinators, will affect male and female reproductive fitness, via subsequent seed

production (Thomson and Plowright, 1980; Zimmerman, 1988). Whilst most studies have found that the majority of pollen will be deposited on the first few flowers, a small number of grains are often deposited over many flowers (Thomson *et al.*, 1982; Harder *et al.*, 2001; Thomson and Eisenhart, 2003). For example, Thomson and Plowright (1980) reported that whilst most *Diervilla lonicera* pollen was deposited or lost from pollinators on the first 10 to 15 flowers, pollen was also deposited on the fifty-fourth flower visited. Floral variability and grooming by pollinating bees between successive flowers may dramatically affect pollen carryover distance and quantity (Waser and Price, 1984; Thomson and Eisenhart, 2003). Waser and Price (1984) found that pollen carryover by hummingbirds increased with variability in the style length and anther position of *Ipomopsis aggregata* flowers.

## 1.5.2 Seed and Fruit Production

### 1.5.2.1 Seed and Fruit Production and Reproductive Success

The potential maximum fruit production of an individual plant is equal to the total number of flowers, and the potential maximum seed production is equal to the total number of ovules (Stephenson, 1981). However, the realised seed set is influenced by numerous internal (e.g. number of flowers pollinated, resource availability) and external (e.g. fruit and seed predation, environmental conditions) factors (Stephenson, 1981; Richardson and Stephenson, 1991; Rathcke, 1992). Plant reproductive theory tells us that the most important limitations on the seed production of flowering plants are pollen availability (via pollinators) and resources (e.g. Bierzychudek, 1981; Stephenson, 1981; Rathcke, 1983; Zimmerman, 1988; Zimmerman and Pyke, 1988b). Resource limits are considered the more important of these two with respect to the development of seed size and number, especially given that trade-offs may exist between these two factors of seed production (Primack, 1987; Aigner, 2006). Haig and Westoby (1988) developed a model that demonstrated that seed production is limited by both pollen and resources, via selection on maternal reproductive effort. Importantly, substantially more research is needed on pollen limitation and potential effects at the plant, population and ecosystem level (Ashman *et al.*, 2004).

Whelan and Goldingay (1989) recognised that to detect a relationship between variation in floral traits (e.g. flower production) and seed production, effective pollination must be limited, resulting in competition among plants. Many studies have found seed

production to vary among plants, as a positive function of both nectar production and floral display (Sections 1.3.1 and 1.3.2; Table 1.2). For example, Conner and Rush (1996) found fruit production increased with increasing flower production, in *Raphanus raphanistrum* (Wild Radish), over three years. As mentioned previously (Section 1.3.2), a review by Rathcke (1992) has thoroughly examined the topic of reproductive success in plants, with respect to nectar production and pollinator behaviour.

#### 1.5.2.2 Limited Fruit and Seed Production

It is widely reported that hermaphroditic plant species commonly have very low fruit-to-flower ratios (Stephenson, 1981; Sutherland and Delph, 1984; Sutherland, 1986; Charlesworth, 1989; Ayre and Whelan, 1989). Furthermore, fruits typically contain fewer seeds than the available number of ovules and more fruits are often initiated than reach maturity (i.e. selective abortion) (Charlesworth, 1989). There are two primary hypotheses suggesting that female reproductive success may be restricted by: insufficient resources (e.g. light, water, nutrients), preventing the production or maturation of seed or fruit; and a lack of pollen due to ineffective or limited pollinator visitation (Pyke, 1981; Sutherland, 1986; Goldingay and Whelan, 1990). Ayre and Whelan (1989) also propose that seed production may be limited by genetically determined prezygotic and postzygotic mortality, due to pollen quality (i.e. selfed or outcrossed pollen), and predation or disease of flowers and/or ovules (i.e. pollen source). For example, Somanathan and Borges (2001) found that fruit set in *Heterophragma quadriloculare* plants was significantly greater from flowers hand-pollinated with outcrossed pollen, than both untreated flowers and flowers hand-pollinated with mixed pollen source.

Given that the resources available to a plant are limited, it has been assumed that an increase in allocation to male function (e.g. pollen production and dispersal) will result in a decrease in allocation to female function (ovule production, fertilisation, and seed development) (Sutherland and Delph, 1984). Despite this prediction, many studies have found positive relationships between female function and flower production (e.g. Zimmerman, 1984; Queller, 1985; Broyles & Wyatt, 1990; Cruzan *et al.*, 1994). For example, seed production on *Iris fulva* plants increased when more flowers were open on plants within the same patch (Cruzan *et al.*, 1994). Broyles & Wyatt (1990) also found significant positive correlations between flower numbers per plant and both seed

production and seeds sired in *Asclepias exaltata* plants. Haig and Westoby (1988) suggest that an evolutionary stable strategy would result in a balance between resource (seed production) and pollen limitation (fertilisation), whereby selection should favour: (1) increased resources to attracting pollinators, when plants are pollen limited; and (2) increased allocation to seed production, when plants are resource limited.

## 1.6 Plant Mating Systems and Reproductive Fitness

Measurements of plant reproductive success (e.g. pollen transfer and seed production) give us an important indication of potential reproductive success. However, if we are to better understand plant-pollinator interactions and plant reproductive fitness, we need to study plant mating systems and patterns of genetic variation among plants (Burczyk and Prat, 1997). Patterns of pollen movement and plant self-compatibility or incompatibility will determine in part how readily a plant may produce seed (e.g. via autogamy or solely reliant on pollinators for pollen transfer) and the genetic structure of plant populations (Spira, 2001; Isagi *et al.*, 2004). For example, in plant species with self-compatible but preferentially outcrossed mating systems, the production of many selfed seeds may ultimately decrease plant fitness, via inbreeding depression. Estimating the outcrossing rate of an individual plant and its progeny (i.e. family outcrossing rate) is one way to ensure that the genetic structure of seeds (i.e. selfed or outcrossed) is considered in an assessment of potential plant fitness.

Xenogamous plant species (i.e. those with primarily outcrossed mating systems) may have greater overall genetic variation than autogamous plant species (Neel *et al.*, 2001). However, they may also suffer more from the negative effects of inbreeding depression, than species that have a history of selfing (Shields, 1982; Schemske and Lande, 1985; Charlesworth and Charlesworth, 1987). Inbreeding depression is the expression of accumulated, recessive alleles, caused by deleterious mutations and increased homozygosity, and may result in less fertile, less viable or smaller offspring (Charlesworth and Charlesworth, 1999; Slate *et al.*, 2004). Theory predicts that within self-compatible xenogamous species, individual plants with higher levels of selfing and biparental inbreeding (mating between related plants) will produce offspring that are less fit, as a result of inbreeding depression driven by genetic load (Barrett and Kohn, 1991; Slate *et al.*, 2004). This prediction is upheld by numerous empirical studies (e.g.

Galen *et al.*, 1985; Dudash, 1990; Krauss, 1994; Kittelson and Maron, 2000; Lui and Spira, 2001; Shi *et al.*, 2005).

For self-compatible species with stable mixed or highly selfing mating systems, reproduction may be assured via autogamy (i.e. without the assistance of pollinators) (Müller, 1883; Baker, 1955; Barrett *et al.*, 1996). Furthermore, the successful establishment of locally adapted genotypes may be generated and maintained within populations (Shields, 1982). The dominance hypothesis of inbreeding depression (referred to as “*partial dominance*” by Charlesworth and Charlesworth, 1987) predicts that intense inbreeding over time, especially in small populations, together with selection against less fit homozygotes, may purge deleterious alleles and limit fitness losses in self-fertilised plants, thereby providing a stable, predominantly self-fertilising mating system (Lande and Schamske, 1985; Charlesworth and Charlesworth, 1987; Barrett and Husband, 1990; Young *et al.*, 1996). A study by Husband and Schamske (1996) supports this hypothesis, and found a significant negative correlation between cumulative inbreeding depression and the selfing rate for 35 species of angiosperms. Therefore, a predominantly self-fertilising mating system may not be an evolutionary disadvantage, with substantial genetic variation available for evolutionary change (Lande and Schamske, 1985).

In fine-scale studies of plant mating systems, seed and maternal plant genotypes may be used to determine family selfing and outcrossing rates, and outcrossing rates subsequently used as conservative estimates of plant fitness (Ritland and Jain, 1981; Ritland, 2002). These estimates will be most useful (with respect to better understanding plant reproductive success) when correlated with other measures of importance within plant mating systems (e.g. floral traits and pollinator activity) (Ritland, 2002). Previous studies conducted at the population level, have found significant positive correlations between floral display size and rates of selfing (e.g. de Jong *et al.*, 1999; Schmidt-Adam *et al.*, 2000; Cascante *et al.*, 2002). For example, Cascante *et al.*, (2002) found that higher levels of selfing (via geitonogamy) were associated with low population density and mass flowering in the tropical tree *Samanea saman*. Schmidt-Adam *et al.* (2000) proposed that mass flowering in the New Zealand Pohutukawa (*Metrosideros excelsa*) promoted selfing via geitonogamous pollen transfer. However, in this species, selection also ensures a predominantly heterozygous

adult population by eliminating self-fertilised offspring (Schmidt-Adam *et al.*, 2000). Previous studies have also found a strong positive relationship between plant density and outcrossing rate, as proposed by Wright (1946) (Murawski *et al.*, 1990; Karron *et al.*, 1995; Franceschinelli and Bawa, 2000). For example, Murawski *et al.* (1990) found that outcrossing estimates increased with the increasing density of flowering plants. Watkins and Levin (1990) also found a weak inverse relationship between population outcrossing estimates for *Phlox drummondii* and plant density. Outcrossing and selfing rates with respect to plant and floral density will be discussed further in Section 1.7.

## **1.7 Plant Size, Density, Environmental Conditions and Photosynthesis**

### **1.7.1 Plant Size and Pollination**

Large plants are often reported as having larger floral displays and greater resource availability, than smaller plants of the same species (Rathcke, 1992; Suzuki, 2000). This in turn, may allow large plants to attract more pollinators, which may result in greater seed production, and potentially greater fitness (reviewed in Rathcke, 1992). Zimmerman (1984) proposed that some long-lived plants do not change substantially in size over a moderate time period. Therefore, large plants will continue to produce more seeds than smaller plants, even if their absolute seed crop decreases over time due to energy or nutrient constraints (Zimmerman, 1984). Numerous studies have reported greater reproductive success in larger plants with greater floral displays (e.g. Vaughton and Ramsey, 1997; Albert *et al.*, 2001; Engel and Irwin, 2003).

The effect of plant size on pollinator foraging activity remains ambiguous, although, many studies have reported that larger plants (with larger floral displays) receive more pollinator visits (e.g. Dreisig, 1995; Lloyd, 1998; Engel and Irwin, 2003). For example, Dreisig (1995) found that honeybee visitation increased with the height of *Viscaria vulgaris* plants. However, the proportion of flowers visited on larger plants may be less than the proportion visited on smaller plants (e.g. Gerber, 1985; Andersson, 1988; Klinkhamer *et al.*, 1989; Klinkhamer and de Jong, 1990). When calculated as a proportion of available flowers, larger plants may not have greater proportional reproductive success (e.g. Andersson, 1988; Susko and Lovett-Doust, 2000; Suzuki, 2000). For example, Susko and Lovett-Doust (2000) found that larger *Alliaria petiolate* plants had significantly greater fruit-set than smaller plants and absolute measures of reproductive success had positive linear relationships with plant size. However,

proportional measures of reproduction were independent of plant size and small plants produced the same proportion of seed as large plants (Susko and Lovett-Doust, 2000). Suzuki (2000) also found that the proportion of pollinated flowers producing fruit was lower on larger *Cytisus scoparius* plants. This was interpreted as a reduction in resource availability due to increased flower production.

The increased attraction of larger plants to pollinators (via greater floral display) is expected to result in increased within-plant pollen movement, which may be a disadvantage to preferentially outcrossed species (as described in Section 1.6). In self-compatible species, pollinators foraging for longer within plants are likely to facilitate increased geitonogamy (Handel, 1983; Klinkhamer and de Jong, 1990; de Jong *et al.*, 1993; Klinkhamer and de Jong, 1993; de Jong *et al.*, 1999; Franceschinelli & Bawa, 2000). In self-incompatible species, pollen may be inefficiently moved within plants, thereby limiting seed production.

### 1.7.2 Plant Density, Nearest Neighbour Distance, and Pollination

The density of plants within a population affects plant attraction to pollinators, intraspecific competition for pollinators and resources (e.g. water and nutrient availability), pollinator foraging behaviour, seed production and rates of selfing and outcrossing (Wright, 1946; reviewed by Handel, 1983; de Jong *et al.*, 1993; reviewed by Ghazoul, 2005). Greater competition for resources (due to increased plant density) may have indirect negative impacts on plant reproduction, if the production of floral rewards is altered and plant attraction to pollinators is decreased. Increased competition for resources, such as nutrients and water, may also directly affect seed production via the quality and production of pollen (reviewed in Delph *et al.*, 1997) and altered resource allocation to seed or fruit production and development (Stephenson, 1981; Campbell and Halama, 1993).

Many studies have found positive relationships between pollinator visits and plant/flower density (e.g. Klinkhamer and de Jong, 1990; Real and Rathcke, 1991; Kunin, 1997). For example, Kunin (1997) found increased pollinator visits and seed set in high density of patches of *Brassica kaber*. However, other studies have found increased pollinator visits to plants in lower density populations, often with more flowers probed per plant (e.g. Mustajärvi *et al.*, 2001; Somanathan *et al.*, 2004). For

example, Mustajärvi *et al.* (2001) found that the rate of bumblebee visits to *Lychnis viscaria* plants in sparse populations was higher, with more flowers probed per plant, and greater reproductive success. They suggested that larger inflorescences and greater flight distances between plants encouraged pollinators to forage for longer on plants in sparse populations (Mustajärvi *et al.*, 2001).

The distance between near neighbours may also affect the rate of pollinator visits, and will determine how far a pollinator must travel to transfer compatible pollen. This, in turn, may influence seed/fruit production and plant outcrossing rates. Mitchell *et al.* (2004) found that more than half of bumblebee visits were between nearest neighbouring (0.8 m) *Mimulus ringens* plants. Furthermore, Gross and Werner (1983) found a significant but weak negative correlation between the percentage of viable *Solidago canadensis* seeds and distance to the nearest flowering neighbour. Allison (1990) also found that seed set of *Taxus canadensis* was significantly correlated with distance to nearest neighbour.

Many studies have found strong relationships between plant density and both outcrossing and selfing rates, as predicted by Wright (1946) (e.g. Murawski *et al.*, 1990; Karron *et al.*, 1995; Franceschinelli and Bawa, 2000; reviewed in Ward *et al.*, 2005). Karron *et al.* (1995) found that the relative frequency of between-plant pollinator flights was significantly greater in higher density patches of *Mimulus ringens*. This pattern of pollinator behaviour was positively correlated with patch outcrossing rate. Murawski *et al.* (1990) found that trees within a cluster of flowering plants had higher outcrossing rates than solitary trees. Cruzan *et al.* (1994) also found that outcrossing rates in *Iris hexagona* plants increased with the number of open flowers on nearby plants.

### 1.7.3 Environmental Conditions and Plant Reproduction

The growing conditions of a plant can affect the number and quality of offspring produced, and consequently plant fitness (Delph *et al.*, 1997). Moreover, it is well recognised that small-scale changes in the physical environment are likely to have an impact on the pollination ecology of plants (Handel, 1983; Corbet, 1990; Kearns and Inouye, 1993). Such changes include: variation in water and nutrient availability, the amount of sunlight versus shade, percent canopy cover, and climatic conditions. The immediate effects of such changes in the physical environment may include variation in



flower opening, nectar production, anther extrusion and dehiscence, style growth, stigma receptivity, pollen production, morphology and germination, pollen deposition onto flower styles, and pollen tube growth (Corbet, 1990; Rathcke, 1992; Primack and Inouye, 1993; Lau *et al.*, 1995; Autio and Hicks, 2004; Marina *et al.*, 2004).

Changes in the physical environment, as a result of climatic variation, may influence various aspects of plant-pollinator systems (e.g. pollinator foraging behaviour, flower production, pollen production and germination). The climatic conditions with the greatest potential affects are low temperature, high humidity, high ultraviolet radiation, high carbon dioxide concentration, and increased wind speed (Kevan and Baker, 1983; Herrera, 1995; Koti *et al.*, 2005). For example, pollinator visits to wild radish plants (*Raphanus sativus*) were reduced by increased cloud cover and greater wind speed (Stanton *et al.*, 1991). It is also recognised that changes in the microhabitat of plants can substantially influence pollinator activity and behaviour, plant floral traits and reproductive success (Herrera, 1995; Albert *et al.*, 2001). For example, Albert *et al.* (2001) found that *Erodium paularense* plants in lithosol microhabitats were larger and had greater flower, fruit and seed production, than their conspecifics in rock microhabitats.

#### 1.7.3.1 Moisture Availability, Drought Stress, and Pollination

Plants require water for numerous pollination processes including floral bud development and growth, flower opening, nectar production, and turgor maintenance of reproductive organs (Mohan Ram and Rao, 1984). Not surprisingly, many studies have found significant relationships between moisture and nectar production (e.g. Zimmerman, 1983; Lee and Felker, 1992; Wyatt *et al.*, 1992; Boose, 1997; Carroll *et al.*, 2001). For example, Zimmerman and Pyke (1988a) found that *Polemonium foliosissimum* plants that were hand watered produced significantly more nectar than control plants.

Evapotranspirational water losses (via inflorescences) may place plants under water stress, especially in arid and dry environments (Galen *et al.*, 1999). Under drought conditions, water allocated to floral parts may limit subsequent development of fruit or seeds, due to reduced carbon supply, resulting from prior stomatal closure (Galen *et al.*, 1999; Galen, 2000). Galen (2000) found that leaf water potential was reduced in

*Polemonium viscosum* plants under drought conditions, compared to plants with excess watering. Galen (2000) also found that plants under drought conditions produced 39-40% fewer fruits than plants with excess watering. Of the seeds that drought-stressed plants produced, their viability was found to be 83-90% lower than that of plants under excess watering (Galen, 2000). Alternatively, some studies have also found increased production of floral traits and reproductive success in plants under water-stressed conditions (Lee and Felker, 1992; Galen, 2000). Galen (2000) found a significant positive relationship between corolla size and pollen production in drought stressed *Polemonium viscosum* plants (and control plants). Lee and Felker (1992) also found that *Prosopis glandulosa* var. *glandulosa* plants under greater water stress (during a drought year) had significantly greater inflorescence production and pod production three times greater, than plants in the wetter year. However, plants in the wetter year produced larger inflorescences and had greater nectar production per inflorescence.

#### 1.7.3.2 Insolation and Pollination

Variation in the availability of sunlight has been found to influence pollinator visits, and measures of floral traits and reproductive success (e.g. Southwick, 1984; Bertin and Sholes, 1993; Herrera, 1995; Suzuki, 2000; Schulz and Zasada, 2004). Boose (1997) found that *Epilobium canum* ramets grown under a 70% reduction in ambient light had 27% lower nectar production rates than control plants. Rathcke and Real (1993) also reported that fruit set of *Kalmia latifolia* was not limited by inadequate pollination in sunny field sites, but was pollination limited in the shaded forest site. Setter *et al.* (2001) found that sugar concentrations in reproductive tissues of maize plants decreased between 20-50% in shaded areas.

#### 1.7.4 Photosynthesis and Pollination

Few studies have examined pollination and photosynthesis directly, but changes in photosynthetic rates have been linked to moisture stress, light availability, floral production and nectar production (e.g. Southwick, 1984; Zhou *et al.*, 1997; Galen, 1999; Setter *et al.* 2001). For example, Setter *et al.* (2001) found that maize plants exposed to water and light deprivation suffered decreased kernel set and leaf photosynthesis. Galen *et al.* (1999) suggested that if growth is limited by photosynthesis, then reproductive costs under dry conditions should be increased due to the water cost of flower production. Pleasants and Chaplin (1983) suggested that nectar production might be a

direct result of the energy produced via photosynthesis. This suggestion supports the finding that nectar production in *Ipomopsis aggregata* was reduced on overcast days (associated with reduced photosynthetic rate) (Pleasants, 1983). Johnsen *et al.* (2003) detected no variation in the ratio of net photosynthesis to stomatal conductance between selfed and outcrossed families of *Picea mariana*. However, they found that mortality was greater among selfed progeny, and suggested that fixed carbon use was modified in surviving selfed progeny (Johnsen *et al.*, 2003).

## 1.8 An Ideal Study System

*Grevillea macleayana* (Proteaceae, formally *G. barklyana* ssp. *macleayana*; see Makinson 2000) represents an excellent model system in which to study intraspecific variation among plants in plant-pollinator interactions, and the consequences of this variation for plant fitness. Earlier research has demonstrated that *G. macleayana* is a self-compatible species (Harriss and Whelan, 1993) that produces a large, but variable, number of inflorescences (Hogbin *et al.*, 1998; Vaughton, 1998; Whelan *et al.*, 2006 - Appendix 1). *Grevillea macleayana* is an obligate seeder and seed production has been found to be very low (Harriss and Whelan, 1993; Hogbin, *et al.*, 1988), and is not pollen limited (Harriss and Whelan, 1993; Vaughton, 1996). Moreover, casual observations indicate that plants produce copious, but variable quantities of nectar, and nectar production is readily measured from such inflorescences (Lloyd *et al.*, 2002). Pollen production is also substantial (Vaughton, 1996), with large pollen caps displayed on each stigma (see Ayre and Whelan, 1989).

*Grevillea macleayana* is visited by a diverse suite of pollinators (e.g. Vaughton, 1996; England *et al.*, 2001; Roberts *et al.*, 2006 - Appendix 1) and populations show dramatic variation in levels of inbreeding (e.g. Ayre *et al.*, 1994; England *et al.*, 2001 - Appendix 1). It is unclear to what extent these characters vary among plants within populations, how mating systems vary within populations, or the extent to which mating systems are dependent on variation in pollinator behaviour. However, the development of species-specific microsatellite primers has allowed England *et al.* (2001) to demonstrate that mating systems can be modified by exclusion of vertebrate pollinators.

*Grevillea macleayana* is a rare (Rare or Threatened Australian Plant database - Briggs and Leigh, 1996), small (i.e. spreading, low-lying to 1 m) to large (i.e. erect, small tree form to 3.5 m) perennial shrub (Figure 1.3a and b), with pink ‘toothbrush’, classically

bird-pollinated inflorescences. The species is highly fragmented (Whelan *et al.*, 2000) and populations are often small (30 to 50 individuals), isolated and associated with road verges and other disturbed sites (Vaughton, 1996; Hogbin *et al.*, 1998; Roberts *et al.*, 2006). *Grevillea macleayana* occurs between Nowra and Ulladulla on the NSW south coast, and extends west to Bundanoon and into Deua National Park (NP) (Olde & Marriott, 1994). The majority of populations occur in Jervis Bay, approximately 120 km south of the city of Wollongong (Figure 1.4). In these locations summers are typically warm to hot and moist, and winters are cool to cold and often wet (Olde & Marriott, 1994). *Grevillea macleayana* occurs in fire-prone coastal heathland, low woodland and open eucalypt forest, typically in nutrient deficient, well-drained sand and/or clay soils (Mills; 1993; Olde & Marriott, 1994). There are three known forms of *G. macleayana*: coastal form, woolly form, and Deua form (Olde & Marriott, 1994). The research presented in this thesis has been conducted on the coastal form.

*Grevillea macleayana* flowers throughout the year, with peak flowering from late winter/early spring (August-September) to mid-summer (December to January) (Olde & Marriott, 1994; Vaughton, 1996). Inflorescences are typically 3 - 8 cm long, 2 - 3 cm wide, and produce abundant nectar (Olde & Marriott, 1994; Lloyd, S., *personal observations* - Figure 1.5). Flowers are hermaphroditic and protandrous, and the number of flowers per inflorescence ranges from 25 to 65, with an average of approximately 50 flowers (Olde & Marriott, 1994; Vaughton, 1996). Typical of many Proteaceae species, pollen is shed from the anther to a modified style (pollen presenter), before flowers open (Vaughton, 1995). Flowers open sequentially from the base to the tip of the inflorescence, taking four to ten days (average of six to seven days) to complete opening (Harriss & Whelan, 1993; Lloyd, S., *personal observations*). However, it is unknown exactly how long inflorescences remain in male and female phases and this requires further investigation. *Grevillea macleayana* has a very low flower-to-fruit ratio (Harriss & Whelan, 1993; Hogbin *et al.*, 1998) and fruit ranges from 12 to 19 mm in length and is approximately 8 mm wide (Olde & Marriott, 1994 - Figure 1.6). Fruit usually matures in approximately two to three months (Vaughton, 1998); however, this may take as little as six weeks in warmer weather (Harriss & Whelan, 1993). Whilst flowers contain two ovules, usually only one seed develops and therefore, fruit set is a reliable indicator of seed set (Harriss and Whelan, 1993; Vaughton, 1995).

(a)



(b)



**Figure 1.3 - Photographs of *Grevillea macleayana* plants (Photo: S. Lloyd).**

Examples of *G. macleayana* plants (as indicated by the yellow borders) as (a) a low-lying sprawling shrub at Greenfields Beach and (b) a large shrub at Chinamans Beach, at Jervis Bay, on the south-east coast of New South Wales.





**Figure 1.4 - A map showing the location of the three study sites in Jervis Bay.**  
The location of Jervis Bay (120km south of Wollongong, N.S.W. Australia) with two study sites (in yellow) at Hyams Beach (Chinamans Beach and Illowra Lane) and one at Vincentia (Greenfields Beach).



**Figure 1.5 - A *Grevillea macleayana* inflorescence with approximately half of its flowers open (Photo: S. Lloyd).**



**Figure 1.6 - Three developing seeds (indicated by yellow stars) on a *Grevillea macleayana* plant at Greenfields Beach (Photo: S. Lloyd).**

European honeybees (*Apis mellifera*) are the most numerous visitors to *G. macleayana* plants, though arguably ineffective due to foraging behaviour and morphological differences. Nectar-feeding birds, in particular honeyeaters (Class Aves, Family Meliphagidae) are considered to be the natural pollinators (Vaughton, 1996; Whelan *et al.*, 2000; England *et al.*, 2001; Beynon *et al.*, *unpublished* - Appendix 1). Most honeyeater visits are from the Eastern Spinebill (*Acanthorhynchus tenuirostris*), New Holland Honeyeater (*Phylidonyris novaehollandiae*) and Red Wattlebird (*Anthochaera carunculata*). However, the White-cheeked Honeyeater (*Phylidonyris nigra*), Little Wattlebird (*Anthochaera chrysoptera*), Fuscous Honeyeater (*Lichenostomus fuscus*), Scarlet Honeyeater (*Myzomela sanguinolenta*), Non-meliphagid Silvereye (*Zosterops lateralis*), and the Noisy Miner (*Manorina melanocephala*) have also been reported to visit the species (Vaughton, 1996; Roberts, 2001; Beynon *et al.*, *unpublished*; Lloyd, S., *personal observations*). Other insects have also been observed visiting the species on occasion, including wasps, ants, butterflies, crickets and beetles (Lloyd, S., *personal observations*). Moreover, Beynon *et al.* (*unpublished*) found some flowers had pollen removed at night, and it is therefore possible that this species also has nocturnal pollinators (e.g. Eastern Pygmy Possum, *Cercartetus nanus*).

Low genetic variation and gene flow, and high levels of inbreeding present in populations of *G. macleayana* are believed to be related to patterns of fragmentation, coupled with elements of the breeding system, such as poor seed dispersal (e.g. Ayre *et al.*, 1994; Hogbin *et al.*, 1998; England *et al.*, 2002 - Appendix 1). However, it has been suggested that the species has always had a fragmented distribution, and that human activity over the past 200 years has further increased fragmentation and decreased population sizes (England *et al.*, 2002). The species appears to lack any obvious characteristic for widespread seed dispersal. Whilst seedlings may occasionally occur in unburnt vegetation, most recruitment occurs from a soil stored seed bank after fire and is often associated with soil disturbance (Ayre *et al.*, 1994; Edwards & Whelan, 1995; Vaughton, 1998; England *et al.*, 2003).

## 1.9 Study Aims & Thesis Design

It has been suggested that among the primary goals of evolutionary plant ecology are: (1) understanding how plant and floral characteristics affect reproductive success; and (2) understanding how individual plants within a population achieve greater fitness,



relative to other plants (Lawrence, 1993; Mitchell, 1994). To contribute to these goals, I quantified intraspecific variation among plants in floral traits, pollinator foraging behaviour, and reproductive success, in a number of populations. Carthew (1993) highlighted the importance of monitoring variation at the level of the individual plant (rather than the whole population), because population measures may mask much of the variation present among individual plants. Furthermore, it is important that variation is monitored and quantified over a number of sites and seasons to potentially provide the most accurate information on the pollination ecology and reproductive success of the species (Carthew, 1993).

It has been proposed that the seed production of individual plants may be limited by two primary factors: effective pollen transfer and resource availability (Pyke, 1981; Zimmerman, 1988; Zimmerman and Pyke, 1988b). Pollen transfer and resource availability may also affect the selection of floral traits, which in turn, may influence plant reproductive success (Caruso *et al.*, 2005). To understand how variation in plant and floral traits are associated with plant reproductive success (as previously described), it is first essential to determine whether pollen or resources limit seed production (Pyke, 1981; Zimmerman, 1988; Zimmerman and Pyke, 1988b). Previous studies on *G. macleayana* have found that, whilst seed production is low, it is not pollen limited (Harriss and Whelan, 1993; Vaughton, 1996). Therefore, seed production is likely to be resource limited (Zimmerman, 1988; Ayre and Whelan, 1989). Secondly, the variability of plant and floral traits must be quantified, tested for the strength of relationships with pollinator foraging behaviour, which, in turn, must be tested for the strength of relationships with pollen transfer and ultimately seed production and quality (Zimmerman, 1988). Variation in plant characteristics and pollinator behaviour (due to strong associations with plant reproductive success and fitness) may determine population growth and influence plant mating systems and evolution (Stearns, 1992; Thompson *et al.*, 2004).

### 1.9.1 Thesis Aims and Questions

The broad aims of the research presented in this thesis were to use *G. macleayana* to: (1) quantify variation among plants in characteristics conferring attractiveness to pollinators (floral traits), and to examine the consequences of such variation for pollinator activity and reproductive success; and (2) to identify the most significant

relationships among the aforementioned components of the *Grevillea macleayana* plant-pollination system. Specifically, the questions I wished to address in my project encompass these five components of pollination ecology:

**(A) Floral Traits**

- (1) How do plants vary with respect to the mean number of flowers per inflorescence (i.e. inflorescence size)?
- (2) How do plants vary with respect to the total number of inflorescences produced over the survey period?
- (3) How consistent are temporal patterns of variation in inflorescence production among plants?
- (4) How do plants vary with respect to mean inflorescence nectar volume and nectar sugar concentration?
- (5) How consistent are patterns of variation in inflorescence nectar volume and sugar concentration among plants over seasons?
- (6) How do plants vary with respect to pollen production?
- (7) How are inflorescence production (both number and size), nectar production (both volume and sugar concentration) and pollen production related among plants (e.g. is there a trade-off between inflorescence and nectar production)?

**(B) Pollinator Foraging Activity**

- (1) How do honeybee and honeyeater visits and/or foraging behaviour differ among plants?
- (2) How do honeybees and honeyeaters differ overall in visits and/or foraging behaviour?
- (3) Are patterns of variation among plants consistent over survey seasons (e.g. do the same plants receive more honeybee visits in consecutive survey seasons)?
- (4) Do nocturnal pollinators visit *G. macleayana* plants and what is their foraging behaviour?
- (5) How is honeybee and honeyeater foraging behaviour associated with variation among plants in floral traits (inflorescence, nectar, and pollen production)?

**(C) Reproductive Success**

- (1) How do plants vary with respect to total seed numbers over the survey period?
- (2) How consistent are temporal patterns of variation in seed production among plants?
- (3) How are inflorescence and seed production related?
- (4) How is inflorescence size related to seed number?
- (5) How do plants vary with respect to pollen deposition?
- (6) How does diurnal and nocturnal pollen deposition vary?
- (7) How consistent are temporal patterns of variation in pollen deposition among plants?
- (8) How is pollen deposition and seed number related among plants?
- (7) How is reproductive success (seed number and pollen deposition) associated with floral visitor foraging behaviour (honeybee and honeyeater), and floral traits (inflorescence, nectar, and pollen production)?

**(D) Family Outcrossing Rates**

- (1) How do plants vary with respect to rates of outcrossing, biparental inbreeding and correlation of paternity?
- (2) How are family outcrossing and inbreeding depression rates associated with floral traits (inflorescence and nectar production), floral visitor foraging behaviour (honeybees and honeyeaters) and reproductive success (i.e. seed number)?

**(E) Non-reproductive Plant Traits and Environmental Variables**

- (1) How do plants vary with respect to size and distance to nearest conspecific (a measure of local density)?
- (2) How do plants vary with respect to percent canopy cover?
- (3) How do plants vary with respect to mean leaf photosynthetic yield and/or leaf moisture content, and are these factors related to plant size, canopy cover, and/or nearest conspecific distance?
- (4) How are measures of plant size, nearest conspecific distance, canopy cover, leaf photosynthetic rate and leaf moisture associated with reproductive success (seed number) and floral traits (inflorescence number)?

### 1.9.2 Thesis Outline

This thesis has five chapters that describe experimental studies, each representing an important component of the plant-pollinator system, as outlined in Sections 1.3 to 1.7 (Figure 1.7). As described above, floral traits are considered a plant's advertising mechanism to potential pollinators. Therefore, studies on various floral traits are provided in Chapter 2. In Chapter 2, I present variation among plants in inflorescence, nectar, and pollen production and examine how these measures are related.

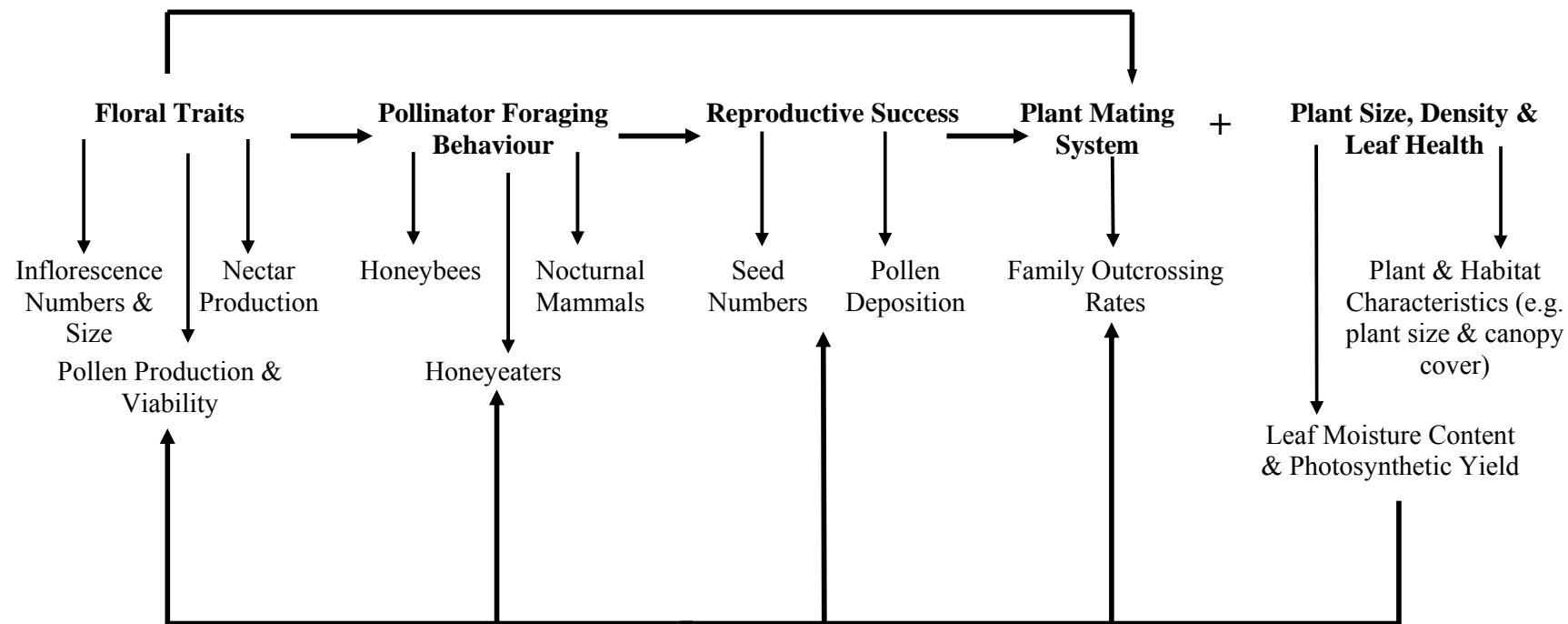
Potential pollinators may respond to floral traits in different ways, depending on the pollinator group (e.g. insect, nectarivore, and mammal). Studies on pollinator observations are provided in Chapter 3. In Chapter 3, I present variation among plants in the foraging activity of honeybees, honeyeaters, and mammals and examine how these measures are related with the three floral traits from Chapter 2.

The reproductive success of a plant may be directly influenced by the foraging behaviour of its pollinators, therefore, experiments on reproductive success are provided in Chapter 4. In Chapter 4, I present variation among plants in seed number and pollen deposition and examined how these measures are related to pollinator activity and floral traits. The quality of seed produced (i.e. whether it is outcrossed or selfed) may also affect plant reproductive success and fitness, depending on the mating system of the plant. I measured family outcrossing rates among plants using microsatellites. These results are presented in Chapter 5. I also examine how outcrossing rates are related to reproductive success, pollinator activity, and floral traits.

Each of these components of pollination ecology may be influenced by, or directly related to non-reproductive plant traits (e.g. plant size and leaf moisture) and/or environmental variables (e.g. canopy cover and plant density). Therefore, experiments on non-reproductive plant traits and environmental variables are provided in Chapter 6. I also test how these measures are associated with plant attraction and floral rewards (i.e. inflorescence number) and reproductive success (i.e. seed number).

In Chapter 7, I bring together the results of these experimental chapters to: (i) identify the traits with the greatest intraspecific variation and (ii) determine the most important relationships and trends (based on the traits I have measured) within the *G. macleayana*

system. I also discuss the importance of this research for pollination ecology and suggest future research needs.



**Figure 1.7 - The five components of the *Grevillea macleayana* plant-pollination system that were used as the basis of this study to: (i) quantify variation among plants and (ii) determine the most important relationships of those identified in this figure.**

As described in the literature review and illustrated in Figure 1.1, plant-pollination systems can be divided into five broad components, these being the basis of this PhD study. The arrows indicate how components (and the individual parts within components) are associated with each other.

**Table 1.1 - Studies that have investigated variation in floral display and/or nectar production and the response of potential pollinators.**

In these studies, the foraging behaviour of potential pollinators is reported as being related to, or affected by, variation in a floral trait (usually interpreted as increased plant attractiveness). With respect to nectar production, the studies reviewed in this Table have been published after 1992, as previous publications have reviewed earlier literature (Zimmerman, 1988; Rathcke, 1992). With respect to floral display, the studies in this Table have been published after 1998, as previous publications have reviewed earlier literature (e.g. Kevan and Baker, 1983; Primack, 1987; Zimmerman, 1988). See Table 1.2 for studies that also investigate pollinator response to variation in floral traits, and subsequent reproductive success.

Floral Trait	Plant Species	Pollinating Organism	Outcome	Reference
Floral Display	<i>Phyla incisa</i>	Several insect species	Pollinators visited larger inflorescences of <i>Phyla incisa</i> more frequently.	Cruzan, Neal, & Willson 1988
	<i>Aralia hispida</i>	Bumblebees ( <i>Bombus</i> sp.)	Bumblebee visits increased with increasing umbel number and size.	Thomson 1988
	<i>Echium vulgare</i>	Bumblebees ( <i>Bombus</i> sp.)	Bumblebee visits increased with increasing flower number per plant.	Klinkhamer & de Jong 1990
	<i>Raphanus raphanistrum</i>	Honeybees ( <i>Apis mellifera</i> )	Honeybees visited larger flowers significantly more and preferentially visited them before smaller flowers.	Young & Stanton 1990
	<i>Phacelia linearis</i>	Solitary bees	Arrival rate of bees increased with the number of flowers and plant.	Eckhart 1991
	<i>Raphanus raphanistrum</i>	Syrphid Flies	Increased flower number and size were correlated with increased fly visits.	Connor & Rush 1996
	<i>Cirsium purpuratum</i>	Bumblebees ( <i>Bombus</i> sp.)	The number of flowers visited on a plant increased with size of floral display.	Ohashi & Yahara 1998
	<i>Mimulus ringens</i>	Bumblebees ( <i>Bombus</i> sp.)	Bumblebee visits per hour increased with increasing flower number per plant. Bumblebees also probed more flowers on plants with larger displays.	Mitchell, Karron, Holmquist & Bell 2004
	<i>Leucadendron xanthoconus</i>	Beetles ( <i>Pria cinerascens</i> )	Plants (of both sexes) with many flowers had an increased number of beetle visits.	Hemborg & Bond 2005
	<i>Echium vulgare</i>	Honeybees ( <i>Apis mellifera</i> )	Plants in patches of high flower number received significantly more pollinator visits.	Leiss & Klinkhamer 2005

Floral Trait	Plant Species	Pollinating Organism	Outcome	Reference
Nectar Production	<i>Hebe stricta</i>	Bees and hover flies	Male plants produced more pollen and nectar than female plants and received more insect visits.	Delph & Lively 1992
	<i>Lobelia deckenii</i>	Sunbird ( <i>Nectarinia johnstoni</i> )	Increased sucrose rewards were related to increased visit rate by sunbirds.	Burd 1995
	<i>Anchusa officinalis</i>	Bumblebees ( <i>Bombus</i> sp.)	The rate of bumblebee visits per plant increased significantly with increased sugar production per flower.	Dreisig 1995
	<i>Echium vulgare</i>	Bumblebees ( <i>Bombus</i> sp.)	Visit duration was significantly longer for flowers on plants with more nectar. Bumblebees visited significantly more flowers on plants with higher nectar standing crop.	Pappers, de Jong, Klinkhamer & Meelis 1999
	<i>Echium vulgare</i>	Bumblebees ( <i>Bombus</i> sp.)	High and low nectar producing plants received equal numbers of approaches when plants were next to each other, but visit length in high plants was 1.65 times more than that of low plants. From 90mins after groups were placed 6m apart, high nectar plants received significantly more approaches than low nectar plants.	Klinkhamer, de Jong, & Linnebank 2001
	<i>Anacardium occidentale</i>	Bees, flies, butterflies, beetles, and ants	The abundance of pollinators coincided with nectar availability.	Bhattacharya, 2004
	<i>Echium vulgare</i>	Bumblebees ( <i>Bombus</i> sp.)	The number of visits per flower was significantly positively correlated with nectar production in sparse populations.	Klinkhamer & van der Lugt, 2004
	<i>Echium vulgare</i>	Honeybees ( <i>Apis mellifera</i> )	Plants in patches of high nectar production received significantly more pollinator visits.	Leiss & Klinkhamer 2005
	<i>Cucurbita pepo</i> L.	Bumblebees ( <i>Bombus terrestris</i> )	An increased frequency of bumblebee visits was correlated with higher volume of nectar.	Roldan-Serrano & Guerra-Sanz, 2005



**Table 1.2 - Studies that reported significant relationships between variation in floral traits, the response of pollinators and reproductive success.**

In these studies variation was often initially quantified in one or more floral traits, with pollinator activity tested against this variation and then linked to some change in reproductive success (see Rathcke, 1992 for further studies concerning nectar production).

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1953a	Alfalfa	Clones varied significantly in nectar production (sugar concentration of nectar & milligrams of sugar per flower)	Significant positive correlation between nectar production & honeybee visits.	Seed production was positively correlated to the number of honeybees per plant.	Results of breeding trials indicate evidence of heritability of nectar production e.g. crossing a high nectar producer with a low nectar producer resulted in progeny of intermediate nectar production.	Pedersen USA
1980b	<i>Combretum farinosum</i>	Inflorescences only secreted nectar on their first day of opening, but plants vary with respect to timing of inflorescence opening.	Hummingbird visits increased with increasing number of nectar producing inflorescences per plant.	Seed production per plant increased with the number of nectar producing inflorescences.	Greater nectar production in this species may have evolved to satiate pollinators & reduce potential territorial behaviour; thereby potentially increasing outcrossed pollen transfer.	Schemske USA (study conducted in Costa Rica)
1980	<i>Diervilla lonicera</i>	Flowers were manipulated to have either 2µL of nectar or only residual traces of nectar.	Bumblebees spent significantly longer foraging on manipulated flowers with greater nectar reward.	Manipulated flowers with a greater nectar reward had significantly more pollen grains deposited per flower.	Authors suggest a “ <i>direct positive feedback</i> ” between nectar production & plant reproductive success.	Thomson & Plowright Canada
1983	<i>Delphinium nelsonii</i>	Experimental plants were hand-watered & produced significantly more nectar than control plants.	Bumblebees visited more flowers per inflorescence & spent more time foraging on flowers of plants with greater nectar, than on control plants.	Plants with greater nectar set significantly more seeds than control plants	Suggest increased seed set due to increased nectar rather than increased water. Female success (i.e. seed set) may be increased by greater resource allocation to nectar production.	Zimmerman USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1986	<i>Erythronium americanum</i> , <i>E. grandiflorum</i> & <i>Linaria vulgaris</i>	Variation in nectar volume & concentration between <i>E. americanum</i> flowers implied, but not described.	Bumblebee time per <i>E. americanum</i> flower increased with nectar volume & significantly with nectar concentration ( $P < 0.01$ ).	<i>Erythronium americanum</i> flowers with greater nectar volume received more pollen grains per visit.	Increased nectar volume may be a selective advantage when increased pollen deposition increases fitness.	Thomson USA
1989	<i>Lupinus argenteus</i>	Purple flowers contain less pollen than yellow flowers.	Pollinator visits increased to plants with larger inflorescences (i.e. more flowers) & pollinators avoided purple flowers (i.e. less pollen). Pollinator visits also decreased to yellow flowers with pollen experimentally removed.	Seed production per flower was significantly lower (in one out of two years) on experimental plants (pollen removed from yellow flowers) than on controls.	Pollinator foraging efficiency may be increased on plants that reliably signal the location of rewarding (yellow) & non-rewarding (purple) flowers (pollinator-tenure mechanism).	Gori USA
1991	<i>Kalmia latifolia</i>	Significant variation between individual plants in mean 24hr nectar production ( $\mu\text{L}$ ). Variation between plants in floral density, statistics not reported.	Significant positive relationship between (1) nectar production & mean visitation rate [(visits/10 mins/100flowers) $P < 0.01$ ] & (2) mean floral density (mean number of open flowers/25cm <sup>2</sup> /day) & mean visitation rate ( $P < 0.05$ ).	Significant positive relationship between visitation rate & percent fruit-set per plant ( $P < 0.01$ ). No significant difference in percent fruit-set between inflorescences from non-augmented (i.e. no hand pollination) plants & augmented plants. Significant increase in percent fruit-set from naturally pollinated to hand-pollinated inflorescences ( $P < 0.05$ ).	No correlation between floral density & nectar production, suggests nectar production not affected by floral density & is property of individual flower. Results suggest that plants doing well one year will do well next. Increased fruit-set may reflect good microsites. Pollinators may act as selective force on floral characteristics.	Real & Rathcke USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1991	<i>Polemoium viscosum</i>	Four phenotype combinations were used, chosen from upper & lower 5% of the frequency distribution of each trait: (1) large corollas & large nectar reward; (2) small corollas & small nectar reward; (3) large corollas & small nectar reward; & (4) small corollas & large nectar reward.	Plants with large corolla & artificially increased nectar reward received significantly longer probe time by bumblebees & larger nectar reward received significantly more flowers probed.	Large corolla & large nectar reward received more pollen grains per flower, but this was not significant.	Extra probe time on large corolla & nectar reward plants may also increase pollen export. Selection should favour larger nectar rewards. If bumble preferences are learned, then “ <i>pollinator preferences can be a source of frequency-dependent selection on floral traits in insect-pollinated plants.</i> ”	Cresswell & Galen USA
1992	<i>Ipomopsis aggregata</i>	Flowers within plants were removed of nectar & received either 1 $\mu$ L or 5 $\mu$ L of 25% sucrose solution.	Probe duration by hummingbirds was significantly greater for flowers with 5 $\mu$ L nectar reward ( $P < 0.01$ ).	Flowers with greater nectar reward received on average ~7 more pollen grains than flowers with lower nectar reward. However, this was not significant. Both pollen deposition ( $P < 0.01$ ) & removal ( $P < 0.01$ ) significantly increased with probe number per flower.	Plants with larger nectar rewards will have greater reproductive success if pollinators: (1) increase the number of probes per flower; (2) visit a larger proportion of flowers per plants; or (3) return to plants more often.	Mitchell & Waser USA
1993	<i>Ipomopsis aggregata</i>	Natural large variation among plants in nectar production rates. Experimental plants received 5 $\mu$ L of 25% sucrose solution.	Hummingbirds probed a larger proportion of flowers at plants with enhanced nectar, than at controls.	Plants with naturally high nectar production rates & experimentally enhanced nectar had greater fluorescent dye dispersal (indicative of pollen) to nearby plants, over three years, than plants with lower nectar production.	Plants with both naturally & experimentally greater nectar production had greater male reproductive success, but no detectable increase in female success (i.e. pollen deposition & seed production).	Mitchell USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1994	<i>Corydalis ambigua</i>	Inflorescence size per plant varied from 1 to 13 flowers (each plant produces one inflorescence).	Plants with larger inflorescences were visited more frequently by bumblebees, than plants with smaller inflorescences.	A significant positive relationship was detected between number of bumblebee visits & seed production per plant. Plants that received at least one long (> 60 s) bumblebee visit also had significantly greater seed production than plants that received only shorter visits.	Plants with larger inflorescences are more attractive to pollinators, provide a larger, more continuous nectar reward & subsequently set more seed.	Ohara & Higashi  Japan
1995	<i>Mirabilis multiflora</i>	Flowers on three plants received one of four treatments over four nights (i.e. each plant received each treatment once): (1) nectar removed; (2) no manipulation; (3) 5µL of artificial nectar added; & (4) 10µL of artificial nectar added.	Significant positive relationship between nectar volume & (1) hawkmoth visits per flower ( $P < 0.01$ ) & (2) the number of flowers visited consecutively ( $P < 0.01$ ).	Significant positive relationship between visits per flower & (1) pollen deposition per stigma ( $P < 0.01$ ) & (2) pollen removal per anther ( $P < 0.01$ ). However, the proportion of self-pollen deposited per plant increased significantly with successive flower visits ( $P < 0.03$ ). Significant positive relationship between nectar volume per flower & (1) pollen deposition per stigma ( $P < 0.01$ ) & (2) pollen removal per anther ( $P < 0.01$ ). Significant positive relationship between flowers per plant & seed production ( $P < 0.01$ ).	Nectar production may be limited due to the subsequent positive effect on hawkmoth visitation & levels of self-pollination (self-incompatible species). Increased self-pollen deposited on stigmas may block outcross pollen from fertilising ovules. Relationship between pollen deposition & seed set may not be linear. Proposed that selection has resulted in nectar production that “balances pollinator needs & behaviour to maximise plant reproductive success.”	Hodges  USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1995	<i>Eichhornia paniculata</i>	Plants were manipulated so that inflorescences comprised three, six, nine, or 12 flowers.	Bumblebees visited more flowers on plants with larger inflorescences, but this was dependent on sizes of nearby inflorescences. Overall, the total number of visits per flower did not differ among inflorescence size treatments.	Seed production did not vary with inflorescence size. Outcrossing decreased & selfing increased with inflorescence size.	Mating cost associated with large floral display due to increased geitonogamy & subsequent pollen discounting. By displaying fewer flowers per day over a longer period a plant may increase its outcrossing rate.	Harder & Barrett Canada
1996	<i>Raphanus raphanistrum</i>	Strong selection for increased flower production in each of three years.	Increased flower number caused significant increase in visits by small bees in one out of three years	Strong positive correlation between fruit production & number of fertilised seeds/fruit in two years, & between fruit production & the proportion of viable seeds in one year. Increased flower number increased fruit number, which was the dominant cause of increased female fitness in all three years.	Flower production was the most important trait in all years & was the main determinant of fruit set. Fruit production was the most important fitness component in determining total seed production.	Conner, Rush, & Jennetten USA
1997	<i>Ipomopsis aggregata</i>	Plants were manipulated to have either four or ten flowers per plant.	Plants with more flowers received significantly more probes per hour ( $P < 0.01$ ) & probes per flower ( $P = 0.02$ ) from birds. However, the proportion of flowers visited decreased as flower number increased.	Plants with more flowers produced significantly more fruits ( $P < 0.01$ ) & set significantly higher percentage of fruits ( $P < 0.01$ ). However, plants with more flowers also had the greatest pre-dispersal seed predation.	Once the probability of being approached over time was included, plants with larger floral display did not have a greater likelihood of flowers being visited. Overall, there was no “disproportionate” increase in fruit set for plants with more flowers.	Brody & Mitchell USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1998	<i>Banksia ericifolia</i>	Significant variation among <i>Banksia ericifolia</i> plants in nectar production per inflorescence & variation among plant in inflorescences per plant.	Significant positive correlation between bird visits per hour & (1) inflorescence number per plant, (2) inflorescence size & (3) plant height ( $P < 0.01$ ).	Significant positive correlation between: (1) rate of honeybee visits & percentage flowers with pollen tubes ( $P < 0.01$ ); (2) rate & duration of honeybee visits & the percentage of flowers with pollen grains ( $P < 0.02$ ); & (3) number of inflorescences visited per plant & time per inflorescence by birds & the percentage of flower with pollen grains ( $P < 0.01$ ).	Bird & insect pollinators varied in foraging behaviour, resulting in differences in pollen removal. Genetic analysis needed to determine whether there are differences in seed from insect or bird visits (i.e. outcrossing rates).	Lloyd Australia
1998a	<i>Wurmbea dioica</i>	Male plants produced larger flowers and significantly more flowers than female plants ( $P < 0.01$ both).	Males received significantly more visits than females ( $P < 0.01$ ) & plants with more flowers received significantly more visits ( $P < 0.01$ ). However, visits were proportional to the number of flowers per plant.	Pollen removal & deposition increased significantly with flower size (not number) three days after flowers opened ( $P < 0.05$ ). Seed number per plant increased significantly with flower number ( $P < 0.01$ ).	Percent seed set decreased significantly with flower size & number, indicating female success is resource limited. Increased floral attraction via male function. Variation among pollinator groups in responses to floral traits may be important for selection that increases attractiveness.	Vaughton, & Ramsey Australia
1999	<i>Prosopis glandulosa</i> var. <i>torreyana</i>	Fixed dimorphic system with half the plants nectarful & the other half nectarless. Nectarless trees produce more pollen grains than nectarful trees ( $P = 0.05$ ).	Nectarful trees were visited 21 times more often than nectarless trees.	Nectarful trees had higher fruit-set & nectarless trees had higher pollen counts. No significant difference between morphs in seed mass or germination.	Overall there was no difference in female function between morphs (despite fruit-set results). Male function was higher for nectarless trees than nectarful trees.	Golubov, Eguiarte, Mandujano, López-Portillo & Montana Mexico

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
1999	<i>Comparenttia falcata</i>	Flowers were injected with 6µL of 20% sucrose.	Visits per flowers per plant were significantly positively correlated with open flower number in one year ( $P < 0.01$ ), no association with nectar. Lower pollinator numbers were associated with rainy days for one year ( $P < 0.01$ ).	No significant difference in pollen removal or fruit-set between treatments.	Reproductive success is often constrained by a combination of pollination & resource limitations. Selection may favour low-nectar phenotypes under current limitations (i.e. increasing nectar did not increase reproductive success).	Salguero-Faria & Ackerman USA
1999	<i>Brassica napus</i>	Nectar was removed & up to 2.0µL sucrose solution was added to 20 flowers. Flowers received one of two pollen treatments: (1) undisturbed & (2) pollen removed.	Significant positive relationship between volume of sucrose solution & bumblebee visit duration ( $P < 0.01$ ).	Flowers with undisturbed pollen had significantly more pollen deposited ( $P < 0.02$ ) than flowers with pollen removed (likely result of self-pollen deposition).	Lack of pollen transfer result of ineffective foraging behaviour by bumblebees. Variation among bumblebees in the duration of visits to unmanipulated flowers resulted from differences in foraging speed.	Cresswell UK
2000	<i>Cistus creticus</i>	Experimental flowers given 3µL of 40% sugar solution. Control flowers treated the same, with no nectar added.	Honeybees spent significantly greater amount of time (s) per flower on nectar-added plants ( $P = 0.04$ ).	Nectar-added flowers had significantly reduced abortion rates. Overall seed yield was higher for nectar-added flowers ( $P = 0.03$ ).	This species is a nectar donor & variation in nectar may modify pollinator behaviour, reproductive success & plant fitness.	Manetas & Petropoulou Greece
2000	<i>Allium cepa</i>	Nectar volume significantly different among hybrid parents in two years (both yrs $P < 0.01$ ).	Positive significant correlation between nectar volume & the number of bee visits in two years ( $P < 0.01$ & $P < 0.03$ ).	Positive significant correlation between the number of bee visits & the amount of seed produced (g) in two years ( $P < 0.01$ & $P < 0.04$ ).	Hybrids that received more bee visits had less variation in nectar volume among individual plants. Selection for flowers with high nectar production may lead to higher pollination rate.	Silva & Dean USA

Year	Plant Species	Floral Trait	Pollinator Response	Reproductive Success	Conclusions	Authors
2001	<i>Heterophragma quadriloculare</i>	Treatments were either small display trees ( $N < 1000$ flowers in 1995 & $N < 400$ in 1996) or large display trees ( $N > 1000$ flowers in 1995 & $N > 400$ in 1996).	Rate of Carpenter Bee visits per tree was positively related to the number of open flowers in both years ( $P < 0.01$ ).	No relationship between bee visitation & fruit-set in 1995, but significant negative relationship in 1996 ( $P < 0.05$ ).	Trees with greater flower production (also of greater girth), produced more fruit in a season. Trees with more flowers “converted a significantly lower proportion of flowers into fruit.”	Somanathan & Borges India
2004	<i>Anacamptis morio</i>	Plants were allocated to either nectar-enriched treatment or control treatment (unmanipulated with no reward).	Bumblebees visited significantly more flowers per inflorescence & spent significantly more time foraging on nectar-enriched plants.	Significantly more pollinaria were removed from nectar-enriched plants than control plants. Self-pollinations via geitonogamy were significantly greater in nectar-enriched plants.	Results support the idea that floral deception is a mechanism to minimise geitonogamy & maximise the efficiency of pollen removal & deposition.	Johnson, Peter, & Ågren South Africa & Sweden
2005	<i>Salvia nipponica</i>	Plants varied in the number of open flowers per raceme & the number of flowering racemes per plant.	Plants with more open flowers per raceme & more flowering racemes received enhanced bumblebee visits, with more probes per visit.	Seed-to-ovule ratios were found to increase significantly with an increase in the number of open flowers per raceme & flowering racemes per plant.	In high-density plots it may be beneficial for plants to increase flower number per raceme & racemes per plant. In low-density plots it may be beneficial to increase raceme number per plant, but not flowers per raceme.	Miyake & Sakai Japan
2005	<i>Chuquiraga oppositifolia</i>	In the third growing season, plants treated with nitrogen had floral displays double that of control plants.	In the third growing season, nitrogen addition plants received twice the number of insect pollinator visits as control plants.	In the third growing season, nitrogen addition plants had three- to four-times the seed production as control plants.	Additional soil nitrogen produced increased bottom-up effects on flower & seed production, in the third season, & significant increases in growth in the second & third season.	Muñoz, Celedon-Neghme, Cavieres, & Arroyo Chile
2006	<i>Disa pulchra</i>	Artificial nectar (sucrose) was added to naturally non-rewarding flowers.	Nectar addition significantly increased the number of flowers probed & the foraging time per flower by flies.	Nectar addition significantly increased the proportion of pollinaria removed per inflorescence.	The level of self-pollination increased with the number of flowers probed per plant. Self-pollinated fruits had half the viable seeds of outcrossed fruits.	Jersáková & Johnson Czech Republic



## Chapter 2 - Variation in Floral Traits

### 2.1 Introduction

#### 2.1.1 Flower, Nectar, and Pollen Production

As described in Section 1.3, the potential reproductive success and subsequent fitness of an animal-pollinated plant is dependent on its ability to attract pollinators, and the ability of those pollinators to transfer compatible pollen (Zimmerman, 1988; Oldroyd, *et al.*, 1997; Carroll *et al.*, 2001). The plant traits most important for attracting pollinators are floral display and nectar rewards (Thomson, 1988; Devlin *et al.*, 1992; Rathcke, 1992; Conner and Rush, 1996). It is expected that plants with greater floral rewards will receive more pollinator visits and produce more seeds, provided there is effective transfer of compatible pollen (Dreisig 1995; Philipp and Hansen, 2000; Roldan-Serrano and Guerra-Sanz, 2005). Specifically, the value of nectar as a reward for pollinators depends on the volume, composition, and sugar concentration (Faegri and van der Pijl, 1979; Simpson and Neff, 1983). A nectar reward also determines the energy return a pollinator receives per unit of foraging time (Simpson and Neff, 1983). By quantifying the natural variation that exists among plants in floral traits, patterns of production may be examined and variation tested against measures of pollinator foraging behaviour and plant reproductive success.

After nectar, pollen is generally considered the next most important floral reward, as it is highly nutritious, containing protein and lipids (Kevan and Baker, 1983; Kearns and Inouye, 1993; van Tets and Hulbert, 1999). Pollen is an essential food source for many pollinating insects, including beetles, flies, butterflies and bee larvae (Kevan and Baker, 1983; Kearns and Inouye, 1993). Pollen is also recognised as an important food source for some non-flying mammals, such as the Eastern Pygmy Possum (*Cercartetus nanus*) and the Sugar Glider (*Petaurus breviceps*) (van Tets and Whelan, 1997; van Tets and Hulbert, 1999). In plant species where reproductive success is limited by inadequate pollen production, plants producing more pollen may have a substantial reproductive advantage, if greater floral display and/or increased pollen production results in more effective pollinator visits and subsequent seed production.

Many plants display temporal variation with respect to floral display and seed production (Copland and Whelan, 1989; Ivey *et al.*, 2003). Some plant species may

also experience trade-offs (due to limited resources) between these two measures (Vaughton and Ramsey, 1998; Vallius, 2000). However, trade-offs may not be apparent in only one flowering season, as resources may be used in one season, at the expense of reproductive success in following seasons (Zimmerman, 1984; Ackerman and Montalvo, 1990; Richardson and Stephenson, 1991). Therefore, patterns of flower and fruit production need to be assessed over consecutive flowering seasons to determine whether there are trade-offs between these measures (Whelan and Goldingay, 1989). Previous studies have also detected significant positive and negative patterns between flower, nectar, pollen and production, indicating that there may be both costs and benefits associated with increased production of floral rewards (e.g. Harder and Cruzan, 1990; Mitchell, 1993; Klinkhamer and van der Veen-van Wijk, 1999; Caruso, 2004). Such positive or negative associations between floral traits will be important in determining overall plant attraction to pollinators, and therefore, potentially plant reproductive success.

### 2.1.2 Study Predictions and Aims

Based on the literature reviewed in this Chapter and Chapter 1, I made several predictions about the likely variation in inflorescence, nectar, and pollen production among *G. macleayana* plants:

- (1) Plants will vary significantly with respect to inflorescence numbers and nectar production. Furthermore, when measured over several seasons, the same plants will consistently produce more inflorescences and/or nectar.
- (2) Plants will not vary with respect to pollen production.
- (3) Given that *G. macleayana* plants are assumed to be resource limited, there may be a negative relationship between inflorescence number and nectar production, indicating a trade-off between these two traits.

In this chapter, I explore the following questions:

#### (A) Inflorescence Production:

- (1) How do plants vary with respect to the mean number of flowers per inflorescence (i.e. inflorescence size)?
- (2) How do plants vary with respect to total inflorescence numbers over the survey period?

- (3) How consistent are temporal patterns of variation in inflorescence production among plants?

**(B) Nectar production:**

- (1) How do plants vary with respect to mean inflorescence nectar volume and nectar sugar concentration?
- (2) How consistent are patterns of variation in inflorescence nectar volume and sugar concentration among plants over seasons?

**(C) Pollen Production:**

- (1) How do plants vary with respect to pollen production?

**(D) Floral Reward Trade-offs:**

- (1) How are inflorescence production, nectar production (both volume and sugar concentration) and pollen production related among plants (e.g. is there a trade-off between inflorescence and nectar production)?

## 2.2 Methods

### 2.2.1 Study Sites

I conducted field experiments on *G. macleayana* at three sites within Jervis Bay, located on the southeast coast of NSW: Chinamans Beach, Greenfields Beach, and Illowra Lane (Figure 1.4). The site referred to as Chinamans Beach (CB) is located in Jervis Bay National Park (JBNP), in woodland, adjacent to a walking track, near CB and Hyams Beach Village (Figure 2.1). The site referred to as Greenfields Beach (GB) is located in JBNP, in woodland and tall open forest, adjacent to a walking track, at GB, Vincentia (Figure 2.2). The site referred to as Illowra Lane (IL) is located on crown land, in heathland and woodland, adjacent to IL, in Hyams Beach Village (Figure 2.3a). The *G. macleayana* populations at all three sites are relatively small, comprising approximately 50 individuals (respectively) at CB and GB and just 30 individuals at IL (Roberts, 2001; Roberts *et al.*, 2006).

In these locations, *G. macleayana* commonly occurs in fire-prone coastal heathland, open woodland and tall open eucalypt forest, typically in nutrient deficient, well drained sand and/or clay soils (Mills, 1993; Olde & Marriott, 1994). Common plant species in the upper storey include: *Corymbia gummifera*, *Eucalyptus sclerophylla*, *E. pilularis*, *Allocasuarina littoralis* and *Banksia integrifolia* in the upper storey. In the mid-storey, common plant species include: *B. ericifolia*, *B. serrata*, *Dodonaea triquetra*, *Kunzea*

*ambigua*, *Hakea teretifolia*, *Acacia longifolia*, and *Leptospermum* spp. Common plant species in the lower storey and ground cover include: *Grevillea macleayana* (also a mid-storey species), *Persoonia* spp., *Pultenaea villosa*, *Lomandra longifolia*, *Pteridium esculentum* and native grasses. The vegetation at each site in JBNP is largely undisturbed (excluding the walking tracks), with very few weeds and only occasional rubbish. However, Jervis Bay is a popular tourist location, and consequently walking tracks and beaches near the three study sites were often busy with tourists, especially in the warmer months.

The Jervis Bay climate is typically warm to hot, moist summers and cool to cold, often wet winters (Olde & Marriott, 1994). Jervis Bay is also prone to bushfires and parts of JBNP experienced bushfires during the summers of 2001, 2002, and 2003. Most of the IL site was burnt in December 2003, during a back-burn exercise conducted by the NSW Rural Fire Service, to protect houses from an advancing bushfire (Figure 2.3b). Given that most seedling recruitment occurs following fire (although occasional seedlings may occur in unburnt vegetation; Lloyd, S., *personal observations*), the number of years since the last fire is a good indicator of plant age (Edwards and Whelan, 1995; Vaughton, 1998). The last fire at CB was in 1986, therefore, I estimated that plants were about 19 years old (Vaughton, 1998). I was not able to obtain information on fires at GB or IL. However, based on the size of plants at GB and IL (compared with the size of plants at CB), I estimated that plants at GB were approximately 15-20 years old and plants at IL were approximately 15 years old.



**Figure 2.1 - A photograph of the Chinamans Beach study site (Photo: S. Lloyd).** Woodland at Chinamans Beach, with a large *Grevillea macleayana* shrub (as indicated by the yellow borders), located in the middle-ground of the photo.



**Figure 2.2 - A photograph of the Greenfields Beach study site (Photo: S. Lloyd).** Tall open forest at Greenfields Beach, with a low-lying *Grevillea macleayana* shrub (as indicated by the yellow borders), located in the middle-ground of the photo.



**Figure 2.3a - A photograph of the Illowra Lane study site (Photo: S. Lloyd).** Woodland at Illowra Lane, with a *Grevillea macleayana* shrub (as indicated by the pink borders), located on the left-hand side of the Lane.



**Figure 2.3b - A photograph of Illowra Lane after a back-burn (Photo: S. Lloyd).** Burnt woodland at Illowra Lane, after the back-burn exercise conducted by the Rural Fire Service in December 2003.



## 2.2.2 Variation Among Plants in Inflorescence Production

### 2.2.2.1 Inflorescence Size

I recorded inflorescence size (flower number per inflorescence) during the nectar production surveys, for eight to nine inflorescences on each of five to six plants per site. Inflorescence size was recorded at CB in January and October 2002, at GB in January 2002, October 2002, January 2003, and November 2003, and at IL in October 2002, and January 2003 (Table 2.1).

### 2.2.2.2 Inflorescence Number

To determine whether there was significant variation among plants in inflorescence production, I recorded the number of inflorescences per plant for two years at CB and GB and 18 months at IL. To ensure I had a broad sample of the population at each site, I recorded the number of inflorescences on approximately half the plants at each site. I commenced the study with 20, 25, and 20 plants at CB, GB, and IL, respectively. However, one plant died at both CB and IL and six plants died at GB, between October 2002 and December 2003, resulting in 19 plants per site (Table 2.1). I documented inflorescence and seed production at CB between June 2002 and May 2004 (24 months), at GB between July 2002 and May 2004 (23 months), and at IL between July 2002 and December 2003 (18 months). I was not able to conduct the last six months of monitoring at IL because the plants were burnt in December 2003.

### 2.2.2.3 Statistical Analysis

Single factor ANOVAs tested for significant variation among plants in mean inflorescence size (flowers per inflorescence) (Question 1). Assumptions of normality were tested using the Shapiro-Wilk test and equal variances were tested for using the O'Brien, Brown-Forsythe, Levene, or Bartlett tests. *A posteriori* Tukey-Kramer HSD tests compared plant means for each ANOVA. Data collected at GB in October 2002 were transformed [square-root ( $x + 0.5$ )] due to some non-normality.

**Table 2.1 - Field and laboratory studies undertaken on three floral traits of *Grevillea macleayana*.**

Field studies and laboratory experiments conducted to quantify variation among plants in inflorescence, nectar, and pollen production. Studies were conducted at three sites in Jervis Bay National Park, between January 2002 and June 2004.

Study	Study Site							
	Chinamans Beach		Greenfields Beach				Illowra Lane	
<b>Inflorescence Production</b>	February 2002 to May 2004		February 2002 to May 2004				July 2002 to December 2003	
Monthly record of inflorescence number	✓		✓				✓	
<b>Nectar Production</b>	January 2002	October 2002	January 2002	October 2002	January 2003	November 2003	October 2002	January 2003
The number of flowers/inflorescence	✓	✓	✓	✓	✓	✓	✓	✓
Inflorescence nectar volume	✓	✓	✓	✓	✓	✓	✓	✓
Sugar concentration of nectar/inflorescence	✓	✓	✓	✓	✓	✓	✓	✓
Amount of sugar (mg) per inflorescence	✓	✓	✓	✓	✓	✓	✓	✓
<b>Pollen Production</b>	-	-	January 2004				-	-

A dash (-) indicates that pollen production was not quantified at CB or IL (Section 2.2.4).



I displayed variation among plants in total inflorescence numbers (as recorded over the survey period) using bar graphs, for each site (Question 2). To determine the consistency of variation in inflorescence production among plants, I used single factor ANOVAs to test for significant variation among plants per site in monthly inflorescence production ranks (Question 3). I tested assumptions of normality and equal variances using the previously described tests. Data for inflorescence production were square-root ( $x + 0.5$ ) transformed for CB and GB, due to some non-normality and heteroscedasticity. *A posteriori* Tukey-Kramer HSD tests compared plant means for each ANOVA. I examined variation in inflorescence production further by plotting the three plants with the best inflorescence production (based on mean monthly ranks) and the two plants with the poorest inflorescence production (based on mean monthly ranks), for each month of the survey period (Question 3).

### 2.2.3 Variation Among Plants in Nectar Production

In order to understand the relationship between nectar production and pollinator behaviour, I first needed to quantify the natural variation in nectar production among plants (Rathcke, 1992). Nectar production can be measured as the amount of nectar accumulated over a set time period (e.g. 24 hr) or estimated from nectar standing crop (Pleasants, 1983; Kearns and Inouye, 1993). Nectar standing crop is the result of patterns of nectar production and prior pollinator foraging activity, and represents the nectar reward encountered by pollinators (Zimmerman and Pyke, 1986). The most appropriate measure for a particular study depends upon on the nature of the questions being addressed (Possingham, 1990; Rathcke, 1992; Kearns and Inouye, 1993).

I decided to quantify nectar production rate rather than standing crop because: (1) measurements of standing crop may not represent consistent interplant variation encountered by pollinators; and (2) measurements of standing crop may result in an inaccurate or underestimated record of nectar availability if pollinator foraging is non-random (Possingham, 1990; Rathcke, 1992; Kearns and Inouye, 1993).

#### 2.2.3.1 Trial Experiments

To determine whether there was variation among plants in nectar production and refine the techniques and study design I proposed to use, I first conducted a trial study. I conducted two trial studies at CB in November 2001. The first used 40 inflorescences (approximately 1900 flowers) on ten plants and the second used 40 inflorescences

(approximately 1850 flowers) on four plants. Inflorescences were randomly chosen and nectar production was quantified as 24 hr measurements for nectar volume ( $\mu\text{L}$ ) on one to two days. All inflorescences had approximately 30% of their flowers open when bagged.

I conducted the first trial on ten plants (four inflorescences per plant) and nectar volume was measured on two consecutive days. Mean ( $\pm$  standard error<sup>3</sup>) inflorescence nectar volume per plant over the two days ranged from 39.95  $\mu\text{L}$  ( $\pm 8.78$ ) to 217.23  $\mu\text{L}$  ( $\pm 55.23$ ) and variation among plants was significant (ANOVA;  $F_9 = 2.75$ ;  $P \leq 0.02$ ). However, nectar volume also varied substantially among inflorescences within plants. In order to reduce within-plant variation, I increased the number of inflorescences per plant (to ten), for the second trial. In doing this, I reduced the number of plants to be included in the study to four because of logistical constraints associated with measuring nectar on all the inflorescences in one day. In the second trial, I also found significant variation among plants in mean nectar volume per inflorescence (ANOVA;  $F_3 = 7.65$ ;  $P \leq 0.01$ ). Mean inflorescence nectar volume ranged from 45.50  $\mu\text{L}$  ( $\pm 11.04$ ) to 137.90  $\mu\text{L}$  ( $\pm 17.36$ ). I conducted a power analysis on the second nectar trial, to determine a minimum sample size. The analysis revealed very strong power (Power = 0.98; Adj Power = 0.94) and a minimum sample size of 18 inflorescences (Least Significance Number = 17.6). I was then able to design subsequent studies, with appropriate numbers of inflorescences and plants based on the results of these preliminary studies.

### 2.2.3.2 *Quantifying Nectar Volume*

I used disposable glass micro-capillary tubes (50  $\mu\text{L}$ ) to remove nectar from inflorescences. This method is commonly used to extract nectar from flowers (e.g. Hocking, 1968; Armstrong and Paton, 1990; Kearns and Inouye, 1993; Lloyd *et al.*, 2002). I removed nectar already present on inflorescences at the start of the experiment, rinsed inflorescences with distilled water, allowed them to dry and bagged them for 24 hr (Kearns and Inouye, 1993). Bags were made of 1  $\text{cm}^2$  course plastic mesh (Gutterguard®) shaped into a cylinder and surrounded by 1  $\text{mm}^2$  aperture fibreglass mesh (insect netting). Bags prevented access by pollinators and the Gutterguard® prevented the bag from collapsing onto the inflorescence. The following day I removed

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<sup>3</sup> All means throughout the thesis are presented with  $\pm$  standard errors (s.e.) unless otherwise specified.

the accumulated nectar using micro-capillary tubes and calculated the volume of nectar present. I also recorded the number of flowers per inflorescence and the number of flowers open each day. I always used inflorescences that had approximately the same proportion of flowers open, given that nectar production may vary depending on the proportion of flowers open per inflorescence (see Section 2.2.3.4).

#### 2.2.3.3 *Measuring the Sugar Concentration of Nectar Samples*

I measured the concentration (%) of sugar in a sample of nectar using a hand-held refractometer (Paton, 1985; Kearns and Inouye, 1993). A refractometer measures the refractive index of a liquid sample, in this case the percentage of sucrose, measured on the BRIX scale (weight of sugar per weight of solution at a given temperature) (Kearns and Inouye, 1993). This measure is then used as an estimate of sugar (i.e. sucrose) concentration (Kearns and Inouye, 1993). I calibrated the refractometer prior to field studies by making sugar solutions of varying concentrations and measuring these on the refractometer.

#### 2.2.3.4 *Quantifying Variation Among Plants*

The amount of sugar in a sample of nectar (mg) is calculated from both the volume of nectar ( $\mu\text{L}$ ) and the sugar concentration (%). I did not want to present the amount of sugar (as the only measure of nectar production) unless it was positively related to both nectar volume and sugar concentration. Nectar volume per inflorescence was significantly positively related to the amount of sugar per inflorescence, explaining up of 98% of variation among plants at CB in January 2002 ( $F_{1,32} = 1462.43$ ;  $P < 0.001$ ). However, the regression between nectar sugar concentration and the amount of sugar explained just 10% and 5% of the variation among plants at CB and GB, respectively. Therefore, I have presented the results for both nectar volume and sugar concentration for the nectar studies in this Chapter.

To quantify variation among plants in nectar production, I initially measured the nectar production of each flower within an inflorescence, for the duration of flowering.

Inflorescences with less than 5% of their flowers open were randomly selected and tagged, with 48 inflorescences (1961 flowers) on six plants at CB and 40 inflorescences (1854 flowers) on five plants at GB, during January of 2002 (Table 2.1). I measured nectar volume and sugar concentration daily (as described above), until all flowers per

inflorescence were open and had ceased nectar production (four to seven days, depending on the inflorescence size). The total number of flowers per inflorescence and the daily number of flowers open per inflorescence were also recorded. However, this method was not feasible for future field seasons because of the large number of consecutive days required in the field (five to eight) and the high frequency of rain (i.e. loss of experimental data). Therefore, in subsequent years, I used a shorter sampling period to measure nectar production.

To ensure that measurements of nectar production recorded over fewer days would accurately represent the total nectar production of an individual inflorescence over its flowering lifetime; I tested the significance of the relationship between lifetime nectar production and that of a shorter survey period. Using the data from January 2002, I found a significant positive relationship between the volume of nectar produced by individual inflorescences over two consecutive days (when the proportion of flowers open on the first day of measurement was  $\geq 25\%$ ) and the total volume of nectar produced from all flowers on the inflorescence ( $r^2 = 0.78$ ;  $df = 26$ ;  $P < 0.01$ ). To ensure an inflorescence would have  $\geq 25\%$  of flowers open on the first day of measurement, data from January 2002 indicated that between 5% and 15% of flowers had to be open the day prior to measurements.

To determine whether there was significance variation among plants in nectar production over two days, I conducted studies on 45 to 50 inflorescences on five to six plants (total of 9750 flowers) at each site in October 2002 and at GB and IL in January 2003 (Table 2.1). Inflorescences with approximately 15% of their flowers open were tagged, and nectar volume and sugar concentration were measured over two to three days (as described above). Plants at CB did not produce enough inflorescences for the study to be conducted in January 2003.

Despite my confidence that measuring nectar production over two days would allow me to accurately quantify variation among plants, I further refined this method for my final field season, and measured nectar production on three to four consecutive days. In November 2003, I measured nectar volume and sugar concentration (as described above), on three to four consecutive days, for 48 inflorescences (2199 flowers), on six plants at GB. Frequent rain (October to December), poor flowering at CB and the loss

of most plants at IL (due to the backburn) prevented me from conducting the study at CB and IL.

#### 2.2.3.5 Statistical Analyses

I used single factor ANOVAs to test for significant variation among plants in mean inflorescence nectar volume ( $\mu\text{L}$ ) and sugar concentration (%), tested separately (Question 1). Nectar volume ( $\mu\text{L}$ ) per inflorescence was summed over the number of days it was measured (e.g. in October 2002 I measured nectar production on two days, and therefore, inflorescence nectar volume was the total of the volumes quantified on those two days). I measured the sugar concentration of nectar on each day I quantified nectar volume (unless the volume was too small to be read by the refractometer). I took a mean of the sugar concentration readings per inflorescence over these days, for use in the ANOVAs. Assumptions of normality and equal variances were tested as described in Section 2.2.2.3. An *a posteriori* comparison among plant means was conducted for each ANOVA using Tukey-Kramer HSD tests. Nectar volume data collected from Illowra Lane in October 2002 and Greenfields Beach in November 2003 were  $\log(x+1)$  transformed due to some heteroscedasticity. Due to poor flowering by some plants at each site, I was unable to use exactly the same plants for each survey season per site. Therefore, I was not able to statistically analyse to investigate the consistency of nectar production over consecutive flowering seasons (Question 2).

### 2.2.4 Variation Among Plants in Pollen Production

#### 2.2.4.1 Quantifying Variation Among Plants

To determine whether there was significant variation among plants in pollen production, I estimated pollen grain number on the plants previously used for the nectar studies, at GB, in January of 2004. Frequent rain and poor flowering prevented this study from being conducted at CB and the December 2003 backburn killed most of the experimental plants at IL.

I sampled nine to 11 inflorescences for each of seven plants at GB and estimated numbers of pollen grains per inflorescence using the following techniques. I haphazardly removed 60 inflorescences (attached to approximately 15cm of branch) from seven plants (previously used in the nectar studies at GB), in late January and early February of 2004. I placed cuttings into zip-lock plastic bags on ice whilst in the field,

and once in the laboratory placed cuttings into sugar solutions (cups of lemonade). There is some evidence to suggest that *G. macleayana* pollen production may vary longitudinally within inflorescences (Cruden, S., *personal communication*). Therefore, to ensure that I generated estimates of pollen production that were representative of the entire inflorescence, I sampled ten flowers from along the length of each inflorescence.

I removed pollen bundles from the ten flowers (per inflorescence) by placing the flower styles upside down in an Eppendorf tube with 100  $\mu$ L of ethanol (70%). I then held the tube in a vortex mixer and the vibration separated the pollen from the style and into the ethanol solution. To ensure all pollen grains had been removed, I examined flower styles under a 50x light microscope. To estimate pollen grain number, I used a hemacytometer (particle counting chamber), which is often used for counting pollen grains (e.g. Pyke *et al.*, 1988; Kearns and Inouye, 1993; Ramsey and Vaughton, 1991; Routley *et al.*, 1999). I removed 10  $\mu$ L of solution from the Eppendorf tube and expelled this into the counting chamber of the hemacytometer ('Improved Neubauer' design by Weber), ensuring the solution was evenly dispersed under the cover slip. I flicked the Eppendorf tube several times before removing the sample, to ensure pollen grains were evenly dispersed in the solution. I placed the hemacytometer onto the microscope stage and examined it under low power. I then counted the number of pollen grains in each of the quarter grids and calculated a mean number of pollen grains from these four grids. With the cover slip in place, the volume of solution over a corner grid is 0.1  $\mu$ L. I multiplied the mean (calculated from the four corner grids) by the dilution factor (10,000) to produce an estimate of pollen grains per ml.

#### 2.2.4.2 *Quantifying Variation in Pollen Viability*

Whilst rarely tested, pollen viability has been reported to vary among individual plants (Oni, 1990). I wanted to quantify the potential viability of pollen grains among *G. macleayana* plants. To do this I used a modified version of the tetrazolium staining technique (Lakon, 1949; Cook and Stanley, 1960) as described by Kearns and Inouye (1993). I did not explore the results of the pollen viability tests statistically, due to my reservations about the usefulness of pollen viability measures as an indication of seed-siring capability, as highlighted by Thomson *et al.* (1994) and Dafni and Firmage (2000). Thomson *et al.* (1994) cautioned that viability tests should only be used if they have a demonstrated correlation with seed-siring capability. I found no relationship

between the mean percentage of coloured (viable) pollen grains per flower and seed production per *G. macleayana* plant (explaining just 0.1% of the variation among plants). Therefore, I have presented the pollen viability study in Appendix 2.

#### 2.2.4.3 Statistical Analyses

I used single factor ANOVAs to test for significant variation among plants in mean inflorescence pollen production (pollen grains per mL). Assumptions of normality and equal variances were tested as described in Section 2.2.2.3. *A posteriori* Tukey-Kramer HSD tests compared plant means for each ANOVA.

### 2.2.5 Floral Reward Trade-offs

I used simple linear correlation and regression analyses to test for significant relationships between measures of inflorescence, nectar, and pollen production (Question 1). I used simple linear correlations analyses to test the significance of the correlations between: (1) inflorescence number (monthly record) and mean inflorescence nectar volume ( $\mu\text{L}$ ) per plant; (2) inflorescence number (monthly record) and mean nectar sugar concentration (%) per plant; (3) inflorescence number (monthly record) and mean inflorescence pollen production (mean number of pollen grains per mL) per plant (at GB only, in January 2004); (4) mean inflorescence size (flowers per inflorescence) and inflorescence number (monthly record) per plant; and (5) mean inflorescence size (flowers per inflorescence) and mean nectar sugar concentration (%) per plant. I used simple linear regressions to test the significance of the relationships between mean inflorescence size (flowers per inflorescence) and mean inflorescence nectar volume ( $\mu\text{L}$ ) per plant.

## 2.3 Results

### 2.3.1 Inflorescence Number and Size

#### 2.3.1.1 Inflorescence Size

There was significant variation among plants in mean inflorescence size (flowers per inflorescence) in two seasons, at each of CB and GB and minimal variation among plants at IL (Table 2.2). In January 2002, there was significant variation among plants in mean inflorescence size, at both CB and GB (Table 2.2). At CB, mean inflorescence size ranged from 33.5 ( $\pm 2.5$ ) on Plant 12 to 50.5 ( $\pm 1.6$ ) on Plant 11 (Figure 2.4). The Tukey-Kramer HSD test revealed that Plant 11 had significantly larger inflorescences

than Plants 8 and 12. At GB, mean inflorescence size ranged from 42.1 ( $\pm 3.1$ ) on Plant 3 to 56.9 ( $\pm 1.4$ ) on Plant 5 (Figure 2.4). The Tukey-Kramer HSD test revealed that Plant 5 was significantly larger than Plants 1, 3, and 4.

In October 2002, I detected significant variation among plants in mean inflorescence size at CB. Substantial (but non-significant) variation was also detected among plants at GB (Table 2.2). At CB, mean inflorescence size ranged from 30.8 ( $\pm 2.8$ ) on Plant 2 to 46.8 ( $\pm 3.4$ ) on Plant N1 (Figure 2.5). The Tukey-Kramer HSD test revealed that Plant N1 and Plant 19 had significantly larger inflorescences than Plants 2, 4, and 12. At GB, mean inflorescence size ranged from 44.1 ( $\pm 2.5$ ) on Plant 1 to 57.2 ( $\pm 0.7$ ) on Plant 10 (Figure 2.5). At IL, mean inflorescence size ranged from 43.8 ( $\pm 2.1$ ) on Plant 16 to 51.8 ( $\pm 3.3$ ) on Plant 8 (Figure 2.5).

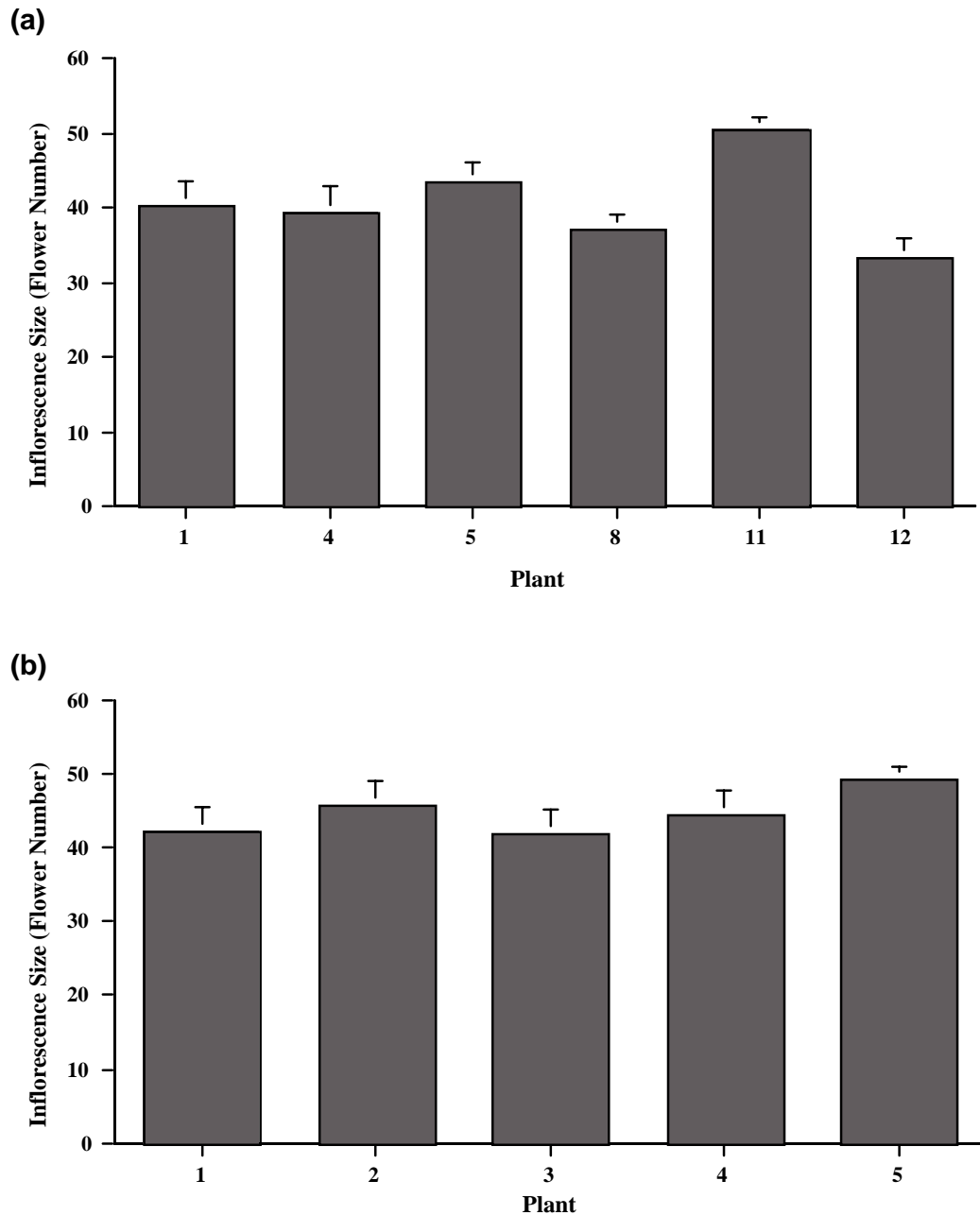
In January 2003, I detected significant variation among plants in mean inflorescence size at GB (Table 2.2). At GB, mean inflorescence size ranged from 41.4 ( $\pm 2.4$ ) on Plant 2 to 54.1 ( $\pm 1.1$ ) on Plant 7 (Figure 2.6). At IL, mean inflorescence size ranged from 36.6 ( $\pm 3.1$ ) on Plant N2 to 46.4 ( $\pm 2.2$ ) on Plant 2. In November 2003, mean inflorescence size ranged from 41.5 ( $\pm 4.0$ ) on Plant N7 to 49.4 ( $\pm 3.4$ ) on Plant 5 and plants were not significantly different (Figure 2.6).



**Table 2.2 - The results of one-way ANOVAs testing for significant variation among *Grevillea macleayana* plants in mean inflorescence size.**

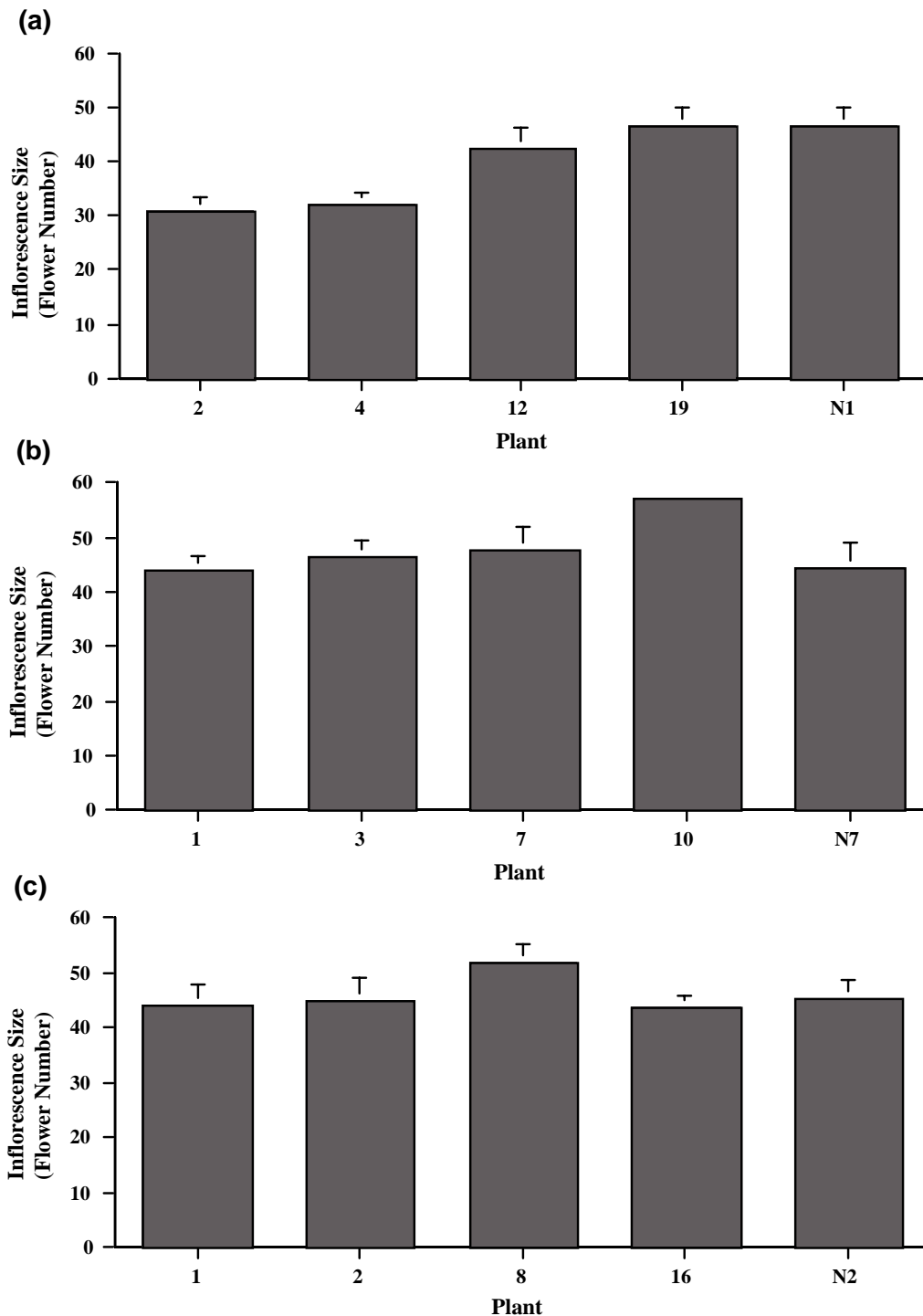
Inflorescence size (flower number per inflorescence) was recorded on eight to nine inflorescences, on each of five to six plants, per site in: (1) January 2002 at Chinamans Beach (CB) and Greenfields Beach (GB); (2) October 2002 at CB, GB and Illowra Lane (IL); (3) January 2003 at GB and IL; and (4) November 2003 at GB. Significant *P* values ( $\alpha < 0.05$ ) are indicated in bold type. An asterisk (\*) indicates ANOVAs comprising square-root transformed data [square-root ( $x + 0.5$ )], due to some non-normality.

Season/Site	Mean Square	<i>F</i> Ratio	df	Probability
<b>January 2002</b>				
Chinamans Beach	270.97	4.95	5	<b>&lt; 0.01</b>
Greenfields Beach	295.53	4.41	4	<b>0.01</b>
<b>October 2002</b>				
Chinamans Beach	542.09	6.86	4	<b>&lt; 0.01</b>
Greenfields Beach*	1.36	2.34	4	0.07
Illowra Lane	88.59	0.92	4	0.46
<b>January 2003</b>				
Greenfields Beach	217.61	5.66	5	<b>&lt; 0.01</b>
Illowra Lane	132.60	2.32	4	0.08
<b>November 2003</b>				
Greenfields Beach	84.84	0.77	5	0.58



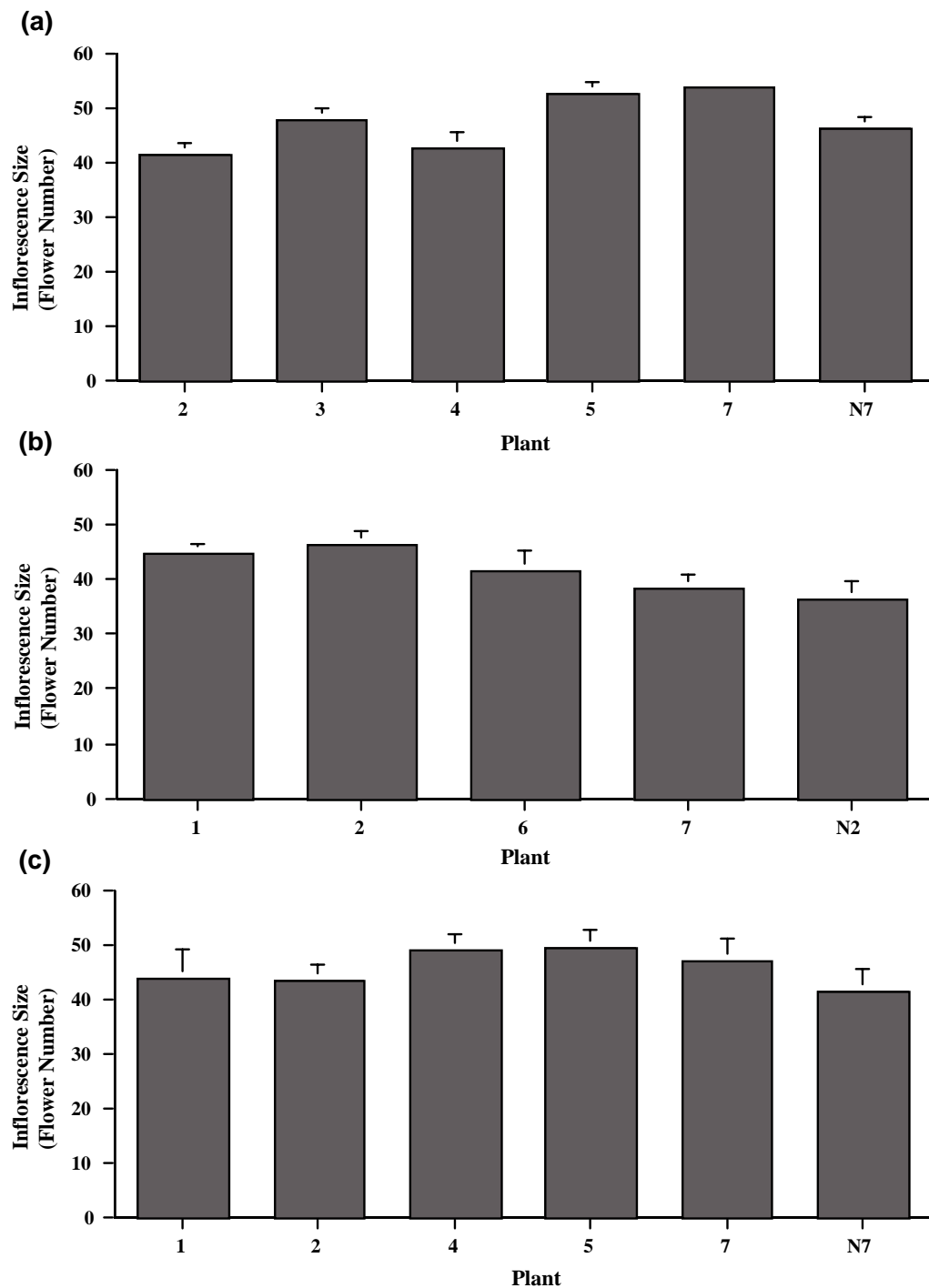
**Figure 2.4 - Mean inflorescence size among *Grevillea macleayana* plants in January 2002.**

The mean inflorescence size (number of flowers per inflorescence) for *G. macleayana* plants at Chinamans Beach (Figure a) and Greenfields Beach (Figure b). Plants are displayed in order of identification code along the x-axis. One-way ANOVAs found significant variation among plants at Chinamans Beach ( $F_5 = 4.95$ ;  $P < 0.01$ ) and Greenfields Beach ( $F_4 = 4.41$ ;  $P = 0.01$ ). Bars indicate plus one standard error.



**Figure 2.5 - Mean inflorescence size among *Grevillea macleayana* plants in October 2002.**

The mean inflorescence size (number of flowers per inflorescence) for *G. macleayana* plants at Chinamans Beach (Figure a), Greenfields Beach (Figure b), and Illowra Lane (Figure c). Plants are displayed in order of identification code along the x-axis. One-way ANOVAs found significant variation among plants at Chinamans Beach ( $F_4 = 6.86$ ;  $P < 0.01$ ). Bars indicate plus one standard error.



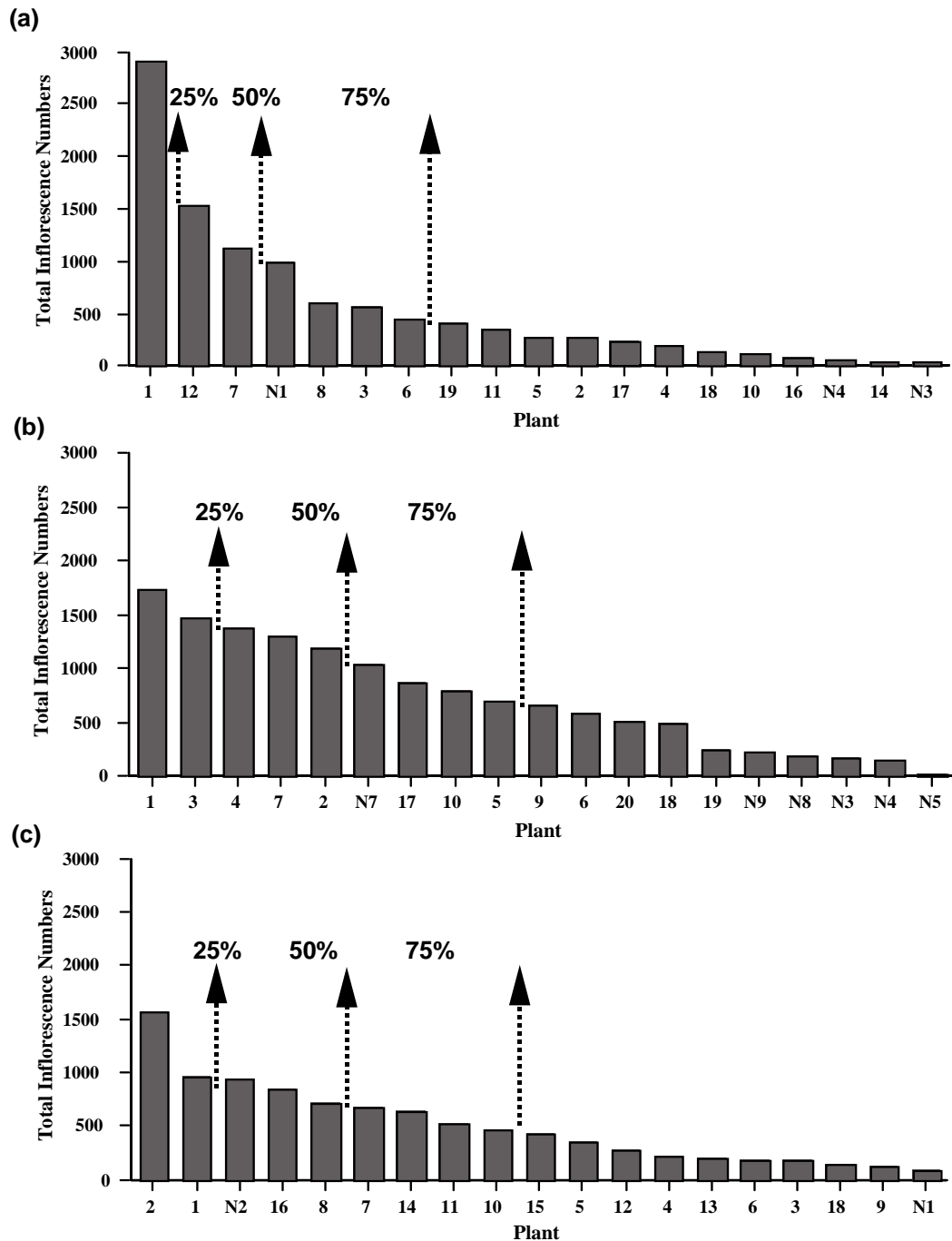
**Figure 2.6 - Mean inflorescence size among *Grevillea macleayana* plants.**

The mean inflorescence size (number of flowers per inflorescence) for *G. macleayana* plants in January 2003 at Greenfields Beach (Figure a) and Illowra Lane (Figure b), and in November 2003 at Greenfields Beach (Figure c). Plants are displayed in order of identification code along the x-axis. One-way ANOVAs found significant variation among plants at Greenfields Beach ( $F_5 = 5.66$ ;  $P < 0.01$ ). Bars indicate plus one standard error.

### 2.3.1.2 *Total Inflorescence Numbers*

There was striking variation among plants at all sites in total inflorescence numbers over the survey period (Figure 2.7). At CB, total inflorescence numbers ranged from just 41 on Plant N3 to 2,912 on Plant 1, a 71-fold difference. At GB, total inflorescence numbers ranged from just 29 on Plant N5 to 1,740 on Plant 1, a 60-fold difference. At IL, total inflorescence numbers ranged from 96 on Plant N1 to 1,566 on Plant 2, a 16-fold difference.

At all sites, I found that three to five plants produced more than 50% of the total survey plant inflorescences and less than half of the survey plants produced more than three-quarters of the total survey plant inflorescences. At CB, Plant 1 produced more than one-quarter (27.6%) of the total survey plant inflorescences. Plants 1, 12, and 7 produced more than half (53.0%) of the total survey plant inflorescences. At GB, Plants 1 and 3 produced nearly one-quarter (23.2%) of the total survey plant inflorescences. Five plants (Plants 1, 3, 4, 7, and 2) produced more than half (51.3%) of the total survey plant inflorescences. At IL, Plants 2 and 1 produced more than one-quarter (26.5%) of the total survey plant inflorescences. Five plants (Plants 2, 1, N2, 16, and 8) produced more than half (52.5%) of the total survey plant inflorescences.



**Figure 2.7 - Total inflorescence numbers per *Grevillea macleayana* plant.**

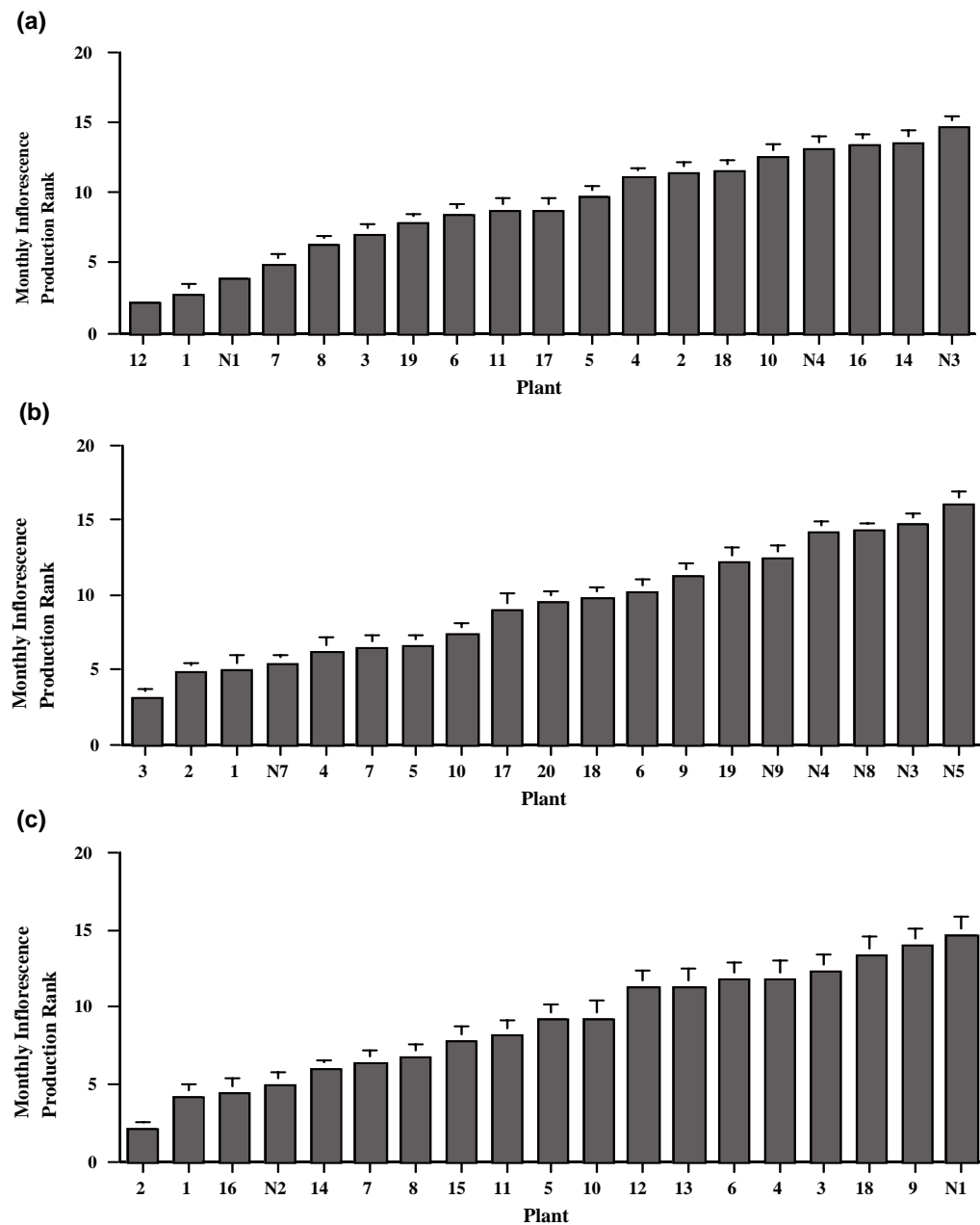
The total number of inflorescences produced per plant for 19 *G. macleayana* plants at Chinamans Beach between June 2002 and May 2004 (24 months - Figure a), Greenfields Beach between July 2002 and May 2004 (23 months - Figure b), and Illowra Lane between July 2002 and December 2003 (18 months - Figure c). Arrows indicate the approximate percentage of survey plant inflorescences produced by the preceding plants. Plants are displayed in descending order of inflorescence production along the x-axis.

### 2.3.1.3 Temporal Patterns of Inflorescence Production

I detected strong patterns of variation among plants at all sites in mean monthly inflorescence production rank, although, the standard errors were very low for all plants (Figure 2.8). These results indicate that whilst plants produced different numbers of inflorescences each month, the rank of each plant per month remained generally constant. I detected significant variation among plants at CB in the mean monthly inflorescence production rank (ANOVA:  $F_{18} = 35.07$ ;  $P < 0.001$ ). The Tukey-Kramer HSD test identified that the plants in the top four positions (P12, 1, N1, and 7) had significantly lower mean monthly ranks than all other plants. I also detected significant variation among plants at GB (ANOVA:  $F_{18} = 25.35$ ;  $P < 0.001$ ), where plants ranked in the top three positions had significantly lower mean monthly ranks than two-thirds of the survey plants. At IL, I also found that significant variation among plants (ANOVA:  $F_{18} = 15.15$ ;  $P < 0.001$ ) and plants ranked in the top two positions had significantly lower mean monthly ranks than two-thirds of the survey plants.

At all sites, in months of good flowering (July to January), the plants that produced the most inflorescences generally ranked very well and the poorest inflorescence producing plants consistently ranked very poorly (Figure 2.9). However, from February to June there was much less differentiation between, because most plants produced very few (if any) inflorescences. I have described these patterns in detail below and have illustrated them using the three plants with the best inflorescence production and the two with the worst, from each site (Figure 2.9).

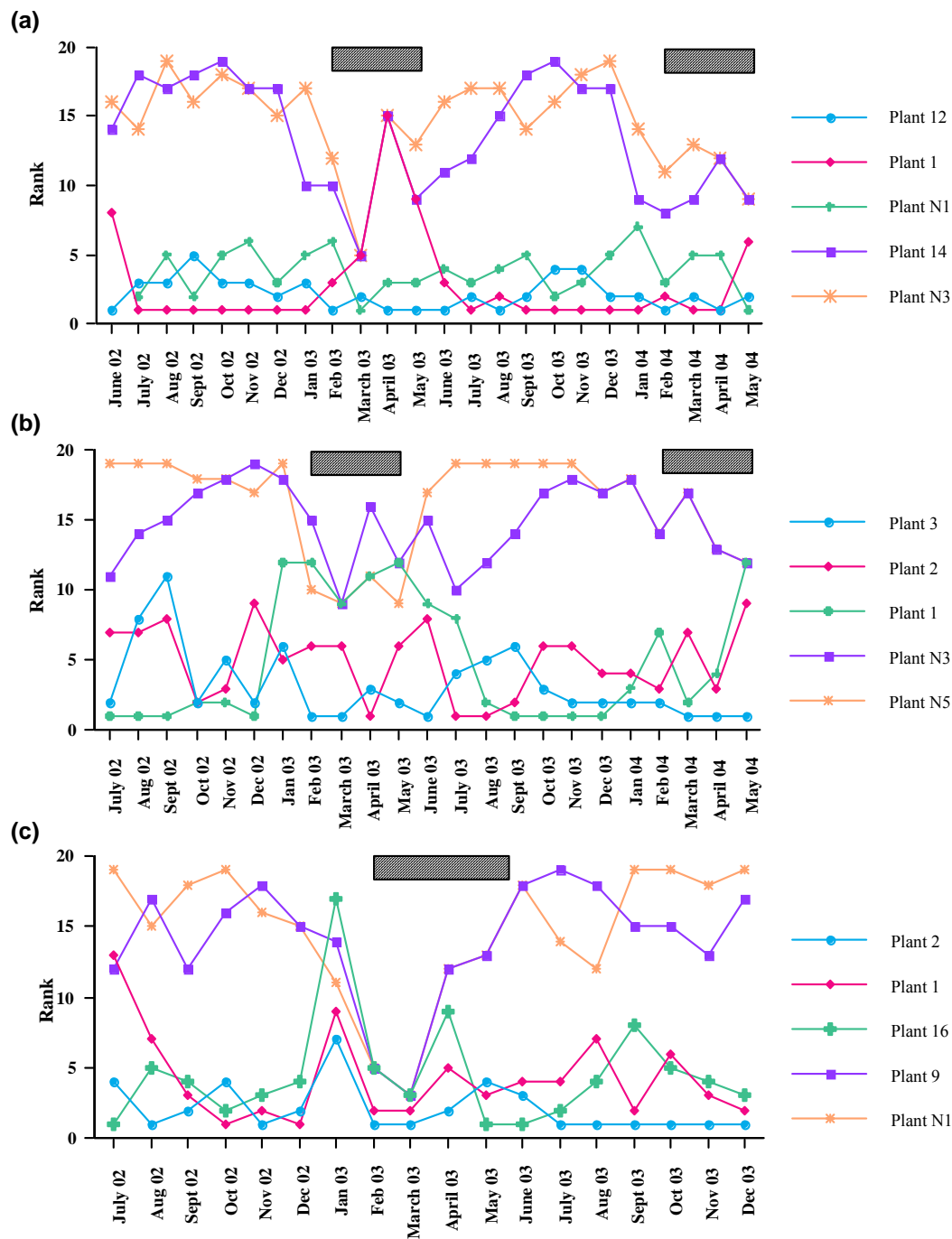
At CB, Plants 1, 12, and N1 (ranked first, second and third) were consistently good inflorescence producers, with the exception of Plant 1 in months of poor flowering, when it did very poorly (Figure 2.9). At GB, Plants 3, 2, and 1 (ranked first, second and third) were consistently good inflorescence producers, in months of good flowering (Figure 2.9). In months of poor flowering, plants generally produced more inflorescences than at CB and IL. At IL, Plants 2, 1, and 16 (ranked first, second and third) were consistently good inflorescence producers, in months of good flowering (Figure 2.9). The plants with the poorest inflorescence production (Plants 9 and N1, ranked second last and last, respectively) consistently did very poorly, regardless of the month.



**Figure 2.8 - Mean monthly inflorescence production rank of *Grevillea macleayana* plants.**

The mean monthly inflorescence production rank of nineteen *G. macleayana* plants at Chinamans Beach between June 2002 and May 2004 (24 months - Figure a), Greenfields Beach between July 2002 and May 2004 (23 months - Figure b) and Illowra Lane between June 2002 and December 2003 (18 months - Figure c). Plants are displayed in ascending order of mean monthly inflorescence production rank, along the x-axis. Significant variation was detected among plants at CB (ANOVA:  $F_{18} = 35.07$ ;  $P < 0.001$ ), GB (ANOVA:  $F_{18} = 25.35$ ;  $P < 0.001$ ), and IL (ANOVA:  $F_{18} = 15.15$ ;  $P < 0.001$ ). Bars represent plus standard error.





**Figure 2.9 - The monthly inflorescence production rank for the three *Grevillea macleayana* plants with the best inflorescence production, and the two plants with the poorest (based on mean monthly ranks).**

Data are shown for plants from Chinamans Beach between June 2002 and May 2004 (Figure a), Greenfields Beach between June 2002 and May 2004 (Figure b) and Illowra Lane between June 2002 and December 2003 (Figure c). Hatched areas indicate months of poor inflorescence production at each site.

### 2.3.2 Nectar Production

#### 2.3.2.1 Nectar Volume

Generally, nectar production began on the first day that the flowers on an inflorescence opened. However, I occasionally measured a small amount of nectar on the day prior to the first flowers opening. Generally between 5% and 15% of flowers opened of the first day, although I did record up to 27% of flowers open on the first day. Nectar production then generally increased for the next two to three days and rapidly decreased thereafter. Nectar production continued for up to two days after all the flowers on an inflorescences were open. However, such nectar production was generally minimal and usually ceased the day after all flowers were open. Whilst I did not measure this specifically, it is also clear that flowers produce some nectar during the start of the female phase. However, this is minimal and generally appeared to last for no longer than one day.

In all seasons and sites, I found that plants varied substantially in mean nectar volume per inflorescence. In January 2002, I quantified nectar production on plants at CB and GB over five to seven days. At CB mean nectar volume per inflorescence ranged from 181.0  $\mu\text{L}$  ( $\pm 36.7$ ) in Plant 8 to 312.5  $\mu\text{L}$  ( $\pm 43.5$ ) in Plant 12, and this variation was marginally significant ( $P = 0.05$ ) (Table 2.3; Figure 2.10). At GB, mean nectar volume per inflorescence ranged from 301.8  $\mu\text{L}$  ( $\pm 45.4$ ) in Plant 2 to 532.5  $\mu\text{L}$  ( $\pm 45.9$ ) in Plant 5, and plants varied significantly (Table 2.3; Figure 2.10). The Tukey-Kramer HSD test revealed that Plant 5 was significantly greater than Plants 1 and 2.

In October 2002, I quantified nectar production on plants at all three sites, over two days. I detected significant variation among plants in mean nectar volume per inflorescence (totalled over two days) at CB and IL (Table 2.3; Figure 2.11). At CB, mean nectar volume per inflorescence ranged from 107.3  $\mu\text{L}$  ( $\pm 27.5$ ) in Plant 4 to 342.4  $\mu\text{L}$  ( $\pm 63.5$ ) in Plant N1 (Figure 2.11). The Tukey-Kramer HSD test revealed that Plant N1 had significantly greater inflorescence nectar production than all other plants at CB. At GB, mean nectar volume per inflorescence was substantially less than at CB, and ranged from just 45.4  $\mu\text{L}$  ( $\pm 9.2$ ) in Plant 1 to 87.1  $\mu\text{L}$  ( $\pm 15.3$ ) in Plant 3 (Figure 2.11). At IL, mean nectar volume per inflorescence varied more among plants than at CB and GB, and ranged from 50.1  $\mu\text{L}$  ( $\pm 8.5$ ) in Plant 8 to 374.4  $\mu\text{L}$  ( $\pm 55.3$ ) in Plant N2 (Figure

2.11). The Tukey-Kramer HSD test revealed that Plant N2 and Plant 1 had significantly greater inflorescence nectar production than Plant 8.

In January 2003, I quantified nectar production on plants at GB and IL over two days, and detected significant variation among plants in mean nectar volume per inflorescence (totalled over two days) at IL (Table 2.3; Figure 2.12). At GB, mean nectar volume per inflorescence ranged from 73.4  $\mu\text{L}$  ( $\pm 9.7$ ) in Plant 2 to 136.8  $\mu\text{L}$  ( $\pm 27.5$ ) in Plant 5 (Figure 2.12). At IL, mean nectar volume per inflorescence ranged from just 91.7  $\mu\text{L}$  ( $\pm 22.0$ ) in Plant 6 to 230.8  $\mu\text{L}$  ( $\pm 54.1$ ) in Plant 2 (Figure 2.12). In November 2003, I quantified nectar production on plants at GB over three days, and detected significant variation among plants, totalled over three days (Table 2.3; Figure 2.13). Mean nectar volume per inflorescence ranged from 65.9  $\mu\text{L}$  ( $\pm 13.6$ ) in Plant 2 to 227.6  $\mu\text{L}$  ( $\pm 32.1$ ) in Plant 4 (Figure 2.13).

#### 2.3.2.2 *Sugar Concentration*

In all seasons and sites, the variation among plants in the sugar concentration of nectar was smaller than the variation in nectar volume. At CB, in January 2002, the mean sugar concentration of nectar per inflorescence ranged from 13.2% ( $\pm 0.4$ ) in Plant 5 to 15.6% ( $\pm 0.54$ ) in Plant 12, and these differences were statistically significant (Table 2.3; Figure 2.10). The Tukey-Kramer HSD test revealed that Plant 12 had significantly greater sugar concentration of nectar than Plant 5. In January 2002 at GB, the mean sugar concentration of nectar per inflorescence varied less among plants than at CB, and ranged from 14.0% ( $\pm 0.4$ ) in Plant 4 to 14.8% ( $\pm 0.5$ ) in Plant 3 (Table 2.3; Figure 2.10).

In October 2002, I detected moderate variation among plants in the mean sugar concentration of nectar per inflorescence. This variation was marginally significant at CB ( $P = 0.05$ ) and significant at GB (Table 2.3; Figure 2.11). At CB, the mean sugar concentration of nectar per inflorescence ranged from 20.2% ( $\pm 1.9$ ) in Plant 4 to 28.6% ( $\pm 2.4$ ) in Plant 12 (Figure 2.11). At GB, the mean sugar concentration of nectar per inflorescence ranged from just 23.8% ( $\pm 1.1$ ) in Plant N7 to 28.9 ( $\pm 1.2$ ) in Plant 7 (Figure 2.11).

In January 2003, I detected significant variation among plants at GB and moderate, but non-significant variation among plants at IL (Table 2.3; Figure 2.12). At GB, the mean sugar concentration of nectar per inflorescence ranged from 25.0% ( $\pm 0.6$ ) in Plant N7 to 30.5% ( $\pm 0.4$ ) in Plant 2 (Figure 2.12). At IL, the mean sugar concentration of nectar per inflorescence ranged from 27.3% ( $\pm 1.0$ ) in Plant N2 to 30.8 % ( $\pm 1.4$ ) in Plant 1 (Figure 2.12). In November 2003, I detected minimal variation among plants at GB in the mean sugar concentration of nectar per inflorescence and no significant variation was detected (Table 2.3; Figure 2.13). The mean sugar concentration of nectar per inflorescence ranged from 22.9 % ( $\pm 1.6$ ) in Plant N7 to 27.3 % ( $\pm 1.0$ ) in Plant 4 (Figure 2.13).

**Table 2.3 - The results of one-way ANOVAs testing for significant variation among *Grevillea macleayana* plants in nectar production.**

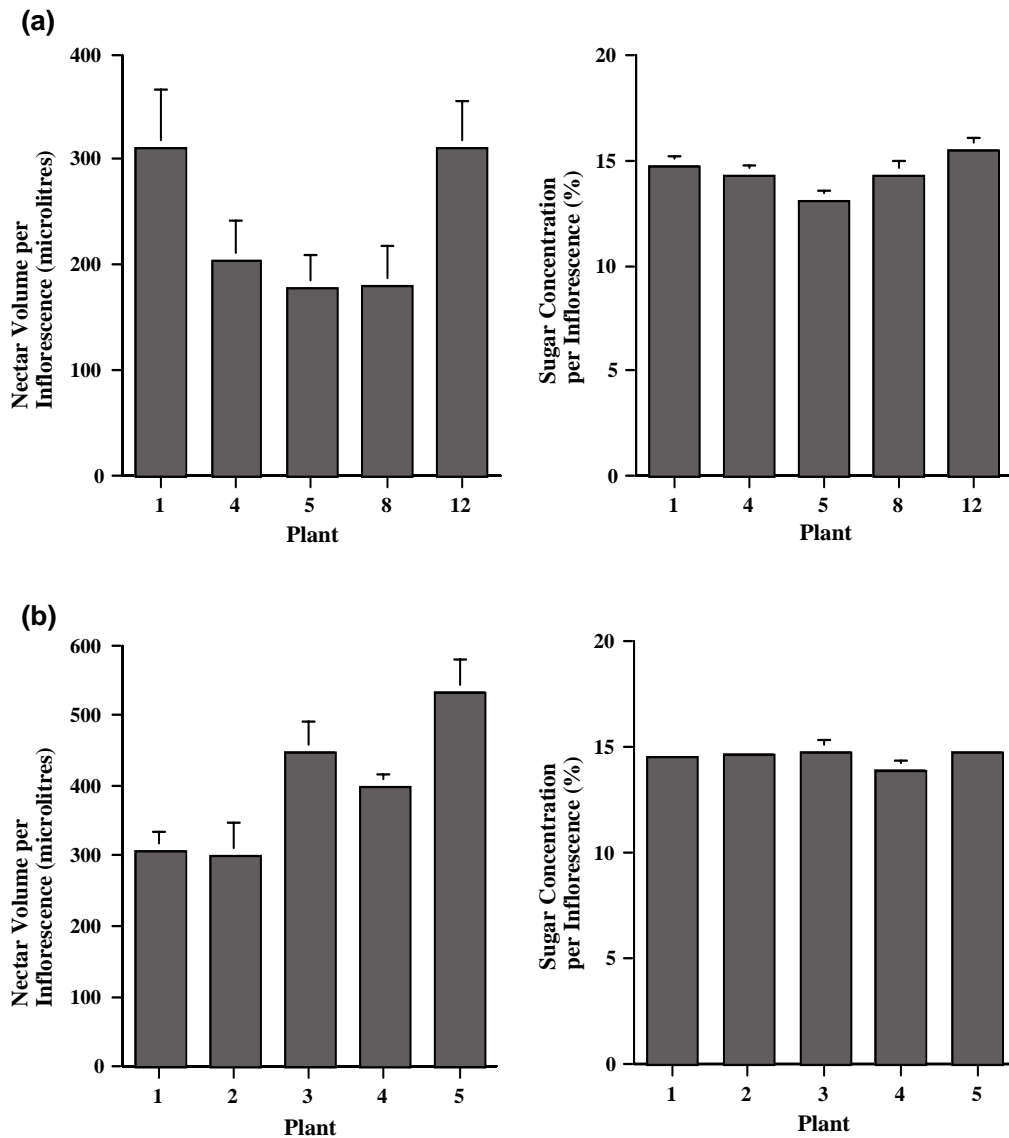
Variation was quantified among plants in (a) mean nectar volume ( $\mu\text{L}$ ) per inflorescence and (b) mean sugar concentration of nectar (%) per inflorescence. Nectar production was measured on five to six plants per site, over: (1) four to seven days in January 2002, (2) two days in October 2002, (3) two days in January 2003, and (4) three days in January 2003. Significant  $P$  values ( $\alpha < 0.05$ ) are indicated in bold type. An asterisk (\*) indicates ANOVAs comprising log transformed data [ $\log(x + 1)$ ], due to some heteroscedasticity.

**(a)**

Season/Site	Mean Square	F Ratio	df (Model)	Probability
<b>January 2002</b>				
Chinamans Beach	31205.4	2.70	4	0.05
Greenfields Beach	70417.3	7.12	4	< <b>0.01</b>
<b>October 2002</b>				
Chinamans Beach	75622.6	5.60	4	< <b>0.01</b>
Greenfields Beach	3013.56	2.48	4	0.06
Illowra Lane*	0.88	4.66	4	< <b>0.01</b>
<b>January 2003</b>				
Greenfields Beach	3805.82	2.33	5	0.06
Illowra Lane	30288.8	3.06	4	<b>0.03</b>
<b>November 2003</b>				
Greenfields Beach*	0.57	8.99	5	< <b>0.01</b>

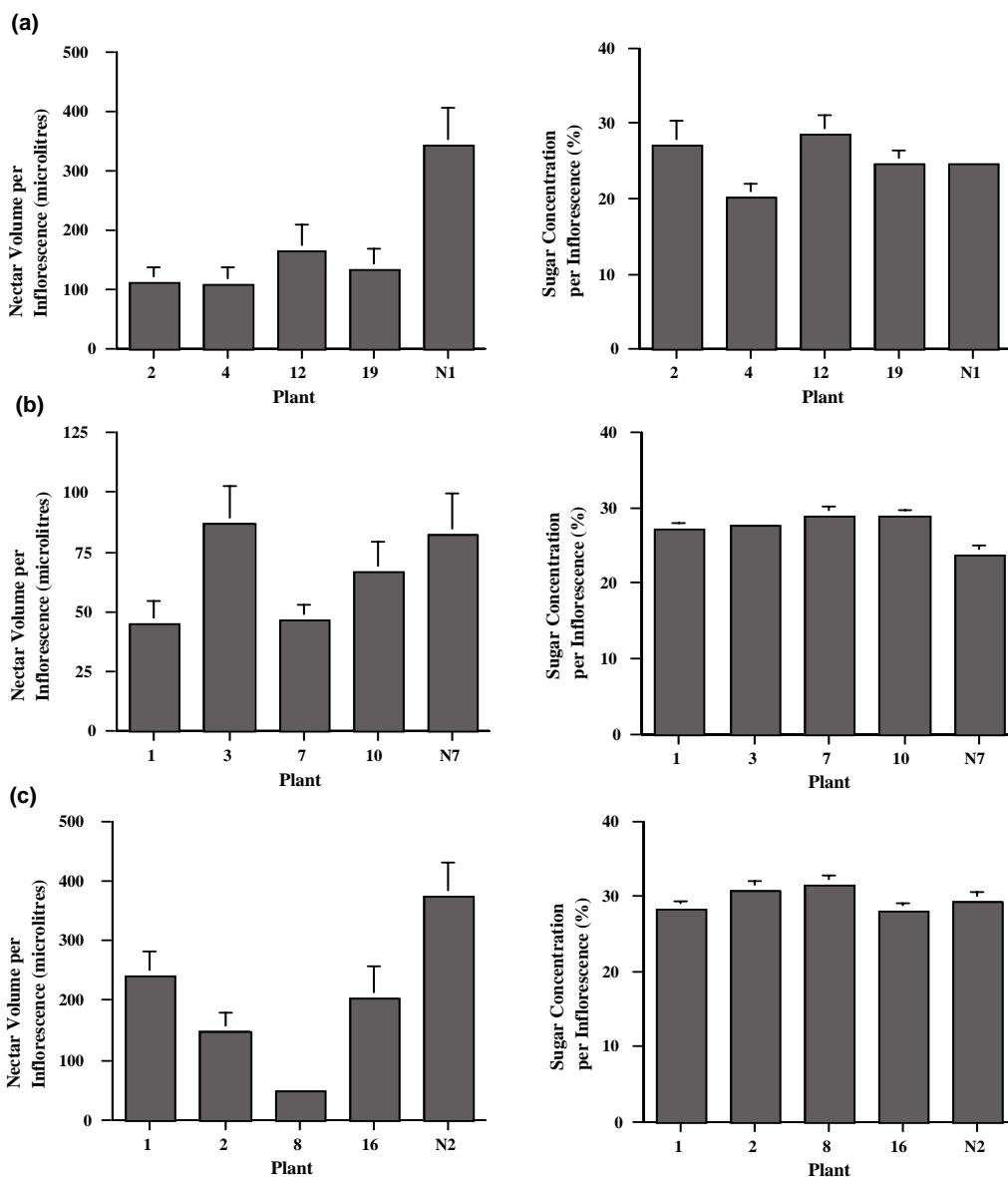
**(b)**

Season/Site	Mean Square	F Ratio	df (Model)	Probability
<b>January 2002</b>				
Chinamans Beach	4.97	2.80	4	<b>0.04</b>
Greenfields Beach	0.92	1.17	4	0.34
<b>October 2002</b>				
Chinamans Beach	81.14	2.61	4	0.05
Greenfields Beach	32.38	4.56	4	< <b>0.01</b>
Illowra Lane	18.06	1.90	4	0.14
<b>January 2003</b>				
Greenfields Beach	30.82	4.96	5	< <b>0.01</b>
Illowra Lane	19.14	2.25	4	0.09
<b>November 2003</b>				
Greenfields Beach	16.20	1.88	5	0.12



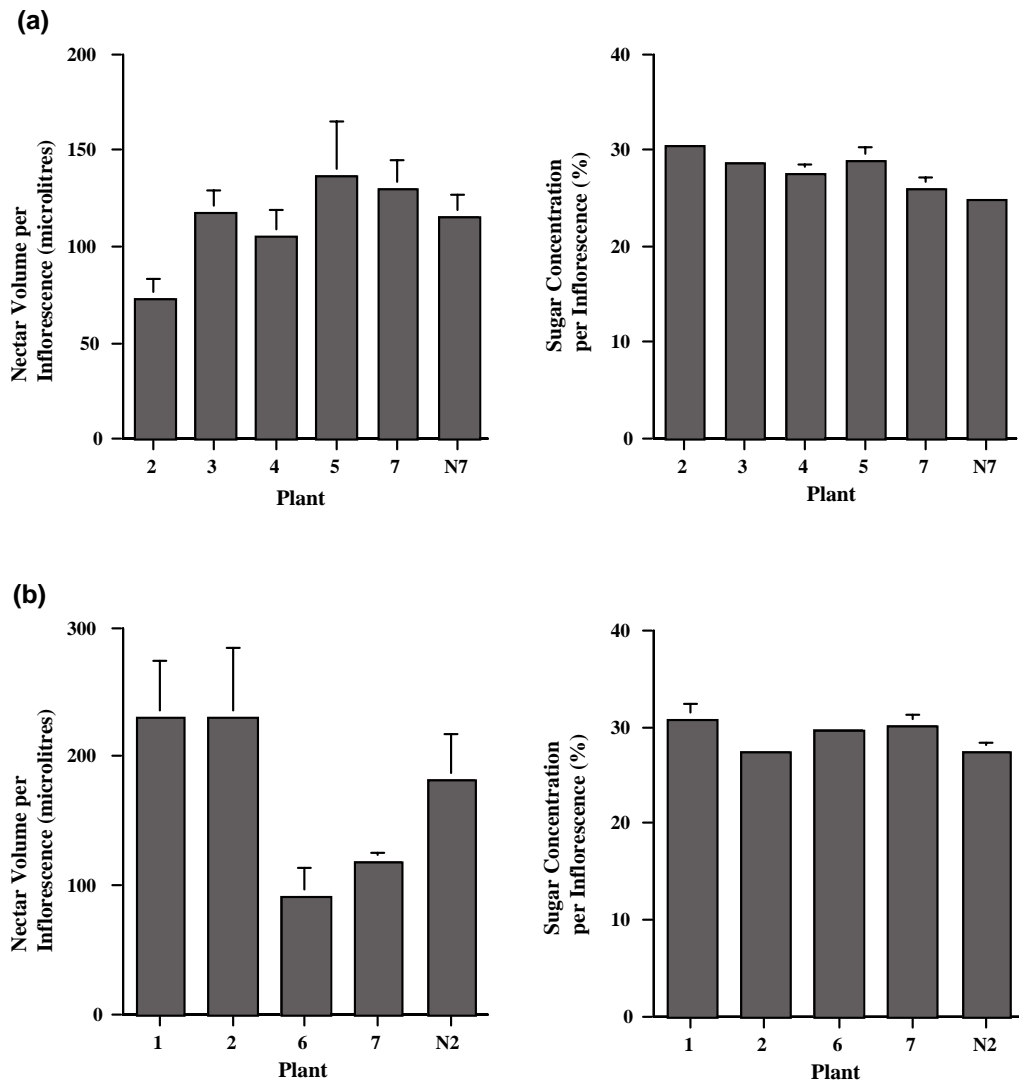
**Figure 2.10 - Mean inflorescence nectar production among *Grevillea macleayana* plants in January 2002.**

The mean nectar volume ( $\mu\text{L}$ ) per inflorescence (left column) and the mean sugar concentration (%) of nectar per inflorescence (right column), quantified on *G. macleayana* plants at Chinamans Beach (CB - Figure a) and Greenfields Beach (GB - Figure b). Plants are displayed in order of identification code, along the x-axis. One-way ANOVAs revealed significant variation among plants in nectar volume at CB ( $F_4 = 2.70$ ;  $P < 0.05$ ) and GB ( $F_4 = 7.12$ ;  $P < 0.001$ ) and in sugar concentration at CB ( $F_4 = 2.80$ ;  $P < 0.04$ ). Bars represent plus one standard error.



**Figure 2.11 - Mean inflorescence nectar production among *Grevillea macleayana* plants in October 2002.**

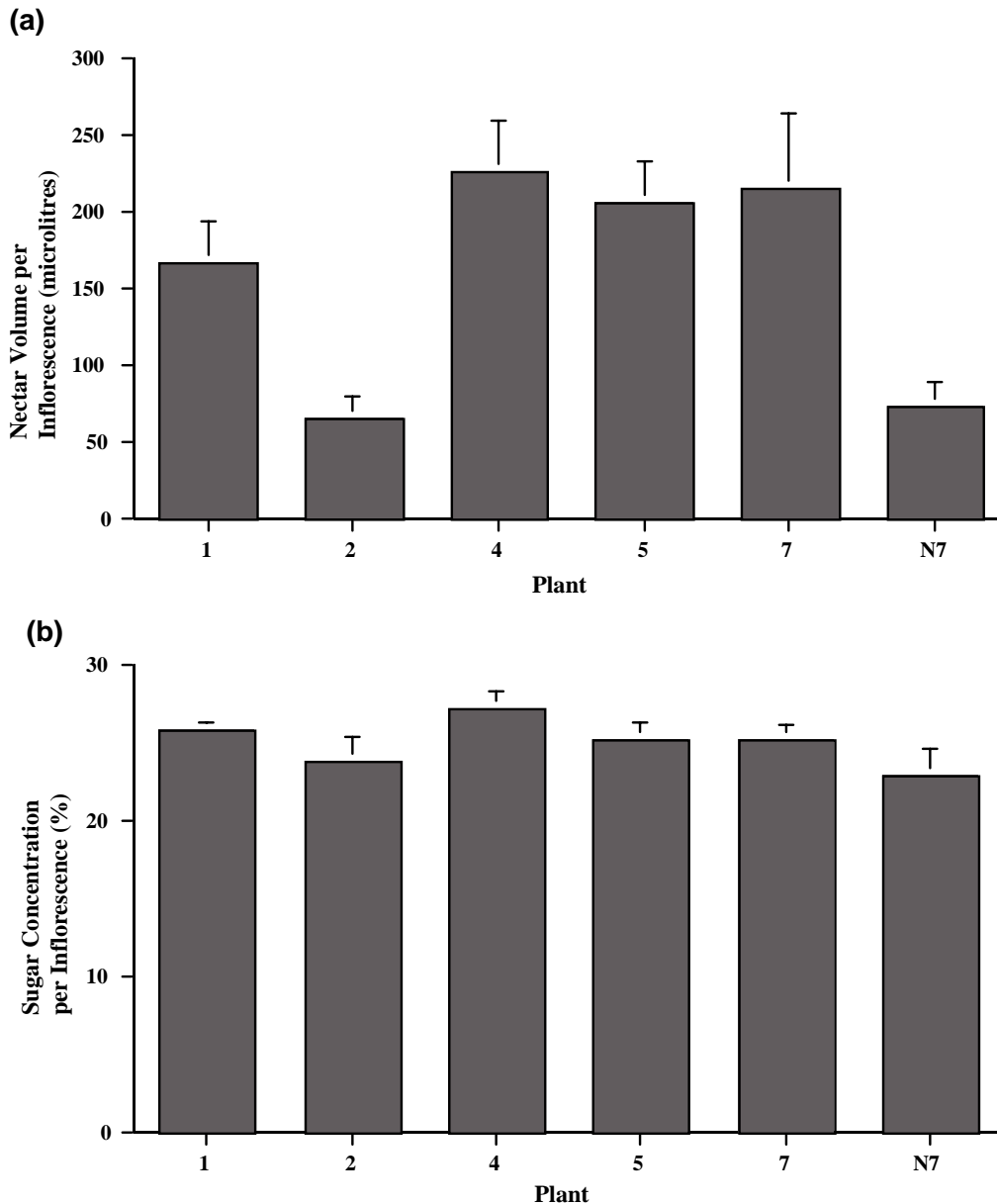
The mean nectar volume (μL) per inflorescence (left column) and the mean sugar concentration (%) of nectar per inflorescence (right column), quantified on *G. macleayana* plants at Chinamans Beach (CB – Figure a), Greenfields Beach (GB – Figure b), and Illowra lane (IL – Figure c). Plants are displayed in order of identification code, along the x-axis. One-way ANOVAs revealed significant variation among plants in nectar volume at CB ( $F_4 = 5.60$ ;  $P < 0.001$ ) and IL ( $F_4 = 4.66$ ;  $P < 0.01$ ) and in nectar sugar concentration at GB ( $F_4 = 4.56$ ;  $P < 0.01$ ). Bars represent plus one standard error.



**Figure 2.12 - Mean inflorescence nectar production among *Grevillea macleayana* plants in January 2003.**

The mean nectar volume ( $\mu\text{L}$ ) per inflorescence (left column) and the mean sugar concentration (%) of nectar per inflorescence (right column), quantified on *G. macleayana* plants at Greenfields Beach (GB – Figure a) and Illowra Lane (IL – Figure b). Plants are displayed in order of identification code, along the x-axis. One-way ANOVAs revealed significant variation among plants in nectar volume at IL ( $F_4 = 3.06$ ;  $P < 0.03$ ) and sugar concentration at GB ( $F_5 = 4.96$ ;  $P < 0.01$ ). Bars represent plus one standard error.



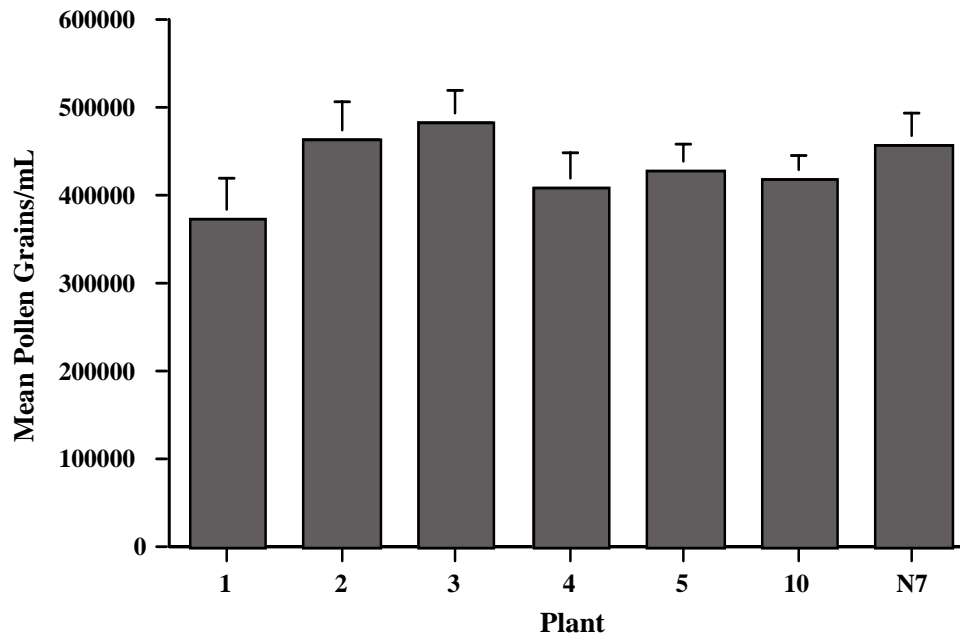


**Figure 2.13 - Mean inflorescence nectar production among *Grevillea macleayana* plants in November 2003.**

The mean nectar volume (μL) per inflorescence (Figure a) and the mean sugar concentration (%) of nectar per inflorescence (Figure b), quantified on *G. macleayana* plants at Greenfields Beach. Plants are displayed in order of identification code, along the x-axis. One-way ANOVAs revealed significant variation among plants in nectar volume ( $F_5 = 5.46$ ;  $P < 0.001$ ). Bars represent plus one standard error.

### 2.3.3 Pollen Production

The mean number of pollen grains per mL, per inflorescence, did not vary significantly among plants, ranging from 372,750 ( $\pm 45,389$ ) in Plant 1 to 482,222 ( $\pm 34,931$ ) in Plant 3 (Figure 2.14).



**Figure 2.14 - The mean number of pollen grains per mL of ethanol solution per inflorescence, for each of seven *Grevillea macleayana* plants.**

Pollen was estimated from fresh pollen bundles, using a hemacytometer and sampled from inflorescences on plants at Greenfields Beach in February 2004. Plants are displayed in order of identification code, along the x-axis. Bars indicate plus one standard error.

### 2.3.4 Floral Reward Trade-offs

I found that eight of the twelve correlations between inflorescence number and both nectar volume and nectar sugar concentration were positive (Table 2.4a). The correlation between inflorescence number and nectar volume, at CB in October 2002, revealed a significant positive correlation ( $r = 0.98$ ;  $n = 5$ ;  $P < 0.01$  - Figure 2.15a). However, this was driven by a single outlier and when removed the correlation was no longer significant ( $r = 0.72$ ;  $n = 4$ ;  $P > 0.05$ ). Therefore, no emphasis will be placed on

this result. The two correlations between inflorescence number and pollen production were not significant and did not reveal the same trend (Table 2.4a).

Whilst I detected no single trend (i.e. positive or negative) between inflorescence size and inflorescence number per plant, I detected negative trends in four out of the six correlations (Table 2.4b). Moreover, two of these negative correlations were significant, at GB in October 2002 ( $r = -0.99$ ;  $n = 5$ ;  $P < 0.01$ ) and at IL in January 2003 ( $r = -0.91$ ;  $n = 5$ ;  $P = 0.03$ ) (Figure 2.15). The remainder of these correlations were not significant and were evenly split between positive and negative correlations.

I found that five of the eight regressions between inflorescence size and mean inflorescence nectar volume were positive (Table 2.4b). Two of these regressions revealed significant positive regressions at GB in January 2003 ( $r^2 = 0.80$ ;  $F_{1,5} = 16.23$ ;  $P = 0.02$ ) and November 2003 ( $r^2 = 0.79$ ;  $F_{1,5} = 15.01$ ;  $P = 0.02$ ) (Figure 2.15). I did not detect significant correlations between inflorescence size and nectar sugar concentration, although, five of the eight correlations displayed positive trends.

**Table 2.4 - Simple linear correlations testing the significance of relationships between measures of inflorescence production, nectar production, and pollen production, as recorded on *Grevillea macleayana* plants.**

Simple linear correlations tested the relationships between inflorescence number per month and: (1) mean inflorescence nectar volume ( $\mu\text{L}$ ), (2) mean nectar sugar concentration (%) per inflorescence and (3) mean inflorescence pollen production (grains per mL) (Table a). Simple linear correlations tested the relationships between mean inflorescence size and: (1) inflorescence number and (2) the mean nectar sugar concentration per inflorescence (Table b). Simple linear regressions tested the relationships between mean inflorescence size and mean inflorescence nectar volume (Table b). Inflorescence number was recorded monthly between February 2002 and May 2004 at Chinamans Beach (CB) and Greenfields Beach (GB), and between July 2002 and December 2003 at Illowra Lane (IL). Studies on nectar production and inflorescence size were conducted at CB and GB in January 2002; at CB, GB, and IL in October 2002; at GB and IL in January 2003; and at GB in November 2003. Studies on pollen production were conducted at GB in January 2004. Significant  $P$  values ( $\alpha < 0.05$ ) are in bold type.

(a)

<b>Inflorescence Number &amp; Mean Inflorescence Nectar Volume (<math>\mu\text{L}</math>) per Plant</b>				
<b>Site/Season</b>	<b><math>r</math></b>	<b><math>n</math></b>	<b>Probability</b>	<b>Trend</b>
<b>Chinamans Beach</b>				
October 2002	0.98	5	<b>&lt; 0.01</b>	Positive
<b>Greenfields Beach</b>				
October 2002	0.08	5	0.90	Slight Positive
January 2003	0.158	6	0.76	Positive
November 2003	0.071	6	0.90	Slight Positive
<b>Illowra Lane</b>				
October 2002	-0.10	5	0.86	Negative
January 2003	-0.20	5	0.74	Negative
<b>Inflorescence Number &amp; Mean Inflorescence Nectar Sugar Concentration (%) per Plant</b>				
<b>Site/Season</b>	<b><math>r</math></b>	<b><math>n</math></b>	<b>Probability</b>	<b>Trend</b>
<b>Chinamans Beach</b>				
October 2002	0.17	5	0.76	Positive
<b>Greenfields Beach</b>				
October 2002	-0.71	5	0.18	Negative
January 2003	0.17	6	0.75	Positive
November 2003	0.44	6	0.39	Positive
<b>Illowra Lane</b>				
October 2002	0.26	5	0.66	Positive
January 2003	-0.41	5	0.49	Negative
<b>Inflorescence Number &amp; Mean Inflorescence Pollen Grains (per mL) per Plant</b>				
<b>Site/Season</b>	<b><math>r</math></b>	<b><math>n</math></b>	<b>Probability</b>	<b>Trend</b>
<b>Greenfields Beach</b>				
January 2004 <sup>a</sup>	0.00	7	0.95	Neutral
January 2004 <sup>b</sup>	0.63	7	0.13	Positive

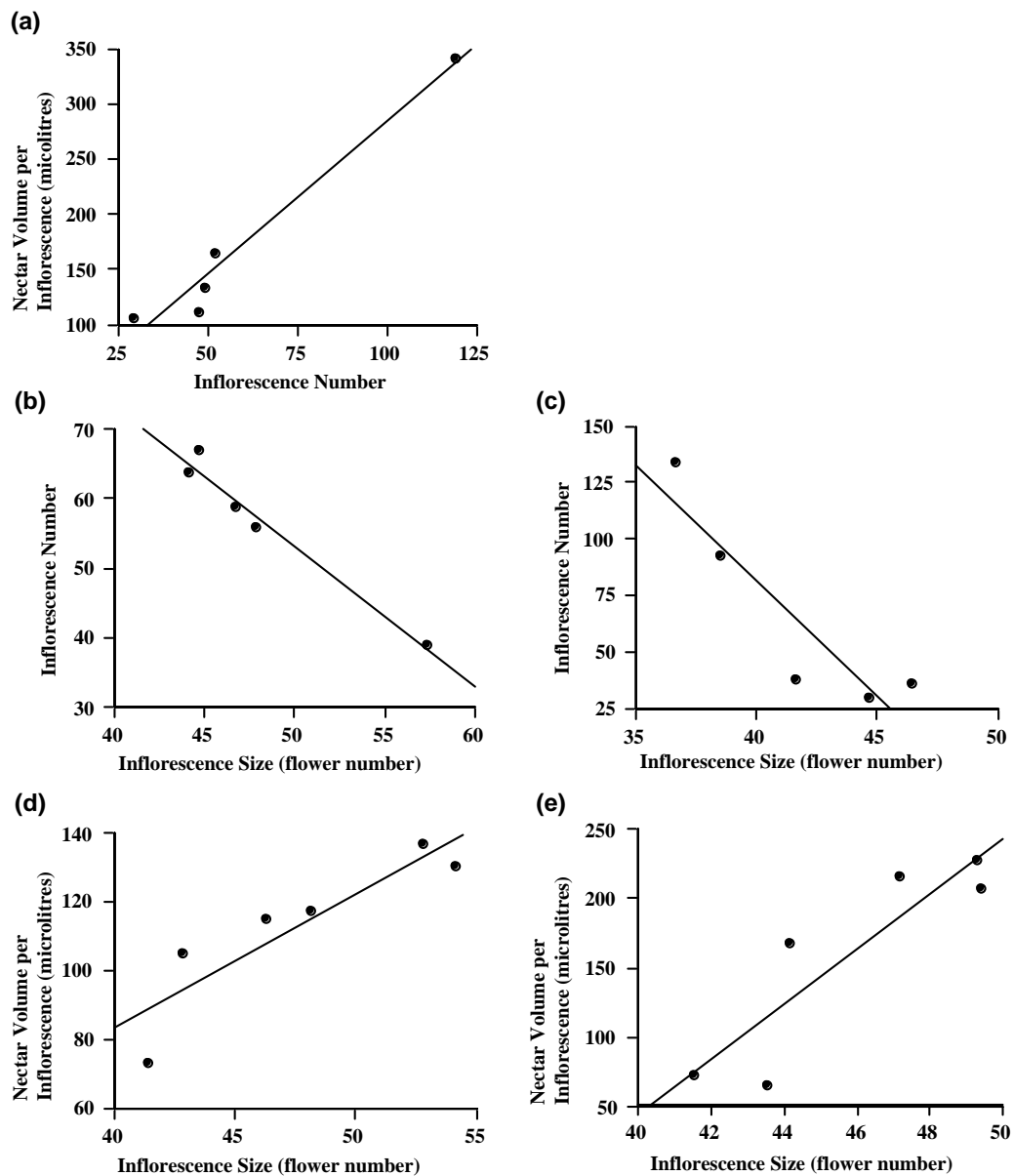
a = Inflorescence production recorded on January 16<sup>th</sup>

b = Inflorescence production recorded on February 14<sup>th</sup>

(b)

Mean Inflorescence Size & Inflorescence Number per Plant					
Site/Season	<i>r</i>	<i>n</i>	Probability	Trend	
<b>Chinamans Beach</b>					
October 2002	0.67	5	0.21	Positive	
<b>Greenfields Beach</b>					
October 2002	-0.98	5	< <b>0.01</b>	Negative	
January 2003	0.24	6	0.65	Positive	
November 2003	-0.2	6	0.69	Negative	
<b>Illohra Lane</b>					
October 2002	-0.32	5	0.60	Negative	
January 2003	-0.91	5	<b>0.03</b>	Negative	
Mean Inflorescence Size & Mean Inflorescence Nectar Volume (μL) per Plant					
Site/Season	<i>r</i> <sup>2</sup>	df*	<i>F</i> Ratio	Probability	Trend
<b>Chinamans Beach</b>					
January 2002	0.25	1, 4	1.00	0.39	Negative
October 2002	0.43	1, 4	2.23	0.23	Positive
<b>Greenfields Beach</b>					
January 2002	0.46	1, 4	2.60	0.21	Positive
October 2002	0.00	1, 4	0.00	0.07	Neutral
January 2003	0.80	1, 5	16.23	<b>0.02</b>	Positive
November 2003	0.79	1, 5	15.01	<b>0.02</b>	Positive
<b>Illohra Lane</b>					
October 2002	0.42	1, 4	2.13	0.24	Negative
January 2003	0.30	1, 4	1.31	0.33	Positive
Mean Inflorescence Size & Inflorescence Nectar Sugar Concentration (%) per Plant					
Site/Season	<i>r</i>	<i>n</i>	Probability	Trend	
<b>Chinamans Beach</b>					
January 2002	-0.88	5	0.05	Negative	
October 2002	-0.10	5	0.90	Negative	
<b>Greenfields Beach</b>					
January 2002	0.2	5	0.74	Positive	
October 2002	-0.58	5	0.31	Negative	
January 2003	0.35	6	0.49	Positive	
November 2003	0.77	6	0.07	Positive	
<b>Illohra Lane</b>					
October 2002	0.78	5	0.12	Positive	
January 2003	0.1	5	0.86	Slight Positive	

\* Model degrees of freedom, Error degrees of freedom.



**Figure 2.15 - Simple linear correlation and regression analyses between measures of inflorescence and nectar production, for *Grevillea macleayana* plants.**

Simple linear correlation analyses between: inflorescence number and mean nectar volume per inflorescence ( $\mu\text{L}$ ) for plants at Chinamans Beach (CB), in October 2002 (Figure a); and between mean inflorescence size (flowers per inflorescence) and inflorescence number for plants at Greenfields Beach (GB), in October 2002 (Figure b) and at Illowra Lane (IL), in January 2003 (Figure c). Significant correlations were detected at CB ( $r = 0.98$ ;  $n = 5$ ;  $P < 0.01$ ), however, this correlation was driven by a single outlier and when removed the correlation was no longer significant. Significant correlations were also detected at GB ( $r = -0.98$ ;  $n = 5$ ;  $P < 0.01$ ), and IL ( $r = -0.91$ ;  $n = 5$ ;  $P = 0.03$ ) (Table 2.4). Simple linear regression analyses detected significant relationships between mean inflorescence size and mean nectar volume per inflorescence ( $\mu\text{L}$ ) at GB in January and November 2003 (Figure d:  $r^2 = 0.80$ ;  $F_{1,5} = 16.23$ ;  $P = 0.02$  and Figure e:  $r^2 = 0.79$ ;  $F_{1,5} = 15.01$ ;  $P = 0.02$ ) (Table 2.4).

## 2.4 Discussion

My analysis of inflorescence production over two years revealed striking, but consistent patterns of variation among plants. Moreover, it is clear that some plants sustained high levels of inflorescence production, and other plants low, over two years. I also found that a small number of plants produced a substantial proportion of the total inflorescence production for the study plants. As predicted, I detected significant patterns of variation in inflorescence nectar volume, in one to two seasons at each site. Importantly, I detected significant variation among plants in nectar volume whether it was quantified over two, three, or up to seven days. This indicates that the variation among plants is not only day-to-day, but also over the lifetime of an inflorescence. As predicted I did not find significant variation among plants with respect to pollen production.

In the following sections, I discuss the variation among plants in inflorescence, nectar, and pollen production, and the potential effects that intraspecific variation may have on pollinator foraging behaviour, plant reproductive success, and fitness. I also examine the common trends between these three floral traits, and discuss the potential implications of any significant relationships with respect to resource allocation, plant reproductive success and fitness.

### 2.4.1 Inflorescence Production and Size

#### 2.4.1.1 Variation in Total Inflorescence Production

Whilst considerable research has been conducted on *G. macleayana*, this is the first study to monitor the inflorescence production of individual plants over consecutive flowering seasons. I detected striking variation among plants in total inflorescence production at all sites, consistent with previous studies on other Proteaceae species (e.g. Carthew, 1993; Krauss, 1994; Lloyd, 1998). I also found that at each site, one to two plants produced more than 25% of the total inflorescence count for the study plants, consistent with the findings of studies on other Proteaceae species (e.g. Carthew, 1993; Whelan and Ayre, *unpublished*).

Previous studies suggest that seed set in *G. macleayana* plants is not pollen-limited, and therefore, is likely to be resource limited (Harriss and Whelan, 1993; Vaughton, 1996). The observable variation among plants in inflorescence production may be due to

variation in resource availability, as a result of climatic and/or microhabitat conditions. Plants with greater water and/or nutrient resources may be able to allocate more resources to the production of inflorescences (and nectar production), as previous studies have demonstrated (e.g. Zimmerman, 1983; Vaughton, 1991; Lee and Felker, 1992; Galen, 2000). It is also interesting to note that the plants at GB (the site with the greatest inflorescence production) are located down-slope in open woodland and tall eucalypt forest. This may be a source of greater water and nutrient runoff, than is present at CB and IL. Manipulation of water and nutrient resource availability may help clarify the observable variation in inflorescence production among these plants.

#### 2.4.1.2 Temporal Patterns of Inflorescence Production

Whilst plants fluctuated in monthly inflorescences production, monthly plant rankings were generally consistent. The low standard errors associated with mean monthly inflorescence ranks indicate little variation in month to month in plant rankings. In months of good flowering, the best inflorescence producers were consistently ranked highly and the poor producers consistently ranked poorly.

Few studies have examined patterns of inflorescence production among plants in this way, although, Carthew (1993) found similar results with another Proteaceae species, *B. spinulosa*. Carthew (1993) found some consistency among *B. spinulosa* plants in flowering patterns over three years. Of the 47 plants monitored, only 21 consistently produced inflorescences each year (Carthew, 1993), and this pattern was confirmed from 1986 to 2006 (Whelan and Ayre, *unpublished*).

### 2.4.2 Nectar Production

#### 2.4.2.1 Variation Among Plants

Intraspecific variation in nectar volume was striking, and this result is consistent with the findings reported in previous studies, on a range of taxa (e.g. Cresswell 1990; Real and Rathcke, 1991; Lloyd, 1998). However, few studies have reported significant variation among Australian plant species (but see Paton, 1982a; Lloyd, 1998). Whilst significant variation in nectar volume was more common, significant variation was present in the sugar concentration of nectar among plants in one season at CB and two seasons at GB. Significant variation in the sugar concentration of nectar samples is less common among plants within species (but see Pedersen, 1953b; Hodges, 1993; Lloyd,



1998). My results support the findings of previous studies that also found substantially greater intraspecific variation in nectar volume than in sugar concentrations (e.g. Real and Rathcke, 1988; Hodges, 1993; Mitchell, 1993; Lloyd, 1998; McDade and Weeks, 2004a). For example, McDade and Weeks (2004a) detected low intraspecific variation in the sugar content of nectar samples, but high intraspecific variation in nectar volume, among plants of 12 hummingbird-pollinated species.

Pyke and Waser (1981) suggested that there is an inverse relationship between nectar sugar concentration and pollinator body size, for both insect and vertebrate pollinators. They found that mean nectar sugar concentration of bee-pollinated plants was 41.6% (156 species), decreasing to 23.4% (49 species) for honeyeater-pollinated plants and 25.4% (202 species) for humming bird-pollinated plants. I found that mean nectar sugar concentration (across sites and seasons) for *Grevillea macleayana* plants was 24.11% ( $\pm 2.18$ ), consistent with the findings of Pyke and Waser (1981) for bird-pollinated plant species.

#### 2.4.2.2 Consistent Patterns of Nectar Production

Whilst I was unable to determine whether the same plants were consistently good or poor nectar producers, some plants continued to produce more nectar than other plants over one or two survey seasons. This variation may be due to variation in localised environmental factors (e.g. moisture availability), variation in non-reproductive plant traits (e.g. such as size), or possibly variation in heritable nectar production traits (Pedersen, 1953a; Boose, 1997; reviewed in Mitchell, 2004). Moreover, studies on other species have found consistency among plants in patterns of nectar production (e.g. Zimmerman and Pyke, 1986; Hodges, 1993). For example, Hodges (1993) found the “relative ranking” of *Mirabilis multiflora* plants remained constant across days and years for mean nectar traits. However, Real and Rathcke (1991) found very little evidence to suggest that plants producing large amounts of nectar in one year will do so again in the following year, with the variation in the first year explaining just 3% of the variation in the second year.

For the plants that I was able to measure in more than one survey season, I found that some tended to produce consistently more nectar (volume) than other plants. For example, at CB, Plant 12 was ranked first and second in January and October 2002,

respectively. At IL, Plant 1 was ranked second in both October 2002 and January 2003. However, there was no consistency among plants in sugar concentration, and plants were ranked haphazardly among seasons. This area of nectar production in *G. macleayana* requires further attention, preferably involving a study that monitors nectar production in the same plants over a number of years.

### 2.4.3 Pollen Production

The lack of variation among plants in the number of pollen grains per flower may reflect that *G. macleayana* plants are not pollen limited (Harriss and Whelan, 1993; Vaughton, 1996) and can set seed via autogamy. Moreover, the presentation of brightly coloured (reddish) flowers, arranged in inflorescences, with large amounts of nectar, indicates adaptation to honeyeaters as pollinators. Honeyeaters are reported to forage primarily on nectar (Paton, 1982a, b), and are not reported to forage for pollen, unlike honeybees and bumblebees, which typically forage for both (Kevan and Baker, 1983; Kearns and Inouye, 1993). Therefore, with respect to attracting honeyeater pollinators, nectar is likely to be the most important floral reward and a plant may gain no reproductive advantage by varying or increasing pollen production. However, pollen is reported as an important food source for some non-flying mammal species (van Tets and Whelan, 1997; van Tets and Hulbert, 1999). Therefore, if mammals are important pollinators in this system, plants may gain some a reproductive advantage by producing more pollen.

#### 2.4.3.1 Pollen Production & Pollen to Ovule Ratios

Cruden (1977) proposed that plants with more efficient pollen transfer mechanisms would produce less pollen than those species with poor pollen transfer, in which much pollen is wasted. Moreover, previous studies have found that xenogamous taxa are likely to produce more pollen than autogamous taxa (reviewed in Cruden, 1977). On assessment of approximately 100 species, Cruden (1977) found that the pollen-to-ovule ratio of a plant also reflected its breeding system. Self-incompatible species were found to have the lowest pollen-to-ovule ratio, followed by autogamous species and outcrossing species, which had the highest pollen-to-ovule ratio.

In the only other study that has examined pollen production in *G. macleayana*, Vaughton (1996) reported a mean of 2,345 ( $\pm 124$ ) pollen grains per flower and a pollen-

to-ovule ratio of 1,172:1. As a self-compatible species adapted for outcrossing, *Grevillea macleayana* would be classified into the facultative xenogamous breeding system, according to Cruden (1977). Plant species in this breeding system had a mean pollen-to-ovule ratio of 796.6: 1 ( $\pm 87.7$ ) (Cruden, 1977), substantially lower than the ratio reported by Vaughton (1996). Moreover, Cruden (2000) reports that species with relatively large pollen grains may have relatively low pollen-to-ovule ratios. *Grevillea macleayana* has relatively large pollen grains and therefore, the pollen-to-ovule ratio reported in Vaughton (1996) is again in conflict with the literature (i.e. Cruden, 1977, 2000). However, as highlighted by Cruden (1977), an elevated level of pollen production may reflect a mixed breeding system (i.e. self compatible) in which flowers produce more pollen to increase the chance of outcrossed seed production. A greater pollen-to-ovule ratio may also indicate that the efficiency of transporting pollen to flowers of conspecifics is low (Ramsey and Vaughton, 1991). In a study on *Banksia menziesii*, Ramsey and Vaughton (1991) found that the mean ( $\pm$  s.e.) number of pollen grains produced per flower was 19,995 ( $\pm 229$ ) and the pollen-to-ovule ratio was 9,998:1. These results were considered consistent with obligate outcrossing breeding systems (Cruden, 1977)

#### 2.4.3.2 Pollen Production and Reproductive Success

Few studies have examined the relationship between pollen production and reproductive success among individual plants (e.g. Stanton *et al.*, 1991; Allison, 1990). Even fewer studies have examined intraspecific variation in pollen production, the response of pollinators and subsequent reproductive success (but see Gori, 1989; Cresswell, 1999; Lau and Galloway, 2004). Given the lack of variation among *G. macleayana* plants in pollen production per flower, inflorescence size and number may give a better estimate of variation in pollen production among plants. As previously described (Section 2.3.1), plants at CB and GB (in three survey seasons over 12 months) varied significantly in inflorescence size, and therefore, plants with larger inflorescences will produce more pollen. Plants with larger floral displays may attract more honeyeaters, and provided pollen transfer is effective, have the capacity to set more seed than plants with smaller floral displays.

#### 2.4.4 Floral Reward Trade-offs

As stated in the aims, I wanted to examine whether there were any consistent trade-offs or significant relationships between inflorescence production (number and size), nectar production (volume and sugar concentration), and pollen production among plants. The results of correlation and regression analyses suggest that, for these sets of *G. macleayana* plants, there are no detectable trade-offs between resource allocation for inflorescence and nectar production. Therefore, resource allocation for nectar and inflorescence production may be independent of one another (Real and Rathcke, 1991). This may be surprising given the likely substantial resource costs of producing the large floral and nectar rewards regularly recorded on *G. macleayana* plants. However, there may be trade-offs with respect to inflorescence number and size (two of the six tests detected significant negative relationships), and this needs to be investigated further. Aigner (2006) suggests that trade-offs may not be apparent “*because a particular phenotype simultaneously optimises several functions*”. Moreover, trade-offs may become more apparent when plants are monitored over a longer time period (Zimmerman, 1984). Given that I only measured pollen production once (at one site); further investigation is required to better establish patterns of variation and potential correlations with other floral traits. Especially considering previous studies have detected significant positive correlations between pollen production and floral size, implying a selective advantage for plants that allocate more resources to male reproduction (Young and Stanton, 1990; Klinkhamer and van der Veen-van Wijk, 1999).

Several previous studies have detected significant correlations and trade-offs between measures of nectar production and flower/inflorescence production (e.g. Harder and Cruzan, 1990; Hodges, 1993; Mitchell, 1993; Caruso, 2004) and flower and pollen production (e.g. Klinkhamer and van der Veen-van Wijk, 1999; Young and Stanton (1990). For example, Caruso (2004) detected negative correlations between flower number and flower size in *Lobelia siphilitica* plants, supporting the common negative trends I found between inflorescence number and size in *G. macleayana* plants. Moreover, several studies have detected positive relationships between several measures of floral rewards, including: nectar production (total sugar) and flower number (Hodges, 1993); nectar production (total sugar) and inflorescence size (Harder and Cruzan, 1990); and nectar production and flower size (Mitchell, 1993). The results of these studies

suggest that these plants suffered no detectable trade-offs between nectar and flower/inflorescence production, supporting the common positive trends I detected between inflorescence and nectar production in *G. macleayana* plants.

### 2.4.5 Floral Traits, Pollinator Activity, and Reproductive Success

In this chapter, I presented evidence for strong variation among plants in inflorescence and nectar production. Moreover, I found that the same plants consistently produced the most or the fewest numbers of inflorescences each month; and a small number of plants produced a large proportion of the inflorescences for the surveyed plants, over two years.

For variation in floral traits to translate into increased plant fitness, plant reproductive success must also increase. For this to occur, pollinators need to respond to this variation with increased effective pollen transfer, resulting in increased seed production. Some studies have found significant positive relationships between various floral traits, pollinator foraging activity, and/or reproductive success (e.g. Zimmerman, 1983; Pyke *et al.*, 1988; Broyles and Wyatt, 1990; Thompson, 2001 - but see Tables 1.1 and 1.2). In the next two chapters, I quantify variation among *G. macleayana* plants in measures of honeybee, honeyeater and nocturnal mammal foraging activity and measures of reproductive success (i.e. seed production and pollen deposition). I then test for consistent trends and/or significant relationships between floral traits, pollinator foraging activity, and reproductive success, in order to determine whether plants with increased floral traits receive greater pollinator visits and have greater reproductive success.

## Chapter 3 - Floral Visitor Foraging Behaviour

### 3.1 Introduction

#### 3.1.1 Plant Attraction and Floral Visitor Foraging Behaviour

As discussed in Chapters 1 and 2, plant reproductive success is a complex function of floral traits (i.e. plant attraction), pollinator activity, resource availability, seed development and plant phenotypic response to environmental variables (Schemske and Horvitz, 1984; Rathcke, 1992; Utelli and Roy, 2000). To understand the important relationships between floral traits and reproductive success for a particular plant species, the visiting patterns and foraging behaviour of the relevant pollinators needs to be quantified (Primack and Inouye, 1993; Pandit and Choudhury, 2001). Whilst long-term studies are limited, there is evidence that many plant species experience variation within and among years in pollinator activity (Herrera, 1989; Utelli and Roy, 2000). Therefore, it is also necessary to observe pollinators at a number of plant populations and over more than one flowering season.

Whilst several different potential pollinators may regularly visit a single plant species, these visitors may vary substantially in effective pollen transfer due to: pollinator fidelity, foraging behaviour, visiting patterns, energy requirements, and/or flower size and morphology (Handel, 1983; Wilson and Thomson, 1991; Castellanos *et al.*, 2003). To gain an understanding of these plant-pollinator relationships, each species that forages on a plant should be monitored for effective pollen transfer (Wilson and Thomson, 1991). *Grevillea macleayana* is visited by a suite of potential pollinators, including invertebrates (primarily European honeybees), birds, and possibly nocturnal mammals (see Section 1.8). However, it is debatable whether flowers visited by honeybees result in effective pollen transfer and seed production (Vaughton 1996; Roberts, 2001). For example, Vaughton (1996) found that when honeybees were given access to *G. macleayana* inflorescences and vertebrates were excluded, inflorescences matured 50% fewer seeds than open inflorescences, to which honeyeaters (nectar-feeding birds) also had access. Since there is no certainty that a flower visitor to *G. macleayana* plants will actually be an effective pollinator (specifically, the honeybee), I refer to the species observed in this chapter as “floral visitors”.

### 3.1.2 Study Predictions

The studies in Chapter 2 revealed striking variation among plants in two of the three floral traits I tested: floral display and nectar production. With respect to nectar production, I found significant inter-plant variation at each site over two to three seasons. Furthermore, I found substantial variation among plants in monthly inflorescence production. These results suggest that, within a population, some plants may have greater floral attraction to honeybees or honeyeaters than other plants. As described in Chapter 1 (Section 1.4), numerous studies have found that pollinator activity increased with increasing floral display size and nectar production (Table 1.1). Therefore, it is reasonable to predict that floral visitors foraging on *Grevillea macleayana* plants will also respond to the variation in inflorescence numbers and nectar production.

In this chapter, I quantify variation among *G. macleayana* plants in the frequency and foraging behaviour of two floral visitors: native honeyeaters and introduced honeybees, at three sites over approximately two years (Table 3.1). I also perform a number of regression analyses to gain an understanding of how variation in floral traits may be related to variation in honeybee and honeyeater foraging behaviour, thus incorporating the results of Chapter 2 with this Chapter.

Based on the literature presented in this Chapter and Chapter 1, I made several predictions about the likely variation in the foraging behaviour of floral visitors among *G. macleayana* plants:

- (1) Honeybee and honeyeater visit frequency and foraging behaviour will vary significantly among plants, and this may be associated with variation in floral traits among *G. macleayana* plants.
- (2) Honeybees and honeyeaters will ‘favour’ the same plants over consecutive flowering seasons, depending upon patterns of floral traits.
- (3) *Grevillea macleayana* plants will be visited by a nocturnal marsupial mammal species, thus supporting previous studies that found nocturnal pollen removal (Beynon *et al.*, unpublished).



**Table 3.1 - Field studies quantifying floral visitor foraging activity among *Grevillea macleayana* plants.**

Field studies conducted to quantify variation among *G. macleayana* plants in honeybee, honeyeater and nocturnal mammal foraging activity, at three sites in Jervis Bay National Park, between 2002 and 2004.

Experimental Work	Study Sites								
	Chinamans Beach					Greenfields Beach			
	March 2002	September – October 2002	February 2003			February 2002	October – November 2002	January 2003	November 2003
<b>Honeybees &amp; Honeyeaters</b>									
Number of honeybees & honeyeaters per plant	✓	✓	✓			✓ *	✓	✓	✓
Number of inflorescences visited per plant	✓	✓	✓			✓	✓	✓	✓
Time per inflorescence	✓	✓	✓			✓	✓	✓	✓
Time per plant	✓	✓	✓			✓	✓	✓	✓
<b>Nocturnal Mammals</b>	February & March 2002	October 2002	February 2003	September - October 2003	January 2004	February 2002		September 2003	
General foraging behaviour	✓	✓	✓	✓	✓	✓		✓	✓

\* - Survey only conducted on honeybees.

### 3.1.3 Study Aims

In this chapter of my thesis I explore the following questions:

- (1) How do honeybee and honeyeater visits and/or foraging behaviour differ among plants?
- (2) How do honeybees and honeyeaters differ overall in visits and/or foraging behaviour?
- (3) Are patterns of variation among plants consistent over survey seasons (e.g. do the plants that receive the most honeybee visits in one survey season also receive the most visits in other seasons)?
- (4) Do nocturnal pollinators visit *G. macleayana* plants and what is their foraging behaviour?
- (5) How is honeybee and honeyeater foraging behaviour associated with variation among plants in floral traits (inflorescence, nectar, and pollen production)?

## 3.2 Methods

### 3.2.1 Honeybee and Honeyeater Foraging Behaviour

Before undertaking the main studies for this chapter, I conducted some preliminary field observations to confirm that I could reliably measure foraging behaviour and to refine the monitoring techniques I proposed to use. I observed in this trial that there was measurable variation among plants in the visiting patterns and foraging behaviour of floral visitors. Furthermore, I refined the techniques I used to monitor honeybees and honeyeaters, to ensure I could record their often quick and inconspicuous movements on a plant.

To determine whether there was significant variation among plants in the visiting patterns and foraging behaviour of floral visitors, I monitored honeybees and honeyeaters on *G. macleayana* plants at three sites. I monitored honeybees for a total of 18 hr over eight days within a 12 month period at CB, for 23 hr over nine days within a 22 month period at GB, and for 12 hr over six days within a four month period at IL (Table 3.1). I monitored honeyeaters for a total of 28 hr over eight days within a 12 month period at CB, for 36 hr over ten days within a 22 month period at GB, and for 20 hr over six days within a four month period at IL (Table 3.1). Observations of floral visitors were conducted on the same five to six plants per site that I had previously used

for the nectar production studies (Chapter 2) and within one week of these experiments (excluding the studies in early 2002, which were delayed due to rain). However, I conducted floral visitor studies at CB in February 2003, even though no nectar studies were conducted due to poor flowering.

On each survey day, I observed honeybees and honeyeaters separately, for between one and three survey periods: morning (7:30 - 10:30); midday (11:30 - 2:00); and afternoon (3:00 - 5:30). Inconsistent weather (i.e. rain) meant that I was only able to conduct one or two surveys on some days. During a survey period, I first observed honeyeaters, for between 15 min and 20 min per plant on every plant, followed by honeybees for between 10 min and 15 min per plant on every plant. The honeyeater monitoring survey periods were longer than those for honeybees because honeyeaters have a lower visit frequency than honeybees. On commencement of a survey period (for an individual plant), I first recorded the number of honeybees and honeyeaters on the plant, depending on whether it was a dedicated honeybee or honeyeater survey period. I then waited for a new honeybee or honeyeater to arrive at the plant, and recorded its foraging behaviour in detail until it left the plant (or, in the case of occasional honeybees, until it was obscured by the plant and unable to be monitored). During the foraging bout of the monitored honeybee or honeyeater, I recorded three variables: the number of inflorescences visited on the plant; the time spent foraging at each of these inflorescences; and the total time spent at the plant. Where possible, I also recorded the plant at which the honeybee or honeyeater next foraged. I continued this process of recording the foraging behaviour of individual honeybees or honeyeaters for the remainder of the survey period per plant. From these data, I examined variation in intra-plant foraging movements among plants and among survey seasons, and I compared the foraging behaviour of honeybees and honeyeaters.

An inflorescence was considered to have received a flower visit if: (1) a honeybee was seen to collect pollen from the pollen presenter and/or forage among open and/or unopened flowers to collect nectar; or (2) a honeyeater probed open flowers.

Observations were made at a distance of 5 - 10 m for honeyeaters and approximately 1 m for honeybees. One site was surveyed per day, plants were surveyed randomly within each survey period and the number of inflorescences per plant was also recorded.

### 3.2.2 Nocturnal Mammal Foraging Behaviour

To determine whether nocturnal floral visitors were visiting *G. macleayana* plants, I conducted spotlighting surveys of plants on nine nights (over 24 months) at CB, three nights (over 20 months) at GB and two nights (over 12 months) at IL (Table 3.1). On a given survey night, I conducted spotlighting surveys at all plants used in the diurnal floral visitor observation and nectar production studies (per site). I spent between 10 and 30 min at an individual plant, depending on whether I detected a potential floral visitor. If I observed a potential visitor, I would then try to follow its foraging movement within the plant and record how long it spent at the plant. Both a video camera and digital still cameras were used to try to record the foraging behaviour of nocturnal mammals. An inflorescence was considered to have received a potential effective pollinator visit if a nocturnal mammal was seen to forage from open flowers. Observations were made at a distance of 5 - 10 m and generally only one site was surveyed per night.

### 3.2.3 Statistical Analysis

I tested for statistically significant variation among plants in honeybee and honeyeater numbers and foraging behaviour using randomisation tests (Question 1). The number of data permutations for each randomisation test was 10 million (Edgington, 1987). More conventional analyses (i.e. ANOVA) could not be used due to significant non-normality and heteroscedasticity of data. The randomisation test was selected on advice from Associate Professor Ken Russell (Statistical Consulting Service, School of Mathematics and Applied Statistics, University of Wollongong). Each survey was tested individually (e.g. January 2003 at GB is one survey), because each survey comprised different plants at different sites and dates.

The following aspects of honeybee and honeyeater foraging activity were tested for variation among plants using randomisation tests: (1) the number of honeybees on a plant at the beginning of a survey period or honeyeaters per survey period; (2) the cumulative number of inflorescences visited during consecutive foraging bouts by monitored honeybees or honeyeaters, within the survey period<sup>4</sup>; and (3) the cumulative

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<sup>4</sup> For example, in a honeybee survey period, if I observed three honeybees that visit two, six, and four inflorescences, respectively, the cumulative number of inflorescences visited by the three monitored honeybees in that survey period equals 12.

foraging time from consecutive foraging bouts by monitored honeybees or honeyeaters, within the survey period<sup>5</sup>.

I used Mann-Whitney Tests to test for significant variation between honeybees and honeyeaters in their foraging behaviour during a single foraging bout (Question 2). I could not use conventional Student's *t*-tests due to non-normality and heteroscedasticity of some data. However, Zar (1984) argued that the Mann-Whitney test is “*one of the most powerful nonparametric tests*” and is appropriate for use when *t*-test assumptions are violated. I compared honeybees and honeyeaters in the following aspects of foraging behaviour: (1) the mean number of inflorescences foraged at per plant (per honeybee or honeyeater); (2) the mean time (s) spent foraging per inflorescence per plant (per honeybee or honeyeater); and (3) the mean time spent foraging per plant (per honeybee or honeyeater).

Spearman Rank Correlations were used to test for significant correlations between honeybees and honeyeaters in the following aspects of foraging behaviour (Question 2): (1) the number of honeybees or honeyeaters present per plant; (2) the mean number of inflorescences visited by an individual honeybee or honeyeater per plant; and (3) the mean foraging time of an individual honeybee or honeyeater per plant. Separate correlations were performed for each survey season per site. No statistical analyses were used to assess temporal patterns of foraging behaviour among plants (Question 3), because the same plants could not be used each season. No statistical analyses were performed on data collected during the spotlighting surveys, due to the very small number of mammal observations (Question 4).

I used multiple regression analyses to test for significant relationships between honeybee and honeyeater foraging behaviour and both inflorescence and nectar production (Question 5). The three measures of honeybee and honeyeater foraging behaviour used were: (1) the mean number of honeybees or honeyeaters; (2) the mean cumulative number of inflorescences visited by monitored honeybees or honeyeaters in a survey period; and (3) the mean cumulative foraging time per plant of monitored honeybees or honeyeaters per survey period. These three measures of foraging

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<sup>5</sup> For example, in a honeyeater survey period, if I monitored two honeyeaters that spend 94 s and 45 s foraging, respectively, then the cumulative foraging time of the two monitored honeyeaters is 139 s.

behaviour were tested independently against two measures of inflorescence production and nectar production. The two measures of inflorescence production used were: (1) inflorescence number per plant and (2) mean inflorescence size (flowers per inflorescence). The two measures of nectar production used were: (1) mean inflorescence nectar volume ( $\mu\text{L}$ ) and (2) the mean sugar concentration (%) of nectar per inflorescence. I also used simple linear regressions to test for significant relationships between the three measures of honeybee and honeyeater foraging behaviour and inflorescence number at CB in February 2003.

### **3.3 Results**

#### **3.3.1 Insect Foraging Behaviour**

##### *3.3.1.1 Honeybee Foraging Behaviour*

I generally observed honeybees only foraging for nectar (i.e. not for pollen). This usually involved the honeybee landing on an inflorescence (on an unopened flower) and burrowing between the perianth segments of individual flowers to reach the nectar at the flower base. The honeybee would then move among open and unopened flowers (in male phase and early female phase) foraging for nectar. This foraging behaviour rarely resulted in the honeybee making contact with the pollen presenter (Figure 3.1a). I observed one true case of nectar robbing (as defined by Inouye, 1980) with a honeybee feeding on nectar through a hole that had been pierced in the base of a flower, on Plant 12 at CB in March 2002. This was the longest honeybee foraging bout I observed at an individual flower. The same honeybee was still at the inflorescence (alive) after 40 min!

I observed honeybees collecting pollen on only a few occasions, two of these during honeybee survey periods, at IL in October 2002 on Plant 8 and at GB in January 2003 on Plant 4. When honeybees foraged for pollen, I observed that they flew immediately above the pollen presenter and hovered there whilst removing pollen, or they landed directly onto the pollen presenter and used their legs to collect pollen into their corbiculae (Figure 3.1b).

(a)



(b)



**Figure 3.1 - Photographs of two honeybees foraging for nectar and pollen at *Grevillea macleayana* inflorescences.**

A European honeybee (*Apis mellifera*) foraging for: (a) nectar between closed flowers and (b) pollen on a *G. macleayana* inflorescence.

When observing the foraging behaviour of individual honeybees, I also attempted to record the next plant it visited. At CB, I observed this for 21 honeybees and of these, 17 flew to another *G. macleayana* plant. At GB, I observed this for 16 honeybees, and at IL, for three honeybees. All of these honeybees flew to another *G. macleayana* plant. It appeared that honeybees were much more likely to fly immediately to another *G. macleayana* plant when plants were clustered together, although, I did not examine this quantitatively.

In the next three sections I outline the variation I observed among *G. macleayana* plants in: (1) the number of honeybees visiting plants; (2) the number of inflorescences visited by honeybees per plant; and (3) the total foraging time of honeybees per inflorescence and per plant. I found striking variation among plants in each of these three foraging variables and also detected substantial variation among survey seasons within sites. I also examine patterns of honeybee foraging behaviour among plants, to determine whether particular plants are receiving lower or higher rates of foraging behaviour, over consecutive survey seasons

#### 3.3.1.2 Number of Honeybees

Overall, I observed 15 honeybees per hour at CB, 19 per hour at GB, and 16 per hour at IL. The number of honeybees foraging at *G. macleayana* plants varied remarkably among survey seasons within sites (Table 3.2). The most extreme variation in total honeybee number was detected at GB, with an approximate 20.5-fold difference between the February 2002 and the January 2003 survey season. GB also had the greatest total number of honeybee visits (438), more than double that of IL and 1.5 times that of CB (Table 3.2). The surveys in January and February 2003 resulted in the greatest number of honeybees at all three sites, contributing to more than 60% of the total number of honeybees observed from all the survey periods combined (Table 3.2). For each site, the greatest mean number of honeybees was 16.3 ( $\pm 1.9$ ) on Plant 1 at CB, 13.2 ( $\pm 1.5$ ) on Plant 3 at GB, and 8.0 ( $\pm 1.6$ ) on Plant 2 at IL, all recorded in January or February 2003.



**Table 3.2 - The total number of honeybees observed foraging on *Grevillea macleayana* survey plants.**

Data are shown for survey seasons at Chinamans Beach, Greenfields Beach and Illowra Lane in 2002 and 2003.

Season	Chinamans Beach		Greenfields Beach		Illowra Lane	
	Honeybees	Honeyeaters	Honeybees	Honeyeaters	Honeybees	Honeyeaters
February 2002			15	3		
March 2002	74	21	-	-	-	-
September - October 2002	21	14	-	-	-	-
October 2002	-	-	-	-	70	8
October - November 2002	-	-	86	8	-	-
January 2003	-	-	309	39	122	10
February 2003	171	122	-	-	-	-
November 2003	-	-	28	7	-	-
<b>Total Number</b>	<b>266</b>	<b>157</b>	<b>438</b>	<b>57</b>	<b>192</b>	<b>18</b>

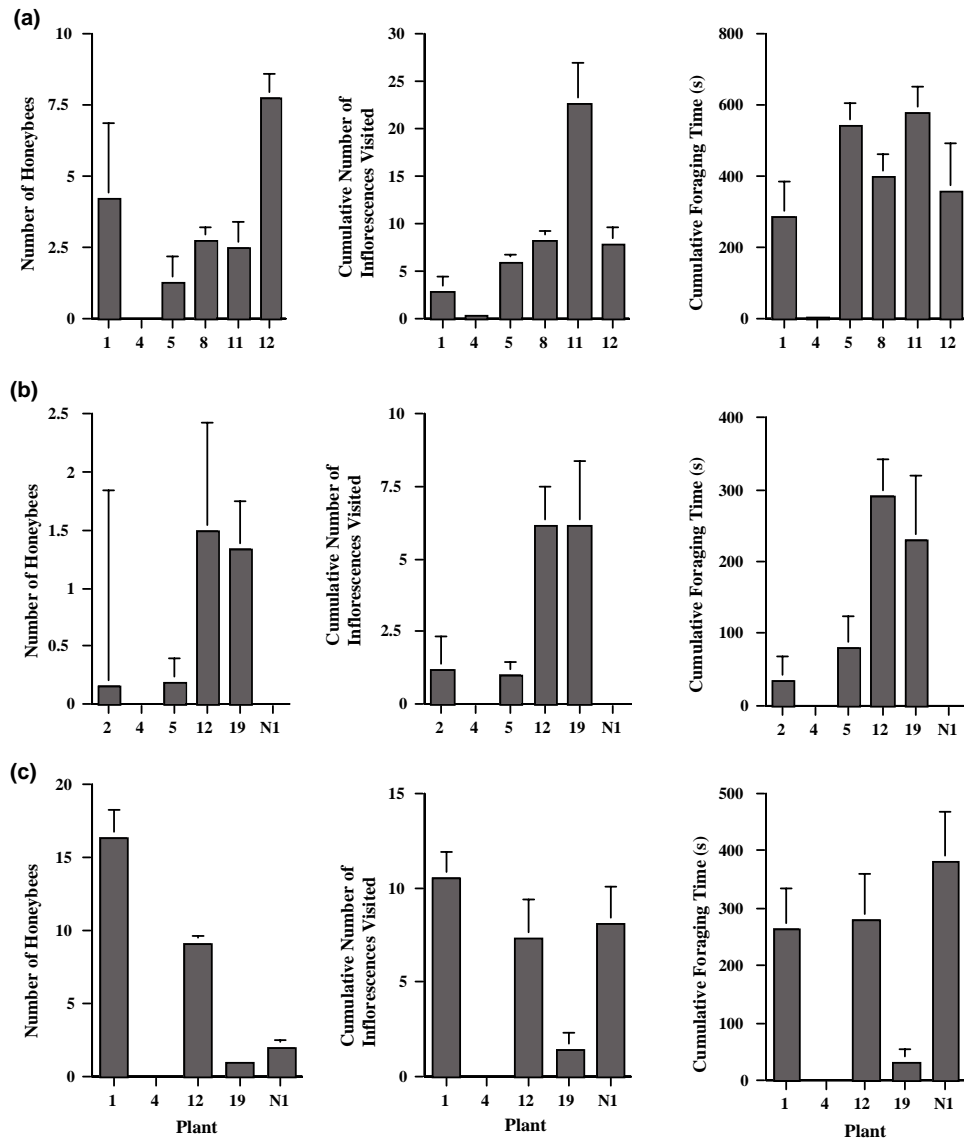
A dash (-) indicates that no observations were made at that site during that survey season.

I detected substantial variation in the mean number of honeybees recorded per plant (at the beginning of a survey period), both among plants per survey season and among sites. The most striking example of variation among plants was a 16-fold difference between the plant with the lowest (P4) and the plant with the highest (P1) mean number of honeybees, at CB in February 2003 (Figure 3.2). At GB, the most extreme example of variation among plants in the mean honeybee number of was an approximate 5.5-fold difference between the plant N7 and P7, in February 2002 (Figure 3.3). At IL, the most striking example of variation among plants in the mean honeybee number was an approximate 15-fold difference between P6 and P2 (Figure 3.4). Moreover, randomisation tests detected significant variation among plants in the mean number of honeybees recorded per plant for each survey season per site (Table 3.3). The only exception was at GB in November 2003, when I recorded very low numbers of honeybees (Table 3.3).

At each site, I detected some strong consistent patterns among plants, with respect to plants with the highest or lowest number of honeybees per survey season. At CB, I found strong consistency among plants with respect to plants with the highest and lowest number of honeybees per survey season. Plants 1 and 12, (used in two and three survey seasons respectively) always had the greatest number of honeybees per survey season and Plant 4 always had zero honeybees, regardless of the survey season (Figure 3.2). At GB, patterns among plants were not consistent for plants with the highest numbers of honeybees, but were strong for the plant with the lowest numbers of honeybees (Figure 3.3). For example, Plants 1, 3, 4 and 7 each had the greatest number of honeybees in one of the four survey seasons (Figure 3.3). However, Plant N7 (used in three survey seasons) always recorded the lowest number of honeybees, regardless of the survey season (Figure 3.3). At IL, patterns among plants were consistent for plants with the highest number of honeybees. The same two plants (Plants 2 and N2) had the greatest number of honeybees in both survey seasons (Figure 3.4).

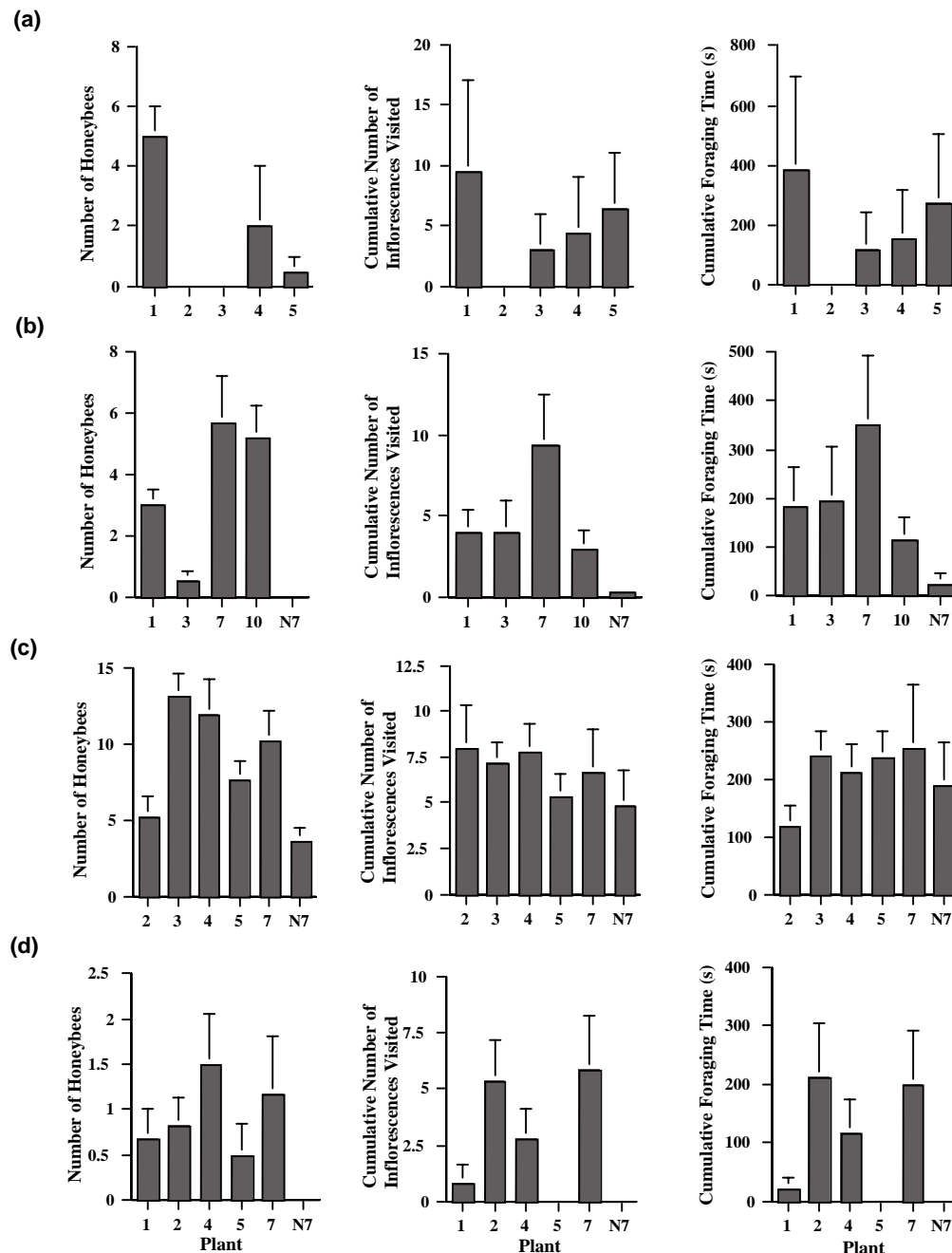
#### *3.3.1.3 Number of Inflorescences Visited*

The mean number of inflorescences visited per plant per honeybee did not vary greatly among seasons or sites, ranging from 3.6 ( $\pm 0.6$ ) at GB in February 2002 to 6.8 ( $\pm 0.6$ ) at GB in October 2002 (Table 3.4). The mean numbers of inflorescences foraged at per plant per honeybee at CB, GB, and IL were 4.6 ( $\pm 0.4$ ), 5.6 ( $\pm 0.4$ ), and 5.9 ( $\pm 0.7$ ), respectively.



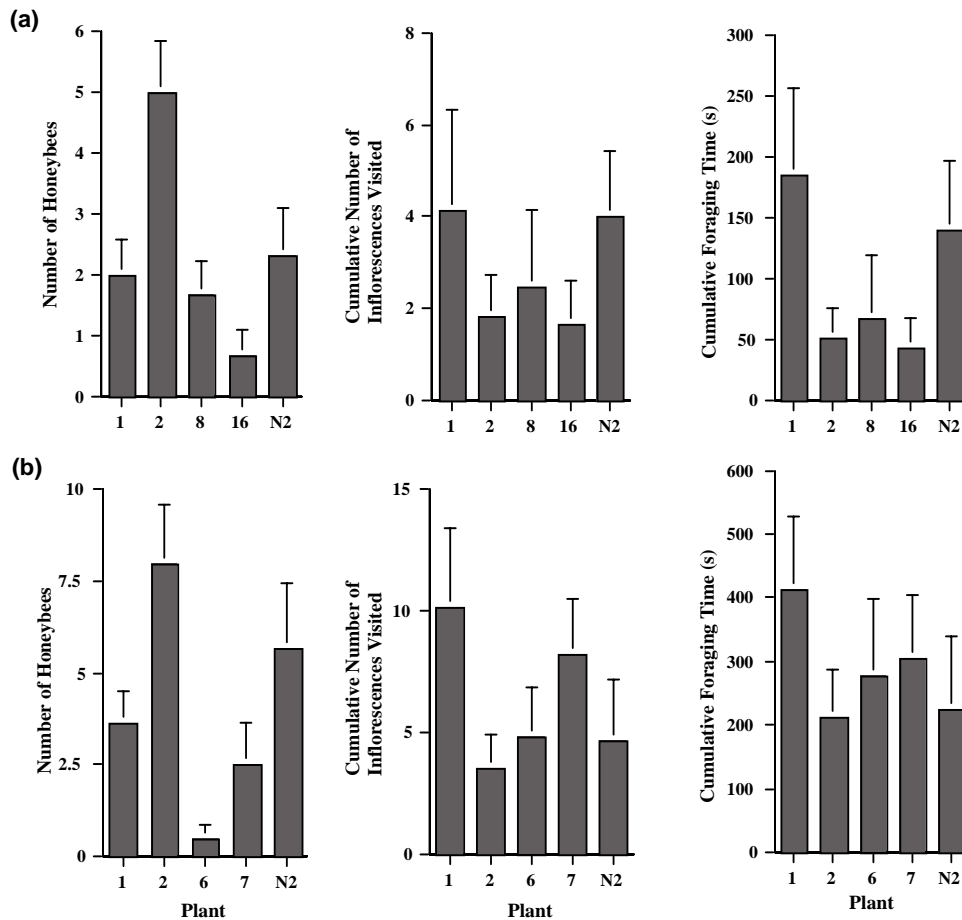
**Figure 3.2 - Honeybee foraging behaviour among *Grevillea macleayana* plants at Chinamans Beach.**

The mean number of honeybees per plant at the beginning of a survey period, the cumulative number of inflorescences visited by consecutive monitored honeybees and the cumulative time (s) consecutive monitored honeybees spent foraging on *G. macleayana* plants per survey period (10 - 15 min). Surveys were conducted at Chinamans Beach, in March 2002 (Figure a), September 2002 (Figure b), and February 2003 (Figure c). Plants are displayed in order of identification code. Bars indicate plus one standard error.



**Figure 3.3 - Honeybee foraging behaviour on *Grevillea macleayana* plants at Greenfields Beach.**

The mean number of honeybees per plant at the beginning of a survey period, the cumulative number of inflorescences visited by consecutive monitored honeybees and the cumulative time (s) consecutive monitored honeybees spent foraging on *G. macleayana* plants per survey period (10 - 15 min). Surveys were conducted at Greenfields Beach, in February 2002 (Figure a), October 2002 (Figure b), January 2003 (Figure c), and November 2003 (Figure d). Plants are displayed in order of identification code. Bars indicate plus one standard error.



**Figure 3.4 - Honeybee foraging behaviour among *Grevillea macleayana* plants at Illowra Lane.**

The mean number of honeybees per plant at the beginning of a survey period, the cumulative number of inflorescences visited by consecutive monitored honeybees, and the cumulative time (s) consecutive monitored honeybees spent foraging on *G. macleayana* plants per survey period (10 - 15 min). Surveys were conducted at Illowra Lane in October 2002 (Figure a) and January 2003 (Figure b). Plants are displayed in order of identification code. Bars indicate plus one standard error.

**Table 3.3 - The results of randomisation tests performed to test for significant variation among *Grevillea macleayana* plants in honeybee foraging activity.**

The foraging activity tested was: (1) the number of honeybees per plant at the start of a survey period; (2) the cumulative number of inflorescences foraged at by consecutive honeybees monitored in a survey period, and (3) the cumulative amount of time (s) spent foraging by consecutive honeybees monitored during a survey period. Surveys were undertaken at Greenfields Beach (GB), Chinamans Beach (CB) and Illowra Lane (IL) in 2002 and 2003. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

Site	Season	Parameter	<i>F</i> Ratio	df <sup>6</sup>	Treatment Sum of Squares	Final Probability
<b>GB</b>	October 02	Number of Bees	9.56	4, 20	408.33	< <b>0.01</b>
	October 02	Inflorescences	2.64	4, 20	769.33	<b>0.03</b>
	October 02	Foraging Time	1.63	4, 20	1253327.83	0.10
	January 03	Number of Bees	8.10	5, 25	3122.67	< <b>0.01</b>
	January 03	Inflorescences	0.53	5, 25	1637.83	0.73
	January 03	Foraging Time	0.56	5, 25	1663855.33	0.72
	November 03	Number of Bees	1.83	5, 25	30.00	0.15
	November 03	Inflorescences	3.58	5, 25	427.17	< <b>0.01</b>
	November 03	Foraging Time	2.86	5, 25	590258.33	<b>0.03</b>
<b>CB</b>	March 02	Number of Bees	4.41	5, 15	374.00	<b>0.02</b>
	March 02	Inflorescences	15.69	5, 15	2779.50	< <b>0.01</b>
	March 02	Foraging Time	6.49	5, 15	4004537.75	< <b>0.01</b>
	September 02	Number of Bees <sup>7</sup>	5.38	5, 20	7.00	<b>0.01</b>
	September 02	Number of Bees <sup>8</sup>	3.51	4, 20	24.33	< <b>0.01</b>
	September 02	Inflorescences	5.84	5, 20	297.00	< <b>0.01</b>
	September 02	Inflorescences	8.03	4, 20	464.50	< <b>0.01</b>
	September 02	Foraging Time	6.67	5, 20	728463.40	< <b>0.01</b>
	September 02	Foraging Time	9.07	4, 20	833900.17	< <b>0.01</b>
	February 03	Number of Bees	59.51	4, 20	2134.83	< <b>0.01</b>
	February 03	Inflorescences	8.43	4, 20	1397.83	< <b>0.01</b>
	February 03	Foraging Time	6.60	4, 20	1777302.50	< <b>0.01</b>
<b>IL</b>	October 02	Number of Bees	10.54	4, 20	226.00	< <b>0.01</b>
	October 02	Inflorescences	0.53	4, 20	274.50	0.70
	October 02	Foraging Time	1.33	4, 20	376663.33	0.28
	January 03	Number of Bees	11.45	4, 20	696.33	< <b>0.01</b>
	January 03	Inflorescences	1.50	4, 20	1364.67	0.19
	January 03	Foraging Time	0.55	4, 20	2632080.17	0.70

<sup>6</sup> Plant degrees of freedom, Residual degrees of freedom.

<sup>7</sup> First test excluding final observation session due to missing data point.

<sup>8</sup> Second test excluding Plant 5 due to missing data point.

**Table 3.4 - The mean number of inflorescences visited by honeybees and honeyeaters on *Grevillea macleayana* plants.**

The mean ( $\pm$  s.e.) number of inflorescences visited per honeybee and honeyeater for individual *G. macleayana* plants. Data are shown for each survey season at Chinamans Beach, Greenfields Beach and Illowra Lane in 2002 and 2003. The results of significant Mann-Whitney Tests comparing honeybees with honeyeaters within a survey season are presented in brackets (underneath the relevant means). No test was conducted for data collected at Illowra Lane in January 2003 due to a lack of honeyeater visits (the mean generated was based on only two honeyeater visits).

	Chinamans Beach		Greenfields Beach		Illowra Lane	
Season	Honeybees	Honeyeaters	Honeybees	Honeyeaters	Honeybees	Honeyeaters
February 2002	-	-	3.61 $\pm$ 0.60	-	-	-
March 2002	3.92 $\pm$ 0.54 (s=1035.5; $P < 0.001$ )	8.0 $\pm$ 1.03	-	-	-	-
September - October 2002	4.05 $\pm$ 0.72	4.31 $\pm$ 1.01	-	-	-	-
October 2002	-	-	-	-	4.65 $\pm$ 0.85 (s=88.5; $P \leq 0.02$ )	11.20 $\pm$ 2.35
October - November 2002	-	-	5.71 $\pm$ 0.91	5.6 $\pm$ 1.81	-	-
January 2003	-	-	6.77 $\pm$ 0.57	7.72 $\pm$ 1.0	6.63 $\pm$ 1.04	2.5 $\pm$ 0.5
February 2003	6.11 $\pm$ 0.79	7.41 $\pm$ 1.12	-	-	-	-
November 2003	-	-	4.63 $\pm$ 0.57 (s=102.5; $P \leq 0.005$ )	14.2 $\pm$ 2.96	-	-

A dash (-) indicates that no observations were made at that site during that month.

The mean cumulative number of inflorescences visited by monitored honeybees per plant per survey period varied greatly across survey seasons and sites. The most striking example of variation among plants was at CB in March 2002, with a 23-fold difference between the plant with the lowest (Plant 4) and the plant with the greatest (Plant 11) mean number of inflorescences visited (Figure 3.2). At GB, the most extreme example of variation among plants in the mean number of inflorescences visited was in February 2002, with an approximate 10-fold difference between Plant 2 and Plant 1 (Figure 3.3). At IL, the greatest example of variation among plants was in January 2003, with a 2.5-fold difference between Plant 2 and Plant 1 (Figure 3.4). The

largest mean cumulative number of inflorescences visited per plant per survey period, was 22.8 ( $\pm 4.2$ ) on Plant 11 at CB in March 2002, 8.0 ( $\pm 2.3$ ) on Plant 3 at GB, and 10.2 ( $\pm 3.2$ ) on Plant 1 at IL, both recorded in January 2003. Randomisation tests detected significant variation among plants in the mean cumulative number of inflorescences visited by consecutive honeybees, for survey seasons at each site. The only exceptions were for GB in January 2003 and IL in October 2002 and January 2003 (Table 3.3).

In contrast to results for the number of honeybees, there were not many strong or consistent patterns with respect to plants with the highest or lowest cumulative number of inflorescences visited by monitored honeybees per survey season (Section 3.3.1.2). At CB, there were no consistent patterns with respect to the plants that received the highest mean number of inflorescences visited (Figure 3.2). However, Plant 4 (used in each survey season) consistently had the lowest number of inflorescences visited (Figure 3.2). At GB, there were no consistent patterns with respect to the plants that received either the highest or lowest mean number of inflorescences visited (Figure 3.3). At IL, there were no consistent patterns with respect to the plants with the lowest number of inflorescences visited (Figure 3.4). However, Plant 1 had the highest mean cumulative number of inflorescences visited in both survey seasons.

#### 3.3.1.4 Honeybee Foraging Time

The mean time individual honeybees spent foraging per inflorescence did not vary greatly among seasons or sites (Table 3.5). There was only a 19 s difference between the highest mean inflorescence foraging time at CB in March 2002 and the lowest, at GB in November 2003. The mean honeybee foraging times per inflorescence for CB, GB, and IL were 44 s ( $\pm 3.0$ ), 34 s ( $\pm 2.0$ ), and 45 s ( $\pm 7.0$ ), respectively. The mean time individual honeybees spent foraging per plant varied two-fold among sites, ranging from 144 s ( $\pm 32.0$ ) at GB in February 2002 to 307 s ( $\pm 40.0$ ) at IL in January 2003 (Table 3.6). The mean honeybee foraging times per plant at CB, GB, and IL were 188 s ( $\pm 15.0$ ), 212 ( $\pm 16.0$ ), and 250 ( $\pm 30.0$ ), respectively.



**Table 3.5 - The mean ( $\pm$  s.e.) time (s) individual honeybees and honeyeaters spent foraging at individual *Grevillea macleayana* inflorescences, for each survey season, at Chinamans Beach, Greenfields Beach and Illowra Lane in 2002 and 2003.**

The results of significant Mann-Whitney Tests comparing honeybees with honeyeaters within survey seasons are presented in brackets underneath the relevant means. No test was conducted for data collected at Illowra Lane in January 2003 due to a lack of honeyeater visits (the mean generated was based on only two honeyeater visits).

Season	Chinamans Beach		Greenfields Beach		Illowra Lane	
	Honeybees	Honeyeaters	Honeybees	Honeyeaters	Honeybees	Honeyeaters
February 2002	-	-	33 $\pm$ 3.0	-	-	-
March 2002	49 $\pm$ 5.0 ( $s=261$ ; $P<0.001$ )	6 $\pm$ 1.0	-	-	-	-
September - October 2002	44 $\pm$ 5.0 ( $s=113$ ; $P<0.001$ )	9 $\pm$ 1.0	-	-	-	-
October 2002	-	-	-	-	37 $\pm$ 9 ( $s=17$ ; $P<0.002$ )	9 $\pm$ 0.0
October - November 2002	-	-	37 $\pm$ 5.0 ( $s=40$ ; $P\leq 0.001$ )	10 $\pm$ 1.5	-	-
January 2003	-	-	33 $\pm$ 3.0 ( $s=331.5$ ; $P<0.001$ )	7 $\pm$ 1.0	48 $\pm$ 10.0	14 $\pm$ 3.0
February 2003	33 $\pm$ 3.0 ( $s=1222.5$ ; $P<0.001$ )	7 $\pm$ 0.5	-	-	-	-
November 2003	-	-	31 $\pm$ 3.0 ( $s=15$ ; $P<0.001$ )	7 $\pm$ 0.5	-	-

A dash (-) indicates that no observations were made at that site during that month.

**Table 3.6 - The mean ( $\pm$  s.e.) time (s) individual honeybees and honeyeaters spent foraging at individual *Grevillea macleayana* plants, for each survey season, at Chinamans Beach, Greenfields Beach and Illowra Lane in 2002 and 2003.**

The results of significant Mann-Whitney Tests comparing honeybees with honeyeaters within survey seasons are presented in brackets underneath the relevant means. No test was conducted for data collected at Illowra Lane in January 2003 due to a lack of honeyeater visits (the mean generated was based on only two honeyeater visits).

Season	Chinamans Beach		Greenfields Beach		Illowra Lane	
	Honeybees	Honeyeaters	Honeybees	Honeyeaters	Honeybees	Honeyeaters
February 2002	-	-	144 $\pm$ 32.0	-	-	-
March 2002	173 $\pm$ 21.0	103 $\pm$ 15.0	-	-	-	-
September - October 2002	178 $\pm$ 28.0 ( $s=126.5$ ; $P<0.001$ )	36 $\pm$ 7.0	-	-	-	-
October 2002	-	-	-	-	162 $\pm$ 29.0	137 $\pm$ 34.0
October - November 2002	-	-	241 $\pm$ 44.0 ( $s=67$ ; $P<0.01$ )	66 $\pm$ 19.0	-	-
January 2003	-	-	243 $\pm$ 23.0 ( $s=398.5$ ; $P<0.001$ )	71 $\pm$ 11.0	307 $\pm$ 40.0	65 $\pm$ 35.5
February 2003	226 $\pm$ 35.0 ( $s=1005.5$ ; $P<0.007$ )	96 $\pm$ 14.0	-	-	-	-
November 2003	-	-	172 $\pm$ 31.0	211 $\pm$ 42.0	-	-

A dash (-) indicates that no observations were made at that site during that month.

The mean cumulative foraging time of monitored honeybees per plant per survey period varied remarkably among plants over survey seasons and sites. At CB, the most striking example of variation among plants in the mean foraging time was in March 2002, with an approximate 600-fold difference between Plant 4 and Plant 11 (Figure 3.2). At GB, the most extreme example of variation among plants in mean foraging time was an approximate 400-fold difference between Plant 2 and Plant 1, in February 2002 (Figure 3.3). However, at IL, there was just a 4-fold difference between the plant with the lowest (Plant 16) and the plant with the greatest (Plant 1) mean foraging time, in the October 2002 survey season (Figure 3.4). Randomisation tests detected significant variation among plants in the mean cumulative foraging time of consecutive monitored honeybees per survey period, for all survey seasons at CB, and for the survey conducted in November 2003 at GB (Table 3.3). The randomisation tests did not reveal any significant variation among plants for either survey season at IL (Table 3.3). The greatest mean cumulative foraging time per survey period per plant, was 578 s ( $\pm 151.0$ ) on Plant 11 at CB in March 2002, 385 s ( $\pm 310.0$ ) on Plant 1 at GB in February 2002, and 413 s ( $\pm 114.0$ ) on Plant 1 at IL in January 2003.

With respect to plants with the highest or lowest mean cumulative foraging time of consecutive honeybees, I found few strong or consistent patterns over survey seasons. At CB, there were no consistent patterns among plants with respect to the highest mean honeybee foraging time (Figure 3.2). However, as with previous foraging variables, Plant 4 had the lowest foraging time for all three survey seasons (Figure 3.2). At GB, there were no consistent patterns among plants with respect to the highest or lowest mean honeybee foraging time (Figure 3.3). At IL, the same plant (Plant 1) had the highest mean honeybee foraging time in both survey seasons, but there was no consistency with respect to the plant with the lowest mean foraging time (Figure 3.4).

#### 3.3.1.5 Other Insect Visitors

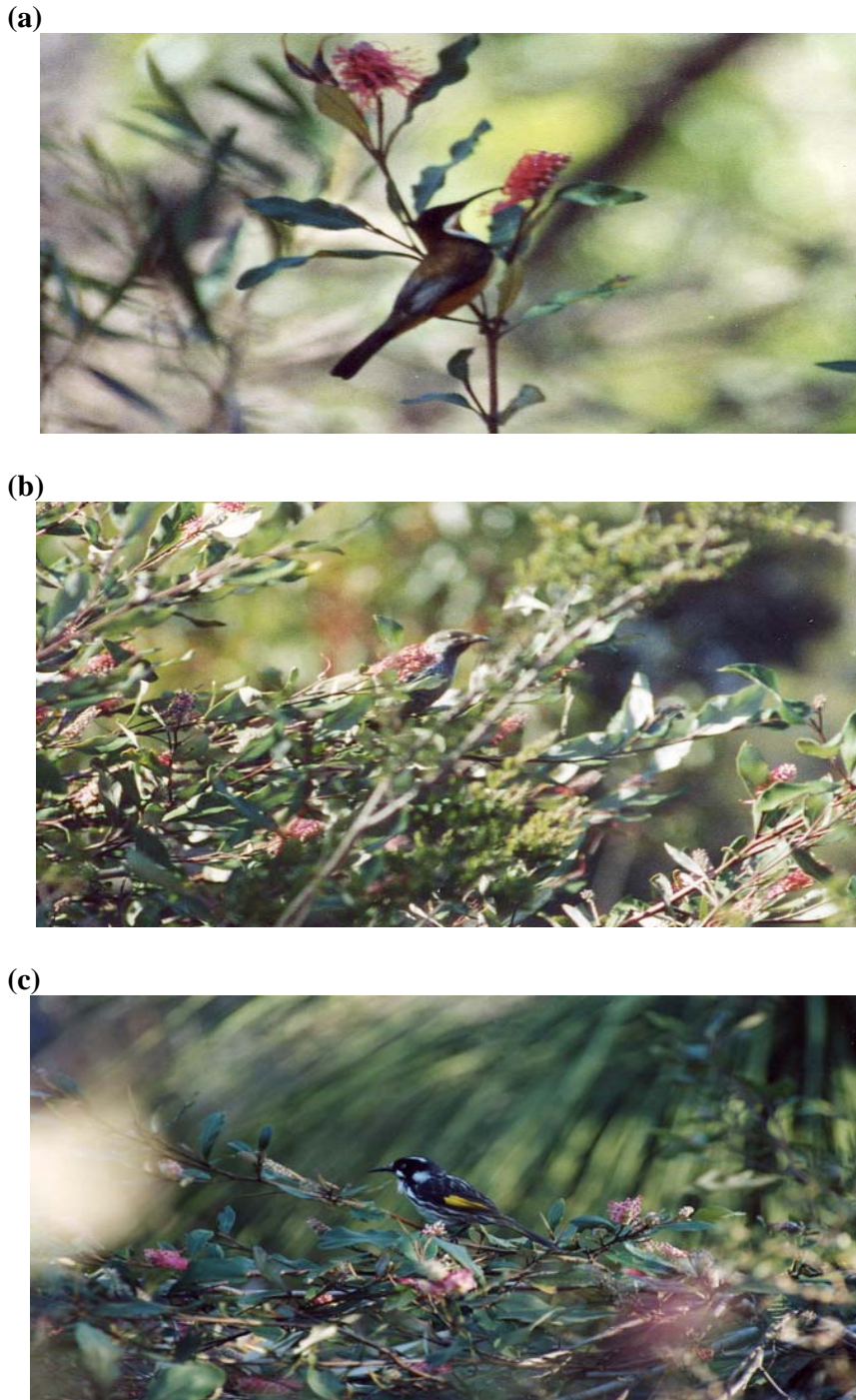
I occasionally observed insects other than honeybees foraging on *G. macleayana* inflorescences. These included several species of butterflies, ants, flies, wasps, and beetles. These insects generally had similar foraging behaviours to honeybees. Flies, wasps, ants and beetles burrowed between flowers to reach the nectary and did not generally contact the pollen presenter. However, butterflies landed near inflorescences and foraged from adjacent open flowers, possibly allowing for pollen to contact their

wings. Due to the scarcity of these visits and the ineffective foraging behaviour I generally observed, I did not consider that these insects contributed substantially to plant reproductive success.

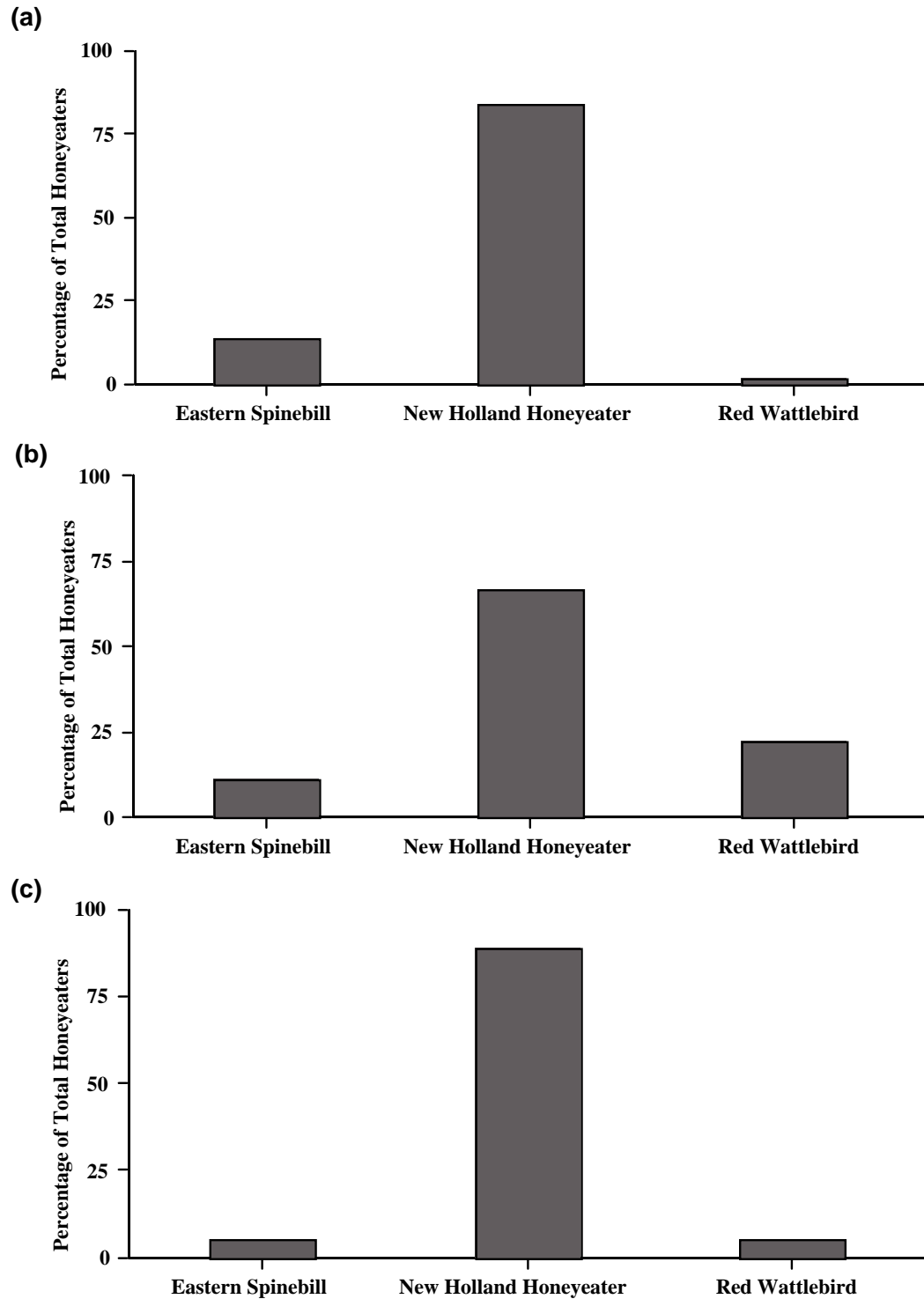
### 3.3.2 Honeyeater Foraging Behaviour

During honeyeater surveys I observed Eastern Spinebills (*Acanthorhynchus tenuirostis*), Red Wattlebirds (*Anthochaera carunculata*), and New Holland Honeyeaters (*Phylidonyris novaehollandiae*) foraging at *G. macleayana* plants (Figure 3.5). However, at other times during fieldwork I also observed Little Wattlebirds (*Anthochaera chrysoptera*) and Silvereyes (*Zosterops lateralis*) foraging for nectar; Crimson Rosellas (*Platycercus elegans*) and Eastern Rosellas (*P. eximius*) feeding on seeds; and Superb Fairy-Wrens (*Malurus cyaneus*) and Eastern Yellow Robins (*Eopsaltria griseogularis*) foraging for insects. Nectar foraging usually involved a honeyeater landing on a stem immediately behind an inflorescence, or close by, and leaning towards the inflorescence to probe for nectar between recently opened (and often unopened) flowers. This foraging behaviour should facilitate pollen transfer, as the forehead, throat and breast of the honeyeater may make contact with pollen presenters, thus enabling either the removal or deposition of pollen. Whilst I did not measure this directly, it is possible that some honeyeater species are more effective pollinators than others due to variation in bill size, foraging behaviour and movements among plants.

The total number of Eastern Spinebills (ESB), New Holland Honeyeaters (NHH), and Red Wattlebirds (RWB) I recorded foraging at *G. macleayana* plants varied greatly among sites. Of the three sites, CB had the greatest number of both ESBs and NHHs, with 22 and 132, respectively. GB had the greatest number of RWBs (12). Overall, NHHs dominated honeyeater foraging activity, with between 66.7% (at GB) and 88.9% (at IL) of all honeyeater visits (Figure 3.6). At CB and IL, the lowest percentages of honeyeater visits were from RWBs, with just 1.9% and 5.6, respectively. At GB, the lowest percentages of honeyeater visits were from ESBs, with 11.1% (Figure 3.6).



**Figure 3.5 - Photographs of honeyeaters visiting *Grevillea macleayana* plants.** An (a) Eastern Spinebill (*Acanthorhynchus tenuirostris*) foraging for nectar on a *G. macleayana* inflorescence, (b) Red Wattlebird (*Anthochaera carunculata*), and (c) New Holland Honeyeater (*Phylidonyris novaehollandiae*) perched on *G. macleayana* plants.



**Figure 3.6 - The percentage (%) of honeyeater visits that were by Eastern Spinebills, New Holland Honeyeaters and Red Wattlebirds.**

Surveys were recorded on *Grevillea macleayana* plants at Chinamans Beach (Figure a), Greenfields Beach (Figure b), and Illowra Lane (Figure c), in 2002 and 2003.

When recording the foraging behaviour of individual honeyeaters, I also recorded the next plant it visited (where possible), after leaving the *G. macleayana* plant I was currently observing. At CB, I observed this for 23 honeyeaters, and of these, 14 (61%) flew to another *G. macleayana* plant. At GB, I observed this for 20 honeyeaters, and of these, 15 (75%) flew to another *G. macleayana* plant. At IL, I observed this for three honeyeaters, and all of these flew to another *G. macleayana* plant. At CB and GB, the other plants that honeyeaters flew to after leaving *G. macleayana* plants were *Banksia* spp., *Eucalyptus* spp., *Kunzea ambigua*, *Lambertia formosa*, and *Dodonaea* spp.

In the next three sections I outline the observed variation among *G. macleayana* plants in: (1) the number of honeyeaters visiting plants; (2) the number of inflorescences visited by honeyeaters per plant; and (3) and foraging time of honeyeaters per inflorescence and per plant. I found substantial variation among plants in each of these three foraging variables and also detected variation among survey seasons within sites. I also examine patterns of honeyeater foraging behaviour among plants, to determine whether particular plants are receiving lower or higher rates of foraging behaviour, over consecutive survey seasons.

### 3.3.2.1 Number of Honeyeaters

Overall, I observed 5.6 honeyeaters per hour at CB, 1.6 per hour at GB, and 1 per hour at IL. The number of honeyeaters I observed foraging at plants varied dramatically among plants within survey seasons, among survey seasons within sites and among sites. The most striking example of variation among survey seasons within a site was at CB, with an approximate 8.5-fold difference between the September-October 2002 and the February 2003 survey seasons (Table 3.2). CB also had the greatest total number of honeyeater visits (157), approximately 3-times that of GB and over 8-times that of IL (Table 3.2). As was the pattern with honeybees, the surveys January and February 2003 surveys resulted in the greatest numbers of honeyeaters at all three sites, contributing to more than 55% of the total honeyeaters observed over all survey periods (Table 3.2). The greatest mean numbers of honeyeaters per survey period per plant per site, were 10.00 ( $\pm 2.0$ ) on Plant 1 at CB, 1.7 ( $\pm 2.9$ ) on Plant 2 at GB, and 1.1 ( $\pm 0.7$ ) on Plant 2 at IL, all recorded in January or February 2003.

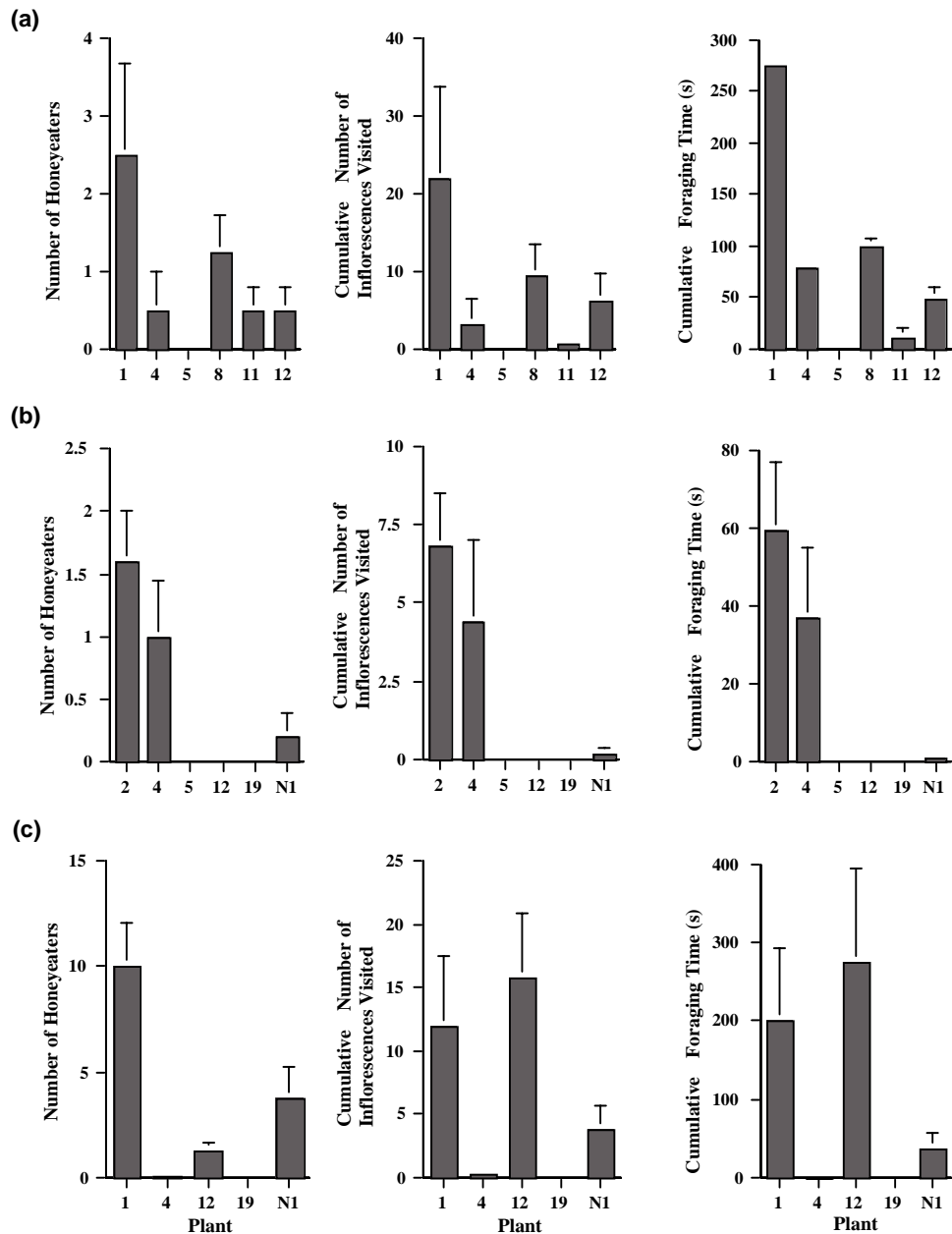
I detected striking variation in the mean number of honeyeaters recorded foraging per plant (during a survey period), both among plants per survey season and among sites. The most striking example of variation among plants was at CB in February 2003, with a 10-fold difference between the plant with the lowest (Plant 19) and the plant with the greatest (Plant 1) mean number of honeyeaters (Figure 3.7). At GB, the most extreme example of variation among plants was detected in January 2003, with an approximate 7-fold difference between Plant N7 and Plant 2 (Figure 3.8). At IL, there was minimal variation among plants, with Plants 1, 6 and N7 receiving no honeyeater visits and Plant 2 having approximately one honeyeater visit (Figure 3.9). Randomisation tests detected significant variation among plants in the mean number of honeyeaters observed during survey periods, conducted in September 2002 and February 2003, at CB (Table 3.7).

At each site, there were some strong patterns of consistency among plants for those with the highest number of honeyeaters per survey season. Patterns were less clear with respect to plants that recorded the lowest numbers of honeyeaters, because many plants did not receive any visits over consecutive survey seasons. At CB, Plant 1 (used in two of three survey seasons) had the highest mean number of honeyeaters per survey period. At GB, Plant 2 (used in two of three survey seasons) had the greatest mean number of honeyeaters per survey period (Figure 3.8). At IL, Plant 2 had the greatest mean number of honeyeaters per survey period (Figure 3.9).

#### 3.3.2.2 *Number of Inflorescences Visited*

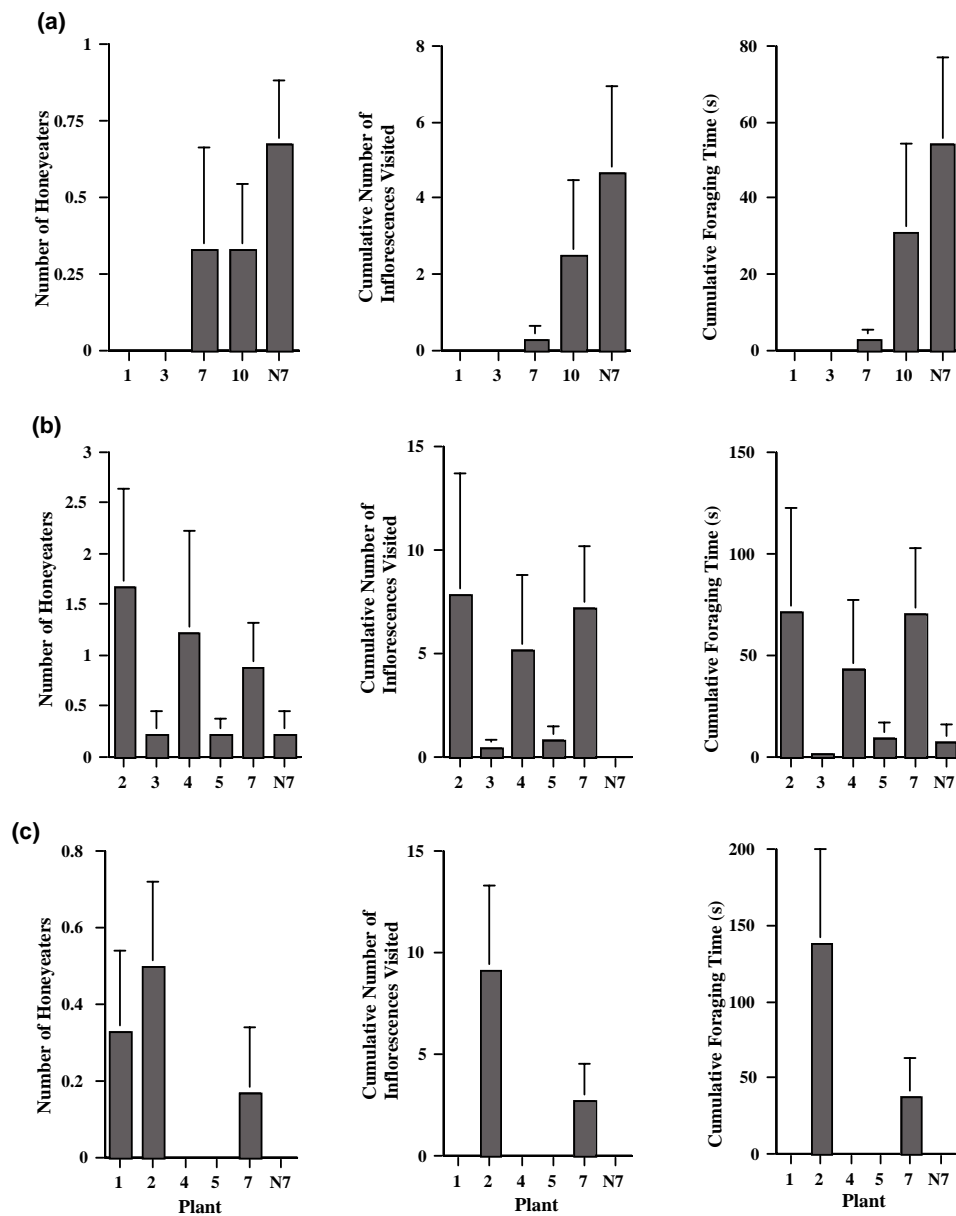
The mean number of inflorescences visited by an individual honeyeater per plant ranged from 2.5 ( $\pm 0.5$ ) in January 2003 at IL (however, this mean was based on only two honeyeaters) to 14.2 ( $\pm 2.96$ ) in November 2003 at GB (Table 3.4). The mean number of inflorescences foraged at per honeyeater per plant varied greatly among survey seasons within sites, approximately two- and three-fold at CB and GB, respectively (Table 3.4). The mean numbers of inflorescences foraged at per honeyeater per plant at CB, GB, and IL were 6.9 ( $\pm 0.7$ ), 8.1 ( $\pm 0.9$ ), and 8.7 ( $\pm 2.3$ ), respectively.





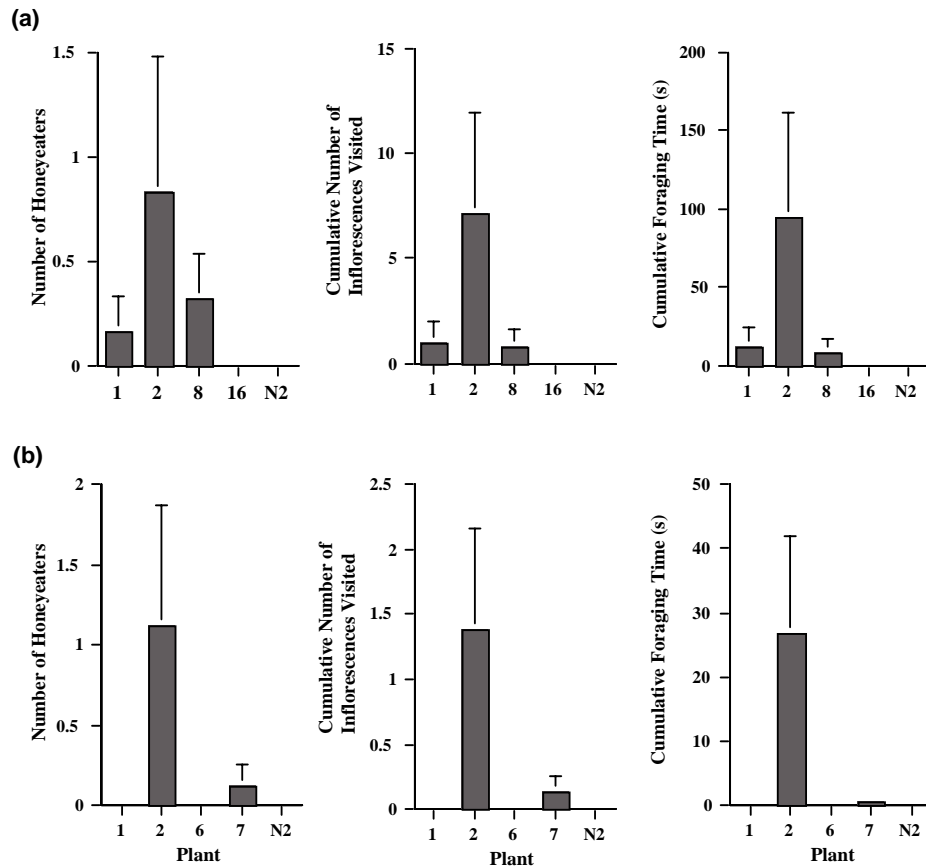
**Figure 3.7 - Honeyeater foraging behaviour among *Grevillea macleayana* plants at Chinamans Beach.**

The mean number of honeyeaters per plant per survey period (15-20 min), the cumulative number of inflorescences visited by monitored honeyeaters, and the cumulative foraging time (s) of monitored honeyeaters on *G. macleayana* plants per survey period. Surveys were undertaken at Chinamans Beach in March 2002 (Figure a), September 2002 (Figure b), and February 2003 (Figure c). Plants are displayed in order of identification code. Bars indicate plus one standard error.



**Figure 3.8 - Honeyeater foraging behaviour among *Grevillea macleayana* plants at Greenfields Beach.**

The mean number of honeyeaters per plant per survey period (15 - 20 min), the cumulative number of inflorescences visited by monitored honeyeaters and the cumulative foraging time (s) of monitored honeyeaters on *G. macleayana* plants per survey period. Surveys were undertaken at Greenfields Beach in October 2002 (Figure a), January 2003 (Figure b), and November 2003 (Figure c). Plants are displayed in order of identification code. Bars indicate plus one standard error.



**Figure 3.9 - Honeyeater foraging behaviour among *Grevillea macleayana* plants at Illowra Lane.**

The mean number of honeyeaters per plant per survey period (15 - 20 min), the cumulative number of inflorescences visited by monitored honeyeaters, and the cumulative foraging time (s) of monitored honeyeaters on *G. macleayana* plants per survey period. Surveys were undertaken at Illowra Lane in October 2002 (Figure a) and January 2003 (Figure b). Plants are displayed in order of identification code. Bars indicate plus one standard error.

**Table 3.7 - The results of randomisation tests performed to test for significant variation among *Grevillea macleayana* plants in honeyeater foraging activity.**

The foraging activity tested was: (1) the number of honeyeaters per plant per survey period; (2) the cumulative number of inflorescences foraged at by consecutive honeyeaters monitored in a survey period, and (3) the cumulative foraging time (s) of consecutive honeyeaters monitored during a survey period. Surveys were undertaken at Greenfields Beach (GB), Chinamans Beach (CB) and Illowra Lane (IL) in 2002 and 2003. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

Site	Season	Parameter	<i>F</i> Ratio	df <sup>9</sup>	Treatment Sum of Squares	Final Probability
<b>GB</b>	October 02	Number of Birds	2.26	4, 20	4.00	0.08
	October 02	Inflorescences	2.15	4, 20	168.83	0.08
	October 02	Foraging Time	2.72	4, 20	23651.50	0.05
	January 03	Number of Birds	1.17	5, 40	46.89	0.33
	January 03	Inflorescences	1.47	5, 40	1283.89	0.17
	January 03	Foraging Time	1.37	5, 40	110542.0	0.15
	November 03	Number of Birds	2.0	5, 25	2.33	0.13
	November 03	Inflorescences	3.74	5, 25	546.83	<b>0.02</b>
	November 03	Foraging Time	4.22	5, 25	122701.5	<b>0.01</b>
<b>CB</b>	March 02	Number of Birds	2.20	5, 15	34.25	0.11
	March 02	Inflorescences	2.14	5, 15	2497.75	0.08
	March 02	Foraging Time	1.87	5, 15	377195.25	0.12
	September 02	Number of Birds <sup>10</sup>	6.06	5, 15	18.75	<b>0.01</b>
	September 02	Number of Birds <sup>11</sup>	6.12	4, 16	18.00	<b>0.01</b>
	September 02	Inflorescences	5.50	5, 15	361.50	<b>&lt; 0.01</b>
	September 02	Inflorescences	5.23	4, 16	328.20	<b>&lt; 0.01</b>
	September 02	Foraging Time	6.10	5, 15	27105.25	<b>0.01</b>
	September 02	Foraging Time	5.51	4, 16	24301.60	<b>0.01</b>
	February 03	Number of Birds	13.15	4, 28	925.125	<b>&lt; 0.01</b>
	February 03	Inflorescences	4.59	4, 28	3233.25	<b>&lt; 0.01</b>
	February 03	Foraging Time	3.49	4, 28	946306.63	<b>0.01</b>
<b>IL</b>	October 02	Number of Birds	1.39	4, 20	5.00	0.23
	October 02	Inflorescences	2.11	4, 20	318.33	0.20
	October 02	Foraging Time	1.85	4, 20	54509.00	0.20
	January 03	Number of Birds	2.10	4, 28	10.25	0.07
	January 03	Inflorescences	2.88	4, 28	15.25	<b>0.02</b>
	January 03	Foraging Time	3.20	4, 28	5780.13	<b>0.04</b>

<sup>9</sup> Plant degrees of freedom, Residual degrees of freedom.

<sup>10</sup> First test excluding final observation session due to missing data point.

<sup>11</sup> Second test excluding Plant 5 due to missing data point.

The mean cumulative number of inflorescences foraged at by consecutive honeyeaters per plant (per survey period) varied dramatically among plants per survey season and among sites. The most striking example of variation among plants in the mean cumulative number of inflorescences visited by honeyeaters per survey period was detected at CB in March 2002, with a 22-fold difference between Plant 5 and Plant 1 (Figures 3.7). At GB, the most extreme example of variation among plants was detected in November 2003, with an approximate 9-fold difference between plants with zero inflorescences visited (Plants 1, 4, 5, N7) and Plant 2 (Figure 3.8). At IL, the most striking example of variation among plants was detected in October 2002, with a 7-fold difference between plants with zero inflorescences visited (Plants 116 and N2) and Plant 2 (Figure 3.9). Randomisation tests revealed significant variation among plants in the mean cumulative number of inflorescences visited by consecutive honeyeaters per survey period for surveys undertaken in September 2002 and February 2003 at CB, November 2003 at GB and January 2003 at IL (Table 3.7). The greatest mean number of inflorescences visited per plant by consecutive honeyeaters (per survey period) at each site were 22.0 ( $\pm 11.71$ ) on Plant 1 at CB in March 2002, 7.9 ( $\pm 5.8$ ) on Plant 3 at GB, and 7.2 ( $\pm 4.7$ ) on Plant 2 at IL in October 2002.

I detected some strong patterns of consistency among plants, for those plants with the highest number of inflorescences visited by honeyeaters per survey season, at GB and IL. Patterns were not as clear for plants that recorded the lowest number of inflorescences visited by honeyeaters, as many plants did not record any inflorescence visits over consecutive survey seasons. At GB, Plant 2 had the greatest mean number of inflorescences visited in the two survey seasons in which it was monitored (Figure 3.8). At IL, Plant 2 had the greatest mean number of inflorescences visited in both survey seasons (Figure 3.9).

### 3.3.2.3 Honeyeater Foraging Time

The mean time individual honeyeaters spent foraging at a single inflorescence per plant varied approximately three-fold among sites, but varied less among survey seasons within sites (Table 3.5). The mean honeyeater foraging times per inflorescence per plant ranged from 6 s ( $\pm 1.0$ ) at CB in March 2002 to 14 s ( $\pm 3.0$ ) at IL in January 2003. However, the mean for IL in January 2003 was generated from only two honeyeater

visits. The mean honeyeater foraging times per inflorescence per plant at CB, GB, and IL were 7 ( $\pm 0.5$ ), 8.0 ( $\pm 0.5$ ), and 11 ( $\pm 1.0$ ), respectively.

The mean time individual honeyeaters spent foraging per plant varied six-fold among sites, ranging from 36 s ( $\pm 7.0$ ) in September 2002 at CB to 211 s ( $\pm 42.0$ ) in November 2003 at GB (Table 3.6). Among survey seasons within sites, the mean foraging time per honeyeater per plant varied approximately two- or three-fold. The mean honeyeater foraging time per plant for CB, GB, and IL was 86 s ( $\pm 9.0$ ), 89 s ( $\pm 12.0$ ), and 117 s ( $\pm 28.0$ ), respectively. The greatest mean honeyeater foraging times per survey period per plant at each site, were 275 s ( $\pm 150.0$ ) on Plant 1 at CB in March 2002, 138 s ( $\pm 62.0$ ) on Plant 2 at GB in November 2003, and 94 s ( $\pm 67.0$ ) on Plant 2 at IL in October 2002.

The mean cumulative foraging time of consecutive honeyeaters per plant per survey period, varied greatly among plants per survey season and among sites. The most striking example of variation among plants was detected at CB in both March 2002 and February 2003; with an approximate 275-fold difference between the plants with the lowest the highest mean foraging time of honeyeaters (Figure 3.7). At GB, intraspecific variation among plants was most notable in November 2003, with an approximate 140-fold difference between plants with no honeyeater visits (Plants 1, 4, 5 and N7) and Plant 2 (Figure 3.8). At IL, intraspecific variation among plants was most notable in October 2002, with a 95-fold difference between plants with no honeyeater visits (Plants 16 and N2) and Plant 2 (Figure 3.9). Randomisation tests found significant variation among plants in the mean cumulative time consecutive honeyeaters spent foraging per plant per survey period, for surveys undertaken in September 2002 and February 2003 at CB, November 2003 at GB and January 2003 at IL (Table 3.7).

I found few strong or consistent patterns over survey seasons, with respect to plants with the highest or lowest mean cumulative foraging time of consecutive honeyeaters per survey period. One to three plants per survey season per site did not receive any honeyeater visits, and often these were the same plants over consecutive survey seasons. At GB, honeyeaters visiting Plant 2 recorded greater mean foraging times in the two survey seasons it was monitored, however, this was only marginal in the January 2003

season (Figure 3.8). At IL, Plant 2 received the greatest mean honeyeater foraging time in both survey seasons (Figure 3.9).

### 3.3.3 Variation between Honeybees and Honeyeaters in Foraging Behaviour

I found significant variation between honeybees and honeyeaters at all three sites, in at least two of the three aspects of foraging behaviour (Section 3.2.3). Mann-Whitney Tests found that honeyeaters visited significantly more inflorescences per plant than honeybees in the three survey seasons of March 2002 at CB, November 2003 at GB, and October 2002 at IL (Table 3.4). Honeyeaters visited twice as many inflorescences per plant than honeybees, in March 2002 at CB and October 2002 at IL (Table 3.4). Honeyeaters also visited three times as many inflorescences per plant than honeybees, in November 2003 at GB (Table 3.4). However, honeybees and honeyeaters visited similar numbers of inflorescences per plant in the four survey seasons of September 2002 and February 2003 at CB and October 2002 and January 2003 at GB (Table 3.4).

With respect to foraging time (s) per inflorescence, I found that individual honeybees spent significantly more time foraging per inflorescence per plant than honeyeaters, in all survey seasons and sites (Table 3.5). The exception to this pattern was observed at IL in January 2003, which I did not test due to a lack of honeyeater visits. At CB, I detected an eight-fold difference in March 2002 and a five-fold difference in September 2002 and February 2003, between honeybees and honeyeaters in mean foraging time per inflorescence per plant (Table 3.5). At GB, I detected a 3.5- to 4.5-fold difference between honeybees and honeyeaters in surveys conducted in October 2002, January 2003 and November 2003 (Table 3.5). At IL, I detected a four-fold difference between honeybees and honeyeaters in the October 2002 survey (Table 3.5). The greatest variation overall was recorded in March 2002 at CB, with honeybees and honeyeaters having a mean foraging time per inflorescence per plant, of 49.3 ( $\pm 5.1$ ) and 6.3 ( $\pm 1.1$ ), respectively.

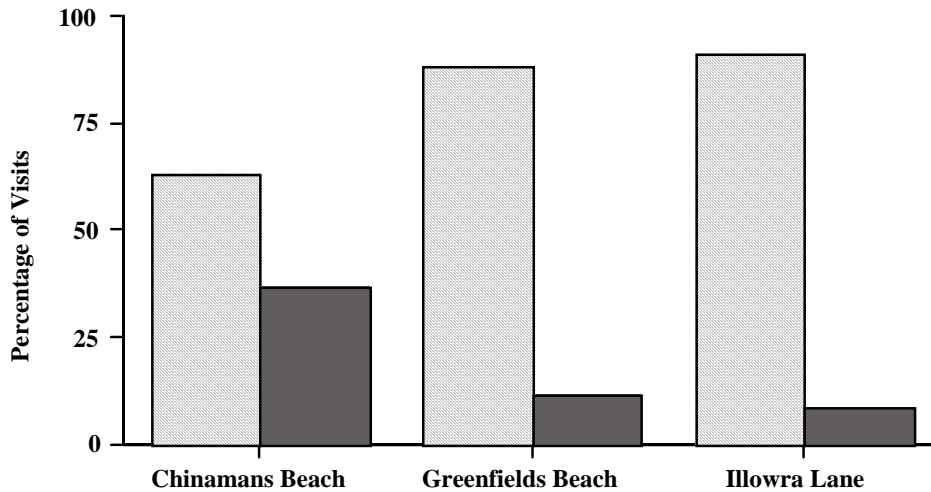
With respect to foraging time (s) per plant, individual honeybees also spent significantly more time foraging per plant than honeyeaters for survey conducted in September-October 2002 and February 2003 at CB, and October-November 2002 and January 2003 seasons at GB (Table 3.6). At CB, the greatest variation between honeybees and honeyeaters in mean foraging time per plant was recorded in February 2003, with 226.0

( $\pm 34.5$ ) and 96.2 ( $\pm 14.5$ ), respectively. At GB, there was a greater than three-fold difference between honeybees and honeyeaters in both the October/ November 2002 and January 2003 surveys (Table 3.6). At IL, there was a minimal 24-second difference in the mean foraging time per plant between honeybees ( $161.9 \pm 29.4$ ) and honeyeaters ( $137.6 \pm 34.3$ ) in October 2002.

Of the 1,128 visits I observed, 79% (896) were honeybees and 21% (232) were honeyeaters. Furthermore, honeybee visits to *G. macleayana* plants were nearly six times as frequent as honeyeater visits, on an hourly basis: 17 honeybee visits per hour compared with 2.8 honeyeater visits per hour. Visits by honeybees comprised more than 60% of all visits regardless of the site (Figure 3.10). Despite this dominance in abundance, there was still a substantial difference among sites (28.6% between CB and IL) in the percentage of honeybee visits (Figure 3.10). Moreover, there was more than a 4-fold difference in the percentage of honeyeater visits between CB, which had the largest percentage (37.1%) and IL, with the smallest percentage (8.6%).

Honeybees foraged throughout the day, and for longer daylight hours than honeyeaters. In the warmer months, I often observed honeybees foraging early in the morning with honeyeaters (i.e. 7:00am) and frequently observed them foraging into the early evening (i.e. 6:00pm). Whilst honeyeaters were active in the morning, I found them to be most active in the early afternoon. This may be because RWBs displayed less aggressive territorial behaviour in the afternoon, allowing the smaller birds (e.g. NHH and ESB) to forage more frequently.



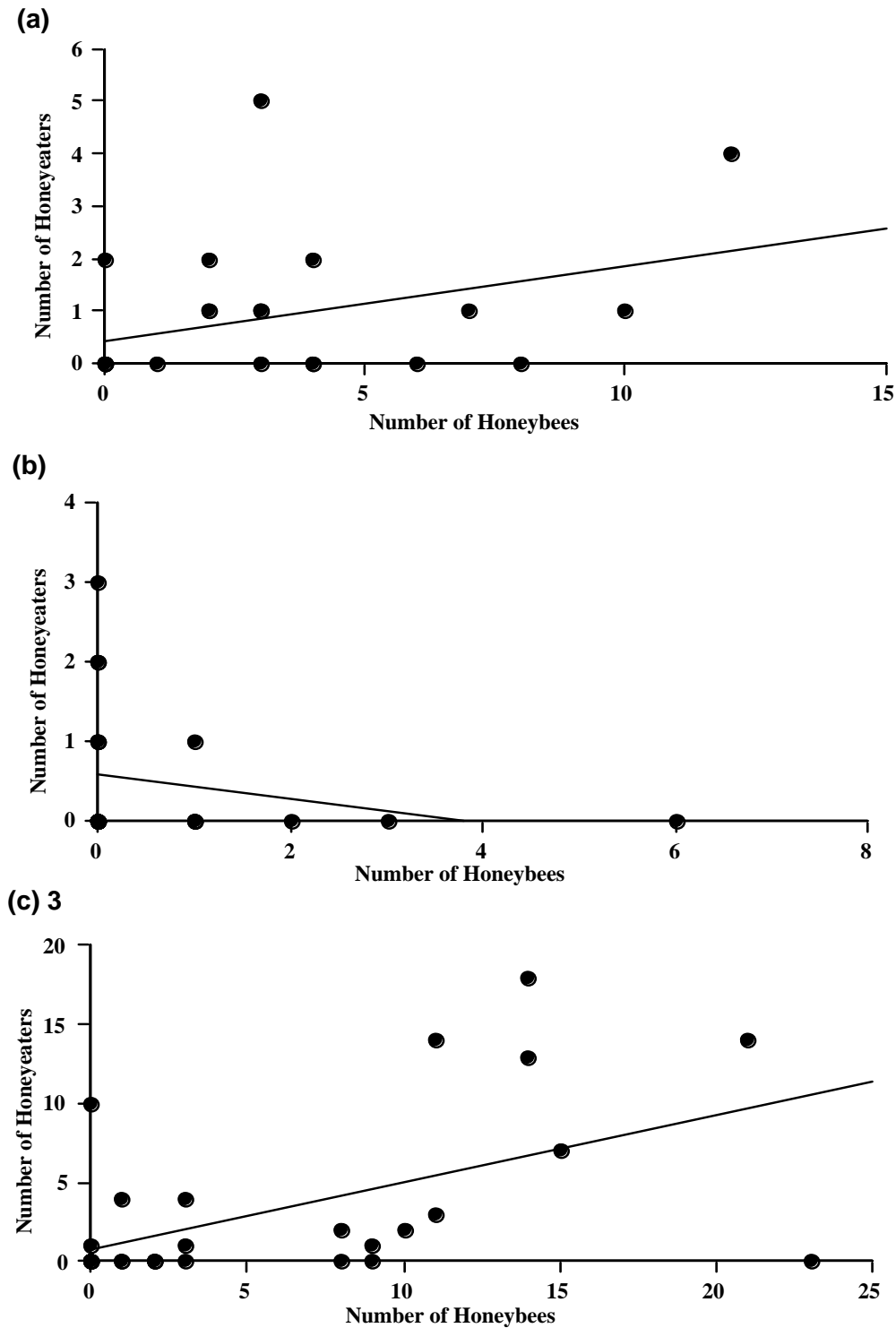


**Figure 3.10 - The percentage (%) of honeybee and honeyeater visits to *Grevillea macleayana* plants at Chinamans Beach, Greenfields Beach, and Illowra Lane.**

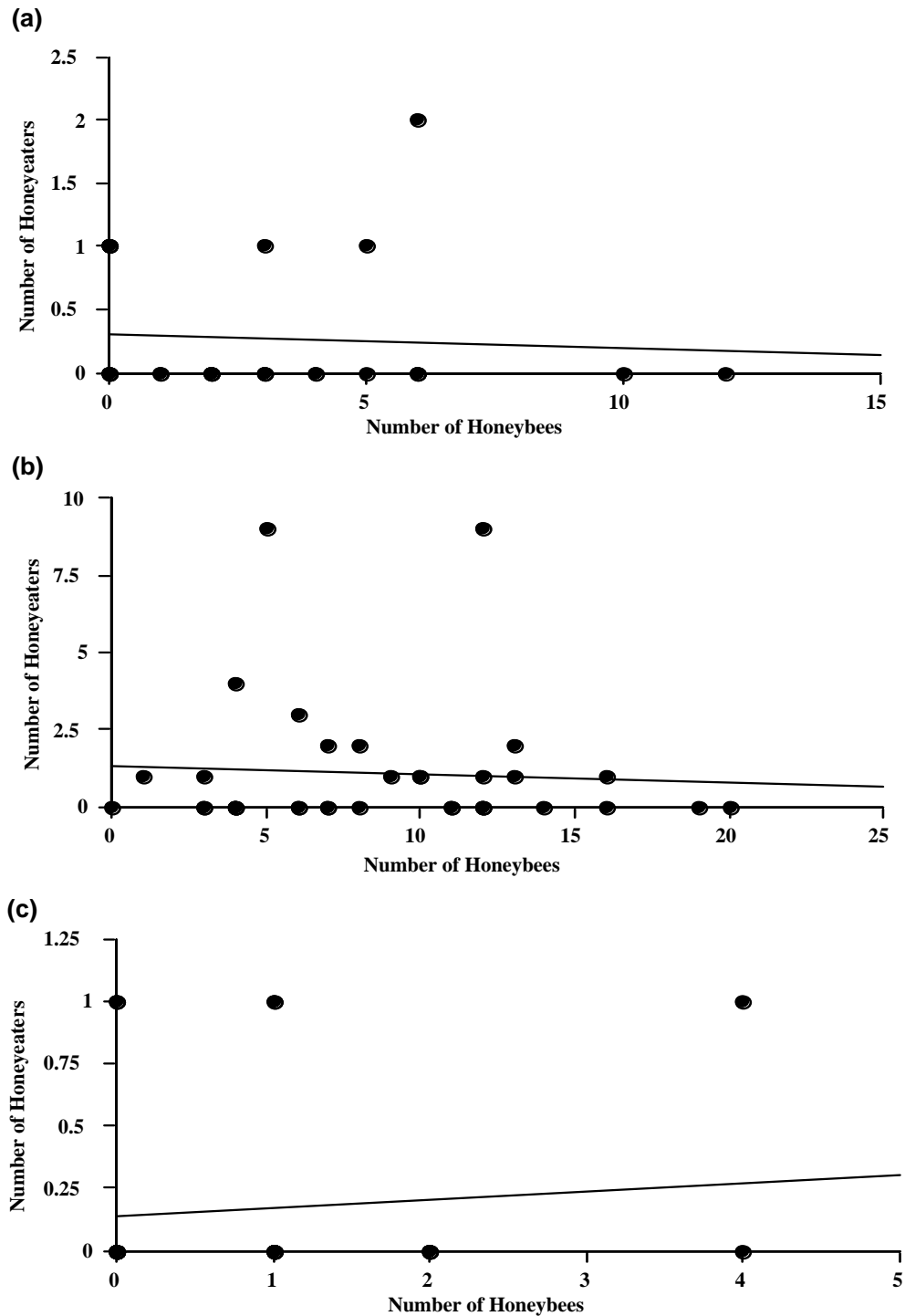
At each site, 38-39% of the survey time was spent monitoring honeybees (diagonal lines) and 61-62% of the survey time monitoring honeyeaters (shaded). Surveys were undertaken in 2002 and 2003.

### 3.3.4 Foraging Patterns of Honeybees and Honeyeaters

Surprisingly, there were few significant correlations between honeybees and honeyeaters in any of the three measures of foraging behaviour: abundance, number of inflorescences visited per plant, and foraging time per plant. With respect to abundance, I found no consistent patterns between honeybees and honeyeaters, except in March 2002 and February 2003 at CB, where positive trends were detected (Figure 3.11 - 3.13). However, only the February survey was significant ( $r_s = 0.54$ ;  $P = 0.002$ ).

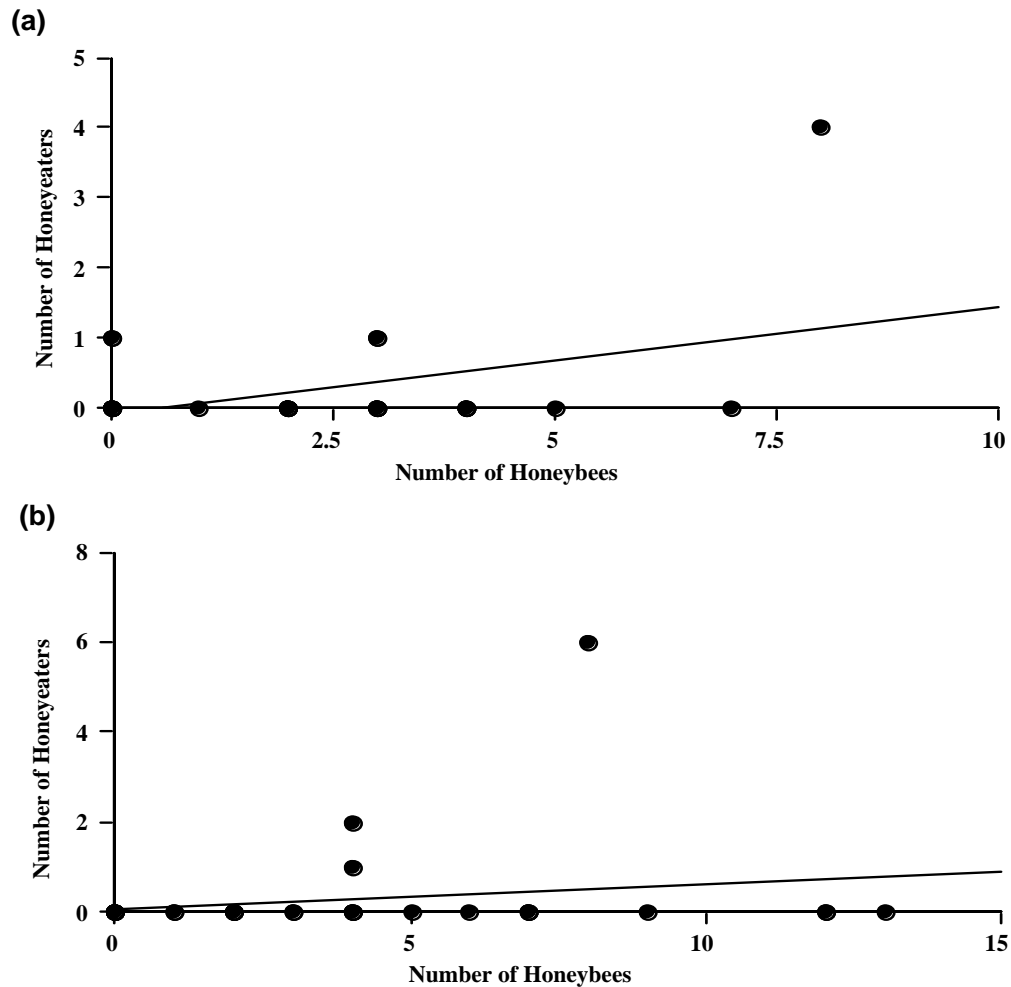


**Figure 3.11 - Spearman Rank Correlations between the total number of honeyeaters and honeybees per plant, per survey period, at Chinamans Beach.** Surveys were conducted on five or six *Grevillea macleayana* plants at Chinamans Beach in March 2002 (Figure a), September 2002 (Figure b), and February 2003 (Figure c). A significant correlation was detected between honeybees and honeyeaters in February 2003 ( $r_s = 0.54$ ;  $P = 0.002$ ).



**Figure 3.12 - Spearman Rank Correlations between the total number of honeybees and honeyeaters per plant, per survey period, at Greenfields Beach.**

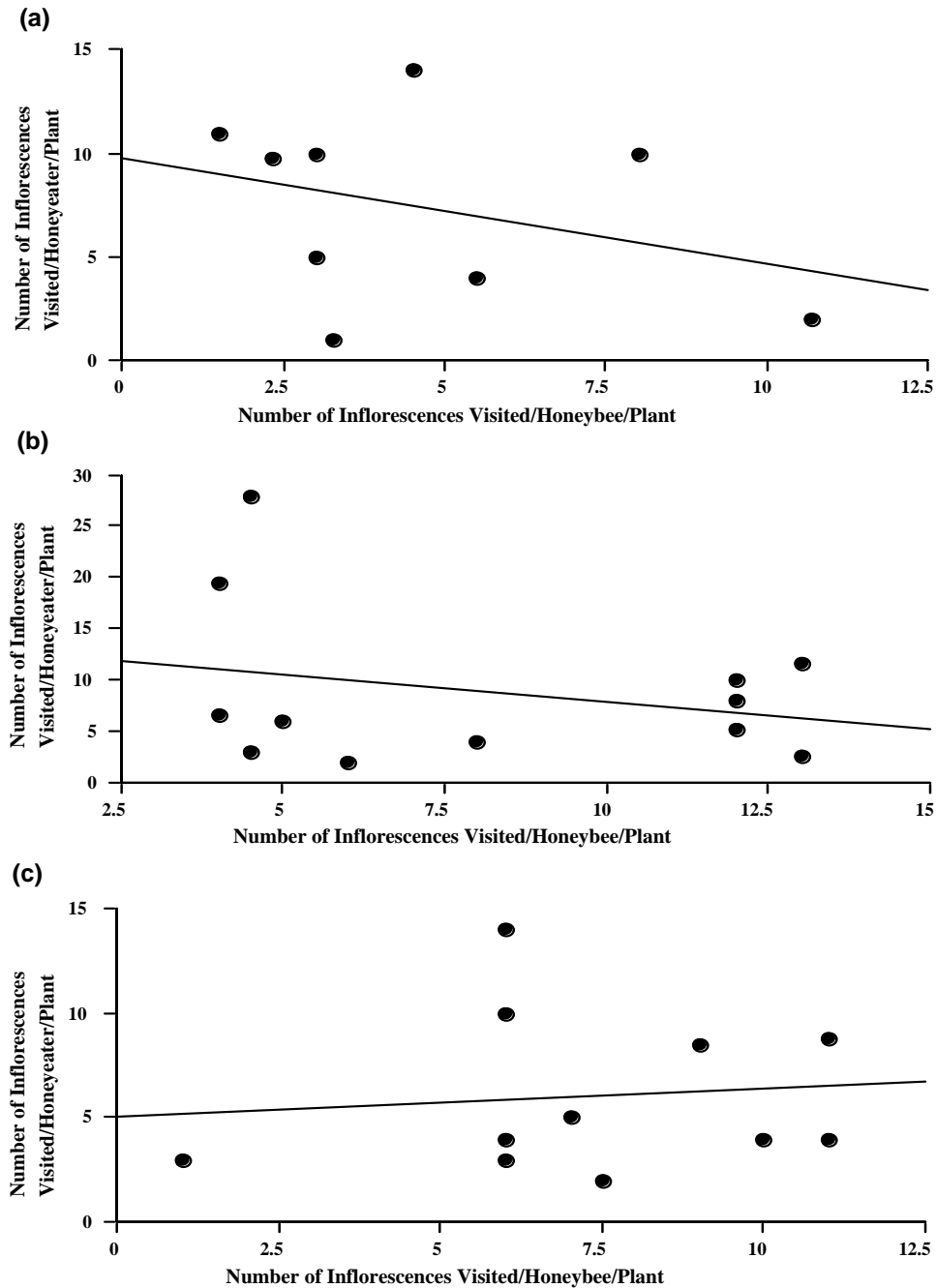
Surveys were conducted on five or six *Grevillea macleayana* plants at Greenfields Beach in October 2002 (Figure a), January 2003 (Figure b), and November 2003 (Figure c). All correlations were judged as not significant (Spearman Rank Correlation  $P > 0.05$ ).



**Figure 3.13 - Spearman Rank Correlations between the total number of honeybees and honeyeaters per plant, per survey period, at Illowra Lane.**

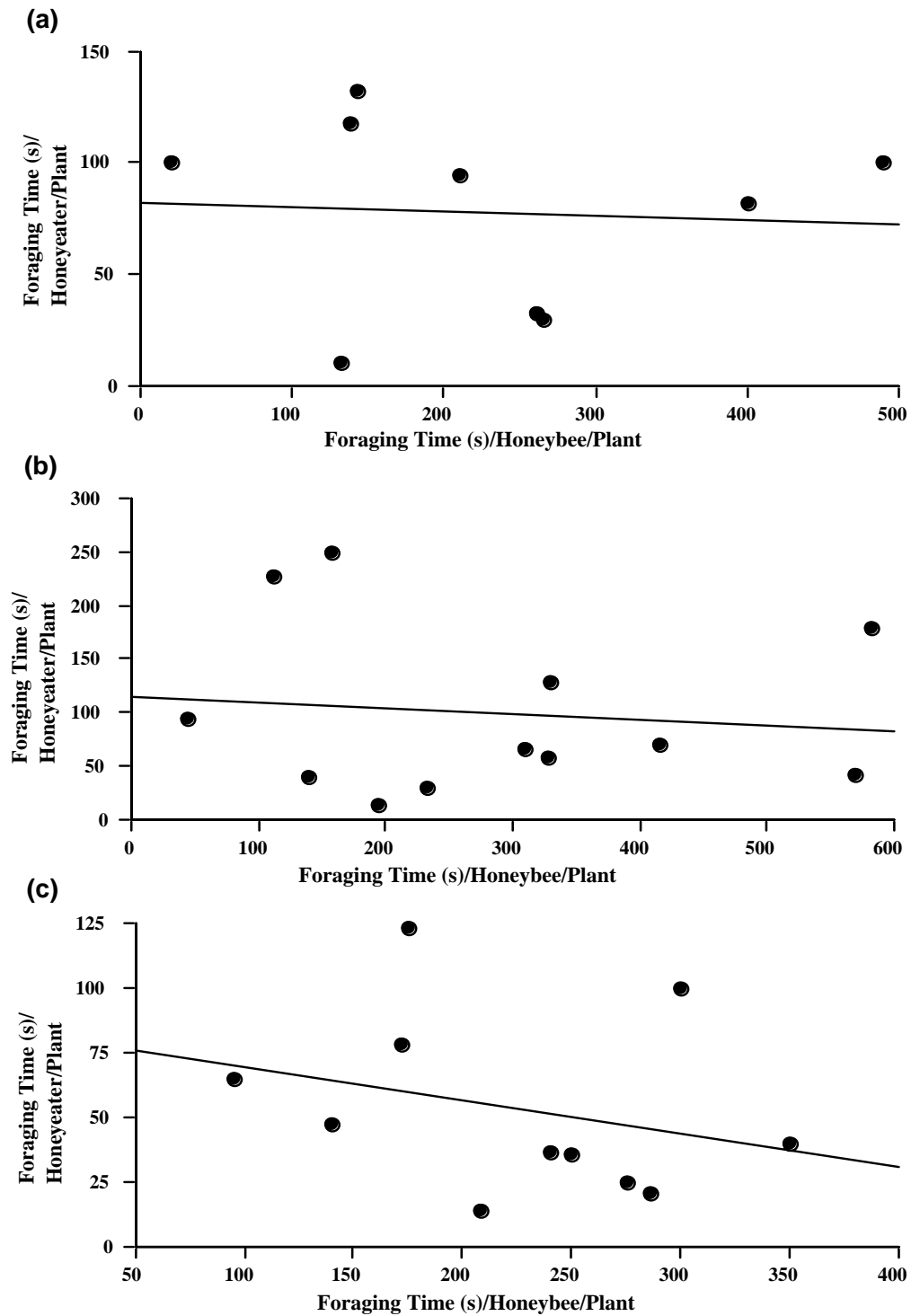
Surveys were conducted on five *Grevillea macleayana* plants at Illowra Lane in October 2002 (Figure a) and January 2003 (Figure b). All correlations were judged as not significant (Spearman Rank Correlation  $P > 0.05$ ).

I was not able to perform Spearman Rank Correlations with respect to the number of inflorescences visited and the total foraging time per plant, for every survey season per site, because of a lack of concurrent honeyeater or honeybee visits in many survey periods. The survey seasons tested were March 2002 and February 2003 at CB and January 2003 at GB. I did not detect any clear patterns or significant correlations between honeybees and honeyeaters for either measure of foraging behaviour (Figure 3.14 and 3.15). However, there were weak negative (non-significant) trends with respect to the number of inflorescences visited per plant, at CB in March 2002 and foraging time per plant at GB, in January 2003.



**Figure 3.14 - Spearman Rank Correlations between individual honeybees and honeyeaters in the mean number of inflorescences visited per *Grevillea macleayana* plant.**

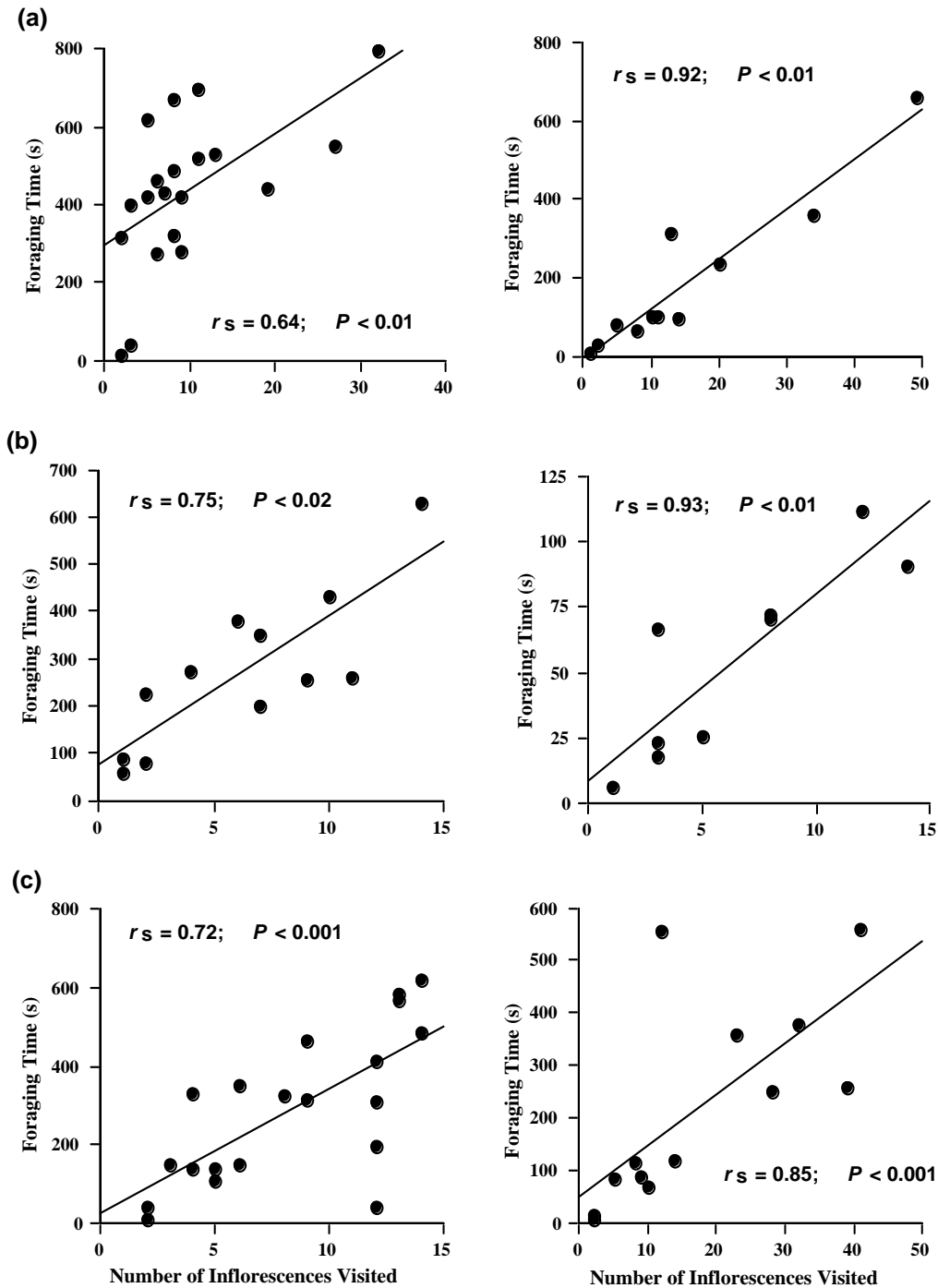
Correlations were performed on data recorded at Chinamans Beach in March 2002 (Figure a), Chinamans Beach in February 2003 (Figure b), and Greenfields Beach in January 2003 (Figure c). All correlations were judged as not significant ( $P > 0.05$ ).



**Figure 3.15 - Spearman Rank Correlations between individual honeybees and honeyeaters in mean foraging time (s) per *Grevillea macleayana* plant.**

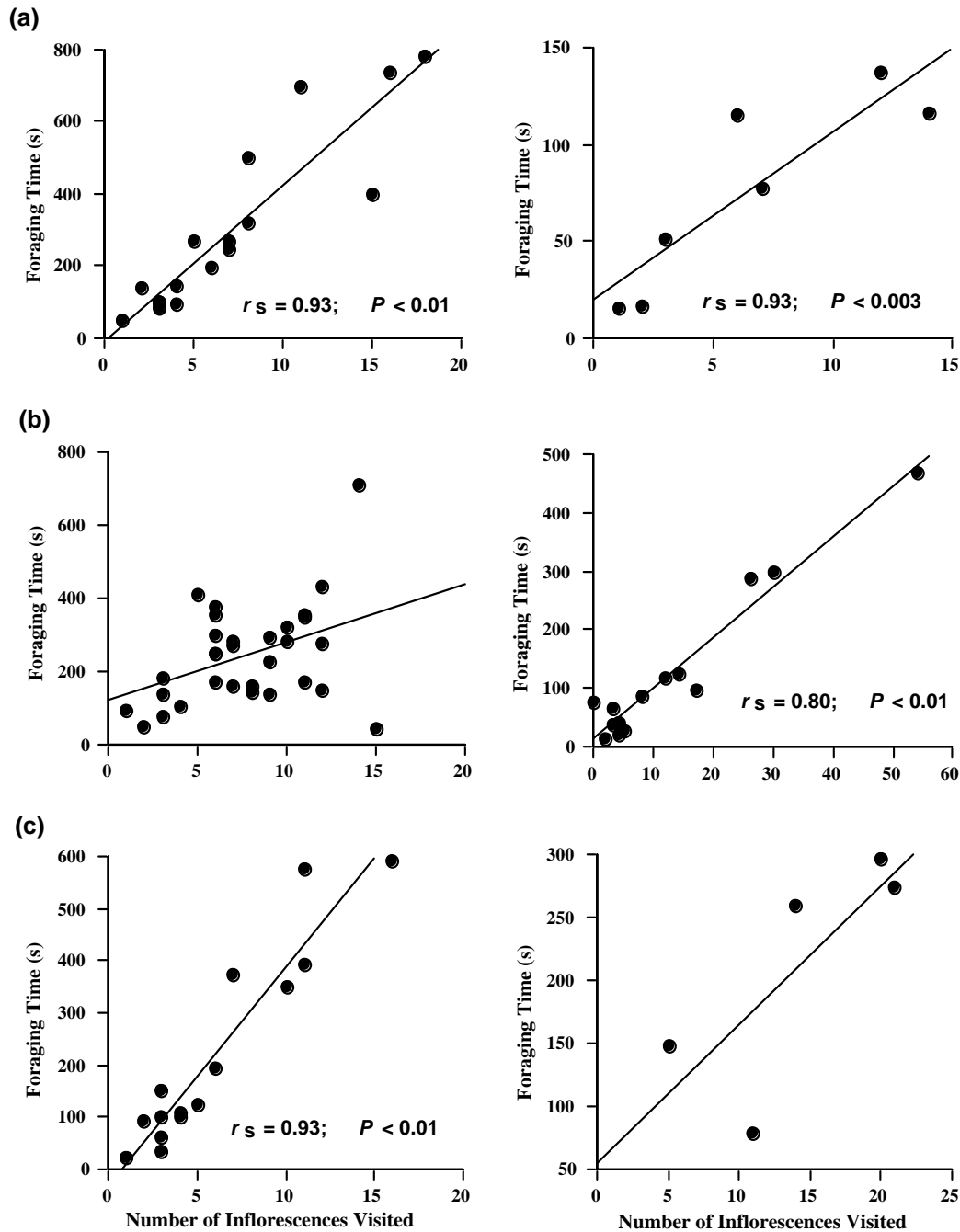
Correlations were performed on data recorded at Chinamans Beach in March 2002 (Figure a), Chinamans Beach in February 2003 (Figure b), and Greenfields Beach in January 2003 (Figure c). All correlations were judged as not significant ( $P > 0.05$ ).

Lastly, I tested for significant correlations between the number of inflorescences visited and foraging time per survey period per plant, for honeybees and honeyeaters (separately), for each survey season (Figure 3.16 – 3.18). As expected, I found that plants that had more inflorescences visited were also foraged on for longer, regardless of floral visitor type. Furthermore, significant positive correlations were detected for both honeybees and honeyeaters for all survey seasons at CB (Figure 3.16). At GB and IL, I detected significant positive correlations for honeybees for all survey seasons (Figure 3.17 and 3.18). Significant positive correlations were also detected for honeyeaters at GB and IL in all survey seasons, except November 2003 at GB and January 2003 at IL (Figure 3.17 and 3.18).

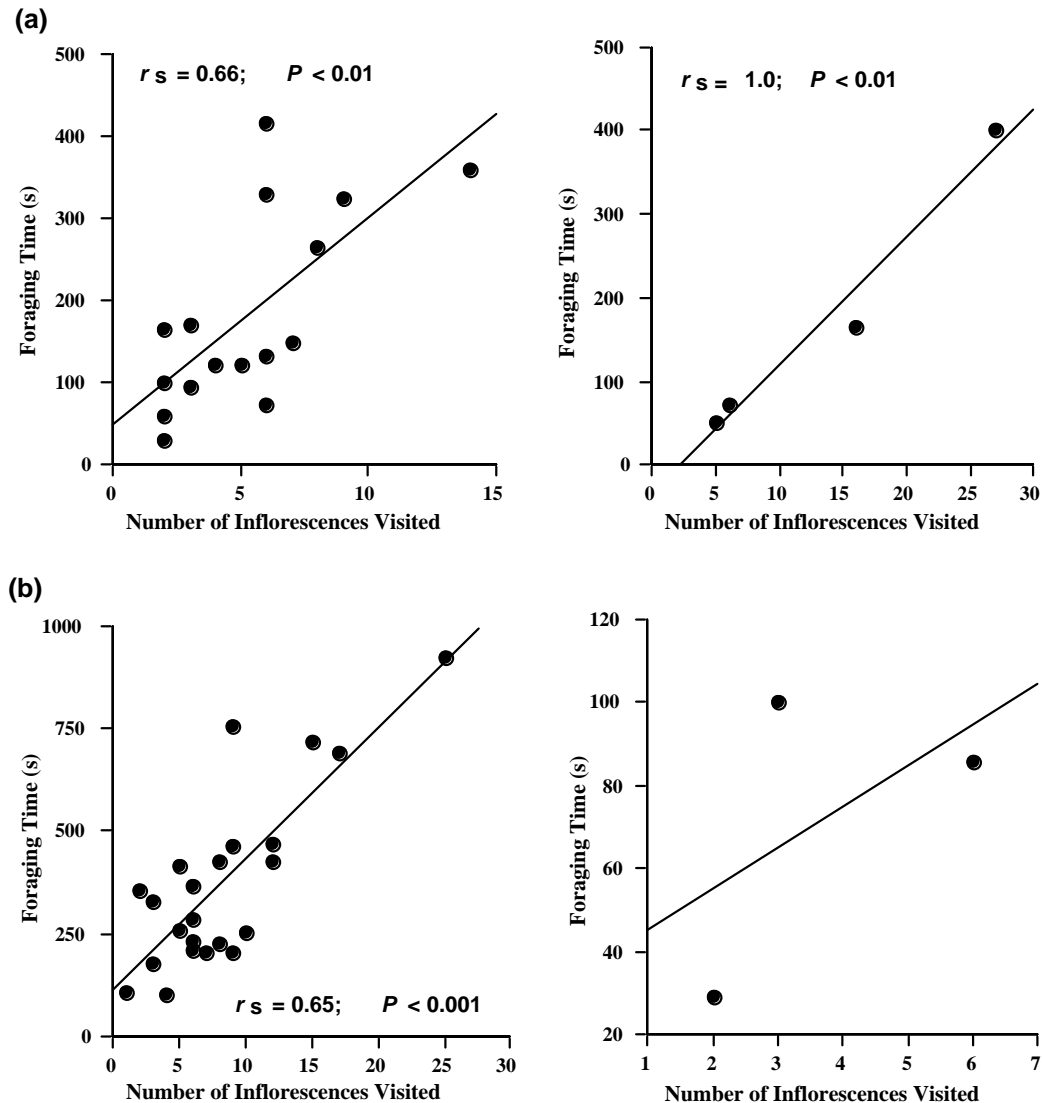


**Figure 3.16 - Spearman Rank Correlations between the number of inflorescences visited and foraging time among *Grevillea macleayana* plants at Chinamans Beach.** Correlations between the number of inflorescences visited and the foraging time (s) per survey period by honeybees (left column) and honeyeaters (right column) on *G. macleayana* plants. Surveys were conducted in March 2002 (Figure a), September 2002 (Figure b), and February 2003 (Figure c). Significant positive correlations were detected for each survey season.





**Figure 3.17 - Spearman Rank Correlations between the number of inflorescences visited and foraging time among *Grevillea macleayana* plants at Greenfields Beach.** Correlations between the number of inflorescences visited and foraging time (s) per survey period by honeybees (left column) and honeyeaters (right column) on *G. macleayana* plants. Surveys were conducted in October 2002 (Figure a), January 2003 (Figure b), and November 2003 (Figure c). Significant positive correlations were detected for each survey season, except for honeybees in January 2003 and honeyeaters in November 2003.



**Figure 3.18 - Spearman Rank Correlations between the number of inflorescences visited and foraging time among *Grevillea macleayana* plants at Illowra Lane.** Correlations between the number of inflorescences visited and the foraging time (s) by honeybees (left column) and honeyeaters (right column) on *G. macleayana* plants at Illowra Lane in October 2002 (Figure a) and January 2003 (Figure b). Significant positive correlations were detected for all survey seasons, except honeyeaters in January 2003.

### 3.3.5 Honeybees, Honeyeaters, and Floral Traits

Multiple regression tests between the number of honeybees or the number of honeyeaters per plant and inflorescence production traits (i.e. inflorescence number and size) explained between 24% and 99% of the variation among plants visited by honeybees, and 35% and 89% of the variation among plants visited by honeyeaters (Table 3.8; Appendix 3, Table A3.1). Furthermore, the whole-model tests were consistently positive and honeybee and whilst most were not significant, positive trends were frequently detected between honeyeater numbers and inflorescence numbers. The regression between honeybee number and inflorescence traits detected significant positive relationships with both inflorescence number and size at IL in January 2003, explaining 99% of the variation among plants (Table 3.8).

Multiple regressions between the number of inflorescences visited by honeybees or honeyeaters and inflorescence production traits explained up to 69% of the variation among plants visited by honeybees and 97% of the variation among plants visited by honeyeaters (Table 3.8; Appendix 3, Table A3.1). The whole-model tests from these regressions were consistently positive and whilst often not significant, positive trends between the number of inflorescences visited by both honeybees and honeyeaters and the number of inflorescences were most common. The regressions between the number of inflorescences visited by honeyeaters and inflorescence traits detected significant positive whole-model and inflorescence number relationships at CB in March 2002 and February 2003, and at GB in October/November 2002, explaining 95%, 92%, and 97% of the variation among plants, respectively (Table 3.8). The regression between the number of inflorescences visited by honeybees and inflorescence numbers detected a significant positive relationship at CB in February 2003, explaining 91% of the variation among plants (Table 3.8). The regression between the numbers of inflorescences visited by honeyeaters and inflorescence traits also detected a significant positive relationship with inflorescence size at GB in October/November 2002, explaining 97% of the variation among plants (Table 3.8).

Tests between honeybee or honeyeater foraging time per plant and inflorescence traits explained up to 80% of the variation among plants in honeybee foraging time and 97% of the variation among plants in honeyeater foraging time (Table 3.8; Appendix 3, Table A3.1). Thirty-two of the 33 whole-model tests were positive. The regressions between

honeyeater foraging time and inflorescence traits detected significant positive relationships with inflorescence number at CB in March 2002 and February 2003, and at GB in October/November 2002, explaining 95%, 93%, and 97% of the variation among plants, respectively (Table 3.8). The regression between honeyeater foraging time and inflorescence traits also detected a significant positive relationship with inflorescence size, at GB in October/November 2002 (Table 3.8). In the non-significant tests, positive trends were also most common between honeyeater foraging time and inflorescence number and between honeybee foraging time and inflorescence size.

Multiple regression tests between measures of honeybee or honeyeater foraging behaviour and nectar traits (i.e. mean nectar volume per inflorescence and the mean sugar concentration of nectar per inflorescence) revealed consistently positive whole-model trends. Regressions between the number of honeybees, the number of inflorescences visited per plant by honeybees and the foraging time per plant by honeybees (tested separately) against nectar traits explained up to 93%, 85% and 96% of the variation among plants, respectively (Table 3.8; Appendix 3, Table A3.1). The regressions between honeybee foraging time and nectar traits detected significant positive relationships with nectar volume at GB and with sugar concentration at IL in January 2003, explaining 83% and 96% of the variation among plants, respectively (Table 3.8). However, regressions between the number of honeyeaters, the number of inflorescences visited per plant by honeyeaters and the honeyeater foraging time per plant (tested separately) against nectar traits did not explain a significant amount of the variation among plants (Appendix 3, Table A3.1). In non-significant tests, negative trends were most common between nectar volume and both the number of honeyeaters per plant and the number of inflorescences visited by honeybees per plant. However, positive (non-significant) trends were more common between nectar volume and foraging time. Positive (non-significant) trends were more common between nectar sugar concentration and all three measures of honeybee foraging behaviour and negative (non-significant) trends were more common between the number of inflorescences visited by honeyeaters and honeyeater foraging time per plant.

**Table 3.8 - Significant simple linear and multiple regression analyses between the three measures of honeybee and honeyeater foraging behaviour, floral traits, and nectar production, for *Grevillea macleayana* plants.**

The foraging behaviours tested were: (1) mean number of honeybees or honeyeaters; (2) the mean cumulative number of inflorescences visited by monitored honeybees or honeyeaters in a survey period; and (3) the mean cumulative foraging time of monitored honeybees or honeyeaters per survey period. These three dependent variables were each tested against two sets of floral traits: (1) inflorescence number per plant and mean inflorescence size (flowers/inflorescence) and (2) mean inflorescence nectar volume ( $\mu\text{l}$ ) and mean sugar concentration (%) of nectar per inflorescence. Simple linear regressions were used to test the significance of relationships between honeybee and honeyeater foraging behaviour and inflorescence production at Chinamans Beach (CB) in February 2003. Significant  $P$  values ( $\alpha < 0.05$ ) are in bold type.

**(a) Chinamans Beach**

<b>March 2002 - Honeyeaters</b>	<b><math>R^2</math> (<math>R^2</math> Adj.)</b>	<b>df*</b>	<b>Mean Square</b>	<b>SE**</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Inflorescences Visited/Plant</b>	0.95 (0.90)	2, 2	136.63	13.81	18.97	<b>0.05</b>	Positive
Inflorescence Number				0.04	37.44	<b>0.03</b>	Positive
Inflorescence Size				0.36	2.41	0.26	Negative
<b>Foraging Time/Plant</b>	0.95 (0.89)	2, 2	20730.1	177.90	17.36	<b>0.05</b>	Positive
Inflorescence Number				0.50	34.70	<b>0.03</b>	Positive
Inflorescence Size				4.59	0.47	0.56	Negative
<b>February 2003</b>	<b><math>r^2</math></b>	<b>df*</b>			<b><math>P</math></b>		<b>Trend</b>
<b>Honeybees Traits vs Inflorescence Number</b>							
Number of Honeybees	0.91	1, 3			<b>0.01</b>		Positive
Inflorescences Visited/Plant	0.60	1, 3			0.13		Positive
Foraging Time/Plant	0.28	1, 3			0.36		Positive
<b>Honeyeaters Traits vs Inflorescence Number</b>							
Number of Honeyeaters	0.41	1, 3			0.24		Positive
Inflorescences Visited/Plant	0.92	1, 3			<b>0.01</b>		Positive
Foraging Time/Plant	0.93	1, 3			<b>0.01</b>		Positive

**(b) Greenfields Beach**

<b>October/November 2002 - Honeyeaters</b>	<b><math>R^2</math> <math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b>SE**</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Inflorescences Visited/Plant</b>	0.97 (0.93)	2, 2	8.21	21.98	28.83	<b>0.03</b>	Positive
Inflorescence Number				0.14	56.24	<b>0.02</b>	Positive
Inflorescence Size				0.29	57.65	<b>0.02</b>	Positive
<b>Foraging Time/Plant</b>	0.97 (0.94)	2, 2	1154.62	236.61	34.90	<b>0.03</b>	Positive
Inflorescence Number				1.52	67.33	<b>0.01</b>	Positive
Inflorescence Size				3.13	69.64	<b>0.01</b>	Positive
<b>January 2003 - Honeybees</b>	<b><math>R^2</math> <math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b>SE**</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Foraging Time/Plant</b>	0.83 (0.72)	2, 3	5128.38	224.04	7.57	0.07	Positive
Nectar Volume/Inflorescence				0.60	12.12	<b>0.04</b>	Positive
Sugar Concentration of Nectar				6.57	0.05	0.84	Positive

**(c) Illowra Lane**

<b>January 2003 - Honeybees</b>	<b><math>R^2</math> <math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b>SE**</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.99 (0.98)	2, 2	16.49	6.04	85.55	<b>0.01</b>	Positive
Inflorescence Number				0.01	154.93	<b>0.01</b>	Positive
Inflorescence Size				0.13	168.64	<b>0.01</b>	Positive
<b>Foraging Time/Plant</b>	0.96 (0.92)	2, 2	12087.4	225.46	23.42	<b>0.04</b>	Positive
Nectar Volume/Inflorescence				0.19	7.27	0.11	Positive
Sugar Concentration of Nectar				7.37	43.20	<b>0.02</b>	Positive

\* Model degrees of freedom, Error degrees of freedom.

\*\* SE = Standard Error

**3.3.6 Nocturnal Mammal Foraging Behaviour**

I observed an individual *Cercartetus nanus* (Eastern Pygmy Possum) visiting two plants at CB, on each of five nights (out of a total of nine at this site) between February 2002 and September 2003 (Figure 3.19). Four of these observations occurred on the one plant (Plant 1). I also identified a *C. nanus* scat near an inflorescence on Plant 3 at CB. No other observations of nocturnal floral visitors were made at either GB or IL.

A single *C. nanus* was observed foraging on Plant 1 at CB, on the first night of nocturnal floral visitor observations (28<sup>th</sup> February 2002). I observed it feeding from

two inflorescences for approximately 3 min. A *C. nanus* was observed on this same plant at CB, one week later (7<sup>th</sup> March 2002). I observed this individual resting on a branch for approximately 3 to 4 min (this was recorded on video camera), although, I was unable to track it once it began to move among inflorescences. A *C. nanus* was also observed on a *G. macleayana* plant at CB (this plant was approximately 19m from Plant 1) on the 11<sup>th</sup> of October 2002. The individual was observed feeding from an inflorescence and it remained still for several minutes (allowing photos to be taken with a digital camera) before it moved into groundcover vegetation (Figure 3.19).

On the 29<sup>th</sup> of September 2003, I observed a *C. nanus* feeding from an inflorescence on Plant 1 for approximately 2 to 3 min before it moved and I was unable to track it. An hour later, at the same plant, I observed a *C. nanus* sitting on a branch for over 5 min (this was recorded on video camera). The next night, I observed a *C. nanus* on the same plant, but it moved very quickly and further spotlighting could not locate it.



**Figure 3.19 - A photograph of an Eastern Pygmy Possum on a *Grevillea macleayana* plant (Photo: S. Lloyd).**

An Eastern Pygmy Possum (*Cercartetus nanus*) observed foraging (just prior to the photograph being taken) from an inflorescence on this *G. macleayana* plant, at Chinamans Beach.

### 3.4 Discussion

The results of the studies presented in this chapter provide strong evidence that both honeybee and honeyeater foraging behaviour varies significantly among *G. macleayana* plants. These results support previous studies on several other species (e.g. Herrera, 1995; Lloyd, 1998; Vaughton and Ramsey, 1998; Somanathan and Borges, 2001). Moreover, my results challenge the expectation (based on a broad range of studies - Tables 1.1 and 1.2) that different pollinators will respond positively to increasing floral traits and rewards (Faegri and van der Pijl, 1979). I predicted that plants that are more popular with honeybees would also be more popular with honeyeaters, due to increased floral rewards. However, I found surprisingly few significant correlations between honeybees and honeyeaters in patterns of foraging behaviour (e.g. plants visited more frequently by honeybees, were not necessarily visited more frequently by honeyeaters). This indicates that honeybees and honeyeaters may be responding differently to variation in the floral traits I measured. I also found patterns of foraging preference for particular plants were stronger for honeyeaters than for honeybees. This may indicate that honeyeaters were responding more than honeybees to variation in floral traits. In the following sections, I discuss the observed variation in foraging behaviour among plants and between honeybees and honeyeaters; the relationships between the foraging behaviour of honeybees and honeyeaters and floral traits (using the results from Chapter 2); and the results of variation in foraging behaviour, with respect to the reproductive success of plants.

#### 3.4.1 Variation Among Plants in Honeybee & Honeyeater Foraging Behaviour

In this study, I found that plants varied specifically with respect to the numbers of honeybee and honeyeater visits and honeybee and honeyeater foraging behaviour (i.e. the number of inflorescences visited, the foraging time per inflorescence and the foraging time per plant). Furthermore, I also detected some consistent patterns with respect to plant preference (i.e. plants visited most often over more than one survey season) that were independent for both honeybees and honeyeaters. For example, Plant 4 at CB received the lowest levels of honeybee foraging behaviour, in each survey season. However, patterns of plant foraging preference were stronger for honeyeaters than for honeybees. For example, at CB and GB, I found that the same plants received the largest number of honeyeater visits, regardless of survey season.



Variation among plants in pollinator foraging behaviour may be influenced by several factors, such as an increase in floral rewards, variation in morphological characteristics, climatic conditions or plant density (Faegri and van der Pijl, 1979; Stanton *et al.*, 1991; Rathcke, 1992) (Table 1.1 and 1.2). Previous studies have reported that variation among plants in plant size (Geber, 1985), sunlight availability (Herrera, 1995), and plant density (Mustajärvi *et al.*, 2001) were all related to variation in pollinator activity. In Chapter 6, I examine variation among plants in non-reproductive plant traits (e.g. plant size), environmental variables (e.g. canopy cover), leaf health (e.g. leaf moisture and photosynthetic yield), and plant distribution (e.g. nearest conspecific neighbour). In Chapter 6, I also examine whether these particular plant characteristics and habitat variables explain variation among plants in inflorescence and seed production, as measures of plant attraction and reproductive success, respectively.

### **3.4.2 Honeybees, Honeyeaters, and Floral Traits**

#### *3.4.2.1 Variation between Honeybees and Honeyeaters in Foraging Behaviour*

It is generally accepted that plants with larger floral displays or greater nectar production will be more attractive to pollinators and will receive more visits (Darwin, 1859; Faegri and van der Pijl, 1979; Zimmerman, 1988; Real and Rathcke, 1991; Klinkhamer and de Jong, 1993; Oldroyd, *et al.*, 1997). In the previous chapter, I reported significant variation among plants in measures of nectar production and inflorescence number, both floral traits associated with pollinator attraction. If honeybees and honeyeaters (as generalist floral visitors) respond similarly to variation in these floral traits, then they should exhibit similar patterns of foraging behaviour among *G. macleayana* plants (e.g. the same plants should receive more visits from both honeybees and honeyeaters). Surprisingly, this was not the case. Only one survey, conducted at CB in February 2003, showed a significant correlation between the abundance of honeybees and honeyeaters on the survey plants. Whilst there were no significant correlations between honeybees and honeyeaters in the number of inflorescences visited, there were some negative trends indicating that as honeybees visited more inflorescences per plant, honeyeaters visited fewer. There were no significant correlations between honeybees and honeyeaters in the foraging time per plant and only one survey indicated a non-significant negative trend. Therefore, my results imply that honeybees and honeyeaters are generally not visiting the same plants more frequently, or for longer periods of time.

These results provide some evidence that honeybees and honeyeaters may be responding to different floral cues, although, such conclusions require further study. One possible explanation is that honeybees and honeyeaters may well be expected to respond differently to variation in floral traits (among plants) because of different evolutionary histories. Specifically, the *Grevillea*-bird system evolved largely in the absence of social pollinating insects (such as honeybees). Furthermore, it is possible that the substantial numbers of honeybees visiting these plants frequently reduced the nectar reward available, thereby altering honeyeater foraging patterns via reduced plant attraction (Schemske and Horvitz, 1984; Ramsey, 1988; Pyke, 1990; Paton, 1993; 1997). If honeybees and honeyeaters are using different floral cues, then selection for floral traits by honeybees may be in a different direction to selection by honeyeaters (Lau and Galloway, 2004). However, it is difficult to predict the significance of honeybee interference, given they generally do not facilitate pollen movement and may influence reproductive success more by altering patterns of honeyeater behaviour, than directly affecting reproductive success.

Whilst I did not specifically measure inter-plant foraging movements of honeybees or honeyeaters, I observed that honeyeaters made more frequent between-plant movements than honeybees. This observation supports previous studies on this, and other *Grevillea* species (e.g. Richardson *et al.*, 2000; Roberts, 2001; Celebrezze, 2002). For example, Roberts (2001) found that honeyeaters visited significantly more *G. macleayana* plants during a single foraging bout than honeybees. This result differed from that reported by Richardson *et al.* (2000) for *G. mucronulata*, who found no significant difference between honeybees and honeyeaters in the number of plants visited during a single foraging bout. Roberts (2001) also found that for both honeybee and honeyeater foraging time at *G. macleayana* plants, more than 84% of movements were between inflorescences on the same plant, and less than 16% were between plants. Moreover, Celebrezze (2002) found that honeyeaters foraging at *G. acanthifolia* moved long distances more frequently than honeybees, with 15% of honeyeaters' inter-plant movements greater than 10m, compared to only 6% for honeybees. Smith and Gross (2002) also found that honeyeaters predominantly moved among inflorescences within *G. beadleana* plants.

### 3.4.2.2 *Floral Visitors and Floral Traits*

The significant positive relationships detected in multiple regression between honeybee and honeyeater foraging behaviour and inflorescence traits suggest that plants with greater floral production will be visited by larger numbers of honeybees and/or honeyeaters, foraging at a greater number of inflorescences for longer periods of time. However, in both significant and non-significant tests, inflorescence number or size were not always positively related to honeybee or honeyeater foraging behaviour. For example, (non-significant) negative trends were most common between the number of inflorescences visited per plant by honeyeaters and inflorescence size. Whilst not significant, these unexpected patterns should not be ignored and may be in part due to competition with honeybees for nectar resource. Specifically, significant regressions and non-significant trends were most common between honeybee foraging time and inflorescence size, thereby likely reducing the available nectar reward for honeyeaters. Further investigation is required to clarify how honeybees and honeyeaters respond to variation in these traits. Overall, these results support many previous studies that have found greater numbers of pollinators or greater foraging activity associated with larger floral displays (see Sections 1.2 - 1.4; Tables 1.1 and 1.2).

The multiple regressions between honeyeater foraging behaviour and nectar traits revealed unexpectedly low R-values and negative trends with respect to honeyeater numbers. This result was unexpected given increased nectar volume returns greater energy to a honeyeater and was therefore predicted to be a favourable nectar trait. The observed trends may be because the consistently large numbers of honeybees depleted nectar resources, thus reducing the nectar reward for honeyeaters and altering predicted foraging patterns (Pyke, 1990; Paton, 1997). With respect to honeybees, the number of inflorescences visited per plant was more often negatively related to nectar volume. Whilst largely not-significant, this unexpected result may reflect the reduced foraging activity required by honeybees that are able to satisfy their energetic requirements from fewer inflorescences. The significant positive regressions and non-significant positive trends detected between honeybee foraging time and nectar volume per plant, reflect the expected increased attraction of plants with a greater nectar reward (see Sections 1.2 - 1.4; Tables 1.1 and 1.2).

Whilst largely not-significant, positive trends were most common between nectar sugar concentration and all three measures of honeybee foraging behaviour. These patterns are not a surprise given that nectar sugar concentration is an important floral attractant and reward for honeybees in the *G. macleayana* system. Positive trends were also expected between nectar sugar concentration and honeyeater foraging activity, and this was not the case. These trends may suggest that honeyeaters provided with nectar rewards of higher sugar concentrations do not need to forage at as many inflorescences, or for as long, to satisfy their energetic requirements. Alternatively, these trends may be a result of increased competition with honeybees for nectar rewards (as discussed above). The positive (non-significant) trends detected between honeybee foraging activity and nectar sugar concentration, and honeybee foraging time and nectar volume support previous studies that have reported greater numbers of pollinators or greater foraging activity associated with greater nectar rewards (see Section 1.3; Tables 1.1 and 1.2).

### 3.4.3 Honeybees, Honeyeaters, and Potential Reproductive Success

The foraging behaviour of honeybees and honeyeaters that I observed was similar to that described in earlier studies on *G. macleayana* (Vaughton, 1996; Roberts, 2001; Beynon *et al.*, *unpublished*). Previous studies observing honeybees foraging for nectar, also described how honeybees burrow between flowers to reach nectaries, and rarely touch pollen presenters (Vaughton, 1996; Roberts, 2001; Beynon *et al.*, *unpublished*). This behaviour can be defined as nectar thieving, whereby a flower is visited by an animal not morphologically suited to the flower design, thereby removing nectar without contacting reproductive parts and pollinating the flower (Inouye, 1980). This, in turn, may reduce plant attraction to potentially effective pollinators, if nectar resources have been substantially depleted. Pettersson (1999) proposed that plants visited by nectar thieves may be selected to provide smaller quantities of nectar in more flowers, thereby discouraging some nectar thieves. The energy saved by reducing nectar production per flower may then be directed into other plant functions, such as seed production (Pettersson, 1999). The potential effects of nectar theft on honeyeater foraging behaviour and subsequent seed production is discussed in Chapters 4 and 5.

Territorial bird behaviour may increase geitonogamy within plants due to the fidelity of birds to a restricted number of plants (Pandit and Choudhury, 2001). However, the

chasing of smaller birds by these larger, more aggressive birds may enhance outcrossing, by increasing inter-plant pollen transfer distances by the less aggressive species (Schmidt-Adam *et al.*, 2000). I found the largest of the three bird species (Red Wattlebird) displayed the most aggressive territorial behaviour, although, the medium sized New Holland Honeyeater dominated in foraging abundance and frequency. The aggressive territorial behaviour of the largest bird species observed in this study is also consistent with the findings of many other studies (e.g. Paton, 1986b; Burd, 1995; Franceschinelli & Bawa, 2000; Schmidt-Adam *et al.*, 2000).

I found that the abundance of honeybees at plants was dramatically greater than those of honeyeaters, as was observed in previous studies, (Vaughton, 1996; Roberts, 2001; Beynon *et al.*, *unpublished*). Beynon *et al.* (*unpublished*) reported that honeybee visits to *G. macleayana* inflorescences were an order of magnitude more frequent than those of honeyeaters. Roberts (2001) reported that honeybees were approximately twice as likely to visit *G. macleayana* inflorescences, as honeyeaters, in bushland sites. However, for plants located within the village of Hyams Beach, the difference was much smaller (Roberts, 2001). This has potentially important consequences if honeybees are substantially altering nectar resources and thereby affecting honeyeater foraging patterns. This, in turn, may reduce pollen movement and outcrossed seed production. The relationships between seed quality (i.e. selfed versus outcrossed), floral traits and the foraging activity of floral visitors are reported in Chapter 5.

A concentration of foraging movements within plants or among near-neighbours may increase geitonogamous pollen movement, resulting in increased selfing or biparental inbreeding and potential inbreeding depression (Charlesworth and Charlesworth, 1987; Barrett and Kohn, 1991; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994; Slate *et al.*, 2004). With respect to within-plant foraging behaviour, I found that honeybees and honeyeaters visited similar numbers of inflorescences per plant for approximately half of the survey seasons. However, the three survey seasons in which significant variation was detected, were due to increases in the number of inflorescences visited per plant by honeyeaters. This increase in the number of inflorescences visited within plants may have two effects on reproductive success of *G. macleayana* plants: (1) increasing pollen transfer, therefore, increasing both the number of seed produced and sired from outcrossed pollen; and (2) increasing geitonogamy and biparental inbreeding

via the transfer of self pollen among flowers. The effect of within-plant pollen transfer on plant reproductive success and fitness is further examined in Chapter 5.

Honeybees spent significantly more time foraging per inflorescence than honeyeaters, in most survey seasons and sites. This is a result of more flowers being visited per inflorescence by honeybees. I also found that honeybees spent significantly more time foraging per plant than honeyeaters. Importantly, this was not because more inflorescences were visited, but rather an increase in the time spent at individual inflorescences. Honeybees frequently spent up to five times the foraging time of honeyeaters per inflorescence and more than double or triple the foraging time of honeyeaters per plant. If honeybees are contacting the stigma (which I rarely observed) then this foraging behaviour may increase geitonogamous pollen transfer and affect plant outcrossing rates (Charlesworth and Charlesworth, 1987; Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994; Ivey *et al.*, 2003). However, the more serious potential impact of extended honeybee foraging time is the likelihood that honeybees will deplete floral resources, thereby decreasing plant attraction to honeyeaters, which may reduce pollen transfer and outcrossed seed production (Schemske and Horvitz, 1984; Ramsey, 1988; Pyke, 1990; Paton 1997).

#### 3.4.4 Nocturnal Mammal Foraging

Another important finding of the research presented in this chapter was the detection of *Cercartetus nanus* (Eastern Pygmy Possum), feeding on *G. macleayana* inflorescences on five occasions. I believe this to be the first study to identify a nocturnal mammal foraging on a *Grevillea* plant, on multiple occasions. My study has already indicated that different floral traits may attract honeyeaters and honeybees differently. This proposal is further complicated by the fact that honeybees are likely to be ineffective pollinators. Moreover, detecting a nocturnal mammal foraging on *G. macleayana* plants indicates that the nature of selection in this plant-pollinator system may be even more complex than previously thought, when just considering honeyeaters and honeybees. These ideas will be examined further in Chapter 4, when nocturnal and diurnal pollen deposition is compared among plants.

*Cercartetus nanus* has an extensive distribution from south-east Queensland to south-eastern South Australia and Tasmania (Strahan, 1983). It has been recorded in several

vegetation types (e.g. rainforest and sclerophyll heath) and is reported to feed primarily on the nectar and pollen of *Banksia* spp. (Strahan, 1983). However, *C. nanus* has also been reported visiting *Acacia* spp., *Eucalyptus* spp., *Telopea speciosissima*, and *Callistemon citrinus* (Goldingay *et al.*, 1987; Cunningham, 1991; Goldingay *et al.*, 1991; Carthew, 1994; Evans and Bunce, 2000). It is rarely detected in fauna surveys (Harris, 2003) and is listed as Vulnerable on Schedule 2 of the *NSW Threatened Species Conservation Act 1995*.

I detected *C. nanus* on five nights out of the nine spotlighting surveys at CB. This indicates that there may be a population of *C. nanus* at CB and that *G. macleayana* may be used as a nectar resource. Whilst I observed *C. nanus* foraging from a few inflorescences, this was very difficult to monitor carefully and I was unable to determine any patterns of foraging behaviour. Therefore, these surveys did not permit me to analyse how *C. nanus* responds to variation in floral traits. Further work is required to determine the potential contribution *C. nanus* makes to the reproductive success of *G. macleayana*. Moreover, very little is known about the general ecology of this rare species and further research may aid in better formulating management plans and conservation strategies.

### 3.4.5 Conclusions

The results presented in this chapter provide strong evidence that *G. macleayana* plants differ significantly in all tested measures of foraging behaviour, for both honeybees and honeyeaters. Surprisingly, there were very few significant correlations between honeybee and honeyeater foraging behaviour at individual plants. This suggests that these floral visitors are not responding similarly to variation among plants in floral cues. In fact, some trends of negative association may even suggest interference by the introduced honeybee. Plants with greater measures of floral traits (measured by the number of inflorescences or nectar production), did not necessarily receive the highest levels of foraging activity from both honeybees and honeyeaters. These results also suggest that it is possible that different plants are specialising in attracting different types of pollinators.

From my observations, it is clear that honeybees foraging on *G. macleayana* plants do not facilitate effective pollen transfer and are therefore removing nectar with no

reproductive gain for the plant. Conversely, the foraging behaviour of honeyeaters appears to provide effective pollen movement. Moreover, it is likely that honeyeaters are primarily responsible for the small level of outcrossing detected in previous studies on this species (Vaughton 1996; Roberts, 2001; Beynon *et al.*, *unpublished* – but see Chapter 5). Furthermore, whilst it is very likely that honeyeaters are in greater abundance than *C. nanus*, the foraging behaviour of this species and its relative contribution to the reproductive success of this species should be investigated further.

Fifty-one regressions between honeybee and honeyeater foraging behaviour and the inflorescence traits of number and size revealed consistent whole-model positive trends and suggest that plants with greater floral production will be visited by larger numbers of floral visitors, which will likely forage at a greater number of inflorescences for a longer period of time. However, inflorescence number or size was not always positively related to honeybee or honeyeater foraging behaviour, and in some cases negative trends were more common for honeyeaters. Given that honeybee numbers and foraging behaviour were frequently positively related to inflorescence number and/or size, it is possible that negative trends, with respect to honeyeater behaviour, may be due to competition for nectar resources.

Forty-five regressions between honeybee and honeyeater foraging behaviour and nectar traits also revealed consistent whole-model positive trends. Moreover, the common positive trends between honeybee behaviour and nectar traits reflect the expected increased attraction of plants with a greater nectar reward. However, trends between foraging behaviour and independent nectar traits were not as consistent as those for inflorescence traits. Specifically, low R-values and negative trends with respect to honeyeater numbers and nectar volume and honeyeater foraging and sugar concentration. As with the floral trait regressions, the negative trends with honeyeaters may be due to competition with consistently large numbers honeybees for nectar resources. However, unexpected negative trends were also detected between the number of inflorescences visited honeybees and nectar volume, and require further research.

Having identified significant inter-plant variation in foraging behaviour, the important consequence of this for plant fitness is how this variation is related to plant reproductive



success. In the next two chapters, I describe experiments I have undertaken on plant reproductive success, including seed numbers and pollen deposition (Chapter 4) and plant outcrossing rates (Chapter 5). In these two chapters, I will integrate the results of previous chapters by testing for consistent trends and/or significant relationships between floral traits, floral visitor foraging behaviour, reproductive success, and plant outcrossing rates.

## Chapter 4 - Variation in Plant Reproductive Success

### 4.1 Introduction

#### 4.1.1 Fruit and Seed Production

It is well recognised that Proteaceae plant species typically have very low fruit-to-flower ratios (reviewed by Ayre and Whelan, 1989). Ayre and Whelan (1989) suggested a number of proximate and ultimate hypotheses worthy of investigation, with respect to limited fruit set. They concluded that factors limiting fruit set might vary spatially and temporally (e.g. flowering intensity, competition with co-occurring species, pollinator abundance and distribution), within and among species. Proposed hypotheses for excess flower production and low fruit-to-flower ratios that may be relevant to Proteaceae species include “*pollinator limitation*”, “*pollinator attraction*”, “*bet-hedging*”, “*selective abortion*” and the “*male function hypothesis*” (Willson, 1979; Sutherland and Delph, 1984; Ayre and Whelan, 1989; Broyles and Wyatt, 1990 – Section 1.3.1.3). Ayre and Whelan (1989) also recognised that resource availability in a given season might be influenced by earlier and future reproductive effort.

Whilst many studies have found positive correlations between flower and seed production (e.g. Johnston, 1992; Conner *et al.*, 1996; Knight, 2003), there are also trade-offs between these measures in some plant species, due to competition for limited resources (Whelan and Goldingay, 1989; Vaughton and Ramsey, 1998; Vallius, 2000 – Section 2.1). Stearns (1992) defined this type of trade-off as a physiological trade-off, whereby resource allocation to one trait (e.g. flower number) should result in less resource allocation to a second trait (e.g. seed production). For example, the proportion of seed set per *Wurmbea dioica* plant was found to have decreased with increased flower size and number, indicating possible trade-offs between floral display and female reproductive success (Vaughton and Ramsey, 1998). Fruit and seed production was also found to decrease with increasing floral production in *Telopea speciosissima* (Whelan and Goldingay, 1989) and *Dactylorhiza maculata* (Vallius, 2000). Therefore, as proposed in Section 2.1, patterns of flower and fruit production need to be assessed over consecutive flowering seasons, to quantify intraspecific variation and determine whether there are trade-offs between these measures (Whelan and Goldingay, 1989).

#### 4.1.2 Pollen Transfer

Pollen removal and deposition ultimately vary as a function of pollinator visit frequency and foraging behaviour (Zimmerman, 1988; Harder and Thomson, 1989; Ohashi and Yahara, 2001). Moreover, pollinators may in turn vary foraging behaviour (as a function of energetic requirements) in response to floral rewards, the spatial arrangement of plants, and weather conditions (Zimmerman, 1988; Harder and Thomson, 1989; Ohashi and Yahara, 2001).

Harder and Thomson (1989) suggest that as pollen removal increases, the proportion of pollen that is deposited into the stigmas of conspecific flowers decreases, therefore, limiting male reproductive success. They proposed that some plants might gain a reproductive advantage if they are able to control pollen removal (Harder and Thomson, 1989). Plants may restrict pollen removal by: (1) “*packaging mechanisms*” that control the amount of pollen presented to individual pollinators for removal and (2) “*dispensing mechanisms*” that restrict how much pollen may be removed by pollinators (Harder and Thomson, 1989). Packaging mechanisms may include staggered opening of flowers on individual inflorescences or inflorescences on plants, so that only a proportion of pollen is available to pollinators at one time (Harder *et al.*, 2001). Therefore, the amount of time that pollen is available for removal by pollinators is increased and may allow more pollinators to disperse pollen to a greater number of conspecifics, potentially increasing male reproductive success (Lloyd and Yates, 1982; Vaughton and Ramsey, 1991).

*Grevillea macleayana* inflorescences are an example of pollen packaging, whereby flowers within an inflorescence open sequentially over approximately seven days. Moreover, for individual plants the commencement of inflorescence flower opening will be staggered, thereby ensuring that during a flowering season most plants will have a continual supply of pollen available to pollinators. Pollen packaging may have evolved to limit the amount of pollen removed per inflorescence by bird and mammal pollinators, who may otherwise remove all pollen by means of nectar foraging, given their large size and energy requirements.

#### 4.1.3 Nocturnal and Diurnal Pollinators and Reproductive Success

Many plant species are visited by both diurnal and nocturnal species of pollinators (Miyake and Yahara, 1999; Hackett and Goldingay, 2001; Young, 2002; Wolff *et al.*,

2003). However, different pollinator groups may vary in pollen transfer efficiency and therefore plant seed production (Wilson and Thomson, 1991; reviewed in Young, 2002). For example, Young (2002) found that flowers of *Silene Alba* exposed only to nocturnal insect pollinators (moths) produced significantly more seeds than those exposed only to diurnal insect pollinators (bees, flies, and wasps). Furthermore, pollen was transferred to significantly more stigmas and significantly greater distances nocturnally, than diurnally (Young, 2002). However, seeds from diurnal visits were significantly heavier than those from nocturnal visits (Young, 2002).

It is known that *G. macleayana* is visited diurnally by honeyeaters and honeybees and nocturnally by the Eastern Pygmy Possum (Chapter 3). Furthermore, previous unpublished work (Beynon *et al.*, unpublished) also reported nocturnal pollen removal on between 8% and 21% of flowers on *G. macleayana* plants. This finding along with my observations indicates likely nocturnal foraging by the Eastern Pygmy Possum, and potentially other nocturnal marsupials such as Sugar Gliders (*Petaurus breviceps*) and *Antechinus* spp.

#### 4.1.4 Study Predictions

In Chapter 3, I identified significant variation among *G. macleayana* plants in measures of honeybee and honeyeater foraging behaviour. Moreover, I found that this intraspecific variation was present over a number of seasons. This indicates that within a population, some plants are visited more than other plants by honeybees or honeyeaters. This variation in foraging behaviour may have important consequences for reproductive success, with respect to both pollen donation and seed production.

In this chapter, I quantify variation among *G. macleayana* plants in two measures of female reproductive success: seed numbers and pollen deposition. As mentioned in Section 1.8, fruit usually contains only one seed that has developed from the two available ovules per flower. Therefore, I have used the word ‘seed’ rather than ‘fruit’ in this and subsequent chapters. I quantify total seed numbers at three sites, over approximately over two years, and intraspecific variation in pollen deposition in five surveys at three sites (Table 4.1). To try and understand how the results of Chapters 2 and 3 are related to the results of this chapter, I have examined the relationships

between floral traits, honeybee and honeyeater foraging behaviour, and reproductive success.

I decided not to use pollen removal as a measure of male reproductive success, given the previous concerns of many authors with respect to how reliably it may predict paternal pollen donation and subsequent seed production (Wilson and Thomson, 1991; Thomson and Thomson, 1992; Klinkhamer *et al.*, 1994 - discussed in Section 1.5.1). The measure of male reproductive success I wanted to use was seed paternity. Whilst I conducted paternity analyses for the seeds genotyped in Chapter 5 ( $n=199$ ), the power of the loci to assign paternity was very low (0.39) and therefore unreliable. This was likely to be due to the high numbers of self-fertilised, homozygous seeds and the presence of common alleles among seeds. Therefore, I did not include the paternity analysis in this Chapter and have instead presented it in Appendix 4.

Based on the literature presented in this Chapter and Chapter 1, I have made several predictions about the likely variation in seed production and pollen deposition among *G. macleayana* plants:

- (1) Plants will have very low, but variable numbers of seeds. Furthermore, when measured over two years, the same plants will consistently produce more seeds.
- (2) Plants will vary significantly with respect to pollen deposition, and the same plants will receive more pollen per site, over two survey seasons.
- (3) There will be significant positive relationships between diurnal and nocturnal pollen deposition per plant.
- (4) Given that pollen deposition determines potential seed production, there will be a positive relationship between pollen deposition and seed number per plant.
- (5) Some measures of floral traits and honeybee/honeyeater foraging behaviour will be positively related to seed number and/or pollen deposition.

**Table 4.1 - Studies quantifying reproductive success among *Grevillea macleayana* plants.**

Studies conducted to quantify variation among *G. macleayana* plants in pollen deposition and seed number, at three sites in Jervis Bay National Park, between February 2002 and 2004.

Study Site					
Experiment	Chinamans Beach		Greenfields Beach		Illowra Lane
Pollen Deposition	September 2003	December 2003	September 2003	January 2004	September 2003
Pollen deposition per flower per plant	✓	✓	✓	✓	✓
Diurnal versus nocturnal pollen deposition	✓	-	✓	-	✓
Inflorescence Size	-	✓	-	✓	-
Seed Number	February 2002 to May 2004		February 2002 to May 2004		July 2002 to December 2003
Monthly record of seed production	✓		✓		✓

A dash (-) indicates that reproductive success studies were not conducted at that site during that particular survey season.

### 4.1.5 Study Aims

In this chapter of my thesis I quantified variation among plants in measures of reproductive success, in order to answer the following questions:

#### (A) Seed Production:

- (1) How do plants vary with respect to the total number of seeds produced over the survey period?
- (2) How consistent are temporal patterns of variation in seed production among plants?
- (3) How are inflorescence and seed numbers related?
- (4) How is inflorescence size associated with seed number?

#### (B) Pollen Deposition:

- (1) How do plants vary with respect to pollen deposition?
- (2) How does diurnal and nocturnal pollen deposition vary?
- (3) How consistent are temporal patterns of variation in pollen deposition among plants?

#### (C) Reproductive Success, Floral Visitors, and Floral Trait Comparisons:

- (1) How is pollen deposition and seed number related among plants?
- (2) How is reproductive success (seed number and pollen deposition) associated with floral visitor foraging behaviour (honeybee and honeyeater), and floral traits (inflorescence, nectar, and pollen production)?

## 4.2 Methods

### 4.2.1 Seed Numbers

#### 4.2.2.1 Quantifying Variation Among Plants in Seed Number

To determine whether there was significant variation among plants in seed number I recorded the number of mature seeds (seeds take approximately eight weeks to mature) per plant for 19 plants per site, every month for approximately two years (Table 4.1). As described in Chapter 2, I began with 20 plants at CB, 25 at GB, and 20 at IL (Section 2.2.1). However, one plant died at both CB and IL and six plants died at GB, between October 2002 and December 2003, resulting in 19 plants per site. I documented seed production at CB between June 2002 and May 2004 (24 months), at GB between July 2002 and May 2004 (23 months), and at IL between July 2002 and December 2003 (18 months).

#### 4.2.2.2 Statistical Analysis

Variation among plants in seed numbers for each site was illustrated using bar graphs of total seed production (as recorded over the survey period) (Question 1). I used single factor ANOVAs to test for significant variation among plants (per site) in monthly seed number ranks (Question 2). Assumptions of normality and equal variances were tested as described in Section 2.2.2.3. Seed production data from CB and GB were square-root ( $x + 0.5$ ) transformed, due to some non-normality and heteroscedasticity. Seed data from IL was  $\log(x + 1)$  transformed, due to some heteroscedasticity. An *a posteriori* comparison among plant means was conducted for each ANOVA using the Tukey-Kramer HSD test. I then examined this variation further by plotting the three plants with the best seed production (based on mean monthly rank) and the two plants with the poorest seed production, per month over the survey period (Question 2).

I used bar graphs to illustrate the variation among plants seed-to-inflorescence ratio (based on total inflorescence and seed number over the survey period per site) and thus seed production efficiency (Question 3). To test the relationship between mean monthly inflorescence rank and mean monthly seed rank (per plant), I used Spearman Rank Correlations (Question 3). I used linear regression analyses to determine the relationship between inflorescence sizes and seed number, recorded approximately eight weeks after inflorescence size was recorded (Question 4).

### 4.2.2 Pollen Deposition

#### 4.2.2.1 Trial Studies

I first performed a series of trial studies to ensure that: (1) the technique I wanted to use for removing pollen was effective; and (2) pollen could be successfully deposited onto the surface of newly cleaned flower stigmas. In the first trial study I wanted to confirm that the technique for removing pollen from pollen presenters, as described by Beynon *et al.* (*unpublished*), was effective. To test this technique I used two garden-variety, hybrid *Grevillea* species: one a large shrub with pink ‘toothbrush’ inflorescences (similar to those of *G. macleayana*) and the other a small tree with large yellow inflorescences, on the campus of Wollongong University in August 2003. I removed pollen from the pollen presenters of one flower per inflorescence, for 20 inflorescences on three of the pink *Grevillea* plants ( $n = 60$ ) and from one flower per inflorescence, for ten inflorescences on three of the yellow *Grevillea* plants ( $n = 30$ ).



To remove pollen from pollen presenters, I used cotton buds moistened with distilled water to gently wipe off the pollen. A clean cotton bud was used for each new pollen presenter. I then removed the style and stored it in an Eppendorf tube and later examined it in the laboratory using a light microscope, to detect any remaining pollen grains. The mean number of pollen grains remaining on pink pollen presenters after cleaning was just 1 ( $\pm 0.0$ ) and only four pollen presenters had more than three pollen grains remaining. The mean number of pollen grains remaining on yellow pollen presenters after cleaning was just 1 ( $\pm 0.0$ ) and only two pollen presenters had more than three pollen grains remaining. Based on the results of this preliminary study, I felt confident that I could effectively remove pollen from the pollen presenters of *G. macleayana* flowers, in field-based studies.

In the second trial study I wanted to confirm that pollinators could readily deposit pollen onto the stigmas of newly cleaned *Grevillea* flowers. For this study I used four flowers from each of four inflorescences, on three of the pink *Grevillea* plants ( $n = 48$ ), on the Wollongong University campus in August 2003. I removed pollen from pollen presenters, as described above, and left these prepared flowers ‘open’ for pollinator visitation for approximately 8 hr. At the end of this 8 hr period, I collected any pollen deposited onto the stigmas of flowers by dabbing the stigmatic surface with a piece of sticky tape ten times, which I then adhered to a labelled glass slide for subsequent microscope examination. I examined glass slides using a light microscope, to quantify the number of pollen grains deposited onto each flower stigma (glass slides were examined randomly). The mean number of pollen grains deposited per flower on Plants 1, 2, and 3 was 29 ( $\pm 22.0$ ), 9 ( $\pm 4.0$ ), and 41 ( $\pm 25.0$ ), respectively. Furthermore, pollen was deposited onto 33 (68.75%) of the 48 cleaned flowers. Having confirmed that pollen could be readily deposited onto cleaned flower stigmas, I set up studies to quantify variation in pollen deposition among plants and between nocturnal and diurnal pollinators.

#### 4.2.2.2 Quantifying Pollen Deposition

In September and October 2003, I conducted the first of two studies to quantify intraspecific variation in pollen deposition among *G. macleayana* plants at Chinamans Beach (CB), Greenfields Beach (GB), and Illowra Lane (IL). On each of six plants per site, I randomly selected 12 inflorescences (with approximately one half of their flowers

open). I randomly assigned six of the inflorescences to the diurnal treatment and six to the nocturnal treatment. For the diurnal treatment, I cleaned the pollen grains from the pollen presenters of five flowers per inflorescence, for each of the six inflorescences per plant, as described in Section 4.2.2.1. I then bagged the six inflorescences per plant whilst I continued to remove pollen from flowers of the remaining plants. Bags were made of coarse plastic mesh shaped into a cylinder and surrounded by fibreglass mesh, as described for quantifying nectar production in Chapter 2 (Section 2.2.3.2). Bags prevented pollinators from visiting plants and thus depositing pollen onto newly cleaned flower stigmas, whilst I was preparing the remaining plants. Once I had prepared all six plants I then removed the bags from all inflorescences and flowers were left 'open' to pollinator visits for approximately 9 hr. I cleaned pollen presenters of pollen from approximately 5:45am to 7:00am, and left them 'open' to pollinators from 7:00am until approximately 5:00pm.

I collected pollen deposited onto the five flower stigmas per inflorescence for each of the six inflorescences per plant, using sticky tape, as described previously. To minimise any variation among plants in the time flowers were 'open' to pollinator visits, I collected pollen from the flowers of three of the six inflorescences for all plants and then returned to collect the pollen from the remaining three inflorescences per plant. Glass slides were stored for later microscope examination of pollen deposition in the laboratory.

To set up the nocturnal treatment, I used the same procedure as the diurnal treatment. I cleaned the pollen from five pollen presenters from each of the six nocturnal inflorescences, bagged these inflorescences whilst I prepared remaining plants, and then left inflorescences 'open' to pollinator visits for approximately 11 hr. I cleaned pollen presenters of pollen from approximately 6:00pm until 7:30pm, and left them 'open' from 7:00pm until approximately 6:00am. I conducted the diurnal and nocturnal treatment for two days and two nights at CB and IL, and one day and two nights at GB (I was unable to use the Day 2 data from GB because of afternoon rain).

In December 2003 and January 2004, I conducted the second pollen deposition study at CB and GB. I was unable to conduct the study at IL because of the backburn in December 2003 that killed most the vegetation at that site. I set up this study using the

same techniques to remove and collect pollen as described previously. However, due to a general lack of variation in pollen deposition between diurnal and nocturnal treatments (described in Section 4.3.2), I quantified pollen deposition over two days (i.e. no nocturnal treatment). I used two flowers from each of ten inflorescences on the same six plants per site, as I used in September 2003 (except at GB where Plant 4 was swapped for Plant 5 due to poor flowering in January 2004).

#### 4.2.2.3 Statistical Analyses

To test the data collected from the first pollen deposition trial, I used randomised block design two-way ANOVAs to test for significant variation among plants (first factor-random) and diurnal/nocturnal treatment (second factor-fixed) in total pollen deposition per inflorescence (Questions 1 and 2). Total pollen deposition was quantified from four of the five flowers examined per inflorescence. On occasion one flower may have been damaged or lost and thus five were not available. To test the data collected from the second pollen deposition trial, I used single factor ANOVAs to test for significant variation among plants in total pollen deposition (from two flowers) per inflorescence (Question 1). Assumptions of normality and equal variances were tested as described in Section 2.2.2.3. Data collected from plants at CB in September 2003, GB in September 2003 and January 2004, and IL on Day and Night 1 in September 2003 were transformed [either square-root ( $x + 0.5$ ) or log ( $x + 1$ )] due to some non-normality or heteroscedasticity. I also used simple linear correlation analyses to test for any significant association between diurnal and nocturnal pollen deposition among plants at each site in September 2003 (Question 2).

#### 4.2.3 Reproductive Success, Floral Visitor, and Floral Trait Comparisons

I used multiple regression analyses to test the significance of relationships between seed number (monthly total per plant) and both diurnal and nocturnal mean inflorescence pollen deposition (the total number of pollen grains from four flowers per inflorescence), as measured at all three sites in September and October 2003 (Question 1). I used simple linear regressions to test for significant relationships between monthly seed numbers and mean diurnal inflorescence pollen deposition (the total number of pollen grains from two flowers per inflorescence), at CB and GB in December 2003 and January 2004. I used measures of seed numbers recorded seven to ten weeks after

pollen deposition was recorded (seeds require six to ten weeks to develop – Harriss and Whelan, 1993; Vaughton, 1998).

I used multiple regression analyses to test for significant relationships between seed numbers (monthly total per plant) and two nectar traits: (1) mean inflorescence nectar volume ( $\mu\text{L}$ ) and (2) mean sugar concentration (%) of nectar per inflorescence (Question). I used multiple and simple linear regressions to test for significant relationships between pollen deposition (the total number of pollen grains from four flowers per inflorescence), inflorescence number (monthly total per plant) and inflorescence pollen production (pollen grains per mL of ethanol solution), where data was available. I used monthly measures of seed numbers recorded six to nine weeks after pollen deposition was recorded (seeds require six to ten weeks to develop - Harriss and Whelan, 1993; Vaughton, 1998).

I used multiple regression analyses to test for significant relationships between seed numbers (monthly total per plant) and the three measures of honeybee and honeyeater foraging behaviour (honeybees and honeyeaters were tested separately) (Question 2). The three measures of honeybee and honeyeater foraging behaviour tested were the: (1) mean number of honeybees or honeyeaters per plant; (2) mean cumulative number of inflorescences visited by consecutive honeybees or honeyeaters during a single survey period; and (3) mean cumulative honeybee or honeyeater foraging time per plant. I used monthly measures of seed numbers recorded six to nine weeks after pollen deposition was recorded.

## **4.3 Results**

### **4.3.1 Seed Numbers**

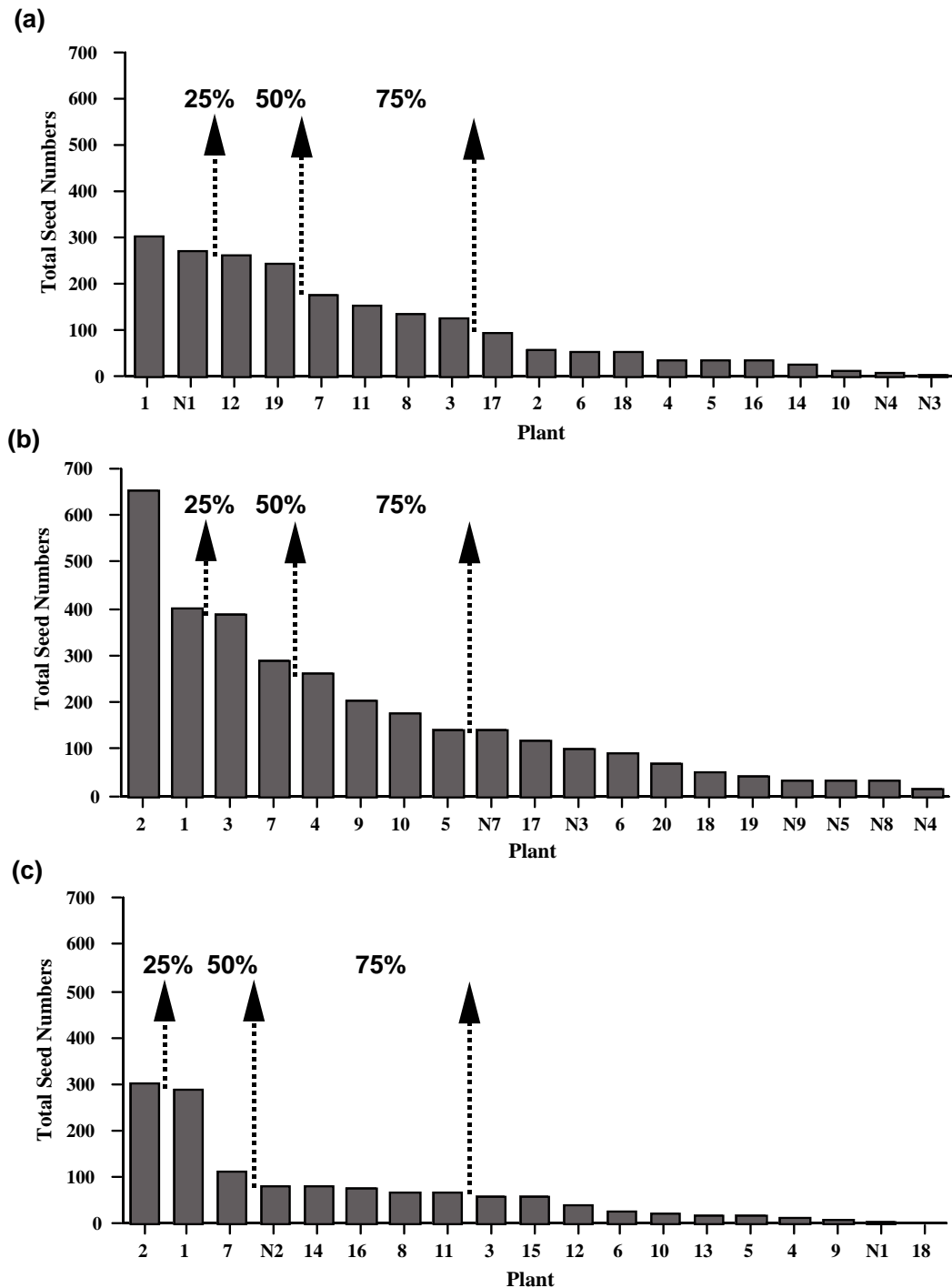
#### *4.3.1.1 Total Seed Numbers*

I found striking variation among plants in total seed numbers over the survey period, at all sites (Figure 4.1). At CB, seed numbers ranged from only 3 on Plant N3 to 302 on Plant 1, a 100-fold difference. At GB, seed numbers ranged from 16 on Plant N4 to 652 on Plant 2, a 41-fold difference. At IL, seed numbers ranged from zero on Plant 18 to 304 on Plant 2.

At all sites, I found that just two or three plants produced more than 40%, and eight plants produced more than three-quarters of the total survey plant seeds. At CB, Plants 1 and N1 produced more than one-quarter (27.8%) of the total survey plant seeds. Four plants (Plants 1, N1, 12, and 19) produced more than half (52.1%) of the total survey plant seeds. At GB, Plants 2 and 1 produced more than one-third (32.3%) of the total survey plant seeds. Four plants (Plants 2, 1, 3, and 7) produced more than half (53.1%) of the total survey plant seeds. At IL, Plant 2 produced nearly one-quarter (22.2%) of the total survey plant seeds. Surprisingly, three plants (Plants 2, 1, and 7) produced more than half (51.6%) of the total survey plant seeds.

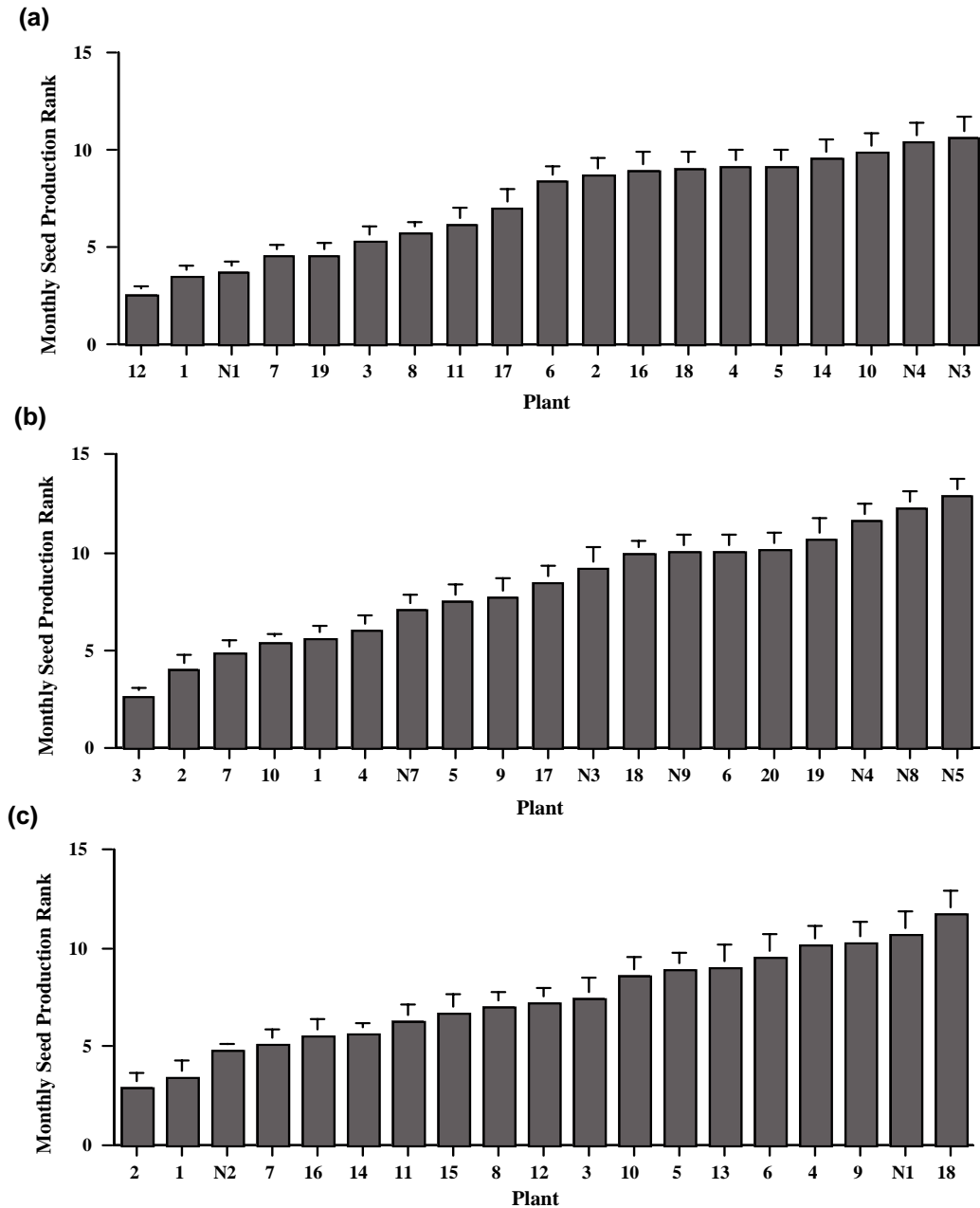
#### *4.3.1.2 Temporal Patterns of Seed Production*

I detected significant variation among plants at all sites, in mean monthly seed production rank (Figure 4.2). At CB and GB, the plants with the greatest seed production (Plant 1 and 2, respectively) were ranked second, based on mean monthly seed production ranks. At IL, the plant with the greatest seed production (Plant 2) was ranked first, based on mean monthly seed production ranks. Significant variation was detected among plants in mean monthly seed production rank at CB (ANOVA:  $F_{18} = 9.69$ ;  $P < 0.001$ ), GB (ANOVA:  $F_{18} = 13.80$ ;  $P < 0.001$ ) and IL (ANOVA:  $F_{18} = 8.30$ ;  $P < 0.001$ ).



**Figure 4.1 - The total number of seeds produced (over the survey period) per plant, for 19 *Grevillea macleayana* plants, at each of three sites.**

Plants were monitored at Chinamans Beach between June 2002 and May 2004 (24 months - Figure a), Greenfields Beach between July 2002 and May 2004 (23 months - Figure b), and Illowra Lane between July 2002 and December 2003 (18 months - Figure c). Arrows indicate the approximate percentage of survey population seeds produced by the preceding plants. Plants are displayed in order of descending seed production, along the x-axis.



**Figure 4.2 - The mean monthly seed production rank for 19 *Grevillea macleayana* plants, at each of three sites.**

Plants were monitored at Chinamans Beach between June 2002 and May 2004 (24 months - Figure a), Greenfields Beach between July 2002 and May 2004 (23 months - Figure b) and Illowra Lane between June 2002 and December 2003 (18 months - Figure c). Plants are displayed in order of ascending mean monthly seed production rank. Significant variation was detected among plants at CB (ANOVA:  $F_{18} = 9.69$ ;  $P < 0.001$ ), GB (ANOVA:  $F_{18} = 13.80$ ;  $P < 0.001$ ), and IL (ANOVA:  $F_{18} = 8.30$ ;  $P < 0.001$ ). Bars represent plus one standard error.

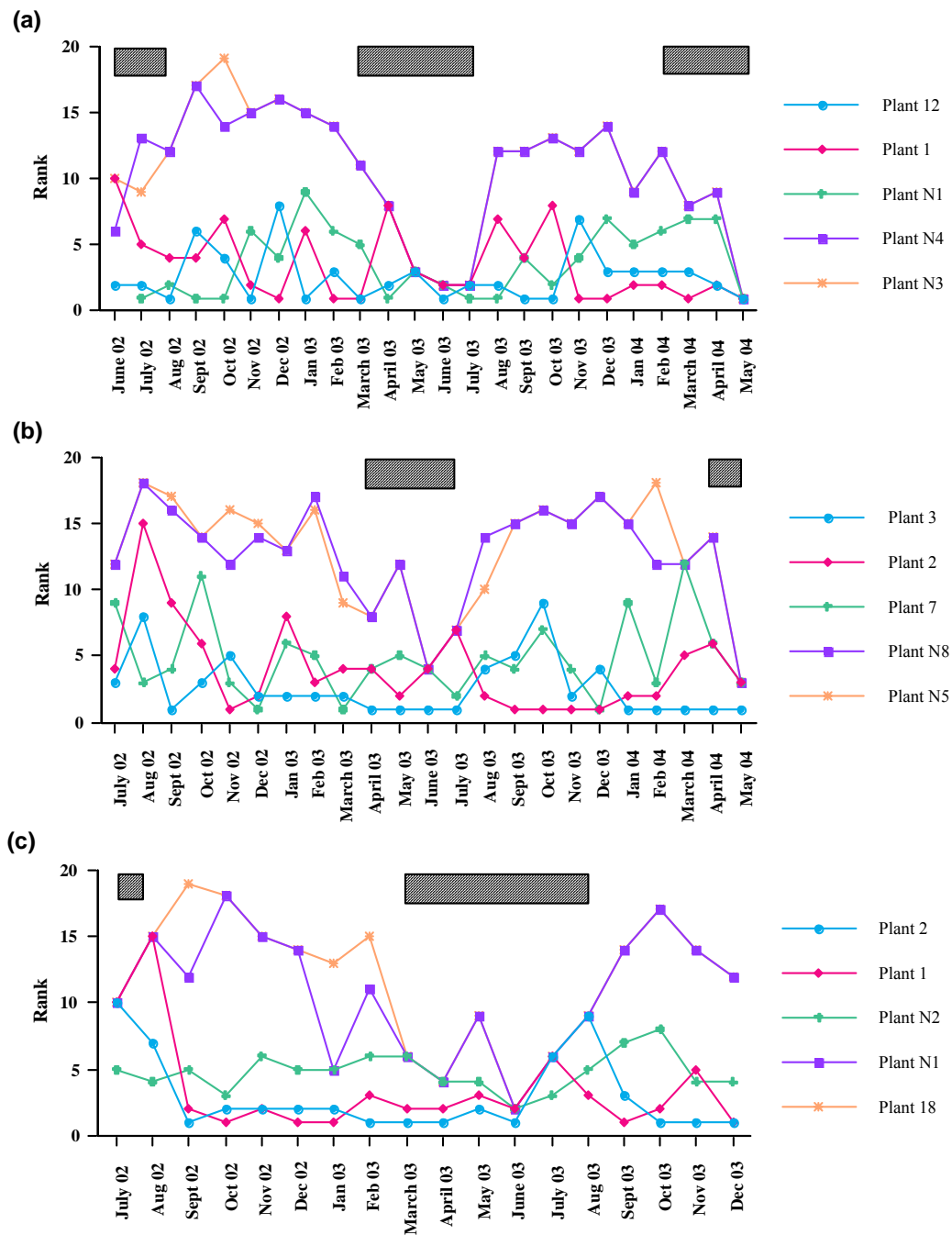
Whilst patterns of seed production among the best and the worst plants showed some consistency, patterns were not as strong as those detected for inflorescence production (Figure 2.9 and 4.3). In months of good seed production (August/September to February) the best seed producers generally ranked well, although, plants fluctuated between first and tenth place per month. The worst seed producers generally ranked very poorly during months of good seed production. From March to August (March to July at GB), most plants had very poor seed production, with many plants producing no seeds. I have illustrated these patterns in detail below and illustrated them using the three plants with the best seed production and the two with the worst, from each site (Figure 4.3).

At CB, whilst Plants 1, 12, and N1 were ranked as the top three seed producers (Figure 4.2), their monthly seed production ranks fluctuated between first and ninth place (Figure 4.3). Plant 12 (ranked first) was ranked in the top three places in 19 out of 24 months. Plants 1 and N1 (ranked second and third, respectively) were ranked in the top three places in 13 and 11 months out of 24, respectively. Plants N4 and N3 (ranked second last and last, respectively) were ranked in the last three places in 24 and 23 months out of 24, respectively.

At GB, whilst Plants 3, 2, and 7 were ranked as the top three seed producers (Figure 4.2), their monthly ranks fluctuated between first and ninth place (Figure 4.3). With the exception of Plant 2, which ranked fifteenth in August 2003 and produced just two seeds (Figure 4.3). Plant 3 (ranked first) was ranked in the top three places in 17 out of 23 months. Plants 2 and 7 (ranked second and third overall, respectively) were ranked in the top three places in 11 and 7 months out of 23, respectively. Plants N8 and N5 (ranked second last and last overall, respectively) were ranked last in 23 and 22 months out of 23, respectively.

At IL, I found that patterns of seed production in good months were more consistent than at CB and GB (Figure 4.3). Plants 2 and 1, (ranked first and second overall, respectively) were both ranked in the top three places in 14 out of 18 months. Plant N2 (ranked third overall) was ranked in the top three places, in three out of 18 months. Plants N1 and 18 (ranked second last and last overall, respectively) were ranked in the last three places in 18 and 17 months out of 18, respectively.





**Figure 4.3 - The monthly seed production rank for the three *Grevillea macleayana* plants with the best seed production and the two plants with the poorest seed production (based on mean monthly rank).**

Data shown are for plants from Chinamans Beach between June 2002 and May 2004 (Figure a), Greenfields Beach between June 2002 and May 2004 (Figure b), and Illowra Lane (Figure c), between June 2002 and December 2003. Hatched areas indicate months of poor seed production at each site.

#### 4.3.1.3 *Seed-to-Inflorescence Ratio*

I detected very low seed-to-inflorescence ratios (based on total seed and inflorescence numbers over the survey period), for all plants, with no plants even close to unity (Figure 4.4). I also detected substantial variation among plants at all sites in seed to inflorescence ratio. At CB, seed-to-inflorescence ratio ranged from just 0.07 for Plant N3 to 0.57 for Plant 19, which also had the greatest mean monthly seed production rank. Plant 1, which had the greatest inflorescence and seed numbers, was ranked seventeenth. At GB, I found the seed-to-inflorescence ratio ranged from 0.11 for Plant N4 (which also had the lowest total seed production) to 1.14 for Plant N5 (Figure 4.4). Plants 1 and 2, which had the greatest inflorescence and seed numbers, respectively, were ranked sixth and third, respectively. At IL, I found the seed-to-inflorescence ratio ranged from zero for Plant 18 (it produced no seed) to 0.32 for Plant 3 (Figure 4.4). Plant 2, which was ranked first for both inflorescence and seed numbers, was ranked third. The mean seed to inflorescence ratio for CB, GB, and IL was  $0.24 (\pm 0.03)$ ,  $0.27 (\pm 0.06)$ , and  $0.13 (\pm 0.02)$ , respectively.

#### 4.3.1.4 *Monthly Inflorescence Rank vs. Monthly Seed Rank*

I detected a strong positive trend between inflorescence and seed production (based on mean monthly inflorescence and seed production rank per plant), at each site (Figure 4.5). At CB, the four plants with the best mean monthly inflorescence production rank also had the best mean monthly seed production rank. Plant N3 (which had the highest seed-to-inflorescence ratio) was ranked last with respect to both mean monthly inflorescence and seed production rank. A significant positive correlation was detected between mean monthly inflorescence and seed production rank per plant ( $r_s = 0.93$ ;  $P < 0.001$ ). At GB, the two plants (Plants 2 and 3) with the best mean monthly inflorescence production rank also had the best mean monthly seed production rank (Figure 4.5). Plants N4, N5 and N8 were ranked in the last four positions with respect to both mean monthly inflorescence and seed production rank. A significant positive correlation was detected between mean monthly inflorescence and seed production rank per plant ( $r_s = 0.87$ ;  $P < 0.001$ ). At IL, the same plants were ranked in the top six positions with respect to mean monthly inflorescence production rank and mean monthly seed production rank (Figure 4.5). Plants 18, 9, and N1 were all ranked in the last three positions with respect to both mean monthly inflorescence and seed

production rank. A significant positive correlation was detected between mean monthly inflorescence and seed production rank per plant ( $r_s = 0.94$ ;  $P < 0.001$ ).

#### 4.3.1.5 Inflorescence Size and Seed Numbers

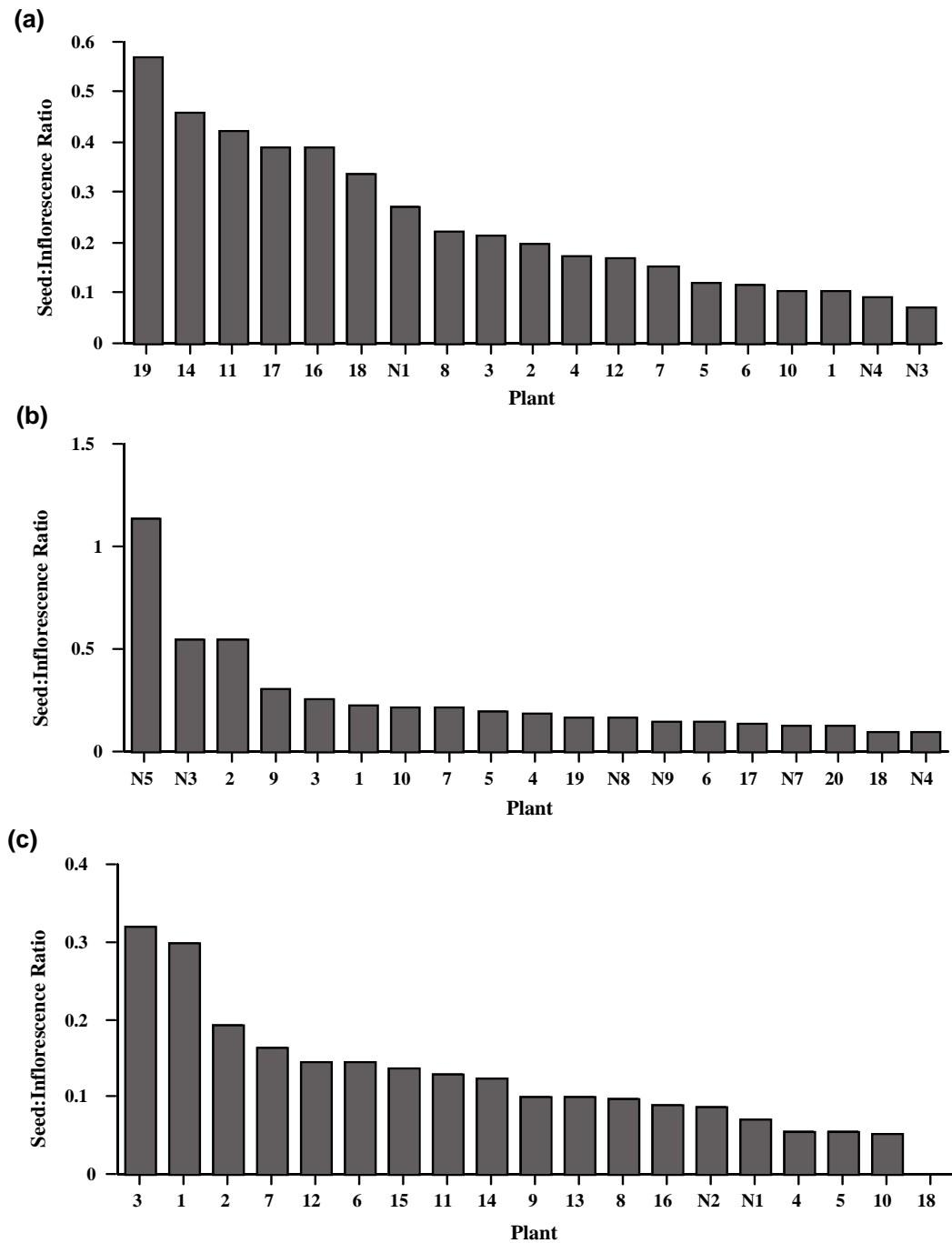
No single trend or significant relationship was detected between inflorescence size and seed numbers per plant (Table 4.2). Positive trends were found between inflorescence size and seed numbers per plant at CB in October 2002, and GB and IL in January 2003. However, negative trends were detected between inflorescence size and seed numbers per plant at GB in November 2003 and IL in October 2003.

**Table 4.2 - The effects of inflorescence size on seed production.**

The results of linear regression analyses testing the significance of relationships between monthly records of inflorescence size (flower number per inflorescence) and seed numbers per *Grevillea macleayana* plant. Surveys were conducted at: (1) Chinamans Beach in October 2002; (2) Greenfields Beach in January 2003 and November 2003; and (3) Illowra Lane in October 2002 and January 2003.

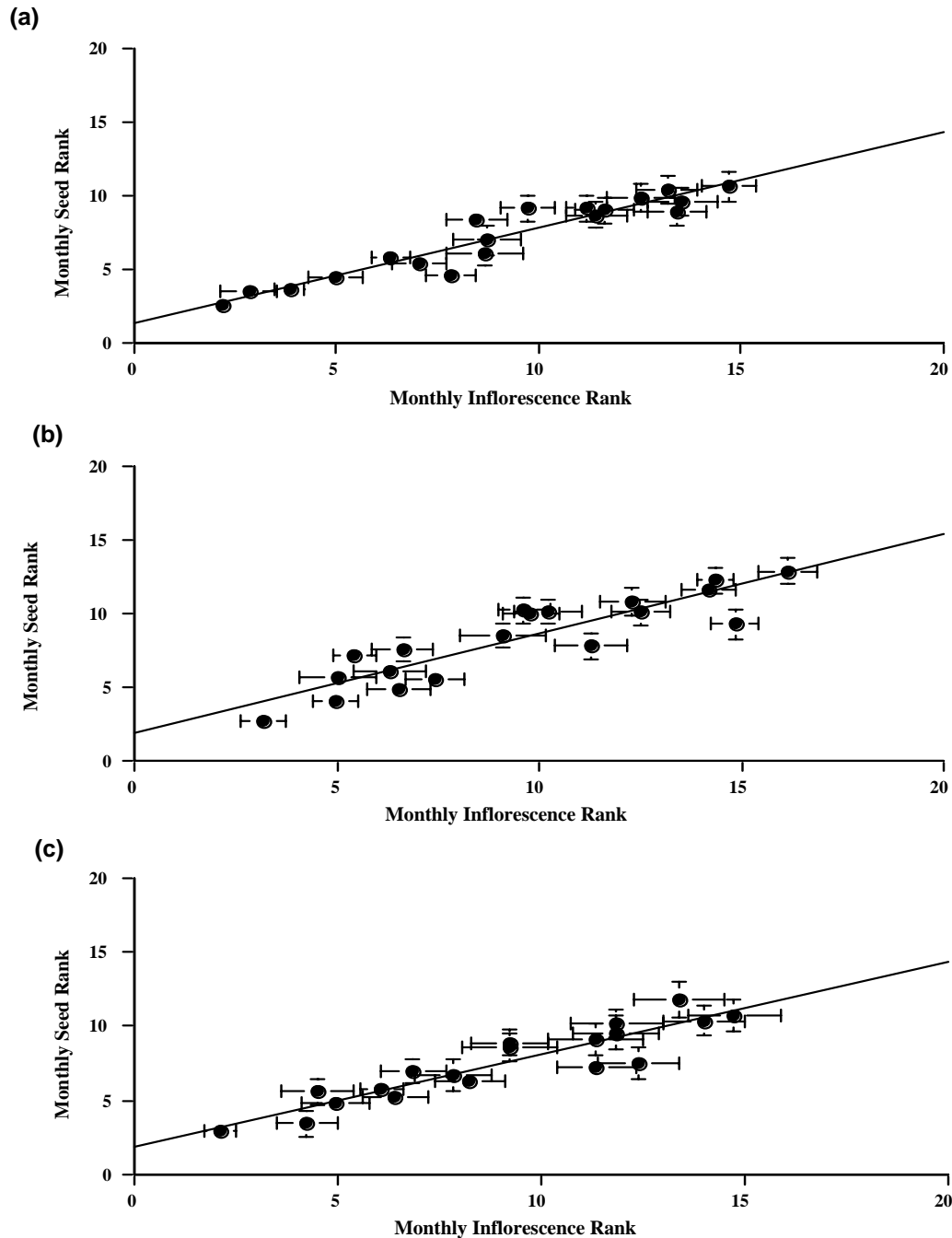
Site/Season	$r^2$	F Ratio	df*	P Value	Trend
<b>Chinamans Beach</b>					
October 2002	0.661	5.86	1, 3	0.094	Positive
<b>Greenfields Beach</b>					
January 2003	0.007	0.03	1, 4	0.872	Slight Positive
November 2003	0.027	0.11	1, 4	0.754	Slight Negative
<b>Illowra Lane</b>					
October 2002	0.111	0.38	1, 3	0.583	Negative
January 2003	0.688	6.62	1, 3	0.082	Positive

\* Model degrees of freedom, Error degrees of freedom



**Figure 4.4 - The seed-to-inflorescence ratio per plant for 19 *Grevillea macleayana* plants, at each of three sites.**

Data shown are for plants at Chinamans Beach (CB - Figure a), Greenfields Beach (GB - Figure b), and Illowra Lane (Figure c). Seed-to-inflorescence ratio is based on total inflorescence and seed numbers over 24 months at CB, 23 months at GB and 18 months at IL. Plants are displayed in order of descending seed-to-inflorescence ratio.



**Figure 4.5 - Spearman Rank Correlations between mean ( $\pm$ s.e.) monthly inflorescence rank and mean ( $\pm$ s.e.) monthly seed rank per *Grevillea macleayana* plant.**

The data shown are based on total inflorescence and seed numbers at Chinamans Beach (CB - Figure a) for 24 months, Greenfields Beach (GB - Figure b) for 23 months and Illowra Lane (IL - Figure c) for 18 months. Mean monthly inflorescence production ranks were positively correlated with mean monthly seed production ranks, per plant, at CB ( $r_s = 0.94$ ;  $P < 0.001$ ), GB ( $r_s = 0.87$ ;  $P < 0.001$ ) and IL ( $r_s = 0.94$ ;  $P < 0.001$ ).

### 4.3.2 Pollen Deposition

#### 4.3.2.1 September - October 2003

Overall, mean pollen deposition per inflorescence varied substantially among plants, but surprisingly significant variation was not detected between diurnal and nocturnal treatments (except at GB). Using two-way ANOVAs, I detected significant variation among plants in overall mean inflorescence pollen deposition at all sites (except at IL on Day and Night 2), but not between diurnal and nocturnal treatments (except at GB on Day and Night 1) (Table 4.3).

At CB on Day and Night 1, I detected a 14-fold and striking 105-fold difference (respectively) between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.6). Diurnal pollen deposition was greater than nocturnal pollen deposition on four of the six plants, Plants 12, 19 and N1, and only marginally on Plant 11 (Figure 4.6). At CB on Day and Night 2, I detected a 15-fold and minimal 4-fold difference (respectively) between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.6). Diurnal pollen deposition was greater than nocturnal pollen deposition on just two of the six plants: Plants 12 and N1 (Figure 4.6).

At GB on Day and Night 1, I detected a 4.5-fold and large 29-fold difference between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.7). Diurnal pollen deposition was greater than nocturnal pollination on all plants (Figure 4.7).

At IL on Day and Night 1, I detected an approximate 7-fold and minimal 2-fold difference (respectively) between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.7). Diurnal pollen deposition was greater than nocturnal pollen deposition on only one of the six plants: Plant 2 (Figure 4.7). At IL on Day and Night 2, I detected a minimal 3-fold and 8-fold difference between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.7). I did not detect significant variation either among plants or between the diurnal and nocturnal treatment (Table 4.3). However, diurnal pollen deposition was greater than nocturnal pollen deposition on four of the six plants: Plants 7, 8, and very marginally on Plants 1 and 16 (Figure 4.7).

**Table 4.3 - One- and two-way ANOVAs testing for significant variation among plants in pollen deposition.**

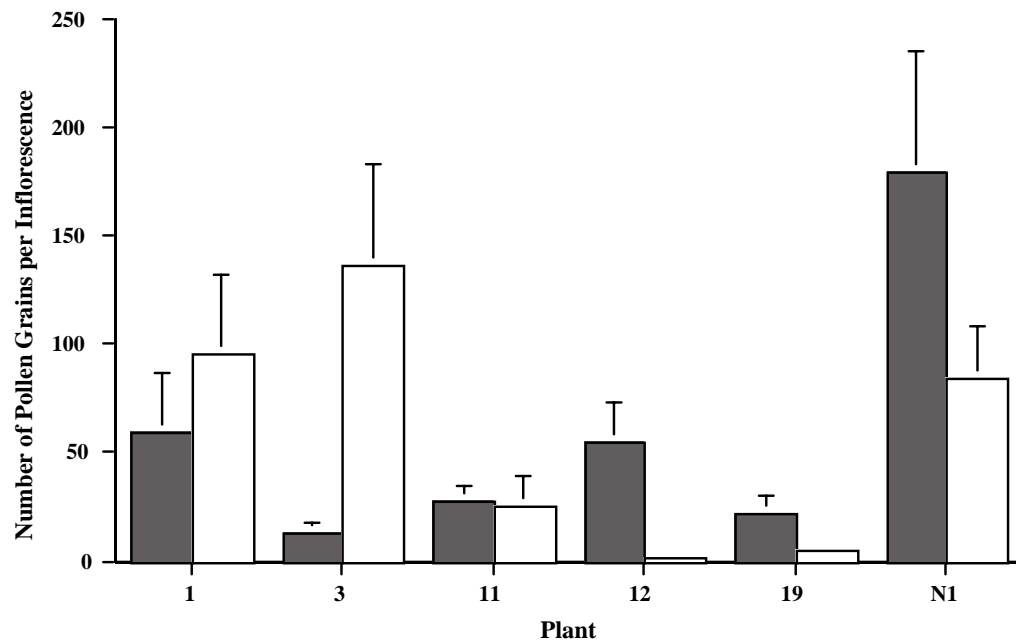
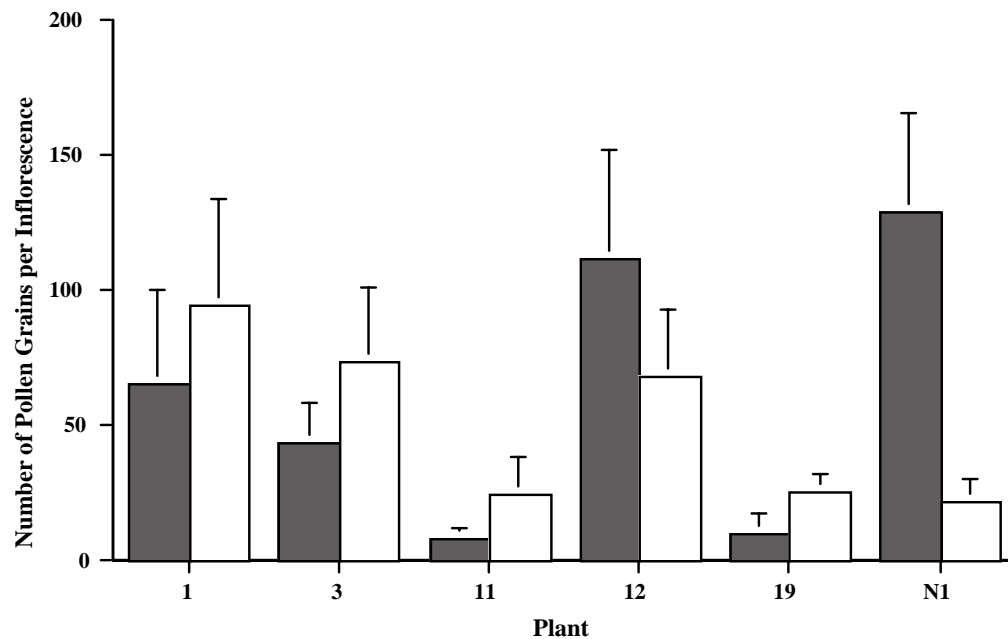
Two-way ANOVAs (Table a) tested for significant variation among plants (first factor) and between diurnal and nocturnal treatments (second factor) in pollen deposition (total from four flowers). Pollen deposition was quantified on six *Grevillea macleayana* plants at Chinamans Beach (CB), Greenfields Beach (GB), and Illowra Lane (IL), over two days and nights in September and October 2003 (Day Two data from GB were unable to be used due to rain). One-way ANOVAs (Table b) tested for significant variation in pollen deposition per inflorescence, among plants (total from two flowers). Pollen deposition was quantified on six plants over two days at both CB and GB, in December 2003 and January 2004. Significant *P* values ( $\alpha < 0.05$ ) are indicated in bold type. Asterisks (\*) indicate ANOVAs comprising transformed data [square-root ( $x + 0.5$ ) or log ( $x + 1$ )], due to some non-normality or heteroscedasticity of data.

**(a)**

Season/Site	Mean Square	<i>F</i> Ratio	df (Model)	Probability (Model)	Probability (Plant)	Probability (Day/Night)
<b>Chinamans Beach</b>						
Day/Night 1*	71.50	4.71	6	<b>0.001</b>	<b>&lt; 0.01</b>	0.64
Day/Night 2*	45.10	3.20	6	<b>0.008</b>	<b>0.004</b>	0.81
<b>Greenfields Beach</b>						
Day/Night 1*	168.69	9.60	6	<b>&lt; 0.01</b>	<b>0.016</b>	<b>&lt; 0.01</b>
<b>Illowra Lane</b>						
Day/Night 1*	21.44	2.24	6	0.05	<b>0.03</b>	0.45
Day/Night 2	2319.00	1.13	6	0.36	0.27	0.69

**(b)**

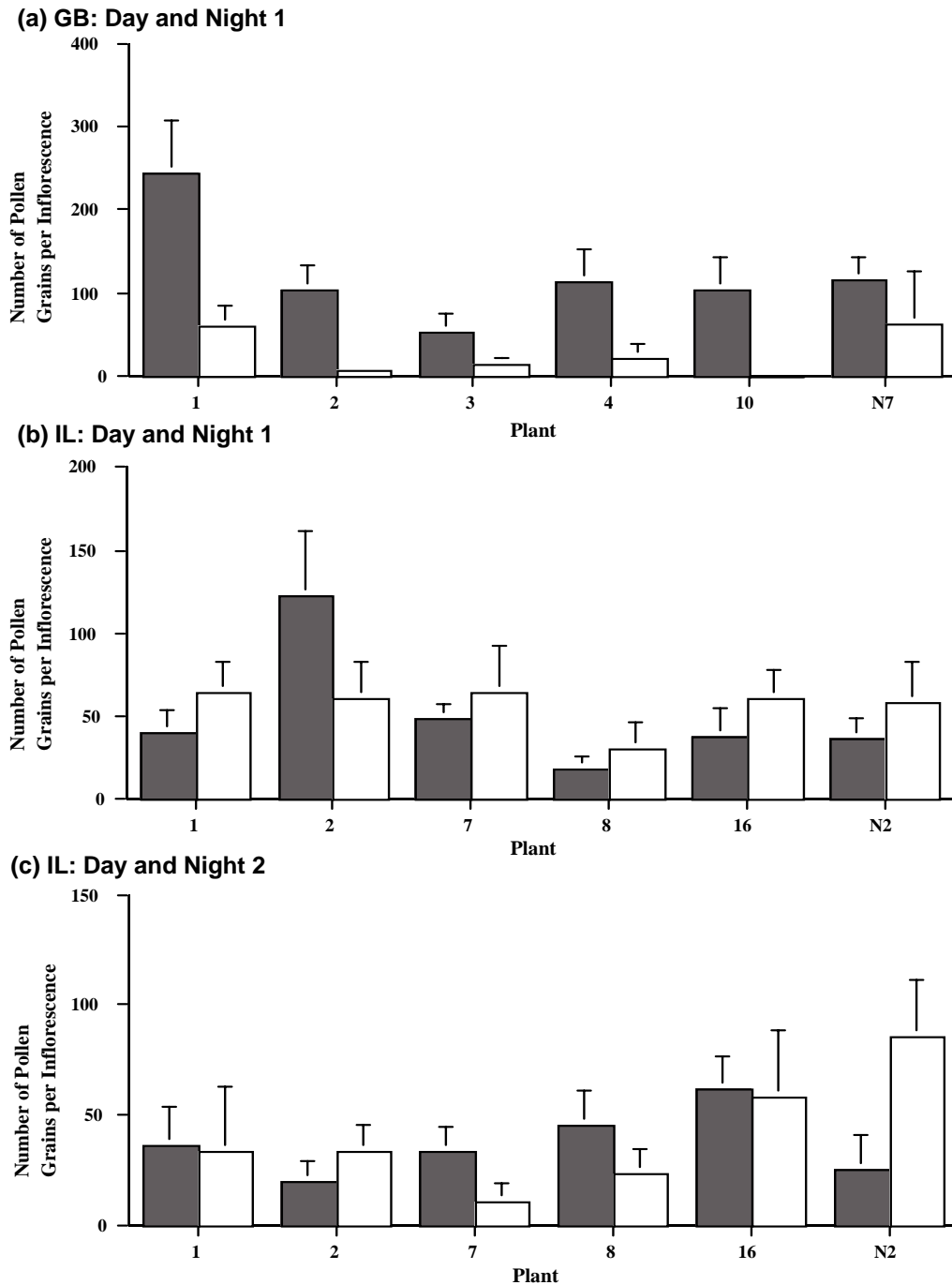
Season/Site	Mean Square	<i>F</i> Ratio	df (Model)	Probability
<b>Chinamans Beach</b>				
Day 1	761.38	1.90	5	0.11
Day 2	106.26	1.38	5	0.25
<b>Greenfields Beach</b>				
Day 1*	21.54	2.56	5	<b>0.038</b>
Day 2*	0.62	2.49	5	<b>0.04</b>

**(a) Day and Night 1****(b) Day and Night 2**

**Figure 4.6 - The mean number of pollen grains per inflorescence recorded on six *Grevillea macleayana* plants, on two days and nights, at Chinamans Beach in September 2003.**

Values for each inflorescence are based on pollen counts for four flowers and plant means are calculated from six inflorescences. Filled columns indicate the diurnal treatment and open columns indicate the nocturnal treatment. Plants are displayed in order of identification code. Bars indicate plus one standard error.





**Figure 4.7 - The mean number of pollen grains per inflorescence recorded on six *Grevillea macleayana* plants at Greenfields Beach and Illowra Lane, in September 2003.**

Pollen deposition was measured on one day and night at GB (Figure a) and over two days and nights at IL (Figures b and c). Day 2 data from Greenfields Beach were not used because of rain. Values for each inflorescence are based on pollen counts for four flowers and plant means are calculated from six inflorescences. Filled columns indicate diurnal treatments and open columns indicate nocturnal treatments. Plants are displayed in order of identification code. Bars indicate plus one standard error.

Whilst positive trends were detected between the diurnal and nocturnal pollen deposition on Day and Night 2 at CB, and Day and Night 1 at GB and IL, no significant correlations were detected (Table 4.4). This is not surprising given that most plants were ranked in different positions for diurnal and nocturnal treatments (i.e. the plants with the greatest diurnal pollen deposition did not have the greatest nocturnal pollen deposition). At CB, whilst Plant N1 received the greatest pollen deposition on Day 1 and 2, Plants 3 and 1 received the greatest pollen deposition on Night 1 and 2, respectively (Table 4.5). At GB, there was greater consistency among plants, with Plants N7 and 1 ranked first or second on Day and Night 1, and Plant 2 ranked fifth on Day and Night 1 (Table 4.5). At IL, Plant 16 ranked fourth, plant N2 ranked fifth and Plant 8 ranked last on Day and Night 1 (Table 4.5).

**Table 4.4 - Correlation analyses between diurnal and nocturnal pollen deposition treatments.**

Pollen deposition was calculated as the total from four flowers per inflorescence. Surveys were conducted at Chinamans Beach, Greenfields Beach, and Illowra Lane in September 2003 for two days and two nights. The Day 2 data from Greenfields Beach were unable to be used due to rain. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

Site/Season	<i>r</i>	<i>n</i>	<i>P</i> Value	Trend
<b>Chinamans Beach</b>				
Day/Night 1	0.00	6	0.97	Neutral
Day/Night 2	0.18	6	0.74	Positive
<b>Greenfields Beach</b>				
Day/Night 1	0.66	6	0.16	Positive
<b>Illowra Lane</b>				
Day/Night 1	0.42	6	0.41	Positive
Day/Night 2	0.01	6	0.98	Neutral

**Table 4.5 - Consistency of pollen deposition for *Grevillea macleayana* plants, for one to two survey seasons per site.**

Inflorescence pollen deposition was measured and plants were ranked in two studies: (1) over two days and nights at Chinamans Beach, Greenfields Beach (GB), and Illowra Lane in September 2003 (the Day Two GB data were unable to be used due to rain); and (2) over two days at CB and GB in December 2003 and January 2004.

Site/Season	First	Second	Third	Forth	Fifth	Sixth
<b>Chinamans Beach</b>						
September 2003 - Day 1	N1	1	12	11	19	3
September 2003 - Day 2	N1	12	1	3	19	11
September 2003 - Night 1	3	1	N1	11	19	12
September 2003 - Night 2	1	3	12	19	11	N1
December 2003 - Day 1	N1	12*	3*	11	1	19
December 2003 - Day 2	1	N1	12	19	11	3
<b>Greenfields Beach</b>						
September 2003 - Day 1	1	N7	4	10	2	3
September 2003 - Night 1	N7	1	4	3	2	10
January 2004 - Day 1	10	5	1	N7	3	2
January 2004 - Day 2	N7	3	10	5	2	1
<b>Illowra Lane</b>						
September 2003 - Day 1	2	7	1	16	N2	8
September 2003 - Day 2	16	8	1	7	N2	2
September 2003 - Night 1	7	1	2	16	N2	8
September 2003 - Night 2	N2	16	1^	2^	8	7

\* Plant 12 and 3 were ranked equal second at CB on Day 1 in December 2003.

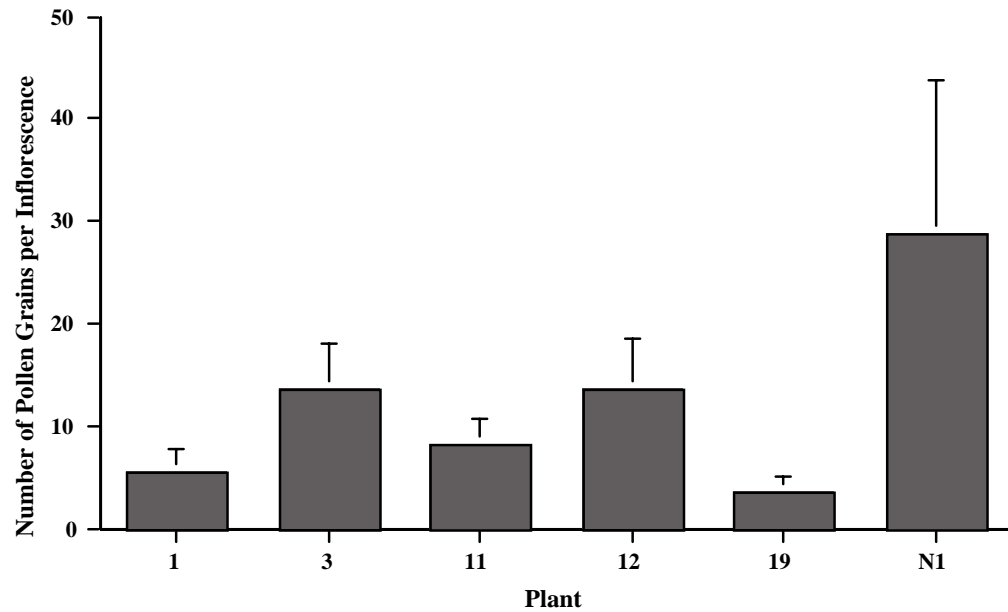
^ Plant 1 and 2 were ranked equal third at IL on Night 2 in January 2004.

#### 4.3.2.2 December 2003 - January 2004

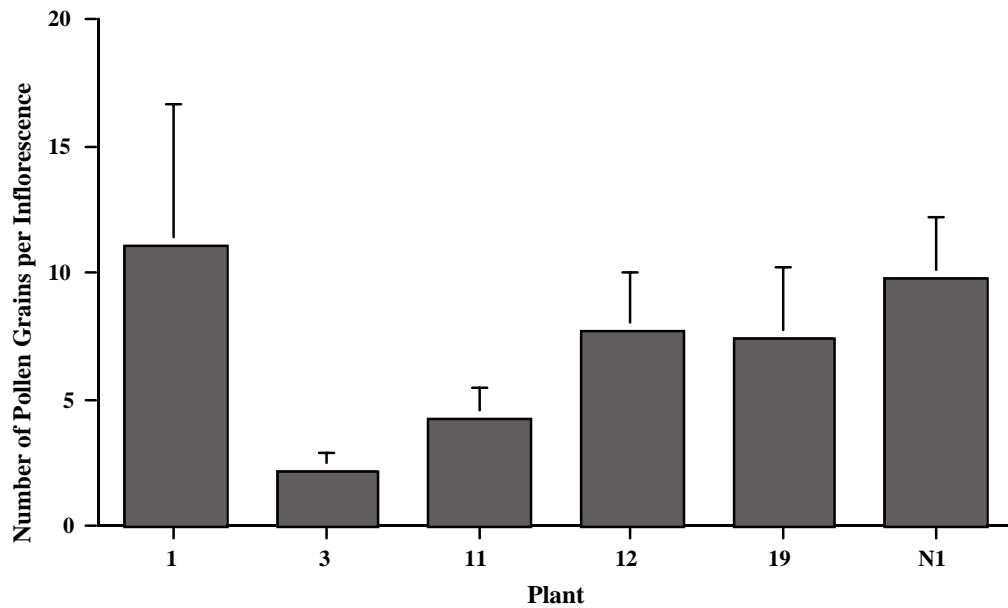
Overall, the diurnal pollen deposition study detected striking variation among plants at GB, but not at CB. At CB on Day 1 and 2, there was a 7-fold and 5-fold difference (respectively) between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.8). At GB on Day 1 and 2, there was an 11-fold and 7-fold difference between the plants with the lowest and the plants with the greatest mean inflorescence pollen deposition (Figure 4.9). Significant variation was detected among GB plants on Day 1 and 2 (Table 4.3).

Some plants ranked in similar positions in the first and second study (Table 4.5). At CB, Plant N1 was again ranked first on Day 1 and second on Day 2 and Plant 12 was again ranked second and third on Days 1 and 2 (Table 4.5). At GB, Plant N7 was again ranked first on Day 2, although, it was ranked fourth on Day 1. Plant 2 was also ranked poorly again, in last and fifth place on Day 1 and 2, respectively (Table 4.5).

(a) Day 1

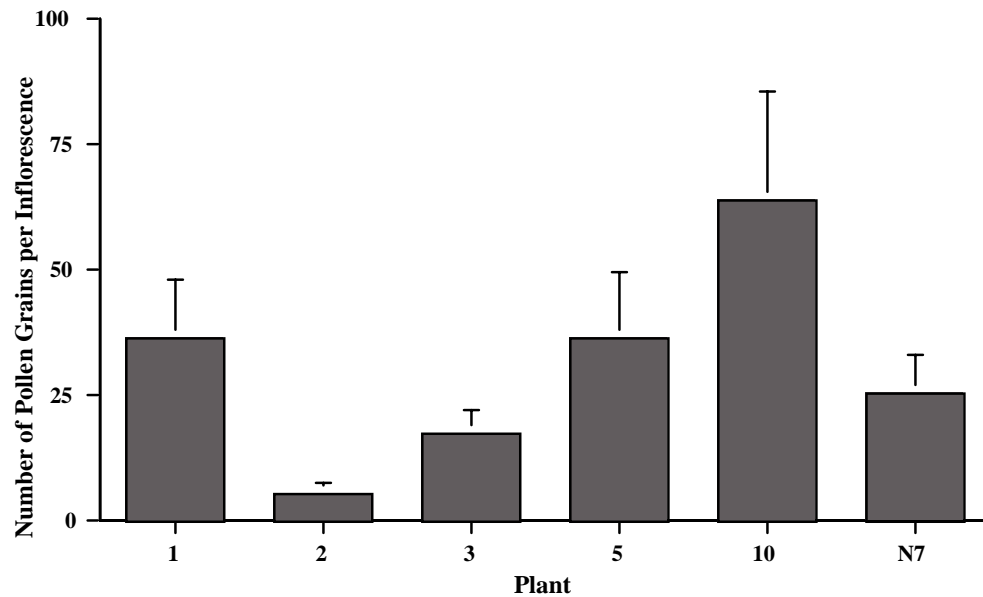
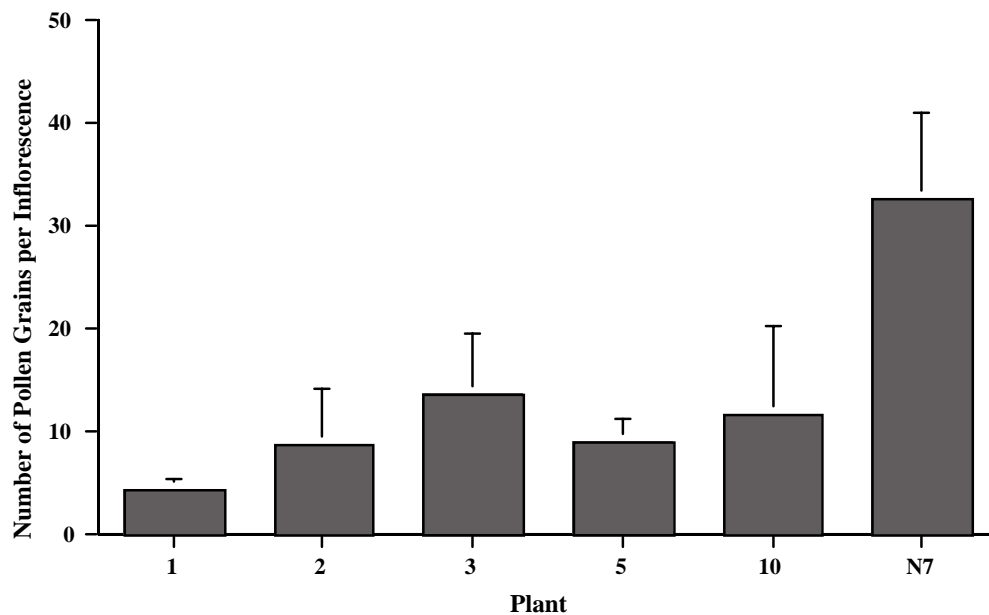


(b) Day 2



**Figure 4.8 - The mean number of pollen grains per inflorescence recorded on six *Grevillea macleayana* plants at Chinamans Beach in December 2003.**

Pollen deposition was measured over two days, Day 1 (Figure a) and Day 2 (Figure b), respectively. Values for each inflorescence are based on pollen counts for two flowers and plant means are calculated from ten inflorescences. ANOVAs did not detect any significant variation among plants. Plants are displayed in order of identification code. Bars indicate plus one standard error.

**(a) Day 1****(b) Day 2**

**Figure 4.9 - The mean number of pollen grains per inflorescence recorded on six *Grevillea macleayana* plants at Greenfields Beach in January 2004.**

Pollen deposition was measured over two days, Day 1 (Figure a) and Day 2 (Figure b), respectively. Values for each inflorescence are based on pollen counts for two flowers and plant means are calculated from ten inflorescences. ANOVAs of transformed data [square-root  $x+0.5$  for (a) and  $\log x+1$  for (b)] detected significant variation among plants ( $F_5 = 2.56$ ;  $P < 0.04$ ) for Figure (a) and ( $F_5 = 2.50$ ;  $P < 0.04$ ). Plants are displayed in order of plant identification code. Bars indicate one standard error.

### 4.3.3 Reproductive Success, Floral Visitors, and Floral Trait Comparisons

#### 4.3.3.1 Seed Number and Pollen Deposition

Whilst the whole-model trends between seed production and pollen deposition were positive, non-significant positive and negative trends were detected between both diurnal and nocturnal pollen deposition and seed numbers. These regressions explained between 47% and 99% of the variation among plants, but significant regressions were only detected on Day 1 at CB and Day 1 and IL (Table 4.6). On Day 1 at CB, the regression explained 80% of the variation among plants and a marginally significant ( $P = 0.05$ ) positive relationship was detected between nocturnal pollen deposition and subsequent seed production (Table 4.6). On Day 1 at IL, the regression explained 99% of the variation among plants and significant positive whole-model and diurnal pollen deposition relationships, and significant negative nocturnal pollen deposition relationships were detected (Table 4.6). The simple linear regression tested on the December 2003 and January 2004 data were less consistent than those from the first season and none were significant, explaining between just 14% and 38% of the variation among plants (Table 4.6).

#### 4.3.3.2 Seed Number, Pollen Deposition, Floral Visitors, and Floral Rewards

The twelve multiple regression tests between seed numbers and nectar traits (volume and concentration) explained between only 7% and 69% of the variation among plants, and none of the tests explained a significant amount of the variation among plants (Appendix 3, Table A3.2). The whole-model tests were consistently positive and (non-significant) positive trends were detected between both nectar volume and sugar concentration and seed numbers in four out of six tests, respectively.

The linear regressions between inflorescence number and pollen deposition were not significant, explaining between 3% (CB, September 2003) and 49% (GB, September 2003) of the variation among plants (Appendix 3, Table A3.2). However, (non-significant) positive trends were detected in five of the nine tests. The two multiple regressions between diurnal pollen deposition and the floral traits of inflorescence number and pollen production both reported non-significant positive whole-model trends (Appendix 3, Table A3.2).

The multiple regressions between seed numbers and the three measures of honeybee and honeyeater foraging behaviour detected positive whole-model trends, for each of the seven and six honeybee and honeyeater tests, respectively (Appendix 3, Table A3.2). However, only the test for CB in September/October 2002 revealed a significant positive relationship, with the number of honeybees significantly related to seed number [ $R^2 = 0.98$ ;  $F_{3,2} = 236.65$ ;  $P = 0.03$  (whole-model);  $P = 0.04$  (honeybee number): Appendix 3, Table A3.2]. Overall, the number of honeybees was evenly split between positive and negative non-significant trends, with respect to seed numbers. The independent variable of inflorescence number visited per plant was (non-significantly) negatively associated with seed number in five of the seven tests. Honeybee foraging time per plant was (non-significantly) positively associated with seed number in six of the seven tests. Despite not being significant, four of the seven honeybee tests explained between 82% and 98% of the variation among plants (February 2003 and September/October 2002 at CB, and October 2002 and January 2003 at IL).

Honeyeater foraging behaviour was not significantly associated with seed number, although four of the six tests explained between 45% and 99% of the variation among plants (Appendix 3, Table A3.2). The non-significant trends between the independent variable of honeyeater number per plant and seed number were positive in two tests and negative in three tests. The number of inflorescences visited per plant by honeyeaters per survey period was (non-significantly) positively associated with seed numbers in two of the six tests. Honeyeater foraging time per plant was (non-significantly) positively associated with seed number in three of the six tests.

**Table 4.6 - Simple linear and multiple regression analyses between seed numbers and pollen deposition, for *Grevillea macleayana* plants.**

Multiple regressions tested the significance of relationships between seed numbers (number per month 7 to 10 weeks after the pollen deposition survey) and (1) diurnal and (2) nocturnal mean inflorescence pollen deposition per plant. Simple linear regressions tested the significance of relationships between seed numbers (number per month 7 to 10 weeks after the pollen deposition survey) and diurnal mean inflorescence pollen deposition (total from two flowers per inflorescence) per plant. Studies were conducted on *G. macleayana* plants at Chinamans Beach (CB) and Greenfields Beach (GB), and Illowra Lane in September-October 2003 and at HB and GB in December 2003-January 2004. Significant *P* values ( $\alpha < 0.05$ ) are in bold type

September - October 2003							
Site	$R^2$ $R^2$ Adj.	df*	Mean Square	SE**	$F$ Ratio	$P$	Trend
Chinamans Beach							
Whole-Model	0.80 (0.67)	2, 3	23.03	1.39	6.13	0.09	Positive
Day 1				0.01	3.15	0.17	Negative
Night 1				0.02	10.68	0.05	Positive
Whole-Model	0.63 (0.38)	2, 3	17.95	2.52	2.51	0.23	Positive
Day 2				0.02	1.28	0.34	Negative
Night 2				0.04	4.43	0.13	Positive
Greenfields Beach							
Whole-Model	0.47 (0.12)	2, 3	9.80	2.62	1.34	0.38	Positive
Day 1				0.03	2.22	0.23	Positive
Night 1				0.06	2.24	0.23	Negative
Illowra Lane							
Whole-Model	0.99 (0.98)	2, 3	47.00	1.35	105.81	<0.01	Positive
Day 1				0.01	177.22	<0.01	Positive
Night 1				0.03	119.14	<0.01	Negative
Whole-Model	0.29 (-0.18)	2, 3	13.93	6.44	0.62	0.60	Positive
Day 2				0.014	0.98	0.40	Negative
Night 2				0.08	0.25	0.65	Negative
December 2003 - January 2004							
Site	$r^2$	df*	$F$ Ratio	$P$	Trend		
Chinamans Beach							
Diurnal Day 1	0.14	1, 4	0.68	0.46	Negative		
Diurnal Day 2	0.21	1, 4	1.04	0.37	Positive		
Greenfields Beach							
Diurnal Day 1	0.33	1, 4	2.01	0.23	Negative		
Diurnal Day 2	0.38	1, 4	2.45	0.19	Positive		

\* Model degrees of freedom; Error degrees of freedom.

\*\* SE = Standard Error



#### 4.4 Discussion

My analysis of seed production revealed striking patterns of variation among plants, over two years. Whilst patterns of seed production among the best and poorest seed producers were not as consistent as I had reported for inflorescence production, in months of good seed production there was a distinction between these two groups. Moreover, two to three plants per site produced over 40% of the total seed production for the study plants. It is clear that whilst the number of seeds produced per plant was always very low, greater inflorescence production was consistently associated with greater seed production, possibly supporting the prediction that excess flower production has evolved for female function (i.e. “*pollinator attraction*” and “*bet-hedging*”). However, I also found very low seed-to-inflorescence ratios for plants at all sites, possibly supporting the “*male function hypothesis*” (Section 1.3.1 - Willson, 1979; Ayre and Whelan, 1989) and/or suggesting that limited resources or lower pollen quality may be restricting seed production (Ayre and Whelan, 1989).

As predicted, I found pollen deposition varied substantially (and often significantly) among plants at all sites, in all seasons. There was some consistency in the first and second study, with respect to the plants with the greatest pollen deposition. Surprisingly, I found no significant variation between diurnal and nocturnal pollination at two of the three sites.

In the following sections, I will discuss the observed variation among plants in seed production and pollen deposition. I will also examine the relationships between these two measures of reproductive success, and the complex relationships between seed production, pollen deposition, floral visitor foraging behaviour (Chapter 3) and floral traits (Chapter 2).

##### 4.4.1 Seed Numbers

###### 4.4.1.1 Variation in Seed Numbers

Whilst considerable research has been conducted on *G. macleayana*, this is the first study to monitor the inflorescence and seed production of individual plants over more than one flowering season. I detected striking variation among plants in total inflorescence and seed numbers (over the survey period) at all sites, consistent with previous studies on Proteaceae species (e.g. Carthew, 1993; Krauss, 1994; Lloyd, 1998).

I also found that a small number of plants at each site (three to five) produced more than 50% the total seed production for the study plants, consistent with the findings of studies on other Proteaceae species (e.g. Carthew, 1993; Whelan and Ayre, *unpublished*).

As discussed in Chapter 2, it has previously been found that for a number of populations and seasons *G. macleayana* plants have not been pollen limited (Harriss and Whelan, 1993; Vaughton, 1996). Therefore, it is probable that my results indicate that the observable variation among plants in seed production is due to variation in resource availability. Plants with greater water and/or nutrient access may be able to allocate more resources to functions such as seed development, as previous studies have demonstrated (e.g. Zimmerman, 1983; Vaughton, 1991; Lee and Felker, 1992; Galen, 2000). Haig and Westoby (1988) suggest that an evolutionary stable strategy would result in a balance between resource (seed production) and pollen limitation (fertilisation), whereby selection should favour: (1) increased resources to attracting pollinators, when plants are pollen limited; and (2) increased allocation to seed production, when plants are resource limited.

#### 4.4.1.2 Temporal Patterns of Seed Production

Whilst the seed production of individual plants varied overtime, there were observable distinctions with respect to plant rankings between the best and worst seed producers. I found very low error rates when testing for significant variation among plants (in mean monthly rank) indicating little month to month variation in plant rankings. I also found that in months of good seed production, the best seed producers ranked highly and the poor seed producers consistently ranked poorly. However, these patterns were not as consistent as for inflorescence production. These findings provide further support for the proposal that resource availability may be the primary cause of variation among plants, given that the same plants were generally the best or worst seed producers.

Few studies have examined patterns of seed production among plants over several years, although, Carthew (1993) found similar results with another Proteaceae species, *B. spinulosa*. Carthew (1993) found some consistency among *B. spinulosa* plants in flowering and fruiting patterns over three years. Of the 47 plants monitored, six plants consistently set some seed and another six consistently failed to set seed (Carthew,

1993). Furthermore, the six plants that set seed each year, also produced a greater proportion of the total number of infructescences produced, and double the seed set of other plants (Carthew, 1993).

#### 4.4.1.3 Relationships between Inflorescence Number, Size, and Seed Number

Not surprisingly, I found very low seed-to-inflorescence ratios and seed numbers for plants at all sites, consistent with previous studies on *G. macleayana* (e.g. Harriss and Whelan, 1993; Vaughton, 1996, 1998; Roberts *et al.*, 2006) and other Proteaceae species (reviewed in Collins and Rebelo, 1987; Ayre and Whelan, 1989). Moreover, previous studies documenting natural levels of fruit and seed production in other *Grevillea* species have also found low levels of production (e.g. reviewed in Hermanutz *et al.*, 1998; Celebrezze, 2002; Llorens, 2004). With the exception of one population of *G. oleoides*, mature fruit-to-flower ratios were less than 0.05, in five populations of five different *Grevillea* species (Hermanutz *et al.*, 1998). Hermanutz *et al.* (1998) also found that most plants lost the majority of initiated fruit and the proportion of fruits that initiated and developed to maturity ranged from 19% (*G. sphacelata*) to 66% (*G. oleoides*).

As described in Section 1.3.1, the “*male function hypothesis*” predicts that fitness increases via female function should plateau or decline (beyond some optimum point) with increasing flower number, due to resource limits on seed production (Willson and Rathcke, 1974; Campbell, 1989). Moreover, male reproductive success will increase with flower number due to increased pollen donation (Sutherland and Delph, 1984). The *G. macleayana* mating system provides some support for the “*male function hypothesis*”, provided that pollen donation increased with inflorescence number per plant. Whilst pollen removal and deposition have been investigated in this species (Vaughton, 1996; Beynon *et al.*, *unpublished*), the results were not correlated with inflorescence production. The results of the multiple regression from Chapter 3 (Table 3.8) provide some evidence that honeyeaters visit plants with more inflorescences more frequently, and therefore, increased pollen removal is expected.

The positive relationship I detected between inflorescence and seed number implies strong selection pressure for greater production of floral traits (with respect to inflorescence production). In fact, these results provide some support for two further

hypotheses that propose excess flower production has evolved for female function: (1) the “*pollinator attraction hypothesis*”, which predicts that increased flower production will increase plant attraction to pollinators and ensure adequate pollinator visits for effective pollen transfer and seed set (Willson and Rathcke, 1974; Stephenson, 1980; Sutherland and Delph, 1984; Ayre and Whelan, 1989; Rathcke, 1992); and (2) the “*bet-hedging hypothesis*”, which predicts that excess flower production has evolved to allow plants to respond to temporal variation in pollinator visits and resources, required for fruit development (Stephenson, 1980; Sutherland and Delph, 1984; Ayre and Whelan, 1989).

Despite low seed production, I found significant positive correlations between mean monthly inflorescence and seed production rank, per plant at all sites. These results are consistent with previous studies that have also found significant positive relationships between flower and fruit production (e.g. Zimmerman, 1984; Firmage and Cole, 1988; Broyles & Wyatt, 1990; Cruzan *et al.*, 1994). Moreover, my results do not provide any evidence that there is a trade-off between these two functions, with respect to the ranking that plants have within the population. Plants that produced more inflorescences in one month also produced more seeds, without any apparent loss in seed production in subsequent months. This pattern is consistent with some previous studies (e.g. Zimmerman, 1984; Sahley, 2001). For example, Zimmerman (1984) found significant positive relationships between plant floral display and the seed production of subsequent years, indicating that plants were not resource limited by the large floral or seed production of previous years. It is also worth noting that in months of poor inflorescence production, all *G. macleayana* plants showed a tendency for lower seed production, regardless of rank.

I found no consistent patterns with respect to inflorescence size and seed number, which is consistent with previous studies that found no evidence of trade-offs between these functions (e.g. Firmage and Cole, 1988; Harder and Barrett, 1995). There is some evidence to suggest that in long-lived perennials, trade-offs may not become apparent until monitoring has been conducted for several years, as plants become older and perhaps more resource limited (Zimmerman, 1984).

#### 4.4.2 Pollen Deposition

##### 4.4.2.1 Variation in Pollen Deposition

My data provide one of the first estimates of natural variation in pollen deposition among plants at various sites and seasons (though see Huang and Guo, 2002), without artificial manipulation of floral rewards such as that performed by Thomson, 1986; Cresswell and Galen, 1991; Vaughton and Ramsey, 1998; Golubov *et al.*, 1999 (Table 1.2). I detected moderate consistency between survey seasons with respect to the plants that received more or less pollen. These patterns were more apparent among high-ranking plants than low-ranking plants. At both CB and GB, at least one plant was ranked in the same position (in at least one out of two days) in the second study, as it was in the first study. Based on the positive relationships detected between pollen deposition and increased floral rewards in previous studies (Table 1.2), it may be possible that the patterns I detected reflect increased floral traits in the high-ranking plants. For example, Plant 12 (at CB), which consistently ranked second or third, also had the second greatest number of inflorescences in both seasons. Furthermore, Plant 3, which ranked last on one day per season, also had the second lowest number of inflorescences in both seasons. However, no such patterns were detected among the highest-ranking plants at either site. The relationships between measures of reproductive success, floral rewards and floral visitors will be discussed further in Section 4.4.3.

##### 4.4.2.2 Nocturnal and Diurnal Pollen Deposition

The results of the nocturnal versus diurnal pollen deposition study were very surprising, with no significant variation between diurnal and nocturnal pollen deposition at two out of three sites (CB and IL). Moreover, at CB and IL, nocturnal pollen deposition was greater than diurnal pollen deposition on up to five of the six plants per night. The lack of variation between diurnal and nocturnal pollen deposition at CB and IL suggests that nocturnal pollinators (likely the Eastern Pygmy Possum *Cercartetus nanus*) are successfully transferring pollen just as regularly as diurnal honeyeaters. This is surprising because whilst honeyeater abundance was not often high, it was reasonably constant and readily observable, compared with *C. nanus*. Whilst I observed *C. nanus* foraging on five nights at CB, this was only on two plants and I made no observations of *C. nanus* at GB and IL. However, all mammal pollinators are very difficult to detect, especially those as small as *C. nanus* (weight of 15-43g, Strahan, 1983). Admittedly, it

is possible that some of the nocturnal pollination activity is attributable to another agent (especially nocturnal moths), although, I did not observe any such activity in many hours of study.

Two previous studies quantifying diurnal and nocturnal pollen removal and deposition have been conducted on *G. macleayana* plants (Vaughton, 1996; Beynon *et al.*, unpublished). Beynon *et al.* (unpublished) found significant variation between diurnal and nocturnal pollen removal on *G. macleayana* plants at three sites in Jervis Bay. Whilst diurnal pollen removal was significantly greater than nocturnal pollen removal at two of the three sites, the results indicated that up to 21% of flowers had some nocturnal pollen removal per site, thus supporting my findings. However, Vaughton (1996) reported negligible nocturnal pollen removal on plants at one site. Clearly, nocturnal pollinator activity on *G. macleayana* plants requires further attention.

Few studies have reported similar levels of diurnal and nocturnal pollinator activity (e.g. Vieira and de Carvalho-Okano, 1996; Hackett and Goldingay, 2001; Wolff *et al.*, 2003). Hackett and Goldingay (2001) found there was no overall difference in pollen removal from newly opened *Banksia* sp. flowers, by nocturnal mammals and diurnal visitors (i.e. birds and insects). A greater number of studies have reported significant variation between nocturnal and diurnal pollinator activity and/or reproductive success, as reviewed in Young (2002). One interesting study using a simulation model and field data collected for *Lonicera japonica* (Miyake and Yahara, 1999), found that diurnal pollinators removed substantially more pollen from flowers than nocturnal pollinators. Furthermore, they concluded that anthesis at dusk resulted in greater overall pollen transfer than anthesis in dawn, because it allowed nocturnal pollinators to make some contribution to pollen transferral, prior to diurnal pollinators removing the majority of pollen. This may also be the case for *G. macleayana*, given that most anthesis occurs overnight (Lloyd, S., *personal observations*).

#### **4.4.3 Reproductive Success, Floral Visitors, and Floral Reward Comparisons**

##### **4.4.3.1 Seed Number and Pollen Deposition**

Despite being largely not significant, the positive trends I generally detected between pollen deposition and seed number support the findings of many previous studies (e.g. Waser and Price, 1990; Quesada *et al.*, 2001; Waites and Ågren, 2004). For example,

Engel and Irwin (2003) found that *Ipomopsis aggregata* plants that received increased pollen loads produced a greater number of seed. However, other studies have found negative trends between pollen deposition and seed number (e.g. Richardson and Stephenson, 1991; Philipp and Hansen, 1999). Moreover, Klinkhamer *et al.* (1994) suggest that with increased pollen deposition a larger proportion of removed pollen will consequently be deposited within the plant, resulting in decreased pollen export and increased geitonogamy (for self-compatible species).

#### 4.4.3.2 Seed Number, Pollen Deposition, and Floral Rewards

Whilst the multiple regressions between seed number and nectar traits (volume and sugar concentration) did not explain a significant amount of the variation among plants, the generally positive trends with both nectar volume and nectar sugar concentration suggest that plants that provide greater nectar rewards may produce more seed. These findings are consistent with many previous studies that have found positive relationships between nectar production and seed production (e.g. Schemske, 1980b; Zimmerman, 1983; Hodges, 1995; Golubov *et al.*, 1999; Manetas and Petropoulou, 2000 - Table 1.2).

In the two multiple regressions between pollen deposition and the floral traits of inflorescence number and pollen production, the (non-significant) trend with inflorescence number was negative. Clearly however, this is not a robust finding since it is not significant and simple linear regressions between pollen deposition and inflorescence number most commonly revealed (non-significant) positive trends (five out of seven tests). Many previous studies have found positive relationships between flower production and pollen removal or deposition (e.g. Vaughton and Ramsey, 1998; Campbell, 1989; Philipp and Thomas, 2000). For example, Philipp and Thomas (2000) found that pollen deposition was positively related to corolla size and floral display size in *Geranium sanguineum*.

#### 4.4.3.3 Seed Number and Floral Visitors

Whilst the whole-model tests for the multiple regressions between seed numbers and the three measures of honeybee and honeyeater foraging behaviour were all positive, only one was significant. The numbers of inflorescences visited by honeybees per plant were more commonly (non-significantly) negatively associated with seed number. A

significant patterns such as this would not be a surprise given honeybees generally remove nectar from inflorescences without transferring pollen, thereby reducing plant attraction to effective honeyeater pollinators (as described in Section 3.4.3). However, given *G. macleayana* sets seed autogamously, I wouldn't necessarily expect a reduction in honeyeater visits (due to reduced plant attraction) unless plants are selecting for outcrossed over self-pollen. Honeybee foraging time was (non-significantly) positively associated with seed production. This pattern deserves further study and may reflect honeybee attraction to plants with favourable floral traits and a greater supply of resources for seed production.

None of the multiple regressions between honeyeater foraging behaviour and seed numbers were significant and many of the trends with independent variables showed no distinct positive or negative trend (i.e. neutral). Pollination ecology theory predicts a positive relationship between honeyeater activity and seed production. Perhaps increased honeyeater movement within plants resulted in substantial geitonogamous pollen movement, which “*clogged*” the stigmatic surface of flowers, preventing effective pollen tube and seed growth (Waser and Fugate, 1986; Waser, 1993b). Honeyeater number and foraging time per plant were each more commonly (non-significantly) positively associated with seed number per plant. Whilst not significant, this pattern is in line with expectations, provided increased honeyeater pollen transfer results in increased seed production. Many previous studies that have found positive relationships between pollinator activity and seed production (e.g. Real and Rathcke, 1991; Ohara and Higashi, 1994; Silva and Dean, 2000 - Table 1.2).

#### 4.4.3.4 Evolutionary Consequences

Variation among individual plants in reproductive success is a “*prerequisite for natural selection*” (Herrera, 1995). Moreover, increased nectar volume (amongst other floral traits) may be a selective advantage if effective pollinator visits are increased and subsequent pollen deposition increases fitness (Thomson, 1986). Given that seven of the nine trends between pollen deposition and seed production were positive, plants with greater reproductive success may possess floral traits with a selective advantage. However, Price and Waser (1979) suggest that realised pollen movement should reflect “*conflicting selection*” between: (1) plants aiming to maximise pollen deposited and received over optimal transfer distances; and (2) pollinators aiming to minimise inter-



plant flight distances whilst ensuring optimal outcrossing. Pollen removal and deposition will ultimately be maximised if limited numbers of pollinators visit a small proportion of flowers per plant, before visiting another conspecific (Klinkhamer *et al.*, 1994). This is unlikely to be the case with *G. macleayana* given the structure of inflorescences comprising up to 50 flowers, although, I observed that honeyeaters often left a plant after visiting just a few inflorescences (Lloyd, S., *personal observations*).

Whilst seed production is a very useful and practical measure of reproductive success, within self-compatible plant species, individual plant fitness gains may also be strongly dependent upon the proportion of outcrossed seed. Therefore, to gain a better assessment of the potential fitness of an individual plant, both the number and quality of seed must be studied (Zimmerman, 1988; Krauss & Peakall, 1998). In Chapter 5, I quantify variation among plants in outcrossing and biparental inbreeding rates.

## Chapter 5 - Variation in Family Outcrossing Rates

### 5.1 Introduction

#### 5.1.1 Family Outcrossing Rates and Plant Mating System Parameters

We know that variation in mating system parameters, such as outcrossing rates, are influenced by a range of environmental and ecological variables (e.g. Hedrick, 1985; Karron *et al.*, 1995; Ritland, 2002). Such mating system parameters can also be driven by heritable variation in life history traits, such as levels of self-compatibility, floral density, synchrony of flowering etc. Despite evidence that plants within populations may display significant variation in mating system parameters and plant reproductive traits (Humphreys and Gale, 1974; Handel, 1983; Neel *et al.*, 2001), there is a lack of information on variation among individuals within populations. Moreover, few studies have attempted to link variation in outcrossing and/or selfing rates at the family level (i.e. individual plants and their progeny) with variation in floral traits, pollinator activity and/or reproductive success (e.g. Motten and Antonovics, 1992; Harder and Barrett, 1995; Neel *et al.*, 2001). Our general understanding of how plant mating system parameters are associated with plant-pollinator systems is still very limited (Section 1.6).

Advances in molecular techniques (e.g. the development of highly variable genetic markers, such as microsatellites) have allowed a more comprehensive examination of plant mating and pollination systems, via more accurate estimates of outcrossing rates, selfing rates, and paternity assignment. In recent years, studies combining field based experiments (e.g. floral production) and molecular markers (e.g. microsatellites) have become more common (e.g. Barrett and Harder, 1996; Klinkhamer and van der Veen-van Wijk, 1999; Richardson *et al.*, 2000; Gaudet and Till-Bottraud, 2003). For example, some studies have explored the relationships between floral display size and rates of selfing (e.g. de Jong *et al.*, 1999; Schmidt-Adam *et al.*, 2000; Cascante *et al.*, 2002). Of the genetic markers available, microsatellites are arguably the most powerful for these types of studies because they display a high level of polymorphism, are codominant, and can be scored consistently and unambiguously (Queller *et al.*, 1993; Barrett and Harder, 1996; van Oosterhout *et al.*, 2004). Microsatellites may also be used to quantify realised patterns of gene flow by accurately assigning paternity to offspring (e.g. Dow and Ashley, 1998; Gerber *et al.*, 2000; Roberts, 2001). Ultimately,

molecular studies, combined with paternity analysis and field-based studies on plant-pollinator systems, should provide us with a better understanding of plant mating and pollination systems and allow us to determine the individuals within populations with the greatest reproductive fitness.

### 5.1.2 Study Predictions and Aims

For many Australian self-compatible, hermaphroditic, bird-pollinated species that are now frequently visited by the introduced honeybees, it is difficult to predict the relative proportion of outcrossed and selfed offspring. It is especially difficult to make these predictions considering that honeybees and honeyeaters have consistently been reported to have substantially different foraging behaviour, and therefore, different effects on reproductive success (discussed in Chapter 3 - Vaughton, 1996; Roberts, 2001; Beynon *et al.*, unpublished). A simple prediction may be that increased attraction to pollinators will increase the frequency of visits, effective pollen transfer, and outcrossed seed. However, increased visits by effective pollinators may also increase self-pollen transfer within a plant, increasing the production of selfed seed and decreasing outcrossing rates. In the case of *G. macleayana*, we may expect honeyeaters to facilitate the production of some outcrossed and selfed seed, but honeybees just to provide for selfed seed or to deplete nectar resources, without effective pollen transfer (Taylor and Whelan, 1988; Vaughton, 1992; Gross and Mackay, 1998; England *et al.*, 2001; Celebrezze and Paton, 2004).

Previous studies using *G. macleayana* plants have reported mixed results with respect to variation in fitness between selfed and outcrossed progeny. Harriss and Whelan (1993) reported that pollen tubes developed from outcrossed hand-pollinations were significantly longer than selfed pollen tubes. Furthermore, percentage seed set per inflorescence was significantly greater for outcrossed compared with selfed flowers. However, Vaughton (1995) reported no significant difference in fruit initiation, maturation or seed weight between selfed and outcrossed treatments. Given the very high levels of selfing consistently reported within populations of this species (e.g. Ayre *et al.*, 1994; England *et al.*, 2001), there may be no significant reproductive disadvantage from selfing. In fact, a stable predominantly self-fertilising mating system, with locally adapted genotypes and limited deleterious alleles, may exist (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Young *et al.*, 1996).

I used *G. macleayana* outcrossing rates as a conservative surrogate of plant fitness and correlated these with floral traits (e.g. inflorescence and nectar production), pollinator foraging behaviour, and reproductive success (i.e. seed number) to determine whether increased floral attraction may have fitness benefits for individual plants. To my knowledge, this is the first study to assess the relationship between the outcrossing rates of individual maternal plants with multiple measures of floral traits, pollinator activity and reproductive success.

In this chapter of my thesis I quantified variation among plants in outcrossing rates, in order to answer the following questions:

- (1) How do plants vary with respect to rates of outcrossing, biparental inbreeding and correlation of paternity?
- (2) How are outcrossing and biparental inbreeding rates associated with floral traits (i.e. inflorescence and nectar production), floral visitor foraging behaviour (i.e. honeybees and honeyeaters) and reproductive success (i.e. seed number)?

## **5.2 Methods**

### **5.2.1 Seed Collection and DNA Extraction**

To determine whether there was variation among plants in outcrossing rates, I examined the genetic composition of a total of 199 seeds collected from eight plants at Greenfields Beach (GB). To allow reliable estimation of the outcrossing rate of each individual plant, I attempted to collect more than 30 seeds per plant (Ritland and Jain, 1981) from the eight plants over a five-month period, between November 2002 and March 2003. I collected between 33 and 62 seeds for six of the eight plants, with the remaining two plants providing only 20 seeds (total collected seeds = 347).

To extract DNA, I used the CTAB (Hexadecyl Trimethyl Ammonium Bromide) extraction protocol described by Doyle and Doyle (1987), modified by the addition of 1% polyvinylpyrrolidone to the extraction buffer (Roberts *et al.*, 2006). I removed seed from their seed coat to avoid contamination by maternal tissue, ground them to a smooth paste before adding extraction buffer, and then ground further to ensure the extraction buffer was mixed thoroughly. For each extraction I tested for the presence of DNA (using electrophoresis on 0.8% agarose gels, staining with ethidium bromide, and

visualisation in UV light) and estimated its crude concentration compared with a known concentration of high molecular weight Aradopsis or Tomato DNA.

The low seed production of plants at Illowra Lane (7 to 42 seed per plant) and Chinamans Beach (0 to 25 seed per plant), over the five-month period, precluded these sites from this component of my study. All *G. macleayana* adult plants at GB ( $n = 51$ ), including the eight from which seeds were collected, had previously been genotyped at ten loci (Roberts, 2001; Roberts *et al.*, 2006) and no further DNA extraction or genetic analysis was required for these eight plants.

### 5.2.2 Microsatellites, Polymerase Chain Reaction and Genotyping

I surveyed variation at six microsatellite loci (*Gm10*, *13*, *25*, *37*, *Gi7*, *9*), using four primers developed for *G. macleayana* (England *et al.*, 1999) and two developed for *G. iaspicula* (Hoebee, 2003) (Table 5.1 and 5.2). These loci were surveyed because they were the most variable of the ten examined in adult plants at all three sites, and therefore, likely to provide the most power in estimating outcrossing rates (Roberts, 2001). Using a multilocus approach minimises the frequency of undetected outcrosses, especially when loci are highly polymorphic.

I conducted the PCR reactions in polypropylene micro-titre trays (Bresatec, Australia) on an MJR Thermal Cycler PTC100 (MJR Research), using the PCR conditions described in England *et al.* (1999): 5 min at 94°C, followed by 30 cycles of 30 s at 94°C (denaturation), 30 s at 55°C (annealing), 1 min at 72°C (extension) and 5 min at 72°C. I used one unit of *Taq* polymerase (Promega) in each 19 µL reaction and the manufacturer supplied the buffer. The reactions were 2.5 mM for  $Mg^{2+}$ , 200 µM of each dNTP, 250 nM for fluorescent dCTP (Perkin Elmer), 10 pmol of each primer and approximately 20 ng of template DNA. To verify the absence of foreign DNA, I included a control reaction with each set of reactions, comprising all PCR components except the template DNA. I did not alter conditions for multiplex reactions.

**Table 5.1 - Microsatellite loci used to genotype 199 seed from eight *Grevillea macleayana* plants, at Greenfields Beach.**

For each locus the following information is shown: the *Grevillea* species for which the microsatellite loci were specifically developed; the repeat motif of the microsatellite region; allele size range (base pairs) and the primer sequence.

Locus	<i>Grevillea</i> Species	Repeat Motif	Allele Size Range	Primer Sequence 5' - 3'
<b>Gm10</b>	<i>Grevillea macleayana</i>	(CT) <sub>21</sub>	144 - 162	CATGTGTGTGCCACATTTCA- TCCACCAAGCTCCCTACAAC
<b>Gm13</b>	<i>Grevillea macleayana</i>	(CT) <sub>12</sub>	138 - 149	CAGACACTGCAAACGAATGG- ACCAGGGAATGTAACCGAAA
<b>Gm25</b>	<i>Grevillea macleayana</i>	(CT) <sub>15</sub>	234 - 264	CGAAACGAGGGAAAATCAAA- GTCCGTCATGTGTGAAAACG
<b>Gm37</b>	<i>Grevillea macleayana</i>	(CT) <sub>8</sub>	133 - 135	TTTGCTGAAAGTCCCCATTC- GTTGTCAAACCCTGCCACTT
<b>Gi7</b>	<i>Grevillea iaspicula</i>	(TG) <sub>2</sub> TA(TG) <sub>7</sub>	220 - 230	TCAACCTCTCTCCCTCTCAC- CCTCCCAACCCATACATAC
<b>Gi9</b>	<i>Grevillea iaspicula</i>	(GA) <sub>12</sub>	185 - 213	GACAAAACCTTCCCAACC- TCCATAATCGCATCTTCC

**Table 5.2 - The allele frequencies for *Grevillea macleayana* plants at six loci (Gm10, 13, 25, 37 and Gi7, 9), estimated from 51 adult plants (Roberts *et al.*, in press).**

I visualised PCR products on a Gel-scan 2000 (Corbett Research), after electrophoresis on 5% denaturing polyacrylamide gels. I sized alleles with reference to the TAMRA-500 (ABI) size standard using the software One Dscan (Scanalytics). To assess whether seeds were likely outcrossed or selfed, I examined the multilocus genotype of each individual seed, and compared them to their maternal parent's genotype. A seed was classified as outcrossed if it carried an allele not possessed by its maternal parent. I then calculated the percentage of outcrossed seed per plant by dividing the number of detectably outcrossed seed by the total number of seed per plant.

### 5.2.3 Outcrossing Rates

To determine whether there was significant variation among plants in family outcrossing rates, I calculated the number of detectable outcrosses per family (as described above). I also used Ritland's Multilocus Estimation program, MLTR (Version 3.0) (Ritland and Jain, 1981; Ritland, 2002) to estimate family outcrossing rates. MLTR should generate a more accurate estimate of outcrossing than counting detectably outcrossed seed, because a proportion of apparently self-fertilised seed (from both homozygous and heterozygous maternal plants) are expected to result from outcrossing with genetically identical individuals (i.e. outcrossing between plants with common alleles)

I used the MLTR program (based on the mixed mating model of Ritland and Jain, 1981 and the correlated matings model of Ritland, 1989) to estimate three mating system parameters: (1) family outcrossing rates, calculated as the multilocus outcrossing rate ( $t_m$ ) and the singlelocus outcrossing rate ( $t_s$ ) (using the Newton-Raphson method); (2) biparental inbreeding rates, calculated as the difference between  $t_m$  and  $t_s$  (Shaw *et al.*, 1980; Ritland and Jain, 1981); and (3) multilocus correlation of outcrossed paternity ( $r_p$ ), which estimates shared paternity among family members (Ritland, 1989; 2002). High levels of random mating (outcrossing) produce  $t_m$  and  $t_s$  values close to one, and high levels of inbreeding produce values close to zero. High levels of biparental inbreeding (calculated as  $t_m - t_s$ ) produce values close to one. For measures of correlated paternity, progeny more closely related than indicated by random mating produce value close to one. Standard deviations were determined using 1000 bootstraps across progeny arrays, and resampling within families. MLTR does not directly test for significant variation among families. However, I determined that means were

significantly different if they were separated by more than  $1.96 \times$  the sum of the standard deviations (Zar, 1984).

#### **5.2.4 Outcrossing Rates, Seed Production, Floral Visitors, and Floral Traits**

I used multiple regression analyses to test for significant relationships between family singlelocus and multilocus outcrossing rates and biparental inbreeding (tested separately) and three floral traits. The three floral traits used were: mean inflorescence size (flowers per inflorescence recorded in January 2003); mean inflorescence nectar volume (total  $\mu\text{L}$  over two days, in January 2003); and mean nectar sugar concentration (mean percent sugar over two days, in January 2003). I used simple linear regressions to test for significant relationships between family singlelocus and multilocus outcrossing rates and biparental inbreeding (tested separately) and both inflorescence and seed number (total per plant between November 2002 and April 2003). I also used multiple regression analyses to test for significant relationships between the singlelocus and multilocus outcrossing rate and biparental inbreeding (tested separately) against the three measures of honeybee and honeyeater foraging behaviour. The three measures of honeybee and honeyeater foraging behaviour tested were the: mean number of honeybees or honeyeaters per plant; mean cumulative number of inflorescences visited by consecutive honeybees or honeyeaters during a single survey period; and mean cumulative honeybee or honeyeater foraging time per plant.

#### **5.2.5 Mendelian Inheritance and Null Alleles**

Interpretation of genetic data generated using microsatellite markers assumes Mendelian inheritance (Ardren *et al.*, 1999). This is rarely investigated, but knowledge of equal allele segregation and independent assortment of alleles at different loci is needed for reliable interpretation of genetic data. Whilst it is a relatively weak test, in eight of the eleven cases where parent plants were heterozygotes for particular loci, the allele frequencies of the self-fertilised seed did not deviate significantly from expectations for Mendelian inheritance (Table 5.3). In two of the three cases where the proportion of genotypes among seed deviated from Mendelian expectations, there was only marginal significance.

Null alleles (non-amplifying alleles) can be the result of substitutions, insertions or deletions within priming sites at microsatellite loci, and may result in incorrect



interpretation of genetic data (Pemberton *et al.*, 1995; Ardren, *et al.*, 1999). If null alleles are present but not accounted for, a heterozygote bearing a null allele may be incorrectly scored as a homozygote, potentially resulting in a deficit in the number of inferred heterozygotes (Pemberton *et al.*, 1995; Ardren, *et al.*, 1999).

I examined the seeds of adult plants that were homozygotes for particular loci and checked that all seeds displayed at least one of the alleles that the maternal plant displayed. I found no evidence of the presence of null alleles in any of the genotyped seeds. These results support previous work conducted on *G. macleayana* by Ayre *et al.* (*unpublished*), involving flowers hand-pollinated with self and outcrossed pollen. This study found that seeds did not differ significantly from the expectations of Mendelian inheritance in 18 of 20 cases, and there was no evidence of the presence of null alleles.

**Table 5.3 - Chi-square test for deviation from Mendelian Inheritance.**

The parental genotype and offspring allele frequencies for all loci where the parent was a heterozygote (the number in brackets is the number of observed alleles). The Chi-square ( $\chi^2$ ) analysis tests for deviation from Mendelian inheritance (CV = 3.84; df = 1).

Plant	Locus	Parental Genotype	Offspring Allele Frequency	$\chi^2$ Value
Plant 1	<i>Gm10</i>	156/158	156/156 (14):156/158 (8):158/158 (6)	9.71**
Plant 1	<i>Gm25</i>	234/244	234/234 (4):234/244 (16):244/244 (8)	1.72
Plant 2	<i>Gm13</i>	138/142	138/138 (9):138/142 (12):142/142 (4)	2.03
Plant 3	<i>Gm10</i>	156/158	156/156 (7):156/158 (14):158/158 (5)	0.46
Plant 3	<i>Gm25</i>	244/252	244/244 (8):244/252 (12):252/252 (7)	0.41
Plant 5	<i>Gm25</i>	244/256	244/244 (3):244/256 (11):256/256 (5)	1.10
Plant 7	<i>Gm10</i>	156/158	156/156 (5):156/158 (12):158/158 (11)	3.15
Plant 10	<i>Gm25</i>	244/254	244/244 (1):244/254 (9):254/254 (4)	2.43
Plant 10	<i>Gi9</i>	189/191	189/189 (0):189/191 (8):191/191 (3)	3.91*
Plant N7	<i>Gm37</i>	133/135	133/133 (7):133/135 (7):135/135 (7)	2.33
Plant N7	<i>Gi9</i>	185/189	185/185 (8):185/189 (5):189/189 (7)	4.4*

\* =  $0.05 > P > 0.01$

\*\* =  $P < 0.005$

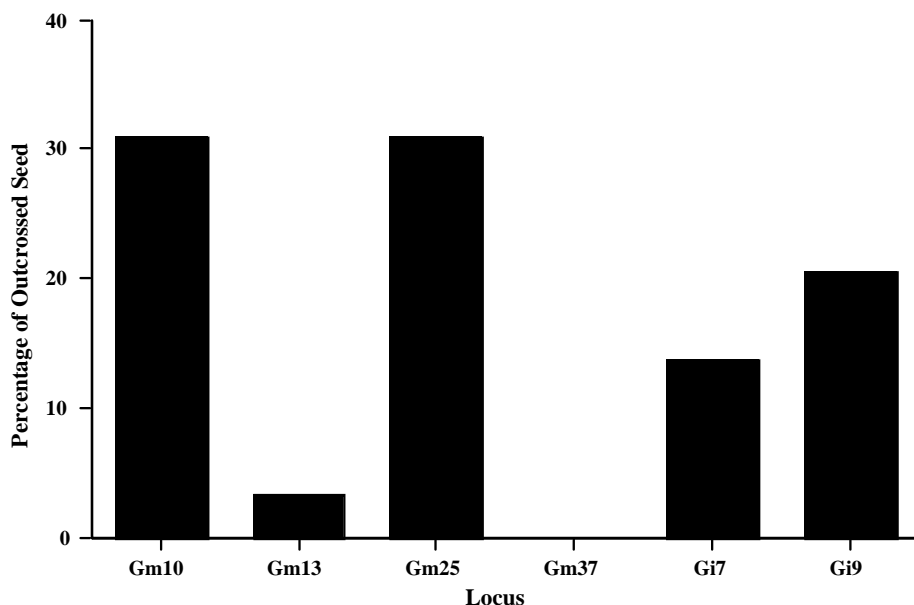
### 5.3 Results

#### 5.3.1 Variation Among Plants in Family Outcrossing Estimates

I detected a total of 18 alleles across the six microsatellite loci, from the survey of 199 seeds. The number of alleles per locus ranged from two to three alleles for all loci, except for *Gm25*, which had six alleles. Seed from each of the eight plants displayed variation for between two and four of the loci, except for Plant 4, which displayed no variation at any of the loci (all seed were homozygous for all loci). The number of detectably outcrossed seed per locus ranged from zero for *Gm37*, to nine (31%) for each of *Gm10* and *Gm25* (Figure 5.1). Of the 199 seed genotyped, 26 seed were detectably outcrossed. The number of detectably outcrossed seed per plant ranged from zero for Plant 4, to seven (27%) for Plant 5 (Figure 5.2).

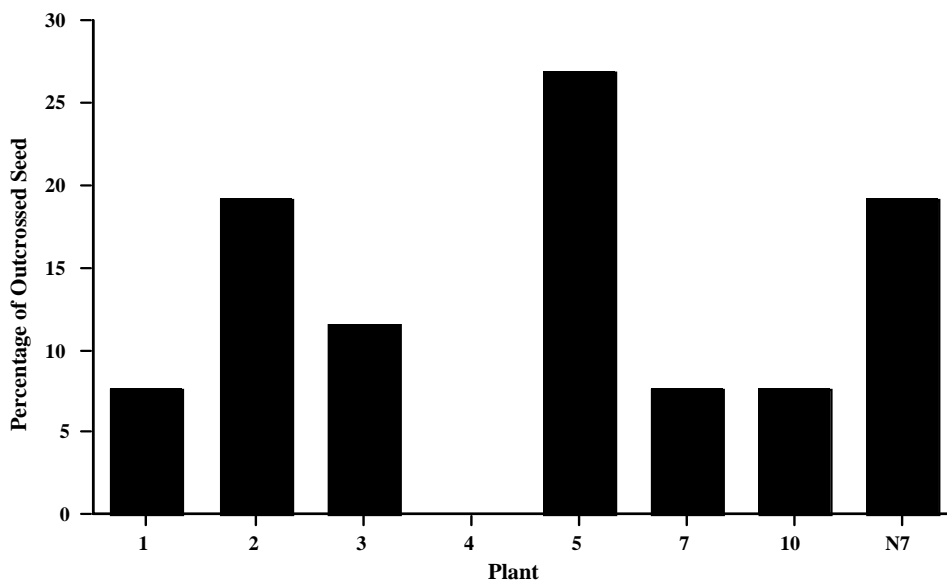
All multilocus and singlelocus outcrossing rates were very low for all families, with a mean ( $\pm$ s.d.) of 0.15 ( $\pm$ 0.09) and 0.10 ( $\pm$ 0.05), respectively. However, estimates of multilocus outcrossing ranged from 0.00 ( $\pm$ 0.0) for Plant 4 to 0.27 ( $\pm$ 0.09) for Plant 5 (Table 5.4). I found Plants 2, 5 and N7 had multilocus outcrossing rates significantly different from zero. Despite estimates for the remaining five plants not being significantly different to zero, some outcrossing was occurring, with the percentage of detectably outcrossed seed ranging from zero in Plant 4, to 11.5% in Plant 3.

Singlelocus outcrossing rates ranged from 0.00 ( $\pm$ 0.00) for Plant 4, to 0.16 ( $\pm$ 0.08) for Plant 2 (Table 5.4). I found Plant 4 had significantly lower multilocus and singlelocus outcrossing rates than Plants 2, 5 and N7.



**Figure 5.1 - The percentage of outcrossed seed for microsatellite loci *Gm10*, *13*, *25*, *37* and *Gi7* and *9*.**

Outcrossed seeds scored from 199 *Grevillea macleayana* seeds, collected from eight plants at Greenfields Beach, between November 2002 and March 2003.



**Figure 5.2 - The percentage of outcrossed seed for each of eight *Grevillea macleayana* plants at Greenfields Beach.**

Seed were genotyped using six microsatellite loci (*Gm10*, *13*, *25*, *37*, and *Gi7* and *9*) and collected between November 2002 and March 2003. Plants are displayed in order of identification code.

**Table 5.4 - Estimated multilocus ( $t_m$ ) and singlelocus ( $t_s$ ) outcrossing rates ( $\pm$  s.d.), and biparental inbreeding ( $t_m-t_s$ ) rates ( $\pm$  s.d.) for *Grevillea macleayana* plants.** Outcrossing and inbreeding estimates generated using MLTR (Ritland and Jain, 1981; Ritland, 2002) from the seed of eight *Grevillea macleayana* plants at Greenfields Beach. Multilocus ( $t_m$ ) estimates of outcrossing rate are estimated from six loci (*Gm10*, *13*, *25*, *37*, *Gi7*, *9*).

Plant	Seed No.	Multilocus Outcrossing Rate	Singlelocus Outcrossing Rate	Biparental Inbreeding
1	30	0.10 (0.07)	0.07 (0.05)	0.03 (0.02)
2	30	0.23 (0.10)	0.16 (0.08)	0.07 (0.03)
3	30	0.15 (0.09)	0.11 (0.06)	0.04 (0.02)
4	10	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)
5	27	0.27 (0.09)	0.13 (0.05)	0.15 (0.05)
7	30	0.10 (0.07)	0.07 (0.050)	0.03 (0.02)
10	16	0.17 (0.11)	0.12 (0.08)	0.05 (0.03)
N7	26	0.19 (0.08)	0.13 (0.06)	0.06 (0.02)

Estimates of biparental inbreeding generated by MLTR were also very low, but variable, with an overall mean ( $\pm$ s.d.) of 0.05 ( $\pm$ 0.04). Rates of biparental inbreeding ranged from 0.0 ( $\pm$ 0.0) in Plant 4, to 0.15 ( $\pm$ 0.05) in Plant 5 (Table 5.4). MLTR generated estimates of multilocus correlation of paternity were also highly variable, with a mean ( $\pm$ s.d.) of 0.45 ( $\pm$ 0.49). Estimates of multilocus correlation of paternity ranged from 0.0 in Plants 3, 10 and 7 to 0.99 in Plants 1, 4 and 5. Given the very low number of outcrossed seed per plant and the high level of selfing within these plants, I would expect the correlation of paternity to be high for all plants (indicating only a few paternal parents). Therefore, the results for the plants with low correlation of paternity values are being treated with caution, and consequently no statistical analysis was performed.

### 5.3.2 Outcrossing Rates, Seed Production, Floral Visitors, and Floral Traits

The results of the three multiple regression analyses between floral traits (inflorescence size, nectar volume and nectar sugar concentration) and the singlelocus outcrossing rate, multilocus outcrossing rate, and biparental inbreeding rate were not significant and explained just 26%, 25%, and 38% (respectively) of the variation among plants (Appendix 3, Table A3.3). However, the whole-model tests were consistently positive

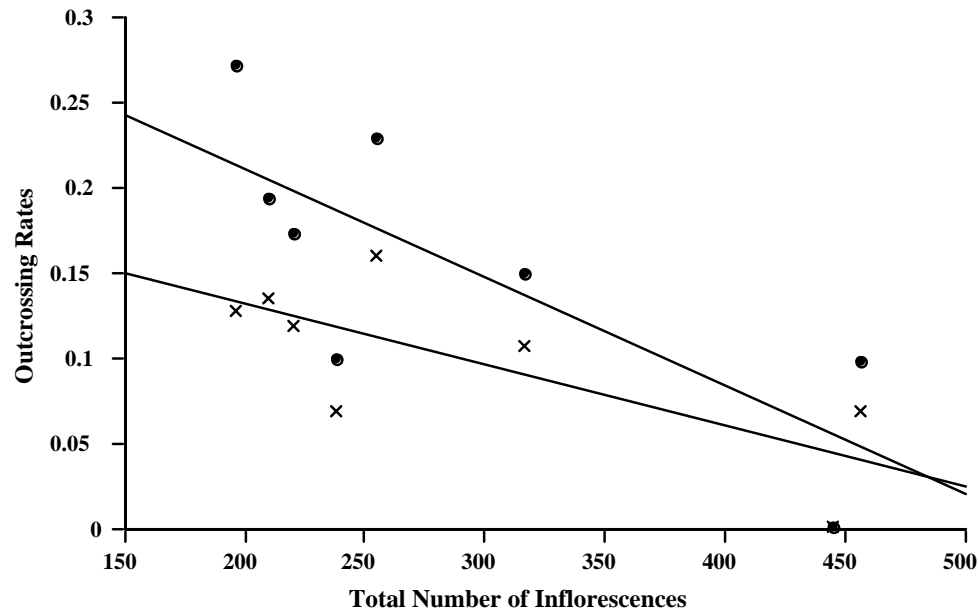
and the independent variables of inflorescence size and nectar sugar concentration displayed non-significant positive trends in all three tests.

Tests between inflorescence numbers and both the singlelocus and multilocus outcrossing rate per plant revealed significant negative relationships ( $r^2 = 0.56$ ;  $df = 1, 6$ ;  $P = 0.03$ ;  $r^2 = 0.60$ ;  $df = 1, 6$ ;  $P = 0.02$ , respectively) and explained 56% and 60% of the variation among plants, respectively (Figure 5.3; Appendix 3, Table A3.3). There was a non-significant negative trend between inflorescence number and biparental inbreeding rates. There were no significant relationships between the total number of seeds produced per plant and estimates of outcrossing or biparental inbreeding (Appendix 3, Table A3.3). Although, seed production had a non-significant positive trend with multilocus outcrossing rates and biparental inbreeding.

The multiple regressions between the multilocus and singlelocus outcrossing rates and the three measures of honeybee foraging behaviour were not significant, explaining up to 51% of the variation among plants (Appendix 3, Table A3.3). However, the cumulative number of inflorescences visited by honeybees (per survey period) and the cumulative honeybee foraging time (per survey period) displayed non-significant negative trends with both singlelocus and multilocus outcrossing rates (Appendix 3, Table A3.3). There were no consistent trends between outcrossing rates and the number of honeybees per plant (Appendix 3, Table A3.3). The multiple regression between biparental inbreeding and honeybee activity displayed (non-significant) negative trends with all three measures of honeybee behaviour (Appendix 3, Table A3.3).

The multiple regressions between the singlelocus and multilocus outcrossing rates and three measures of honeyeater foraging behaviour were not significant, explaining very little of the variation among plants (Appendix 3, Table A3.3). However, the whole-model trends were positive in both tests, as were the independent variables of honeyeater numbers per survey period per plant and cumulative honeyeater foraging time per survey period per plant. There were no consistent trends between outcrossing rates and the cumulative number of inflorescences visited by honeyeaters per survey period per plant (Appendix 3, Table A3.3). The multiple regression between biparental inbreeding and honeyeater behaviour explained very little of the variation among plants

and did not reveal consistent trends with the three measures of honeyeater behaviour (Appendix 3, Table A3.3).



**Figure 5.3 - The relationship between the total number of inflorescences and the MLTR (Ritland and Jain, 1981; Ritland, 2002) generated multilocus and singlelocus outcrossing estimate, for eight *Grevillea macleayana* plants, at Greenfields Beach.**

Total inflorescence number per plant was calculated from monthly records between November 2002 and April 2003. Family outcrossing rates were generated using six microsatellite loci (*Gm10*, *13*, *25*, *37* and *Gi7* and *9*). Both the multilocus (circles, top line) and singlelocus (crosses, bottom line) outcrossing rates were significantly negatively related to inflorescence number ( $r^2 = 0.60$ ;  $df = 1, 6$ ;  $P = 0.02$ ,  $r^2 = 0.56$ ;  $df = 1, 6$ ;  $P = 0.03$ ).

#### 5.4 Discussion

My analysis of seed genotypes revealed consistently very low family outcrossing rates. However, I detected significant variation among plants in both multilocus and singlelocus estimates of outcrossing and several plants had outcrossing rates that were significantly different from zero. Strikingly, I found that multilocus outcrossing rates were significantly negatively correlated with inflorescence number per plant. This may indicate that plants with more inflorescences receive more honeyeater visits, resulting in greater within-plant pollen movement and geitonogamous pollen transfer, thereby reducing outcrossing rates.

In the following sections, I discuss the observed variation among plants in family outcrossing rates. I will also discuss the observed patterns between measures of outcrossing and biparental inbreeding and seed production (Chapter 4), floral visitor foraging behaviour (Chapter 3) and floral traits (Chapter 2).

#### 5.4.1 Variation Among Plants in Family Outcrossing Rates

The very low outcrossing and biparental inbreeding rates estimated for *Grevillea macleayana* plants in this study are consistent with the results of previous studies, which detected very low (but occasionally highly variable) outcrossing rates at the population level (Ayre *et al.*, 1994; England *et al.*, 2001; Roberts, 2001; England *et al.*, 2003). Whilst the level of selfing detected in *G. macleayana* plants is unusually high for a long-lived species (Barrett *et al.*, 1996), we know that plants have large numbers of inflorescences with male- and female-stage flowers open at the same time, therefore, geitonogamy may be common and limiting to high outcrossing rates (Vaughton, 1996). Overall, the low outcrossing rates indicate that whilst this is a mixed mating system, these individuals are predominantly self-fertilised.

The outcrossing rates of some plants were both significantly different from zero and from each other. There are several possible reasons for variation in outcrossing rates, including heritable variation among plants in the level of self compatibility (Kahler *et al.*, 1975) and genetic structuring within the population (Hamrick, 1982). Variation among individual plants in outcrossing rates may be due to variation in the amount of outcrossed pollen received and the ability of plants to discriminate between outcrossed and self-pollen. Few other studies have found such substantial variation among plants within a population, in family outcrossing or self-fertilising rates (e.g. Humphreys and Gale, 1974; Brown *et al.*, 1975; Murawski *et al.* 1994; Isagi, *et al.*, 2004).

The biparental inbreeding rates for all plants were very low, suggesting low levels of pollen transfer between related plants. Four of the eight study plants (Plants 2, 4, 7, and 10) were located very close to other *G. macleayana* plants (i.e. less than one metre), and it may have been reasonable to predict moderate biparental inbreeding rates due to pollen transfer between closely related near neighbours. Very fine scale genetic (less than 5 m) structuring has been detected within these and other *G. macleayana* populations using spatial autocorrelation analysis (England *et al.*, 2003; Roberts *et al.*,

*unpublished*). However, the low levels of biparental inbreeding estimated are consistent with observations that honeyeaters make some movements among neighbouring plants, but are more likely to fly among conspecifics separated by more than 5 m.

Surprisingly, estimates of multilocus correlation of paternity were highly variable, indicating that the number of paternal parents approximated infinity for some plants and were very low for others. This is most likely due to MLTR being unable to compute reliable estimates, due to the low number of outcrossed seeds. Given the very low outcrossing rates, I would have expected the correlation of paternity estimates to be close to unity for all plants (O'Connell *et al.*, 2001; Alves *et al.*, 2003).

The evolution of tolerance to selfing may indicate adaptations to geographic isolation of fragmented populations, unreliable pollinator activity or altered fire regimes (Barrett *et al.*, 1996; England *et al.*, 2002). Under such conditions, selfing provides some assurance of reproduction (Shields, 1982; Barrett *et al.*, 1996). Given the small population sizes and high level of inbreeding in *G. macleayana* populations, it is possible that deleterious alleles have long ago been eliminated and a stable, predominantly self-fertilising mating system (possibly maintained by selection) has been established (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Young *et al.*, 1996; Porcher and Lande, 2005).

#### **5.4.2 Outcrossing Rates, Floral Traits, Pollinator Activity, and Seed Number**

The results of the multiple regression analyses between outcrossing rates and floral traits (inflorescence size, nectar volume and nectar sugar concentration) did not explain a significant amount of the variation among plants. However, the consistent non-significant positive trends with inflorescence size and nectar sugar concentration suggest that further study is needed. It may be expected that plants with greater nectar and inflorescence production have greater attraction to pollinators and therefore, greater potential for outcrossed seed production. However, it is also important to note that greater nectar rewards may encourage honeyeaters to forage for longer, thereby facilitating increased within-plant pollen transfer (i.e. geitonogamy) and decreasing outcrossing rates (Klinkhamer and de Jong, 1993; Klinkhamer *et al.*, 1994).

Interestingly, the plant with the greatest multilocus outcrossing and biparental inbreeding rate (Plant 5) also had the greatest nectar production, but the lowest



inflorescence production and only moderate seed production, illustrating the complexity of these relationships.

The tests between outcrossing rates and inflorescence numbers revealed significant negative relationships. These results support previous studies on other species and suggest that highly attractive plants (i.e. with large floral displays) at low densities encourage a greater number of honeyeaters to forage among a greater number of inflorescences within plants, thereby increasing geitonogamy and decreasing pollen export (e.g. Handel, 1983; de Jong *et al.*, 1993; Klinkhamer and de Jong, 1993; de Jong *et al.*, 1999). The regressions between outcrossing and biparental inbreeding rates and seed number per plant did not detect any significant trends. This may not be a surprise given that *G. macleayana* sets seed autogamously and therefore does not rely on cross-pollen for fertilisation. Seed production may more accurately reflect plant access to resources for seed development, rather than honeyeater foraging activity and outcrossing rates (Zimmerman, 1988).

The multiple regressions between outcrossing rates and honeybee behaviour did not detect any significant relationships, although negative non-significant trends were common. Whilst these negative trends were not significant, tests such as these revealing significant patterns would not be a surprise given that the large numbers and intense activity of honeybees likely removes most of the nectar, thereby reducing plant attractiveness to honeyeaters (being the floral visitor most likely to facilitate outcrossing) and increasing the chance of autogamy and thus, selfing (Pyke, 1990; Paton, 1997). As with honeybees, multiple regression tests with honeyeaters did not detect any significant relationships (although non-significant positive trends were common). I had expected significant positive relationships between honeyeater behaviour and outcrossing rates, given the effectiveness of honeyeater pollen transfer (as previously discussed). These trends need to be examined further, especially given that outcrossing rates were significantly negatively related to inflorescence number and honeyeaters that visit more inflorescences per plant may be facilitating geitonogamous pollen movement and reduced outcrossing rates.

A very small number of studies have detected a relationship between plant floral display and/or pollinator foraging behaviour and family selfing or outcrossing rates (reviewed

for floral display in Snow *et al.*, 1996; Brunet and Eckert, 1998; Karron *et al.*, 2004; Hingston and Potts, 2005; Miyake and Sakai, 2005). However, the negative relationships I detected between inflorescence numbers and outcrossing rates are consistent with studies that found negative relationships between floral display and outcrossing rates among *Aquilegia caerulea* plants (Brunet and Eckert, 1998) and positive relationships between floral display and selfing rates among *Mimulus ringens* plants (Karron *et al.*, 2004). It is important to note that most previous studies have been conducted at the population or sub-population level and not at the level of individual plants (e.g. de Jong *et al.*, 1999; Franceschinelli and Bawa, 2000; Schmidt-Adam *et al.*, 2000; Gaudeul and Till-Bottraud, 2003). However, the findings of this study (and others conducted at the plant level) demonstrate that there are clearly significant relationships between important components of the pollination system that are likely to be missed at the population level.

Previous studies and field observations on *G. macleayana* have proposed that the foraging activity of honeybees largely removes nectar and facilitates very little effective pollen movement (Harriss and Whelan, 1993; Vaughton, 1996; England *et al.*, 2001; Lloyd, S., *personal observations*). Moreover, any pollen movement that does occur is likely to be within a plant, therefore, facilitating geitonogamy (de Jong *et al.*, 1993). It has also been proposed that the foraging behaviour of honeybees is at such a high level that honeyeater foraging behaviour has an insignificant impact on the outcrossing rate of plants (England *et al.*, 2001). However, seed production and outcrossing rates have both been significantly reduced by excluding honeyeaters from *G. macleayana* plants, and therefore, honeyeaters are considered important in maintaining some level of outcrossed pollination within and between populations (Vaughton 1996; England *et al.*, 2001). Population genetic evidence from previous studies suggests that selfing has occurred in populations in the past (Ayre *et al.*, 1994; England *et al.*, 2002; England *et al.*, 2003), and long-distance gene flow is likely to have been limited historically (England *et al.*, 2002). This evidence may support the idea that that some *G. macleayana* populations have established a stable, predominantly self-fertilising mating system, in which honeyeaters provide the minimal level of outcrossing detected. This mating system may be unexpected given that few woody plants are reported with high selfing rates, due to a longer life expectancy increasing genetic load and inbreeding depression (Barrett *et al.*, 1996).

### 5.4.3 Non-reproductive Plant Traits and Environmental Variables

Plant pollination systems are influenced by numerous factors outside of the immediately obvious components of floral traits, pollinator foraging behaviour, reproductive success and mating systems. Traits such as plant size and density, environmental conditions, photosynthetic rate and canopy cover all play a potentially important role in the pollination of many plant species (Handel, 1983; Mohan Ram and Rao, 1984; de Jong *et al.*, 1993; Vaughton and Ramsey, 1997; Setter *et al.* 2001; Engel and Irwin, 2003). In Chapter 6, I quantify variation among plants in measures of plant size, distance to nearest conspecific, canopy cover and leaf health. I also test for significant relationships between these measures and both floral traits (i.e. inflorescence production) and reproductive success (i.e. seed production).

## Chapter 6 - Non-reproductive Plant Traits and Environmental Variables

### 6.1 Introduction

#### 6.1.1 Non-reproductive Plant Traits, Environmental Variables and Pollination

Variation in reproductive success is not only a reflection of pollinators, resources and heritable variation, but also phenotypic plasticity in response to external influences, such as the climatic and microhabitat conditions present within a population. Many non-reproductive plant traits and environmental variables are known to significantly affect reproductive success and other important components of pollination systems, such as the production of floral traits, pollinator foraging activity, and rates of outcrossing and selfing (see Section 1.7). These variables include (but are not limited to): plants size; plant density; nearest neighbour distances; environmental and climatic variables; and photosynthetic processes (Handel, 1983; Mohan Ram and Rao, 1984; Corbet, 1990; Rathcke, 1992; Galen *et al.*, 1999). However, determining the potential impacts of changes in individual variables on plant-pollination systems is very complicated, given that many variables are inter-related (Corbet, 1990; Primack and Inouye, 1993). For example, high light availability is usually associated with higher temperatures (Primack and Inouye, 1993).

Based on my observations in the field, I already know that there is substantial variation among plants in size and distance to nearest conspecific. It is also likely that variables such as canopy cover, water availability and photosynthetic rate vary substantially among plants. This variation may have important consequences for plant attraction, pollinator activity and plant reproductive success. To understand how this intraspecific variation may affect plant attraction and reproductive success, variation in non-reproductive plant traits and environmental variables needs to be quantified and tested with the results of previous chapters.

#### 6.1.2 Study Predictions and Aims

In Chapter 1, I identified what I believe to be the five key components of plant-pollination systems: (1) floral traits; (2) pollinator foraging activity; (3) reproductive success; (4) plant mating systems and fitness; and (5) other non-reproductive plant traits and environmental variables (e.g. plant size and climatic conditions). In Chapters 2 to 5, I detected significant variation among plants in each of these components, with the

exception of plant fitness, which was not directly tested. Furthermore, some of the variation among plants was explained by relationships between floral traits, floral visitor behaviour, reproductive success and/or plant mating system parameters. It is likely that there is substantial variation among plants in many other non-reproductive plant traits and environmental variables (as described above). The variation in these variables, when tested with variation among plants in other components of the pollination system, is likely to have important consequences for plant reproductive success and fitness.

There are many plant morphological, physiological and distributional (e.g. density) characteristics, as well as environmental and climatic variables, that are relevant to plant-pollination systems and can be measured. I wanted to include examples of plant size, density, and environmental conditions, as I believe the literature outlines some of these as the most important for pollination systems (see Section 1.7). Due to time constraints, I limited my measurements to: plant height and area (as measures of size); distance to nearest conspecific (as a measure of density); percent canopy cover (as an indication of shade), leaf moisture (as an indication of plant moisture availability) and leaf photosynthetic rate. This is one of the few studies to examine such a variety of morphological, density, environmental, and physiological variables, in the context of previously quantified variation in the key components of a pollination system.

Based on the literature presented in this Chapter and Chapter 1, I have made several predictions about the likely variation among *G. macleayana* plants in the some measures outlined above:

- (1) Plants will exist either in small clumps (an indication of a soil stored seed bank) or as individuals (which may indicate local soil movement).  
*Grevillea macleayana* has a soil stored seed bank, no reported facilitated mode of seed dispersal, and seed germination may be triggered by soil disturbance (Appendix 1).
- (2) Plants will vary significantly in leaf moisture, due to likely variation in local water resource availability.
- (3) Positive relationships will exist between leaf moisture content and plant size (indicating that larger plants have greater access to water resources).  
Negative relationships will exist between: (1) leaf moisture content and distance to nearest conspecific (indicating that plants closer together may be

competing for water resources) and (2) leaf photosynthetic rate and canopy cover (indicating that plants exposed to more light have greater photosynthesis).

- (4) Measures of inflorescence production and reproductive success may be positively related to plant characteristics such as plant size and leaf photosynthetic rate.

Specifically, I asked the following questions:

- (1) How do plants vary with respect to size and distance to nearest conspecific?
- (2) How do plants vary with respect to percent canopy cover?
- (3) How do plants vary with respect to mean leaf photosynthetic yield and/or leaf moisture content, and are these factors related to plant size, canopy cover, and/or nearest conspecific distance?
- (4) How are measures of plant size, nearest conspecific distance, canopy cover, leaf photosynthetic rate and leaf moisture associated with reproductive success (seed number) and floral traits (inflorescence number)?

## **6.2 Methods**

### **6.2.1 Plant Size and Distance to Nearest Conspecific**

I measured plant height (cm) from the base of the plant to the top of the tallest branch. Area ( $\text{m}^2$ ), I calculated as the area of an ellipse:  $\pi * (0.5 \text{ width } 1) * (0.5 \text{ width } 2)$ . I measured the width of the plant at the widest point and measured width 2 at  $90^\circ$  to width 1. I measured distance to nearest conspecific as the shortest distance between two conspecifics. I recorded these measurements for a total of 57 plants (19 per site), between April and November 2003, with survey months dependent upon the site (Table 6.1).

**Table 6.1 - Studies quantifying variation in plant size, distance to nearest conspecific, canopy cover, and leaf health among *Grevillea macleayana* plants.**

Field and laboratory experiments conducted to quantify variation among *G. macleayana* plants in morphological characteristics (height, area and distance to nearest conspecifics), percent canopy cover, leaf photosynthetic yield, and leaf moisture at three sites in Jervis Bay National Park, between 2002 and 2003.

Experiment	Field Site		
	Chinamans Beach	Greenfields Beach	Illowra Lane
<b>Morphological Characteristics</b> Height, area, distance to nearest <i>Grevillea macleayana</i> plant	April to November 2003	April to September 2003	July to November 2003
<b>Percent Canopy Cover</b>	November 2003	November 2003	November 2003
<b>Leaf Photosynthetic Yield</b>	November 2002	November 2002	-
<b>Leaf Moisture</b>	October – November 2003	November 2003	October 2003

Leaf photosynthetic yield was not conducted at IL due to a bushfire threat.

### 6.2.2 Percent Canopy Cover

I estimated the percent canopy cover of *G. macleayana* plants using a spherical densiometer. This instrument comprises a reflective dome (radius of approximately 3 cm) with a square grid imprinted into it (Figure 6.1). By visualising four quarters to each square on the grid, I counted the number of *open* quarters (i.e. not occupied by canopy foliage) for each square on the dome. I repeated this measure facing three to four different directions per plant. Counts are converted to percentage canopy cover by multiplying the count of open quarters by 1.04, and subtracting this from 100. I generated an estimate of mean percent canopy cover per plant using the measures taken from different directions per plant. I recorded percent canopy cover on the same 57 plants used to quantify variation in plant size and distance to nearest conspecific (Section 6.2.1), in November 2003.



**Figure 6.1** - A spherical densiometer, used to measure percent canopy cover, above *Grevillea macleayana* plants (Photo: S. Lloyd).



### 6.2.3 Leaf Photosynthetic Yield and Leaf Moisture

#### 6.2.3.1 Leaf Photosynthetic Yield

To measure optimum photosynthetic activity, I used a Mini Pulse Amplitude Modulated (Mini PAM) chlorophyll fluorometer (H. Walz, Germany). This measuring system records chlorophyll fluorescence signals in the presence of actinic light, and can be used with attached or detached leaves. Dark-adapted measures of fluorescence are reported to be a reliable indicator of the maximum photon-use efficiency of photosynthesis in a range of plant taxa (Bjorkman and Demming, 1987). Moreover, this is the most appropriate way to take measurements to be used for comparisons among plants, since it is a measure of maximum or optimal efficiency of photosynthesis.

This study comprised ten plants at CB and eight plants at GB. Plants from IL were unable to be used due to a bushfire threat. I collected 15 to 20 leaves (evenly distributed) from each plant on the morning of the 26<sup>th</sup> of November 2002, and took measurements that afternoon. The leaves I collected were fully grown and were all of the same age class. This is important because photosynthetic rate is known to decline with leaf age (Thiagarajah *et al.*, 1981). I stored leaves in zip lock plastic bags and placed them on ice whilst in the field. When back in the laboratory, leaves were dark-adapted for 20 mins (under black plastic), and subsequent measures of leaves were made in the dark.

I used the Mini PAM to record three measurements: (1) fluorescence ( $F_o$ ) emitted in the dark, when photosystem II centres are open; (2) maximum fluorescence ( $F_m$ ), produced by a saturating flash ( $5000\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ ), which closes photosystem II centres; and (3) yield (optimal maximum quantum efficiency of photosystem II centres), which can be used as an estimate of plant photosynthetic health. Yield is calculated as  $(F_m - F_o) / F_m$  and represents the maximum yield of photochemical energy conversion.

Settings on the Mini PAM optimised for *G. macleayana* plants for the intensity of measuring light = 4, for the electronic signal gain = 3, for the electronic signal damping = 1.0 s, for the saturating pulse intensity = 9 and for the width of saturating pulse = 0.6s. To record these measurements using Mini PAM, I used the protocol described in the instruction manual.

### 6.2.3.2 Leaf Moisture Content

I conducted the leaf moisture survey on the same ten plants from CB and eight plants from GB, used in the photosynthesis study, and an additional seven plants from IL. I collected leaves from plants at CB, GB and IL in the morning on the 30<sup>th</sup> of October, 21<sup>st</sup> of November and 21<sup>st</sup> of October 2003, respectively. I collected between 20 and 25 leaves per plant evenly distributed on the plant. As with the photosynthesis measurements, leaves were fully grown, because leaf moisture content is known to vary with leaf age (Hurley, 2000). Leaves were placed in zip-lock plastic bags, and stored on ice whilst in the field. In the laboratory, I measured the fresh weight of each leaf. I then placed leaves in individually labelled aluminium cups on oven trays in an oven preset to 80°C. Ovens trays were rotated within and between ovens every 24 hr and reweighed daily until they reached constant weights (at 4 to 5 days). I calculated the moisture content of each leaf as a percentage difference between the fresh weight and the Day 4 or Day 5 dry weight.

### 6.2.4 Statistical Analyses

I used a one-way ANOVA to test for significant variation among plants in mean percent canopy cover at CB (Question 2). Assumptions of normality and equal variances were tested as described in Section 2.2.2.3. I used non-parametric ANOVAs (Kruskal-Wallis Test) to test for significant variation among plants in percent canopy cover at GB and IL (Question 2). I could not use a parametric ANOVA in these cases due to some heteroscedasticity of data.

I tested for significant variation among plants in both leaf photosynthetic yield and leaf moisture content using non-parametric ANOVAs (Kruskal-Wallis Test), because of non-normality and heteroscedasticity of data (Question 3). I used multiple regression analyses to test for significant relationships between measures of plant size, distance to nearest conspecific and percent canopy cover and (1) leaf photosynthetic yield, and (2) leaf moisture content (Question 3).

I used multiple regression analyses to test for consistent or significant relationships between plant height, plant area, mean canopy cover and distance to nearest conspecific and (1) inflorescence number (total over two years) and (2) seed number (total over two years). I also used multiple regressions to test for consistent or significant relationships

between plant photosynthetic yield and leaf moisture and: (1) inflorescence number (monthly record); and (2) seed number (recorded 8 weeks after the study was conducted). I restricted the dependent variables to inflorescence and seed production because inflorescence production represents both a floral trait and reward to floral visitors and seed production represents plant reproductive success.

## 6.3 Results

### 6.3.1 Variation Among Plants in Size and Nearest Conspecific Distance

#### 6.3.1.1 Plant Height

Plants at all sites varied greatly in height. I detected the greatest variation at CB, with a 5-fold difference in height between Plant N4 (73 cm) and Plant 1 (355 cm) (figures are located in Appendix 5). At GB, I detected a 3-fold difference in height between Plant 19 (107 cm) and Plant 17 (355 cm) (Appendix 5). At IL, I also detected a 3-fold difference in height between plants, ranging from Plant 9 (100 cm) to Plant 2 (290 cm) (Appendix 5). Mean plant height at CB, GB and IL was 194.5 cm ( $\pm 17.8$ ), 196.0 cm ( $\pm 16.0$ ), and 162.3 cm ( $\pm 12.0$ ), respectively.

#### 6.3.1.2 Plant Area

Plants at all sites varied more in area than in height. As for plant height, the greatest variation occurred at CB, with a 25-fold difference in area between the smallest (1.88 m<sup>2</sup> - Plant 16) and the largest (47.42 m<sup>2</sup> - Plant 1) plants (Appendix 5). At GB, I detected an 11-fold difference between Plant 7 (2.86 m<sup>2</sup>) and Plant 17 (33.09 m<sup>2</sup>). At IL, I detected just a 4-fold difference between the plant with the smallest (4.57 m<sup>2</sup> - Plant 3) and the plant with the largest (18.68 m<sup>2</sup> - Plant N2) area (Appendix 5). The mean plant area for CB, GB, and IL was 3.9 m<sup>2</sup>  $\pm 2.6$ , 16.0 m<sup>2</sup>  $\pm 2.0$ , and 9.4 m<sup>2</sup>  $\pm 1.0$ , respectively.

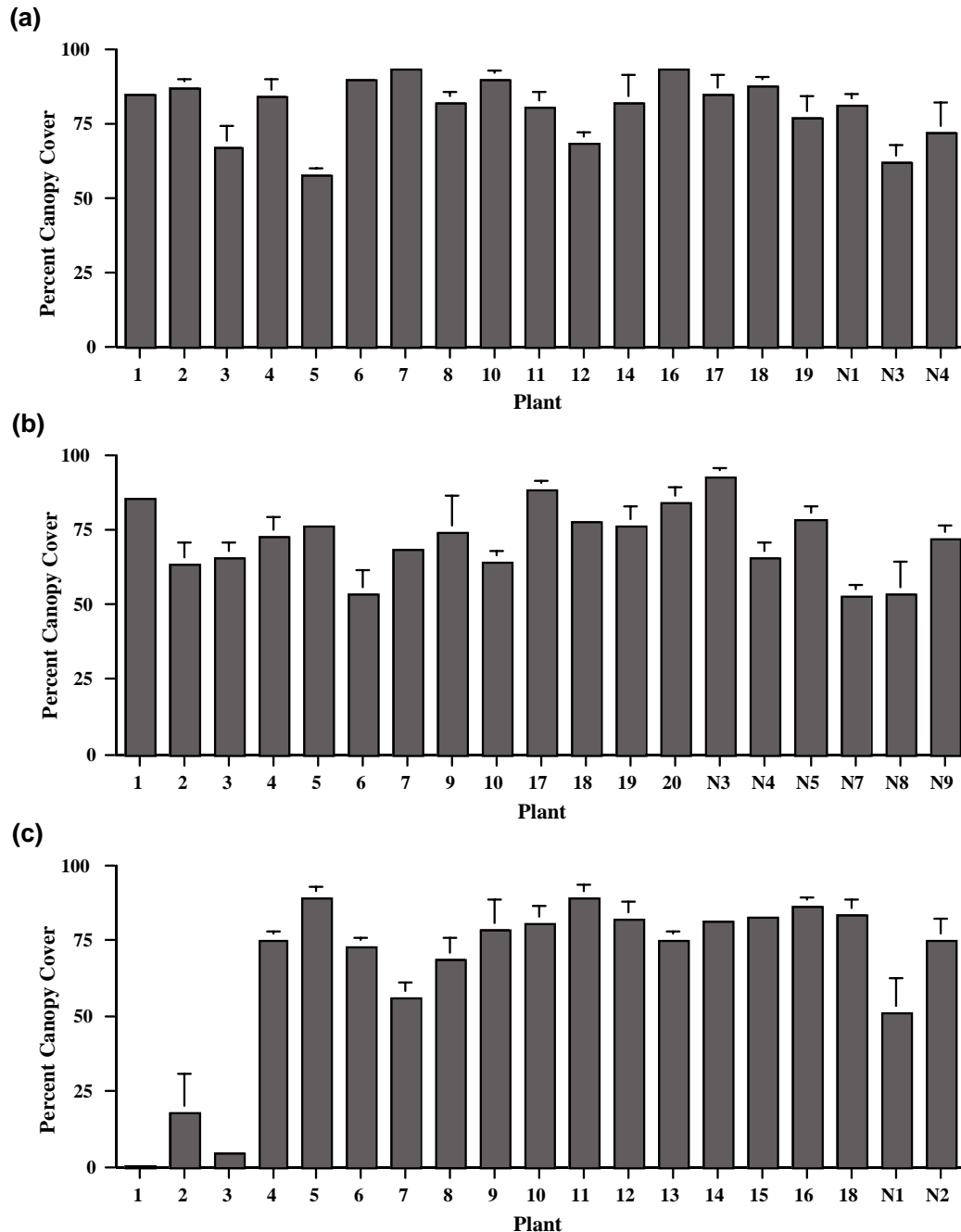
#### 6.3.1.3 Distance to Nearest Conspecific

Plants at all sites varied substantially in distance to nearest conspecific, with the greatest distances at IL. At CB, I found that the distance to the nearest conspecific ranged from zero (where plants were located in small clumps) to 777 cm (Plant 2), an individual plant (Appendix 5). At GB, 11 of the study plants were located immediately next to another *G. macleayana* plant, and Plant 18 (an individual) was located the furthest from a conspecific, at 1175 cm (Appendix 5). At IL, just three plants were located adjacent

to other *G. macleayana* plants (zero nearest conspecific distance) and Plant 9 was located the furthest from a conspecific, at 2000 cm (Appendix 5). The mean distance to the nearest conspecific at CB, GB and IL was 169.0 cm ( $\pm 39.2$ ), 147.6 cm ( $\pm 75.4$ ), and 372.4 cm ( $\pm 116.3$ ), respectively.

### 6.3.2 Variation Among Plants in Percent Canopy Cover

Moderate variation was detected among plants at all sites in mean percent canopy cover (Figure 6.2). At CB, mean percent canopy cover ranged from Plant 5 with 57.9% ( $\pm 2.2$ ) to Plant 16 with 93.1% ( $\pm 0.4$ ), and significant variation was detected among plants (ANOVA:  $F_{18} = 4.63$ ;  $P < 0.001$ ). A Tukey-Kramer HSD test revealed that four plants (Plants 7, 16, 6, and 10) had significantly greater mean percent canopy cover than up to four other plants. Eight plants were significantly greater than Plant 5. At GB, mean percent canopy cover ranged from Plant N8 with 52.5% ( $\pm 11.1$ ) to Plant N3 with 92.2% ( $\pm 3.3$ ), and significant variation was detected among plants ( $\chi^2_{19} = 43.47$ ;  $P < 0.001$ ). At IL, mean percent canopy cover ranged from Plant 1 with just 0.2% ( $\pm 0.0$ ) to Plant 11 with 89.3% ( $\pm 4.3$ ). Significant variation was detected among plants ( $\chi^2_{19} = 49.60$ ;  $P < 0.001$ ).



**Figure 6.2 - The mean percent canopy cover above *Grevillea macleayana* plants at three sites.**

Data were collected from plants in November 2003 at Chinamans Beach (CB - Figure a), Greenfields Beach (GB - Figure b), and Illowra Lane (IL - Figure c). One-way ANOVA detected significant differences among plants at CB ( $F_{18} = 4.63$ ;  $P < 0.001$ ), non-parametric ANOVAs (Kruskal-Wallis) detected significant differences among plants at GB ( $\chi^2 = 43.47$ ;  $df = 19$ ;  $P < 0.001$ ) and IL ( $\chi^2 = 49.60$ ;  $df = 19$ ;  $P < 0.001$ ). Plants are displayed in order of identification code. Bars represent plus one standard error.

### 6.3.3 Variation Among Plants in Leaf Photosynthetic Yield and Moisture Content

#### 6.3.3.1 Leaf Photosynthetic Yield

I detected slight, but significant, variation among plants at CB and GB in mean leaf photosynthetic yield (Table 6.2). At both CB and GB, I detected a difference of just 0.03 in mean leaf photosynthetic yield between the plants with the smallest measure (CB: Plant 8; GB: Plant 4) and the plants with the largest measure (CB: Plant 3; GB: Plant 5). Despite this very small amount of variation, I found significant variation among plants at both sites (CB:  $\chi^2_9 = 29.77$ ;  $P < 0.001$ ; GB: ( $\chi^2_7 = 37.51$ ;  $P < 0.001$ ). Mean leaf photosynthetic yield for plants at CB and GB was 0.82 ( $\pm 0.003$ ), 0.81 ( $\pm 0.004$ ), and 0.82 ( $\pm 0.009$ ), respectively.

**Table 6.2 - The mean photosynthetic yield of leaves collected from *Grevillea macleayana* plants at two sites.**

Data on leaf photosynthetic yield were collected from plants in November 2002 at Chinamans Beach (a) and Greenfields Beach (b). Non-parametric ANOVAs (Kruskal-Wallis) revealed significant variation among plants at CB ( $\chi^2 = 29.77$ ;  $df = 9$ ;  $P < 0.001$ ) and GB ( $\chi^2 = 37.51$ ;  $df = 7$ ;  $P < 0.001$ ). Data on mean leaf photosynthetic yield and standard error (SE) are presented.

**(a) Chinamans Beach**

Plant	Mean Yield	SE
1	0.826	0
2	0.823	0
3	0.831	0
4	0.814	0.01
5	0.816	0
8	0.803	0.01
11	0.811	0.01
12	0.816	0
19	0.815	0.01
N1	0.808	0.01

**(b) Greenfields Beach**

Plant	Mean Yield	SE
1	0.803	0.01
2	0.81	0.01
3	0.818	0.01
4	0.785	0.01
5	0.82	0
7	0.809	0.01
10	0.815	0
N7	0.81	0.01

#### 6.3.3.2 Leaf Moisture Content

I detected slight, but significant variation among plants in mean leaf moisture content, at CB and GB (Table 6.3). I found a difference of 6.8%, 2.7%, and 3.9% moisture content between the plant with the lowest and the highest mean leaf moisture content at CB, GB, and IL, respectively. This variation was significant at CB ( $\chi^2_9 = 58.23$ ;  $P < 0.001$ )

and GB ( $\chi^2_7 = 60.14$ ;  $P < 0.001$ ). No significant variation was detected among plants at IL. Measures of mean leaf moisture for plants at CB, GB and IL were 44.9% ( $\pm 0.6$ ), 51.9 ( $\pm 0.4$ ), and 47.3 ( $\pm 0.5$ ), respectively.

**Table 6.3 - The mean leaf moisture (%) of leaves collected from *Grevillea macleayana* plants at three sites.**

Data were collected from plants at Chinamans Beach (a) in October 2003, Greenfields Beach (b) in November 2003 and Illowra Lane (c) in October 2003. Non-parametric ANOVAs (Kruskal-Wallis) revealed significant variation among plants at CB ( $\chi^2 = 58.23$ ; d.f = 9;  $P < 0.001$ ) and GB ( $\chi^2 = 60.14$ ; df = 7;  $P < 0.001$ ). Data on mean leaf moisture (%) and standard error (SE) are presented.

**(a) Chinamans Beach**

Plant	Mean Leaf Moisture (%)	SE
1	46.03	2.21
2	50.22	0.51
3	49.04	0.57
4	51.04	0.41
5	48.62	0.6
8	49.47	0.35
11	52.81	0.35
12	50.8	0.42
19	50.68	0.3
N1	50.16	0.55

**(b) Greenfields Beach**

Plant	Mean Leaf Moisture (%)	SE
1	50.9	0.31
2	53.4	0.32
3	51.41	0.6
4	50.76	0.23
5	51.33	0.58
7	51.13	0.49
10	52.99	0.33
N7	53.45	0.44

**(c) Illowra Lane**

Plant	Mean Leaf Moisture (%)	SE
1	49.06	2.49
2	45.49	3.07
6	48.68	3.23
7	47.23	3.28
8	47.81	2.92
16	45.65	3.17
N2	46.81	3.06

### 6.3.4 Relationships Between Leaf Health, Plant Size, and Canopy Cover

#### 6.3.4.1 Leaf Photosynthetic Yield

The multiple regression analyses I used to explore the relationship between mean leaf photosynthetic yield and the measures of plant height, area, percent canopy cover and distance to nearest conspecific, did not detect any significant relationships. However, the whole-model trends were positive and explained up to 61% of the variation among plants (Table 6.4). I found that plant area and distance to nearest conspecific displayed (non-significant) positive trends with leaf photosynthetic yield at both sites. Mean canopy cover displayed (non-significant) negative trends with leaf photosynthetic yield at both sites. I found no consistent pattern with respect to leaf photosynthetic yield and plant height.

#### 6.3.4.2 Leaf Moisture Content

I detected significant whole-model relationships between mean leaf moisture content and the measures of plant height, area, mean percent canopy cover and distance to nearest conspecific, at IL, and marginally at CB (Table 6.5). Mean leaf moisture was significantly negatively related to plant area at CB ( $P \leq 0.01$ ). This regression explained 80% of the variation among plants and was marginally significant ( $F_{4,5} = 1.98$ ;  $P = 0.05$ ). At GB, I detected a (non-significant) negative trend between mean leaf moisture and plant area, however, a weak positive trend was detected at IL. At IL, I found that mean leaf moisture was significantly negatively related to plant height ( $P = 0.02$ ). This regression explained 99% of the variation among plants ( $F_{4,2} = 33.54$ ;  $P = 0.03$ ). At GB, I also detected a (non-significant) negative trend between mean leaf moisture and plant height, however, a (non-significant) positive trend was detected at IL. There were no significant or consistent trends with respect to mean percent canopy cover or distance to nearest conspecific (Table 6.5).



**Table 6.4 - Multiple regression analyses testing for the significance of relationships between mean leaf photosynthetic yield per *Grevillea macleayana* plant and measures of plant size, distance to nearest conspecific, canopy cover, and leaf health.**

The plant traits included in the analyses were: (1) plant height (cm); (2) area (m<sup>2</sup>); (3) mean percent canopy cover; and (4) distance to nearest conspecific (NC) (cm). Measurements were taken on ten plants at Chinamans Beach and eight plants at Greenfields Beach. Measurements of leaf photosynthetic yield were recorded in November 2002 and measurements of plant parameters were recorded between April and November 2003 (Table 6.1).

Study Site/ Parameter	$R^2$	$R^2$ Adj.	Mean Square	df (Model, Error)	F Ratio	SE*	Probability (Whole-model)	Probability (Parameter)	Trend
<b>Chinamans Beach</b>	0.61	0.30	0.00	4, 5	1.98	0.02	0.24	-	Positive
Height					0.20	0.00		0.67	Negative
Area					2.35	0.00		0.19	Positive
Mean Percent Canopy Cover					2.52	0.00		0.25	Negative
Distance to NC					1.67	0.00		0.17	Positive
<b>Greenfields Beach</b>	0.40	-0.40	0.00	4, 3	0.50	0.04	0.74	-	Positive
Height					0.34	0.00		0.60	Positive
Area					0.15	0.00		0.73	Positive
Mean Percent Canopy Cover					1.60	0.00		0.41	Negative
Distance to NC					0.93	0.00		0.29	Positive

\* SE = Standard Error

**Table 6.5 - Multiple regression analyses testing for the significance of relationships between mean leaf moisture content (%) per *Grevillea macleayana* plant and measures of plant size, distance to nearest conspecific, canopy cover, and leaf health.**

The plant traits included in the analyses were: (1) plant height (cm); (2) area (m<sup>2</sup>); (3) mean percent (%) canopy cover; and (4) distance to nearest conspecific (NC) (cm). Measurements were recorded on ten plants at Chinamans Beach, eight plants at Greenfields Beach, and seven plants at Illowra Lane. Measurements of leaf moisture were recorded in October and November 2003 and measurements of plant parameters were recorded between April and November 2003 (Table 6.1). Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

Study Site/ Parameter	<i>R</i> <sup>2</sup>	<i>R</i> <sup>2</sup> Adj.	Mean Square	df (Model, Error)	<i>F</i> Ratio	SE*	Probability (Whole-model)	Probability (Parameter)	Trend
<b>Chinamans Beach</b>	0.80	0.65	5.81	4, 5	5.12	2.95	0.05	-	Positive
Height					2.22	0.01		0.20	Positive
Area					15.43	0.05		<b>0.01</b>	Negative
Mean % Canopy Cover					0.70	0.04		0.45	Positive
Distance to NC					0.68	0.00		0.44	Positive
<b>Greenfields Beach</b>	0.66	0.20	1.53	4, 3	1.44	3.49	0.40	-	Positive
Height					0.26	0.01		0.64	Negative
Area					0.34	0.07		0.60	Negative
Mean % Canopy Cover					0.00	0.05		0.23	Negative
Distance to NC					2.20	0.00		0.96	Slight Positive
<b>Illowra Lane</b>	0.99	0.96	2.83	4, 2	33.54	1.55	<b>0.03</b>	-	Positive
Height					46.49	0.00		<b>0.02</b>	Negative
Area					2.00	0.00		0.29	Positive
Mean % Canopy Cover					3.83	0.01		0.19	Negative
Distance to NC					8.18	0.00		0.10	Negative

\* SE = Standard Error

### 6.3.5 Patterns with Inflorescence and Seed Number

#### 6.3.5.1 Plant Size, Distance to Nearest Conspecific & Canopy Cover

Overall, inflorescence numbers were positively related to plant size (height and area) and negatively related to mean percent canopy cover and distance to nearest conspecific, although these tests were not always significant (Table 6.6; Appendix 3, Table A3.4). Moreover, the multiple regression analyses detected significant positive whole-model relationships at each site, specifically with respect to at least one measure of plant size (Table 6.6). At CB, the multiple regression explained 87% of the variation among plants ( $F_{4, 14} = 22.98$ ;  $P < 0.01$ ), and detected a significant positive relationship between inflorescence number and plant area ( $P < 0.01$ ). At GB, the multiple regression explained 58% of the variation among plants ( $F_{4, 14} = 4.87$ ;  $P = 0.01$ ), and detected a significant positive relationship between inflorescence number and plant area ( $P = 0.01$ ). At IL, the multiple regression explained 71% of the variation among plants ( $F_{4, 14} = 8.53$ ;  $P < 0.01$ ) and detected a significant positive relationship between inflorescence number and plant height ( $P = 0.01$ ) and a significant negative relationship with canopy cover ( $P \leq 0.03$ ).

As was observed with inflorescence number, seed numbers displayed significant positive relationships with plant size (height) and both significant and non-significant relationships with canopy cover (Table 6.6). Trends between seed number and distance to nearest conspecific were not consistent among sites (Table 6.6; Appendix 3, Table A3.4). At CB, the multiple regression explained 76% of the variation among plants ( $F_{4, 14} = 11.34$ ;  $P < 0.01$ ), and detected a significant positive relationship between seed number and plant height ( $P = 0.01$ ). At IL, the multiple regression explained 77% of the variation among plants ( $F_{4, 14} = 11.47$ ;  $P < 0.01$ ), a significant positive relationship was detected between seed number and plant height ( $P = 0.01$ ) and a significant negative relationship with canopy cover ( $P < 0.01$ ).

#### 6.3.5.2 Leaf Photosynthetic Yield & Moisture Content

The linear regressions between leaf photosynthetic rate and both inflorescence and seed numbers did not detect any significant relationships (Appendix 3, Table A3.4). However, at both sites, there was a (non-significant) positive trend between leaf photosynthetic rate and inflorescence number. The regressions between leaf photosynthetic rate and seed number detected differing trends at each site.

The linear regressions between leaf moisture content and both inflorescence and seed numbers detected negative trends at each site (Appendix 3, Table A3.4). A significant relationship was detected at CB test between leaf moisture content and inflorescence number ( $P < 0.02$ ), explaining 61% of the variation among plants (Table 6.6; Figure 6.3). However, this regression was driven by a single outlier and when removed the relationship was no longer significant ( $r^2 = 0.09$ ;  $df = 1, 7$ ;  $P > 0.05$ ). Therefore, no emphasis will be placed on the results of the original regression. The remaining non-significant tests explained 13% of the variation among plants at GB and 37% of the variation at IL (Appendix 3, Table A3.4). The tests between leaf moisture content and seed number explained between 1% (GB) and 23% (CB) of the variation among plants (Appendix 3, Table A3.4)

**Table 6.6 - Significant simple linear and multiple regression analyses testing for the significance of relationships between inflorescence and seed numbers (tested separately) and measures of plant size, distance to nearest conspecific, canopy cover, and leaf moisture content, for *Grevillea macleayana* plants.**

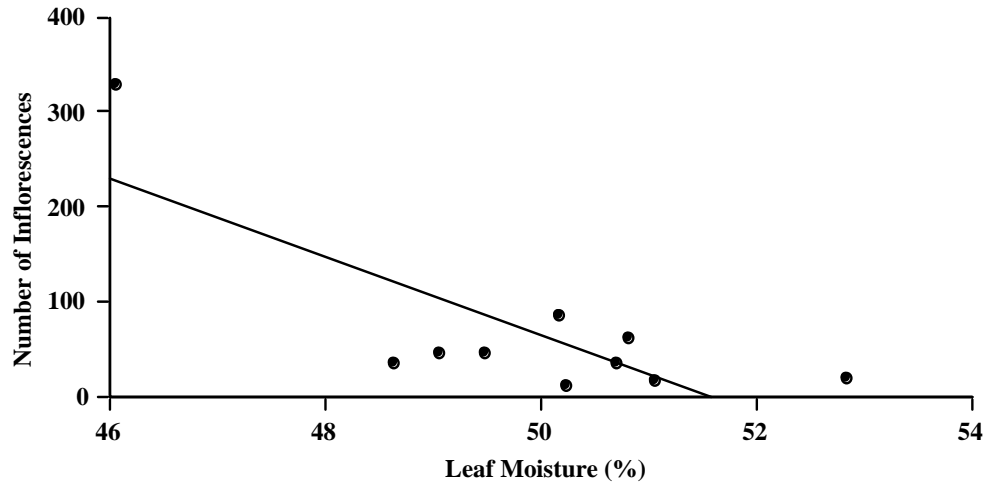
Multiple regression analyses tested for significant relationships among *G. macleayana* plants in total inflorescence and seed number, over two years, (tested independently) and the measures of: (1) plant height (cm); (2) plant area (m<sup>2</sup>); (3) distance to nearest conspecific (cm); and (4) mean percent canopy cover (Table a). Measurements were recorded on 19 *G. macleayana* plants at each site. Simple linear regressions tested for the significance of relationships between mean leaf moisture content and both (1) monthly inflorescence number and (2) monthly seed number (recorded eight weeks after the study was conducted) (Table b). Leaf moisture surveys were conducted in October and November 2003. Significant  $P$  values ( $\alpha < 0.05$ ) are in bold type.

<b>Inflorescence Number</b>								
<b>Study Site/ Parameter</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>MS</b>	<b>df*</b>	<b><math>F</math> Ratio</b>	<b>SE**</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Chinamans Beach</b>	0.87	0.83	1917214	4, 14	22.98	5.35.83	< <b>0.01</b>	Positive
Height					3.09	1.60	0.10	Positive
Area					15.41	10.60	< <b>0.01</b>	Positive
Canopy Cover					0.37	7.18	0.56	Slight Negative
Distance to NC					2.86	0.42	0.11	Negative
<b>Greenfields Beach</b>	0.58	0.46	680269	4, 14	4.87	605.95	<b>0.01</b>	Positive
Height					3.07	1.40	0.10	Positive
Area					8.61	10.17	<b>0.01</b>	Positive
Canopy Cover					0.57	8.56	0.46	Negative
Distance to NC					3.08	0.30	0.10	Negative

<b>Inflorescence Number</b>								
<b>Study Site/ Parameter</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>MS</b>	<b>df*</b>	<b>F Ratio</b>	<b>SE**</b>	<b>P</b>	<b>Trend</b>
<b>Illohra Lane</b>	0.71	0.63	476861	4, 14	8.53	269.09	< <b>0.01</b>	Positive
Height					10.51	1.35	<b>0.01</b>	Positive
Area					1.85	14.94	0.20	Positive
Canopy Cover					6.05	2.03	<b>0.03</b>	Negative
Distance to NC					0.01	0.13	0.91	Neutral
<b>Seed Number</b>								
<b>Study Site/ Parameter</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>MS</b>	<b>df*</b>	<b>F Ratio</b>	<b>SE**</b>	<b>P</b>	<b>Trend</b>
<b>Chinamans Beach</b>	0.76	0.70	33508.2	4, 14	11.34	100.86	< <b>0.01</b>	Positive
Height					8.18	0.30	<b>0.01</b>	Positive
Area					0.97	2.0	0.34	Positive
Canopy Cover					0.97	1.35	0.34	Negative
Distance to NC					4.24	0.08	0.06	Negative
<b>Illohra Lane</b>	0.77	0.70	32574.4	4, 14	11.47	60.64	< <b>0.01</b>	Positive
Height					8.68	0.30	<b>0.01</b>	Positive
Area					4.28	3.37	0.06	Positive
Canopy Cover					16.87	0.46	< <b>0.01</b>	Negative
Distance to NC					0.51	0.03	0.49	Positive
<b>Mean Leaf Moisture Content</b>								
<b>Study Site</b>	<b><math>r^2</math></b>		<b>df (Model, Error)</b>				<b>P</b>	<b>Trend</b>
<b>Chinamans Beach*</b>								
Inflorescence	0.61		1, 8				<b>0.01</b>	Negative
Seed	0.23		1, 8				0.16	Negative

\* Model degrees of freedom; Error degrees of freedom

\*\* SE = Standard Error



**Figure 6.3 - The relationship between inflorescence number per plant and leaf moisture, for *Grevillea macleayana* plants at Chinamans Beach, in October/November 2003.**

The simple linear regression between inflorescence number (total from October and November 2003) mean leaf moisture (%) revealed a significant relationship ( $r^2 = 0.61$ ;  $df = 1, 8$ ;  $P = 0.01$ ) (Table 6.6). However, it is important to note that this regression was driven by a single outlier and when removed it is no longer significant. Therefore no emphasis will be placed on this result.

#### 6.4 Discussion

The studies presented in this chapter revealed substantial variation among plants in plant size (height and area) and distance to nearest conspecific and significant variation among plants in canopy cover, photosynthetic yield, and leaf moisture. I found significant negative relationships between leaf moisture and plant size, perhaps indicating a lack of water availability. Leaf moisture was also significantly negatively related to inflorescence number at CB (although non-significant trends were present between moisture and both inflorescence number at all sites), this result was unexpected and requires further investigation. Inflorescence and seed number were significantly positively related to plant size (area or height) at all sites (except GB for seeds), indicating that larger plants may have greater access to resources or may receive more effective honeyeater visits. Inflorescence and seed number were significantly negatively related to percent canopy cover at IL, perhaps indicating that increased

canopy cover decreases photosynthetic energy available for inflorescence and seed production.

#### **6.4.1 Plant Size, Distance to Nearest Conspecific, and Percent Canopy Cover**

##### *6.4.1.1 Plant Size*

It is clear that there is substantial variation in size among plants at all sites, and this may be the result of a number of factors. Whilst I estimated plants to be of approximately the same age, some plants may have germinated after the last fire, due to soil disturbance (Edwards and Whelan, 1995). Therefore, these plants would be younger and smaller than other plants. Moreover, the observed variation may also be due to seed mass variation, genetic variation, environmental variation, or an interaction among all three (Section 1.7.1). Variation in life history traits (such as rates of plant growth), are commonly observed to be the result of genetic and environmental interactions (Stearns, 1992).

In a previous study on *G. macleayana*, Hogbin *et al.* (1998) found that roadside plants were larger (this was not significant) and produced significantly more inflorescences and seed than non-roadside plants. Furthermore, similar levels of genetic variation were detected between roadside and non-roadside populations (Hogbin *et al.*, 1998).

Therefore, it was suggested that variation in plant growth and reproduction may be due to environmental factors such as increased water and nutrient runoff and reduced competition in roadside populations (Hogbin *et al.* (1998). In a previous study on *G. caleyi*, Llorens (2004) found striking variation among plants and populations in mean crown volume, despite plants being of similar age. Llorens (2004) suggests that whilst this was most likely to have been from environmental variation (e.g. variation in shading, water and nutrient availability), there was also the possibility it was due to genetic factors. Llorens (2004) also found a negative relationship between fixation indices and plant size in *G. caleyi* and *G. longifolia* populations, indicating that inbreeding depression may have reduced growth.

##### *6.4.1.2 Distance to Nearest Conspecific and Percent Canopy Cover*

I detected striking variation among plants at all sites with respect to the distance to nearest conspecific and percent canopy cover. As mentioned in Chapter 1 (Section 1.7), variation in plant distribution and shading may influence pollinator attraction,

subsequent reproductive success, and levels of selfing and outcrossing (e.g. Firmage and Cole, 1988; Klinkhamer *et al.*, 1989; Bosch and Waser, 1999; Suzuki, 2000). Previous studies have found that shaded and isolated plants receive fewer pollinator visits, and isolated plants may have a greater proportion of flowers visited (Klinkhamer *et al.*, 1989). This pollinator behaviour on isolated plants may increase geitonogamy, which may be a disadvantage for optimally outcrossing species (Klinkhamer *et al.*, 1989). However, isolated plants may also receive pollen from further away (i.e. from less related plants) and this may offset potential inbreeding depression (Klinkhamer *et al.*, 1989). Therefore, variation among *G. macleayana* plants in nearest conspecific distance and canopy cover are important because subsequent honeyeater behaviour may be influenced, thereby affecting pollen movement and outcrossing rates (Chapter 5).

#### **6.4.2 Leaf Photosynthetic Yield and Moisture Content**

I found no significant relationships between leaf photosynthetic yield and any of the four plant or environmental variables tested at CB or GB. However, there were non-significant positively trends with plant area and distance to nearest conspecifics, and negative trends with canopy cover. Whilst these results are not significant, tests revealing significant relationships such as these may not be surprising given that plant growth is dependent on light availability and photosynthesis, and photosynthesis has been significantly related to plant growth and size (Zimmerman and Pyke, 1988; Fichtner *et al.*, 1995; Pereira, 1995). Photosynthesis may also decrease with prolonged and increasing competition for resources among plants, such as nitrogen (Pereira, 1995). As the density of *G. macleayana* plants increases, so to does the potential for increased competition for essential resources, such as water supply (e.g. Mustajärvi *et al.*, 2001; Setter *et al.*, 2001; Llorens, 2004).

Plant area and height were significantly negatively related to leaf moisture at CB and IL, respectively. These results may indicate plant moisture decreased with increasing plants size, due to greater allocation of limited water resources. Similarly, measures of plant moisture may increase as plant density decreases (i.e. increasing distance to nearest conspecific), due to a subsequent decrease in competition for water resources (Hogbin *et al.*, 1998).



### 6.4.3 Relationship to Floral Traits and Reproductive Success

The multiple regressions revealed significant positive relationships between inflorescence number and plant size (area or height) at each site. These relationships are supported by previous studies that attributed greater inflorescence and flower production in large plants to greater resource availability (reviewed in Rathcke, 1992; Albert *et al.*, 2001; Suzuki, 2000). One multiple regressions also detected a significant negative relationship between inflorescence number and canopy cover at IL (non-significant negative trends were also detected at CB and GB). This pattern is expected given that changes in photosynthetic rate have been linked to floral production (e.g. Galen *et al.*, 1999); and increased shade should decrease photosynthetic yield, and therefore, energy available for inflorescence production. No significant relationships were detected between inflorescence number and distance to nearest conspecific (although non-significant negative trends were detected at CB and GB). Positive trends were predicted, indicating that plants further apart would not have to compete as greatly for limited resources. These expectations were based on previous studies, reporting that plant growth and reproductive processes are reduced when there is increased competition for resources such as light, water and nutrients (e.g. Lau *et al.*, 1995; Galen, 2000; Schulz and Zasada, 2004; Somanathan *et al.*, 2004).

As with inflorescence number, seed number was significantly positively related to plant height at CB and IL (although non-significant positive trends were found with area at all sites and height at GB). Many previous studies have found similar patterns between plant size and seed production, presumably as a result of increased attraction to effective pollinators and/or greater access to resources (e.g. Schemske, 1980b; Emms *et al.*, 1997; Vaughton and Ramsey, 1997; Albert *et al.*, 2001; Engel and Irwin, 2003). However, Suzuki (2000) proposes that whilst larger plants often have a disproportionately larger floral display than smaller plants (via available resources), and may subsequently produce more seeds/fruits, the proportion of fruit production to pollinated flowers may actually be less than for smaller plants.

As the regression revealed, seed number was significantly negatively related to canopy cover IL, although non-significant negative trends were also detected at CB and BG (as explained above with respect to photosynthetic energy). In addition to the growth advantage, seed production may also be greater for plants in sunny areas due to

increased pollinator visits. This is presumably because pollinators prefer to forage in areas where the ambient temperature is higher (Klinkhamer *et al.*, 1989; Rathcke and Real, 1993; Suzuki, 2000). With respect to distance to nearest conspecific, I had expected a negative relationship with seed number, primarily due to increased pollinator attraction to the greater floral display of grouped plants. Many previous studies have reported that plants in higher densities have greater reproductive success (e.g. Firmage and Cole, 1988; Kunin, 1997; Bosch and Waser, 1999; Cascante *et al.*, 2002). No significant relationships were detected between distance to nearest conspecific and seed number.

#### 6.4.3.1 Leaf Photosynthetic Yield & Moisture Content

Despite my prediction, no significant relationships were found between inflorescence number and photosynthesis (although, non-significant positive trends were detected at both sites). Given that photosynthesis provides energy to plants for growth and other functions, such as nectar production (Southwick, 1984), I had also expected a positive relationship with seed number, although none were detected. Very few studies have examined the relationship between photosynthesis and pollination functions (e.g. Southwick, 1984; Zhou *et al.*, 1997; Galen, 1999; Setter *et al.* 2001 Johnsen *et al.*, 2003). Although, previous studies have reported that water stressed (e.g. Zhou *et al.*, 1997; Setter *et al.*, 2001) and light stressed plants (e.g. Setter *et al.*, 2001) suffered decreased photosynthesis and seed set. Clearly, there is a great need for further research into some of the most basic relationships between photosynthesis and pollination processes.

Whilst I detected a significant negative relationship between leaf moisture content and inflorescence number at CB, this regression was driven by an outlier and when removed the relationship was no longer significant. Few studies have found negative relationships between moisture levels and either floral traits or reproductive success (Lee and Felker, 1992; Galen, 2000). Although, Turner (1993) found that white clover (*Trifolium repens* L.) plants under long-term stable levels of water deficit resulted in increased inflorescence production, but also increased floret abortion and premature death of flower heads. Given the minimal difference among plants in leaf moisture (only a 6.8% difference between plants), the trends detected may not be indicative of potential increases in inflorescence and seed growth with substantially increased water

availability. Ideally, an experiment manipulating water availability and monitoring subsequent inflorescence and seed growth would provide more reliable results.

#### **6.4.4 Conclusions**

The results of this chapter illustrate the complex relationships that exist between key component of plant-pollinator systems (i.e. floral display and seed production) and other important non-reproductive plant traits and environmental variables, such as plant size and distribution, light and moisture availability. Whilst there is plenty of scope for future research in these areas, the inter-related nature of these variables does make it very difficult to be certain of the affect of different variables, on particular aspects of plant-pollination systems. Controlled glasshouse experiments may the most effective way of determining the affect of individual morphological, physiological and environmental variables on plant-pollinator systems.

## Chapter 7 - General Discussion

In this chapter, I bring together the results of the five experimental chapters and discuss the most important relationships (based on the traits I have measured) within the *G. macleayana* pollination system. I outline the most important results of the study and discuss these with respect to our understanding of plant-pollinator systems in general. I also suggest future research needs.

### 7.1 Significance of the Study

In Chapter 1, I identified that the primary goals of evolutionary plant ecology were to understand: (1) how plant and floral characteristics affect reproductive success; and (2) how individual plants within a population achieve greater fitness, relative to other plants (Lawrence, 1993; Mitchell, 1994). The purpose of my PhD study was to use a holistic approach to studying a plant-pollinator system, in order to make a significant contribution to the aforementioned goals, and therefore, better understand how the various components of the system are linked together. In using this holistic approach, my study has made an important contribution to our understanding of intraspecific variation in plant-pollination systems and how this variation is associated with plant reproductive success. Furthermore, my study has challenged some of the widely held beliefs about plant attraction and added to our limited knowledge of some important plant processes and their role in this system (e.g. family outcrossing rates, photosynthetic rates). Importantly, the results of this study may also contribute to better understanding the plant-pollinator systems of other long-lived, self-compatible, perennial shrubs, especially other Proteaceae species.

Few studies have tried to address the complex system that is pollination ecology, rather preferring to study just one or two components (e.g. floral traits and pollinator behaviour – but see Table 1.2). I believe this study to be one of the few Australian studies to address the three major components of pollination ecology (i.e. floral traits, pollinator activity, and reproductive success), with respect to within species variation and reproductive success (but see Vaughton and Ramsey, 1998a and Lloyd, 1998, both outlined in Table 1.2). Most previous studies have been conducted on northern hemisphere species, primarily in the U.S.A (Table 1.2).

In trying to determine the most important relationships among the numerous components of the *G. macleayana* system, I have actually revealed a very complex plant-pollinator system. Whilst some of the relationships I found were as predicted, trends were not always consistent and it is clear that patterns of floral attraction, pollinator behaviour and reproductive success are not always intuitive.

The most important individual findings of this study are:

(1) Importantly, my results revealed significant negative relationships between inflorescence display and outcrossing rates. These results provide evidence that highly attractive plants (via increased floral display) encourage honeyeaters to forage more within plants, thereby increasing geitonogamy and selfing rates. To my knowledge, this is the first study to assess the relationship between family outcrossing rates with multiple measures of floral traits, pollinator activity and reproductive success. Most previous research has studied these relationships at the population level, which I believe ignores significant variation and relationships at the plant level.

(2) Surprisingly, I found very few similarities between honeybees and honeyeaters in patterns of foraging behaviour (i.e. plants with more inflorescences were not necessarily receiving the most visits from both honeybees and honeyeaters). These results provide some evidence that honeybees and honeyeaters may be responding to different floral cues. Moreover, my results challenge the widely held belief that different pollinator groups will respond positively to the same suite of floral traits, primarily greater floral display and nectar rewards (Faegri and van der Pijl, 1979). Whilst these results require further investigation, one possible explanation is that honeybees and honeyeaters have different evolutionary histories, and therefore, may respond differently to variation in floral cues among plants.

(3) At each site, I found that a very small number of plants (three to five) produced over half of the inflorescences and seeds for the study plants, over the survey period. Moreover, greater inflorescence production was consistently associated with greater seed production, with no apparent trade-off. These results provide some support for the “*pollinator attraction hypothesis*” and “*bet-hedging hypothesis*”, which propose that excess flower production has evolved primarily for female function. However, the very low seed-to-inflorescence ratios also provide some support for the “*male function*

*hypothesis*”, which proposes excess flower production evolved to improve male reproductive success.

(4) I found that trends between floral rewards (i.e. inflorescence display and nectar production) and floral visitors (especially honeyeaters) were not always positive. This was unexpected given that these floral traits are generally considered the most important floral rewards. I believe the very large nectar reward that many of these plants provided and the competitive ability of large numbers of honeybees to deplete nectar sources, may be affecting “typical” honeyeater behaviour.

(5) Interestingly, I found no significant variation between diurnal and nocturnal pollen deposition at two of the three sites, indicating that nocturnal pollinators may play an important role in pollinating *G. macleayana* plants. Furthermore, I observed an Eastern Pygmy Possum visiting two *G. macleayana* plants on five occasions. I believe this to be the first study to identify a nocturnal mammal foraging on a *Grevillea* plant. These observations support previous publications detailing the importance of nocturnal marsupial mammals as pollinators of Australian plants (reviewed in Carthew and Goldingay, 1997 and Goldingay, 2000). My observations have triggered a more intensive survey at the CB site, to be undertaken in February and March 2006.

(6) I detected slight, but significant variation among plants in leaf photosynthetic yield and moisture content. Moreover, I found photosynthetic yield was positively related to plant size and negatively related to canopy shade, indicating that larger plants may have greater carbon stores and that plants with less sunlight have lower rates of photosynthesis. Whilst we are aware of the vital role that both photosynthesis and moisture play in plant-pollinator systems, these two processes are very rarely incorporated into pollination studies.

## **7.2 Overview of Specific Results**

### **7.2.1 Intraspecific Variation in Components of the Pollination System**

In this study, I investigated variation among plants in characteristics conferring attractiveness to pollinators (floral traits), and examined the consequences of this variation for pollinator activity and reproductive success. I also identified the most significant relationships among the various components of the plant-pollination system.

I have summarised below the major results of my studies that quantified variation among plants in components of the plant-pollination system (Table 7.1).

The major results of the Chapter 2 (floral traits) studies were:

- Substantial variation among plants in the total number of inflorescences produced, over the survey period;
- At each site, a small number of plants contributed to more than half the total inflorescence production of the survey population;
- In months of good inflorescence production, the best inflorescence producers generally ranked well and the poor producers consistently ranked poorly; and
- Significant variation among plants in inflorescence nectar volume in one to two survey seasons per site, much less variation in nectar sugar concentration (significant at two sites, in one to two survey seasons).

The major results of the Chapter 3 (floral visitor foraging behaviour) studies were:

- Significant variation among plants in at least one feature of honeybee foraging behaviour, for each survey season and site;
- Significant variation among plants in honeyeater foraging behaviour, for one or two survey seasons per site;
- Very few similarities between honeybees and honeyeaters in patterns of foraging behaviour;
- Some evidence to suggest that the same plants are visited more frequently, or for longer, than other plants over consecutive survey seasons; and
- Observation of an Eastern Pygmy Possum on two different *G. macleayana* plants at CB, on five different nights.

The major results of the Chapter 4 (reproductive success) studies were:

- Substantial variation among plants in the total number of seeds produced, over the survey period;
- At each site, a small number of plants contributed to more than half the seed production of the survey population;
- In months of good seed production, the poor producers consistently ranked very poorly, but there were monthly fluctuations among the best seed producers;
- Very low, but variable seed to inflorescence ratio among plants;

- The best inflorescence producers were generally also the best seed producers;
- Significant variation among plants in pollen deposition at each site, for one to two seasons; and
- No variation between diurnal and nocturnal pollen deposition at two of the three study sites.

The major results of the Chapter 5 (family outcrossing rates) studies were:

- Very low outcrossing estimates across all families, and some plants were significantly different from zero and each other; and
- Very low biparental inbreeding rates across all families;

The major results of the Chapter 6 (non-reproductive plant traits and environmental variables) studies were:

- Substantial variation among plants in height, area, and distance to nearest conspecific and significant variation among plants in canopy cover;
- Slight, but significant, variation among plants in mean leaf photosynthetic yield and leaf moisture at CB and GB (but not at IL);
- Plant area and distance to nearest conspecific were positively related to leaf photosynthetic yield;
- Canopy cover was negatively related to leaf photosynthetic yield; and
- No consistent patterns between leaf moisture and plant size, distance to nearest conspecific or canopy cover, but area and height were significantly negatively related at two sites.

### 7.2.2 Significant Relationships and Common Trends

In Chapter 1, I outlined the relationships within the *G. macleayana* plant-pollination system that form the basis for this study (see Figure 1.7). Here, I re-evaluate these relationships and identify the ones I believe to be most important with respect to plant reproductive success (Table 7.1); based on the results outlined in Section 7.2 and the key results of the analyses testing the relationships between the five components of the pollination system (see Appendix 6).

With respect to floral traits, nectar and inflorescence production (flower number per inflorescence) were most commonly positively associated with each other (although this



was not always significant). This may not be surprising given that nectar production will be greater for larger inflorescences. However, a trade-off may have also been expected between these traits, due to the resources required to produce a large number of inflorescences and nectar rewards, over many months of the year.

Honeybee foraging behaviour was positively associated with all measures of inflorescence and nectar production, in almost all tests, indicating the general attractiveness of these floral rewards. However, honeyeater foraging behaviour was much less consistent, being more commonly positively associated with inflorescence traits and negatively associated with nectar traits. These patterns were not expected and require further investigation, especially with respect to potential interference from the introduced honeybee.

Seed and inflorescence numbers were significantly positively correlated, indicating that plants with more inflorescences also produced more seeds. Seed number was more commonly positively related to nectar production, indicating that plants with inflorescences that produced more nectar also produced more seeds. Patterns between seed numbers and floral visitors were inconclusive, with marginally more positive trends detected with both honeybees and honeyeaters. However, the positive trends between honeybee visits and seed production were not expected (based on the observed foraging behaviour and lack of effective pollen transfer), and most likely reflect autogamous seed production and honeyeater foraging behaviour.

Outcrossing rates were significantly negatively related to the number of inflorescences per plant and may reflect increased selfing due to geitonogamous pollen movement by honeyeaters. Outcrossing rates were also negatively related to some aspects of honeybee behaviour. This relationship may reflect the reduced attraction of plants to effective honeyeater pollinators, due to depleted nectar rewards by honeybees. However, outcrossing rates were more commonly positively related to some aspects of honeyeater behaviour, suggesting honeyeaters do facilitate outcrossed pollen movement.

Photosynthetic yield was positively related to plant area and negatively related to canopy cover, indicating that larger plants have a greater capacity for photosynthesis and increased shade inhibits photosynthesis. Leaf moisture was more commonly

negatively related to plant size. Whilst this result was unexpected, the variation among plants in leaf moisture was minimal and the relationship needs to be investigated further.

Both inflorescence and seed numbers were positively related to plant size and negatively related to canopy cover, suggesting that larger plants have more resources for inflorescence and seed production and that reduced sunlight (and rates of photosynthesis) may inhibit production. Inflorescence number was more commonly positively related to photosynthetic yield, suggesting that plants with greater photosynthetic yield produce more inflorescences.

**Table 7.1 - Summary table of the variables with substantial intraspecific variation and the strongest relationships between variables.**

The five components of the *Grevillea macleayana* plant-pollination system that I studied as part of my PhD: (1) Floral Traits; (2) Floral Visitor Foraging Behaviour; (3) Reproductive Success; (4) Family Outcrossing Rates; and (5) Non-reproductive Plant Traits and Environmental Variables. A pink tick indicates substantial or significant variation among plants. Green boxes indicate the strongest relationships within the plant-pollinator system (based on the results of the regression and correlation analyses - Section 7.2; Appendix 6).

[illegible]

### 7.3 The *Grevillea macleayana* Plant-pollinator System

#### 7.3.1 Floral Traits & Reproductive Success

*Grevillea macleayana* plants have very large inflorescence displays and nectar rewards for apparently very little effective pollen transfer and outcrossed seed production. In a self-compatible species such as *G. macleayana*, the realised costs and benefits of larger floral displays will depend on the effect of geitonogamy, highly selfed populations, and how effective potential pollinators are at pollen removal and donation (Klinkhamer *et al.*, 1989). Whilst the results of tests for inbreeding depression in this species are inconclusive (Harriss and Whelan, 1993; Vaughton, 1995), large floral rewards must have evolved from some reproductive advantage, such as increased pollinator attraction and pollen transfer. However, the introduction of the honeybee may have altered the honeyeater-plant relationship to such a point that pollen transfer by honeyeaters is now negligible (England *et al.*, 2002).

The foraging behaviour of effective pollinators may result in selection for an “*optimal*” floral display size (Andersson, 1988). However, this selection process may be stronger for floral display sizes that maximise the female contribution to plant fitness (Schemske, 1980a; Wyatt, 1980, 1982). Broyles and Wyatt (1990) found that flower number explained more of the variation in female reproductive success than in male success. They concluded that female reproductive success was at least as important as male success, with respect to selection for floral display (Broyles and Wyatt, 1990). They also suggested that large floral displays might have evolved as the result of selection for increased overall reproductive success, and not just for male function as proposed by the “*male function hypothesis*” (e.g. Campbell, 1989; Broyles and Wyatt, 1990). Moreover, Harder and Thomson (1989) proposed that selection to increase male reproductive success may result in increased pollen production. However, such an increase may also result in a trade-off with female function, if the resources available for reproduction are limited (Whelan and Goldingay, 1989; Stearns, 1992; Vaughton and Ramsey, 1998). The large floral displays, very low seed-to-inflorescence ratios, but positive inflorescence and seed production relationships that I detected in the *G. macleayana* system provide an ideal opportunity to study hypotheses of excess flower production and variable reproductive success further.

My results demonstrate the importance of evaluating individual plant variability within a population, rather than simply using population means or totals (Carthew, 1993). This is especially important in the *G. macleayana* system because a very small number of plants contributed to more than half of the maternal reproductive success of the survey population. Ayre and Whelan (1989) and Carthew (1993) highlighted various reasons why particular plants may have greater fecundity, including: (1) favourable climatic and microhabitat conditions in particular locations (e.g. Herrera, 1995; Albert *et al.*, 2001); (2) increased rates of pollinator visits to plants with larger floral displays, resulting in greater pollen transfer and set seed (Willson and Rathcke, 1974; Rathcke, 1992); (3) limited reproductive success and thus fecundity in some plants, due to greater sensitivity to disease and predation (Ayre and Whelan, 1989); and (4) genetically superior plants due to pollen source (e.g. selfed or outcrossed) or limited reproductive success in plants due to inbreeding depression (Barrett and Kohn, 1991; Slate *et al.*, 2004). However, it is often difficult to determine which of these reasons might best explain the observed variation in male and female reproductive success (Carthew, 1993).

### 7.3.2 Honeybees and Honeyeaters as Floral Visitors

The results of the honeybee and honeyeater behaviour studies are particularly interesting in that they challenge some of the traditional views in pollination ecology about generalist plant-pollinator relationships. Specifically, different classes of pollinators (birds and bees) visiting *G. macleayana* plants responded differently to variation among plants in various floral traits. As a result, honeybees (as exotic pollinators) are likely interfering with existing, coevolved plant-pollinator relationships, although the extent of honeybee interference is unclear. Further study quantifying the visit patterns and foraging behaviour of honeybees and honeyeaters is required to better understand these results.

The honeybee foraging behaviour I observed (i.e. rarely making contact with pollen presenter) is not conducive to effective pollen transfer or pollination of this species. This conclusion is consistent with previous studies on *G. macleayana* and many other Australian, vertebrate-pollinated plants (Collins *et al.*, 1984; Taylor and Whelan, 1988; Vaughton, 1992, 1996; Paton, 1993, 1996, 1997; Gross and Mackay, 1998; Celebrezze and Paton, 2004; Roberts *et al.*, 2006; Beynon *et al.*, *unpublished*). Moreover, many

studies have also reported a greater abundance and/or frequency of honeybees than native pollinators, both in Australia (e.g. Collins *et al.*, 1984; Ramsey, 1988; Richardson *et al.*, 2000; Hackett and Goldingay, 2001; Celebrezze, 2002; Rymer *et al.*, 2005) and overseas (e.g. Aizen and Feinsinger, 1994; Barthell *et al.*, 2001; Dupont *et al.*, 2004). For example, Celebrezze (2002) found that honeybees were the most frequent forager to *G. sphacelata* and *G. acanthifolia* plants, and Richardson *et al.* (2000) found they were the most frequent forager at *G. mucronulata* flowers. As was previously discussed in Section 1.4.5, the consequences of introduced honeybees foraging in native plant-pollinator systems will depend on several important factors including, but not limited to: (1) whether honeybees can effectively transfer pollen; (2) whether honeybees are depleting floral resources that would otherwise be available for native pollinators; (3) whether honeybees are displacing native pollinators; and (4) the mating systems of the plants involved (i.e. preferentially outcrossed). Further research is required on this plant-pollinator system to better determine the affect of honeybees, preferably in an environment where honeybees can be excluded, but native pollinators (including native bees) are allowed access to plants. Given *G. macleayana* can set seed via autogamy (i.e. reproductive success is assured without pollen transfer), honeybee interference may be affecting plant reproductive success and outcrossing rates due to “altered” honeyeater behaviour more than total seed numbers.

#### 7.4 Future Research

Further research is needed to investigate some of the results and issues raised in this study. I have outlined below the most important of these.

(1) Lifetime Fitness: Waser (1993a, b) emphasised the need to compare the lifetime fitness of outcrossed and selfed progeny, given that the expression of genetic load and inbreeding depression may vary among life history stages (e.g. germination versus growth and reproduction), and therefore, have a delayed component (Schmidt-Adam *et al.*, 2000). For *G. macleayana*, it is unclear whether there is a significant reproductive disadvantage (i.e. inbreeding depression) suffered by selfed progeny, with previous studies reporting conflicting results (Harriss and Whelan, 1993; Vaughton, 1995). Whilst it is very difficult to study lifetime fitness in long-lived perennial species, selfed and outcrossed *G. macleayana* seed (from hand pollinations) could be used in a germination and growth study to determine if inbreeding depression was evident in

selfed progeny, or if outcrossed progeny were more fit. Furthermore, it would be possible to monitor variation in floral and seed production when plants were of reproductive age, thereby addressing important aspects of plant reproductive fitness. Such a study would also allow a more accurate assessment of whether there is a stable, predominantly self-fertilised system in place. If this were the case, then there should be no evidence for reduced fitness among selfed progeny, when compared with outcrossed progeny over several years.

(2) Honeybee and Honeyeater Attraction to Floral Traits: I found very few similarities between honeybees and honeyeaters in their foraging behaviour. An ideal study would implement three trials: (1) with both floral visitors; (2) excluding honeyeaters (e.g. using mesh bags); and (3) excluding honeybees (which is extremely difficult unless set up in a laboratory environment). The study could provide controlled floral rewards and monitor changes in honeybee and honeyeater behaviour, and reproductive success. Other floral visitors (e.g. native bees) could also be added to the system and the subsequent reproductive success monitored. Floral visitor behaviour could then be compared with reward quality and type.

(3) Heritability of Traits: Very little is known about the heritability of plant and floral traits. However, it would be possible in a controlled environment (i.e. greenhouse) to grow progeny plants using the seed from adults that vary in several important floral and plant traits. The progeny could be studied over a number of years to determine whether there were any relationships between adult and progeny plants in the amount or quality of floral traits and reproductive success.

(4) Pollen Removal: If particular species of floral visitors reduce plant reproductive success, then selection should favour floral traits that effectively exclude these visitors from removing floral rewards, especially pollen (Feinsinger, 1983). Whilst I observed pollen removal by honeybees only rarely, previous studies have observed honeybees removing pollen more frequently (Roberts, 2001; Vaughton, 1996). It is therefore possible, that pollen-removing honeybees (by reducing the amount of pollen available for effective honeyeaters to transfer) may have a greater impact on plant fitness via reduced pollen donation and male reproductive success. The ideal study would monitor pollen removal and subsequent reproductive success before and after the removal of

honeybees from the natural *G. macleayana* system. Pollen movement could be monitored using coloured dyes and stains (Thomson, 1986; Peakall, 1989; Thomson and Thomson, 1989). However, this would be logistically very difficult in a natural setting, unless using a small number of isolated plants. Paternity analysis may be a more effective technique for determining how pollen flow changed with the removal of honeybees from the system.

(5) Seed Mass and Seedling Size: Seed mass may influence seedling size and thus plant size, since larger seeds may become established before smaller seeds, and therefore, have a growth advantage (Vaughton and Ramsey, 1998). Furthermore, the nitrogen and phosphorus content of seeds may increase linearly with mass, indicating that greater seedling size may be a result of greater seed nutrient content (Vaughton and Ramsey, 1998). It would be interesting to quantify the seed mass and seedling size variation of progeny and compare this with the production of floral traits and reproductive success of maternal plants, to determine if there were any advantages (i.e. faster progeny growth) or disadvantages (i.e. trade-offs with floral display) from producing larger seeds.



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## Appendix 1

**Table A1** - A summary of the publications on the rare species *Grevillea macleayana* (formerly *G. barklyana* ssp. *macleayana*).

Year	Work Conducted	Major Outcomes	Conclusions	Reference
1993	Investigated the breeding system, including the presence & length of pollen tubes, fruit set & selective abortion, using various pollination treatments (eg. selfed, outcrossed, autogamous & open).	<p>High proportions of hand-pollinated flowers produced pollen tubes.</p> <p>No significant difference between self &amp; outcrossed hand-pollinated flowers in the percentage of flowers with pollen tubes.</p> <p>Outcrossed pollen tubes were significantly longer than selfed tubes. Autogamy had fewest flowers with pollen tubes.</p> <p>Low fruit set overall &amp; significantly higher fruit set for outcrossed flowers (<math>P &lt; 0.05</math>).</p>	<p><i>G. barklyana</i> is self compatible.</p> <p>Pollen movement may be necessary for germination or pollen tube growth.</p> <p>Results imply that timing of pollination &amp; the pollen source determine which flowers develop into fruit.</p> <p>May be selective development of outcrossed fruit through: (1) pollen tubes from outcrossed flowers reaching ovules before those from selfed flowers (2) fruits that develop 1<sup>st</sup> deplete limited resources &amp; (3) fruit initiated later abort when others fruits are developing.</p>	<p>Harris, F &amp; Whelan, R. J.</p> <p><i>Australian Journal of Botany</i> 41: 499-509</p>
1994	Single-locus electrophoretic survey of at least ten maternal plants from each of four sites & their progeny arrays to test the prediction that <i>G. barklyana</i> has a 'preference' for outcrossed pollen & would thus produce higher levels of outcrossing.	<p>Plants within three out of four populations were almost completely selfed.</p> <p>Mean (<math>\pm</math> SE) outcrossing rates in these populations ranged from 0.07 (<math>\pm</math> 0.03) to 0.33 (<math>\pm</math> 0.08) &amp; showed little variation among yrs.</p> <p>Virtually no exchange of genes between immediately adjacent plants in 1 population (i.e. selfing). Fourth population highly outcrossed (<math>0.85 \pm 0.2</math> - Honeymoon Bay).</p>	<p>Understanding optimal &amp; realised mating systems is necessary to the conservation of threatened plant populations (i.e. minimum population size &amp; level of genetic diversity).</p> <p>The realised mating system for three out four sites differs from expectations based on previous experiments &amp; pollinator movements.</p> <p>Different mating systems could reflect genetic variation or variation in pollinator behaviour (i.e. pollen transfer).</p> <p><i>G. barklyana</i> may be tolerant of high levels inbreeding &amp; a good colonist of disturbed sites.</p> <p>Work needs to be done to determine the optimal mating system for this species.</p>	<p>Ayre, D. J.; Whelan, R. J. &amp; Reid, A.</p> <p><i>Heredity</i> 72: 168-174</p>

Year	Work Conducted	Major Outcomes	Conclusions	Reference
1990	As for Edwards and Whelan, 1995		As for Edwards and Whelan, 1995	Edwards, W. <i>Honours Thesis</i> University of Wollongong
1995	Investigated seed-bank dynamics (e.g. seed density) & dormancy characteristics (e.g. viability & germination) of seeds from 40 soil cores (20 'covered' & 20 'open') from each of three sites.	No seeds were retrieved from 'open' samples. Overall seed numbers/sample were small (0.7 – 2.25/sample). Total seeds from sites one & two were much larger than site three. 75% of seeds were innately dormant, due to a hard seed-coat. 21% of initial seeds germinated on moist filter paper without pre-treatment. All seeds that cracked from heating germinated. 85% of scarified seeds germinated. Significantly more seeds germinated when buried at 2cm rather than 4cm.	<i>G. barklyana</i> does develop a soil-stored seed-bank, but only under established individuals (i.e. populations cannot increase in range over one generation). Both laboratory & glasshouse experiments indicated polymorphism in germination behaviour. Increased levels of polymorphism are considered to increase likelihood of establishment in systems where the fire regime allows for two or more generations within the inter-fire period. Break in seed dormancy is through rupture of the seed-coat rather than heat.	Edwards, W. & Whelan, R. <i>Australian Journal of Ecology</i> 20: 548-555
1995	Hand cross, self & mixed pollinations were conducted on 15 plants from each of two populations. Fruit initiation, maturation & abortion were scored.	Significantly fewer fruits were initiated ( $P < 0.001$ ) & matured ( $P < 0.01$ ) at Honeymoon (outcrossed) than Abraham's (selfed). 37% & 46% of initiated fruits matured at Abraham's & Honeymoon respectively. No difference in fruit initiation & maturation or seed weight between treatments at either site. Similar numbers of self & crossed fruits were initiated & matured on mixed inflorescences. Most fruits were aborted within 2wks of initiation.	Self fruits were not selectively aborted. No evidence for inbreeding depression (i.e. self & crossed progeny may be equally vigorous). Likely that many populations have experienced a history of inbreeding. An alternative explanation is that there is lack of genetic variation within populations.	Vaughton, G. <i>International Journal of Plant Science</i> 156 (4): 417 - 424

Year	Work Conducted	Major Outcomes	Conclusions	Reference
1996	Observations of honeybee foraging behaviour, pollen removal by birds & honeybees, efficiency of honeybees as pollinators. Monitoring of flowering phenology, pollen production, stigma receptivity & pollen limitation.	<p>76% of honeybees collected pollen only, 21% nectar only &amp; 3% both.</p> <p>Nectar collecting honeybees didn't contact the pollen or stigma.</p> <p>Pollen collecting honeybees preferred new flowers &amp; could remove all pollen in one visit.</p> <p>Pollen removal at night was negligible</p> <p>Caged inflorescences initiated &amp; matured 50% fewer fruits than open inflorescences &amp; fewer than bagged inflorescences.</p> <p>Fewer than 2% of flowers matured fruits in all treatments.</p> <p>96% of pollen grains stained with acetocarmine (i.e. high levels of initial pollen fertility).</p> <p>Pollen adherence increased with time after flowers opened until 48h &amp; then declined after 72h.</p> <p>No significant difference between open &amp; bagged inflorescences in fruit initiation &amp; maturation (i.e. fruit-set not pollen limited).</p>	<p>Honeybees were less efficient pollinators than birds because of (1) distance between reproductive parts &amp; nectary (19-28mm) &amp; (2) bees collected all pollen but deposited little (i.e. returned to the hive &amp;/or didn't visit older, receptive flowers).</p> <p>Bees removed most pollen within 6h flower opening, but maximal stigma receptivity occurs at 48h (i.e. contact between bees &amp; receptive stigmas unlikely).</p> <p>Male fitness may be reduced if efficient pollen transfer is reduced.</p> <p>Many <i>G. barklyana</i> populations are small &amp;/or isolated &amp; may be unable to maintain bird numbers.</p> <p>Honeybees are likely to be present &amp; subsequent decreased fruit-set may threaten long-term survival of populations.</p>	Vaughton, G. <i>Plant Systematics &amp; Evolution</i> 200: 89 - 100
1998	Assessed the fitness, genetic variability & links among road verge & non-road verge populations. RAPDs were used to assess genetic variation. Inflorescence & fruit-set was measured for six months.	<p>80% of variation was among individuals within populations, 16.7% was among populations within groups &amp; 3.3% was between verge &amp; non-verge populations.</p> <p>Road verge populations produced significantly more inflorescences &amp; seed than non-verge populations (<math>P &lt; 0.05</math> &amp; <math>P &lt; 0.025</math> respectively).</p>	<p>Road verge plants may have greater reproductive success (i.e. fitness).</p> <p>Little evidence of local population genetic subdivision.</p> <p>Contrast with Ayre <i>et. al.</i> (1994) may be due to difference in age of plant material.</p>	Hogbin, P. M.; Ayre, D. J. & Whelan, R. J <i>Heredity</i> 80: 180 - 186

Year	Work Conducted	Major Outcomes	Conclusions	Reference
1998	The development of techniques to determine the relative contribution of groups of pollinators to gene flow and seed dispersal among populations of <i>G. macleayana</i> . To do this chloroplast markers were developed and then techniques were developed for genotyping small amounts pollen loads.	The CTAB extraction technique used to extract plant tissue DNA in previous studies on <i>G. macleayana</i> was successful in extracting pollen DNA, although greater amplification was needed. A two-step pre-amplification procedure using the same PCR protocol gave the best results. However, contaminated DNA was amplified instead of template DNA when low concentrations of DNA were used. Therefore, contamination would have to be eliminated before this technique could be used.	There was no correlation between the concentration of genotypes before amplification and the amount of PCR product. Therefore, the amount of PCR product was not a reliable indicator of the relative amounts of each genotype in a mixed pollen load. However, so long as the results are verified by amplifying the DNA of plants of known genotypes and any contamination can be eliminated, then pollen carried by pollinators can be amplified using the double amplification technique.	Usher, A. <i>Honours Thesis</i> University of Wollongong, NSW, Australia
1998	Seed & inflorescence production was examined for six populations 2-29 years after fire. Pre- & post-dispersal seed predation was examined in two populations. Senescence & adult mortality was assessed in all populations in the 1 <sup>st</sup> year & two populations in the 2 <sup>nd</sup> year. Density & survival of seedlings was assessed in one population following fire. Seed bank size, viability & germination were assessed for each population.	Inflorescence, seed production & seed bank size increased 15-16 years after fire & thereafter remained constant for inflorescences & declined for seeds. Parrots destroyed 1-28% of flowers. Initiated fruits aborted (42-69%), eaten by parrots (9-40%) or matured (4-41%). 34-42% of initiated fruits survived to maturity when parrots were excluded (5-9% when parrots had access). Plant size increased for 16 years after fire & thereafter remained constant. Senescence was less than 10% in populations 2-16 years after fire. 16% of plants produced flowers & fruits two years after fire. Seed bank size was a quadratic function of time since fire. An average of 85.7% of seeds were viable.	Average 1% flowers mature to fruits. Seed production low in first few years of flowering because plants are small & produce few inflorescences. Relatively high % flowers initiated & matured fruits in two year old population, this may offset low inflorescence production of young plants (increased fruit-set likely due to greater nutrient availability & reduced predation by parrots after fire) Little recruitment in absence of fire. After fire two-thirds of seeds in the seed bank emerged as seedlings. Management strategies must consider seed bank properties. <i>G. barklyana</i> may be limited in resilience to fire, especially if intervals are very long (20-25 years) or short (10-12 years).	Vaughton, G. <i>Australian Journal of Ecology</i> 23: 375 - 384

Year	Work Conducted	Major Outcomes	Conclusions	Reference
1999	Development of nine microsatellites for <i>G. macleayana</i> .	Successful transfer of microsatellite primers from <i>G. macleayana</i> to other <i>Grevillea</i> sp. will increase opportunities for ecological genetic research in genus.	Microsatellite development necessary due to low allozyme variability (Ayre <i>et. al</i> 1994).  In NSW 18 <i>Grevillea</i> sp. are listed on TSC Act & 13 listed as ROTAP (Briggs & Leigh 1996).	England, P. R.; Ayre, D. J. & Whelan, R. J. <i>Molecular Ecology</i> 8: 685 - 702
2000	Review of <i>Grevillea</i> studies, focusing on population fragmentation & consequences for conservation, including mating systems, genetic tools, seed banks & pollinator activity etc	See conclusions	Studies to date imply that molecular/genetic tools will be needed to understand the consequences of variation in mating systems & gene flow, & to understand fine-scale gene flow within populations. Use of genetic markers may provide a better understanding of maintenance of genetic variation & consequences of variation in pollen quality & source transferred between & among populations for fitness.	Whelan, R. J.; Ayre, D. J.; England, P. R.; Llorens, T. & Beynon., F. In: <i>Genetics, Demography &amp; Viability of Fragmented Populations</i> .
2001	Assessed pollinator visitation, pollen removal & deposition during peak (spring) & non-peak (winter) flowering at three sites.	Honeybees were the primary visitor & visits were lower during winter. Honeybee visits were approximately an order of magnitude more frequent than birds. Honeymoon Bay (HB) plants received significantly more bird visits/day ( $P < 0.00$ ) & significantly more visits between rather than within plants ( $P < 0.00$ ). More flowers had pollen removed during the day, than at night at all sites. Pollen removal was similar regardless of treatment (i.e. caged or not), indicating some removal by honeybees. Pollen deposition was similar for treatments at two sites, but at HB was significantly higher for 'open' flowers (i.e. birds).	Suggest that birds are “ <i>responsible for effective pollen (gene) dispersal &amp; outcrossing</i> ”, thus the difference among populations. Vegetation structure may alter pollinator foraging behaviour. In semi-cleared sites (i.e. 2 selfed populations), bird visitation rates were lower & movement between plants uncommon (i.e. little pollen movement). The closed site (i.e. HB the most outcrossed population), bird visitation rates & movement between plants were higher. Factors such as plant density, plant distance, plant height & canopy presence may influence pollinator behaviour.	Beynon, F. M; Ayre, D. J. & Whelan, R. J. <i>Unpublished</i>

Year	Work Conducted	Major Outcomes	Conclusions	Reference
2001	Compared outcrossing rates of open' vs. 'bird excluded' inflorescences (using cages), in six to 13 plants from three sites. Seed were collected, genotyped using microsatellites & outcrossing rates calculated.	Seed predation & low fruit set prevented comparison of the two treatments. Inbreeding occurred in all three populations. Outcrossing rates in 'open' seeds were very low (0.062 – 0.225). Outcrossing was significantly lower when birds were excluded (pooling data). The proportion of detectable outcrosses for Honeymoon Bay (HB) were close to outcrossing rates, for Abraham's Bosom (AB) & Elmoos Road (ER) rates were lower. Fixation index (F) for seeds was always high for AB & ER, for HB values were low in 1990-1991, but high in 1994-1995. F for adult plants was always lower, significant for ER & HB (1994-1995).	Difficult to determine the effect of various pollinators, due to " <i>inherent plasticity of the realised mating system</i> ". Populations experience asynchronous/temporal fluctuations in outcrossing levels. High levels of selfing may be consequence of honeybees rather than within-plant bird movements. This & earlier studies (Ayre <i>et. al.</i> 1994) suggest pollen movement is limited within populations. Estimate honeybees have been present in this system for between 4 & 20 years. Adult genotypes consistently display greater levels of heterozygosity than current seed, implying that outcrossed seedlings are the fittest. Suggest that bee activity is so high that the contribution of birds may be " <i>relatively trivial</i> ".	England, P. R.; Beynon, F.; Ayre, D. J. & Whelan, R. J. <i>Conservation Biology</i> 15 (6): 1 - 11
2001	The genetic diversity, genetic differentiation, mating system, reproductive success, and pollinator activity of <i>G. macleayana</i> plants was studied with respect to plants in urban gardens, relative to nearby natural populations. Microsatellite loci were used for the genetic work.	Overall, garden plants contained a greater number of alleles (including private alleles), than natural populations. On average, allele frequencies varied significantly among populations. Detectable outcrosses in seeds from garden plants and one of the natural populations were very low. In both populations, the seed examined revealed very high levels of inbreeding. Honeyeaters visits were similar among populations, but honeybees were more frequent visitors to two natural populations.	Garden plants were as reproductively successful as nearby natural populations (as based on inflorescence and seed production). Greater genetic diversity in garden plants may be because populations comprise individuals from a variety of sources. Therefore, garden plants provide some conservation value for this rare, fragmented species. Although, there is a risk of garden plants becoming hybrid and hybrid pollen moving to the natural populations.	Roberts, D. <i>Honours Thesis</i> University of Wollongong, N.S.W. Australia



Year	Work Conducted	Major Outcomes	Conclusions	Reference
2002	Leaf tissue samples were taken from plants at six populations & six microsatellite loci were used to determine genetic diversity.	Genetic diversity was low for all populations. Genotypic composition of all populations was consistent with predicted effects of inbreeding (e.g. multilocus heterozygote deficits & high indirect fixation indexes). High estimates of gene exchange between nearby populations. Significant population differentiation & moderate gene structure.	Effective genetic size may be much smaller than the census population. Populations in the past may have been large & genetically homogeneous. Distribution of allele sizes suggests that geographic differentiation is driven by mutation. Natural patterns of pollen/seed dispersal, together with patchy fire distribution may have restricted long distance gene flow in past.	England, P. R.; Usher, A. V.; Whelan, R. J. & Ayre, D. J. <i>Molecular Ecology</i> 11 (6): 967 - 977
2003	Compared genetic structure of plants in two undisturbed populations to plants in two disturbed populations.	High levels of selfing levels at all sites. Spatial clustering of genes at $\leq 10$ m in undisturbed populations. Weak spatial autocorrelation at disturbed sites (absence of fine-scale structure).	High levels of selfing & limited pollen & seed dispersal (greater at undisturbed sites). Mixing of seed bank at disturbed sites elevates naturally low seed dispersal and selfing.	England, P. R.; Whelan, R. J. & Ayre, D. J. <i>Heredity</i> 91: 475 - 480
2006	Assessed the morphological & genetic diversity of garden plants.	There were two groups of plants (as distinguished by multivariate & genetic analysis): (1) similar to nearby bushland plants & (2) morphologically distinct. Flowering phenologies overlapped, indicating potential for gene flow.	Garden plants contributed to the genetic variation of an “urban/bushland metapopulation”. However, the morphologically distinct plants may contaminate the genetic make-up of the bushland populations. Management measures are suggested.	Whelan, R. J., Roberts, D. G., England, P. R. & Ayre, D. J. <i>Biological Conservation</i> 128: 493 - 500
2006	Evaluation of the potential conservation value, inflorescence and seed production, pollinator visitation & plant genotypes, compared between three populations of bushland plants & one population or urban plants.	No significant differences between urban & bushland plants in mean monthly inflorescence production. Urban plants initiated & matured significantly more fruits than bushland plants, at one site. Honeybee & bird visits didn't vary significantly between bushland & urban plants. At each site, birds visited significantly more inflorescences per plant. Expected heterozygosity & the number of alleles per locus were greater for the urban population than the bushland populations.	Remnant plants within an urban environment can maintain genetic diversity greater than plants in bushland environments. The introduced honeybee may be the greatest factor inhibiting gene flow among populations & may increase levels of inbreeding due to its foraging behaviour. Possible that remnant plants in urban gardens could be used to contribute to recovery plans for endangered and vulnerable plant species.	Roberts, D. G., Ayre, D. J. & Whelan, R. J. <i>Conservation Biology</i> <i>In Press</i>

## Appendix 2 - Pollen Viability

Whilst rarely tested, pollen viability has been reported to vary among individual plants (Oni, 1990). I wanted to quantify the potential viability of pollen grains among *G. macleayana* plants. To do this I used a modified version of the tetrazolium staining technique (Lakon, 1949; Cook and Stanley, 1960) as described by Kearns and Inoyue (1993). Tetrazolium is a redox dye, and a change from colourless to coloured indicates the presence of redox enzymes (Stanley and Linsken, 1974). The presence of this enzyme activity in pollen grains is used as an indication of cellular respiration and pollen viability (Stanley and Linsken, 1974). I sampled twenty-eight inflorescences from the seven Greenfields Beach (GB) plants studied during the pollen production experiment (one of the plants did not have enough inflorescences to include it in the study). I counted numbers of stained pollen grains from one flower on each inflorescence, on each of the seven GB plants, in February 2004.

To the extent that staining tests are an indication of pollen viability, I found that the mean percentage of coloured pollen grains per flower was at least 90% for all plants, except Plant 5 and Plant 4, in which 82.97% ( $\pm 4.51$ ) and 62.87% ( $\pm 3.12$ ) of pollen grains were coloured, respectively. Vaughton (1996) found similar percentages of stained *G. macleayana* pollen grains (using acetocarmine), with a mean of 96% ( $\pm 1.0$ ) per flower. Smith and Gross (2002) found that the pollen viability of *G. beadleana* flowers (using the tetrazolium technique) approached 100% at anthesis, remained high until pollen age exceeded 24 hr, and then decreased to between 84 – 77% as pollen aged to 72 hr.

I did not explore the results of the pollen viability tests statistically, due to my reservations about the usefulness of pollen viability measures as an indication of seed-siring capability, as highlighted by Thomson *et al.* (1994) and Dafni and Firmage (2000). Thomson *et al.* (1994) cautioned that viability tests should only be used if they have a demonstrated correlation with seed-siring capability. Whilst I did not hand-pollinate seeds, most seeds from these plants are selfed (investigated in Chapter 5, but see Ayre *et al.*, 1994; Hogbin *et al.*, 1998; England *et al.*, 2002) and therefore a positive relationship between pollen viability and seed production might be predicted. I found no relationship between the mean percentage of coloured (viable) pollen grains per

flower and maternal seed production per *G. macleayana* plant (explaining just 0.1% of the variation among plants). These results indicate that pollen viability is not a reliable indicator of seed-siring ability (even though seeds were not hand-pollinated with self pollen)

### A3.1 Analyses between Measures of Floral Visitor Foraging Behaviour (Chapter 3) and Floral Traits (Chapter 2)

**Table A3.1 – Simple linear and multiple regression analyses testing the significance of relationships between the three measures of honeybee and honeyeater foraging behaviour, floral traits, and nectar production, for *Grevillea macleayana* plants.**

The foraging behaviours tested were: (1) mean number of honeybees or honeyeaters; (2) mean cumulative number of inflorescences visited in a survey period; and (3) mean cumulative foraging time per survey period. These three dependent variables were each tested against two sets of floral traits: (1) inflorescence number per plant and mean inflorescence size (flowers/ inflorescence) and (2) mean inflorescence nectar volume (µl) and mean sugar concentration (%) of nectar per inflorescence. Simple linear regressions were used to test the significance of relationships between honeybee and honeyeater foraging behaviour and inflorescence production at Chinamans Beach in February 2003. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

#### (a) Chinamans Beach

March 2002 - Honeybees	<i>R</i> <sup>2</sup>	<i>R</i> <sup>2</sup> Adj.	df*	Mean Square	<i>F</i> Ratio	<i>P</i>	Trend
<b>Number of Honeybees</b>	0.66	0.34	2, 2	11.95	1.97	0.34	Positive
Inflorescence Number					0.61	0.52	Positive
Inflorescence Size					3.67	0.20	Negative
<b>Number of Honeybees</b>	0.71	0.41	2, 2	12.75	2.42	0.29	Positive
Nectar Volume/Inflorescence					0.46	0.57	Positive
Sugar Concentration of Nectar					0.40	0.59	Positive
<b>Inflorescences Visited/Plant</b>	0.29	-0.43	2, 2	6.39	0.40	0.71	Positive
Inflorescence Number					0.25	0.67	Negative
Inflorescence Size					0.44	0.57	Negative
<b>Inflorescences Visited/Plant</b>	0.06	-0.87	2, 2	1.42	0.07	0.94	Positive
Nectar Volume/Inflorescence					0.10	0.78	Negative
Sugar Concentration of Nectar					0.13	0.75	Positive
<b>Foraging Time/Plant</b>	0.05	-0.89	2, 2	4251.60	0.06	0.95	Negative
Inflorescence Number					0.06	0.83	Negative
Inflorescence Size					0.07	0.82	Positive
<b>Foraging Time/Plant</b>	0.17	-0.66	2, 2	13419.0	0.20	0.83	Positive
Nectar Volume/Inflorescence					0.15	0.74	Positive
Sugar Concentration of Nectar					0.37	0.61	Negative
March 2002 - Honeyeaters	<i>R</i> <sup>2</sup>	<i>R</i> <sup>2</sup> Adj.	df*	Mean Square	<i>F</i> Ratio	<i>P</i>	Trend
<b>Number of Honeyeaters</b>	0.89	0.78	2, 2	1.69	8.18	0.11	Positive
Inflorescence Number					16.32	0.06	Positive
Inflorescence Size					1.57	0.53	Negative
<b>Number of Honeyeaters</b>	0.26	-0.48	2, 2	0.50	0.35	0.74	Positive
Nectar Volume/Inflorescence					0.32	0.63	Positive
Sugar Concentration of Nectar					0.01	0.92	Negative
<b>Inflorescences Visited/Plant</b>	0.95	0.90	2, 2	136.63	18.97	0.05	Positive
Inflorescence Number					37.44	<b>0.03</b>	Positive
Inflorescence Size					2.41	0.26	Negative

<b>March 2002 – Honeyeaters continued</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Inflorescences Visited/Plant</b>	0.40	-0.21	2, 2	57.13	0.66	0.60	Positive
Nectar Volume/Inflorescence					0.61	0.52	Positive
Sugar Concentration of Nectar					0.03	0.89	Negative
<b>Foraging Time/Plant</b>	0.95	0.89	2, 2	20730.1	17.36	<b>0.05</b>	Positive
Inflorescence Number					34.70	<b>0.03</b>	Positive
Inflorescence Size					0.47	0.56	Negative
<b>Foraging Time/Plant</b>	0.32	-0.36	2, 2	7045.6	0.47	0.68	Positive
Nectar Volume/Inflorescence					0.54	0.54	Positive
Sugar Concentration of Nectar					0.06	0.83	Negative
<b>September/October 2002 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.24	-0.52	2, 2	0.27	0.31	0.76	Positive
Inflorescence Number					0.58	0.53	Negative
Inflorescence Size					0.46	0.57	Positive
<b>Number of Honeybees</b>	0.37	-0.26	2, 2	0.41	0.59	0.63	Positive
Nectar Volume/Inflorescence					0.28	0.65	Negative
Sugar Concentration of Nectar					0.98	0.43	Positive
<b>Inflorescences Visited/Plant</b>	0.29	-0.43	2, 2	5.89	0.40	0.71	Positive
Inflorescence Number					0.74	0.48	Negative
Inflorescence Size					0.60	0.52	Positive
<b>Inflorescences Visited/Plant</b>	0.39	-0.22	2, 2	8.00	0.64	0.61	Positive
Nectar Volume/Inflorescence					0.36	0.61	Negative
Sugar Concentration of Nectar					1.03	0.42	Positive
<b>Foraging Time/Plant</b>	0.19	-0.63	2, 2	7186.0	0.23	0.81	Positive
Inflorescence Number					0.44	0.58	Negative
Inflorescence Size					0.30	0.64	Positive
<b>Foraging Time/Plant</b>	0.40	-0.20	2, 2	15480.9	0.67	0.60	Positive
Nectar Volume/Inflorescence					0.29	0.65	Negative
Sugar Concentration of Nectar					1.15	0.40	Positive
<b>September/October 2002 - Honeyeaters</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeyeaters</b>	0.47	-0.06	2, 2	0.48	0.89	0.53	Positive
Inflorescence Number					0.04	0.87	Positive
Inflorescence Size					1.22	0.38	Negative
<b>Number of Honeyeaters</b>	0.22	-0.55	2, 2	0.23	0.29	0.78	Positive
Nectar Volume/Inflorescence					0.50	0.55	Negative
Sugar Concentration of Nectar					0.04	0.86	Negative
<b>Inflorescences Visited/Plant</b>	0.52	0.03	2, 2	10.25	1.07	0.48	Positive
Inflorescence Number					0.00	0.97	Neutral
Inflorescence Size					1.24	0.38	Negative
<b>Inflorescences Visited/Plant</b>	0.30	-0.39	2, 2	6.02	0.44	0.70	Positive
Nectar Volume/Inflorescence					0.78	0.47	Negative
Sugar Concentration of Nectar					0.05	0.85	Negative
<b>September/October 2002 – Honeyeaters continued</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Foraging Time/Plant</b>	0.52	0.03	2, 2	767.83	1.07	0.48	Positive
Inflorescence Number					0.00	0.97	Positive
Inflorescence Size					1.24	0.38	Negative

<b>September/October 2002 – Honeyeaters continued</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Foraging Time/Plant</b>	0.30	-0.40	2, 2	447.58	0.43	0.70	Positive
Nectar Volume/Inflorescence					0.79	0.47	Negative
Sugar Concentration of Nectar					0.03	0.88	Negative
<b>February 2003</b>	<b><math>r^2</math></b>		<b>df*</b>			<b><math>P</math></b>	<b>Trend</b>
<b>Honeybees Traits vs Inflorescence Number</b>							
Number of Honeybees	0.91		1, 3		<b>0.01</b>		Positive
Inflorescences Visited/Plant	0.60		1, 3		0.13		Positive
Foraging Time/Plant	0.28		1, 3		0.36		Positive
<b>Honeyeaters Traits vs Inflorescence Number</b>							
Number of Honeyeaters	0.41		1, 3		0.24		Positive
Inflorescences Visited/Plant	0.92		1, 3		<b>0.01</b>		Positive
Foraging Time/Plant	0.93		1, 3		<b>0.01</b>		Positive

**(b) Greenfields Beach**

<b>February 2002 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.51	0.02	2, 2	4.58	1.04	0.49	Positive
Inflorescence Number					1.36	0.36	Positive
Inflorescence Size					0.17	0.72	Positive
<b>Number of Honeybees</b>	0.28	-0.44	2, 2	2.52	0.39	0.72	Positive
Nectar Volume/Inflorescence					0.49	0.56	Negative
Sugar Concentration of Nectar					0.20	0.70	Negative
<b>Inflorescences Visited/Plant</b>	0.35	-0.29	2, 2	9.08	0.55	0.65	Positive
Inflorescence Number					1.08	0.41	Positive
Inflorescence Size					0.57	0.53	Positive
<b>Inflorescences Visited/Plant</b>	0.03	-0.94	2, 2	0.79	0.03	0.97	Positive
Nectar Volume/Inflorescence					0.04	0.86	Positive
Sugar Concentration of Nectar					0.03	0.88	Negative
<b>Foraging Time/Plant</b>	0.28	-0.44	2, 2	12217.6	0.39	0.72	Positive
Inflorescence Number					0.78	0.47	Positive
Inflorescence Size					0.44	0.58	Positive
<b>Foraging Time/Plant</b>	0.02	-0.95	2, 2	1015.3	0.02	0.98	Positive
Nectar Volume/Inflorescence					0.05	0.85	Positive
Sugar Concentration of Nectar					0.04	0.96	Negative

<b>October/November 2002 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.63	0.26	2, 2	8.48	1.69	0.37	Positive
Inflorescence Number					1.48	0.35	Negative
Inflorescence Size					0.93	0.44	Neutral
<b>Number of Honeybees</b>	0.81	0.61	2, 2	10.89	4.17	0.19	Positive
Nectar Volume/Inflorescence					1.92	0.30	Negative
Sugar Concentration of Nectar					2.54	0.25	Positive
<b>Inflorescences Visited/Plant</b>	0.69	0.37	2, 2	14.70	2.20	0.31	Positive
Inflorescence Number					4.39	0.17	Neutral
Inflorescence Size					4.21	0.18	Neutral
<b>Inflorescences Visited/Plant</b>	0.64	0.28	2, 2	13.67	1.77	0.36	Positive
Nectar Volume/Inflorescence					0.65	0.50	Negative
Sugar Concentration of Nectar					1.27	0.38	Positive
<b>Foraging Time/Plant</b>	0.80	0.60	2, 2	23239.0	3.94	0.20	Positive
Inflorescence Number					7.85	0.11	Neutral
Inflorescence Size					7.81	0.11	Neutral
<b>Foraging Time/Plant</b>	0.61	0.22	2, 2	17773.5	1.56	0.39	Positive
Nectar Volume/Inflorescence					0.51	0.55	Negative
Sugar Concentration of Nectar					1.20	0.39	Positive
<b>October/November 2002 - Honeyeaters</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Number of Honeyeaters</b>	0.65	0.30	2, 2	0.10	1.84	0.35	Positive
Inflorescence Number					3.62	0.20	Neutral
Inflorescence Size					3.68	0.20	Neutral
<b>Number of Honeyeaters</b>	0.25	-0.49	2, 2	0.01	0.34	0.75	Positive
Nectar Volume/Inflorescence					0.00	0.99	Neutral
Sugar Concentration of Nectar					0.54	0.54	Negative
<b>Inflorescences Visited/Plant</b>	0.97	0.93	2, 2	8.21	28.83	<b>0.03</b>	Positive
Inflorescence Number					56.24	<b>0.02</b>	Positive
Inflorescence Size					57.65	<b>0.02</b>	Positive
<b>Inflorescences Visited/Plant</b>	0.51	0.01	2, 2	4.30	1.03	0.49	Positive
Nectar Volume/Inflorescence					0.12	0.76	Positive
Sugar Concentration of Nectar					1.14	0.40	Negative
<b>Foraging Time/Plant</b>	0.97	0.94	2, 2	1154.62	34.90	<b>0.03</b>	Positive
Inflorescence Number					67.33	<b>0.01</b>	Positive
Inflorescence Size					69.64	<b>0.01</b>	Positive
<b>Foraging Time/Plant</b>	0.48	-0.04	2, 2	569.35	0.92	0.52	Positive
Nectar Volume/Inflorescence					0.13	0.75	Positive
Sugar Concentration of Nectar					0.98	0.43	Negative

<b>January 2003 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.53	0.22	2, 3	19.00	1.7	0.32	Positive
Inflorescence Number					3.14	0.17	Positive
Inflorescence Size					0.01	0.95	Positive
<b>Number of Honeybees</b>	0.18	-0.36	2, 3	6.52	0.33	0.74	Positive
Nectar Volume/Inflorescence					0.61	0.49	Positive
Sugar Concentration of Nectar					0.37	0.59	Positive
<b>Inflorescences Visited/Plant</b>	0.53	0.21	2, 3	2.25	1.67	0.33	Positive
Inflorescence Number					1.52	0.31	Positive
Inflorescence Size					2.56	0.21	Negative
<b>Inflorescences Visited/Plant</b>	0.50	0.17	2, 3	2.15	1.52	0.35	Positive
Nectar Volume/Inflorescence					1.05	0.38	Negative
Sugar Concentration of Nectar					0.51	0.53	Positive
<b>Foraging Time/Plant</b>	0.71	0.51	2, 3	4324.83	3.56	0.16	Positive
Inflorescence Number					0.70	0.46	Positive
Inflorescence Size					5.13	0.11	Positive
<b>Foraging Time/Plant</b>	0.83	0.72	2, 3	5128.38	7.57	0.07	Positive
Nectar Volume/Inflorescence					12.12	<b>0.04</b>	Positive
Sugar Concentration of Nectar					0.05	0.84	Positive
<b>January 2003 - Honeyeaters</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeyeaters</b>	0.35	-0.08	2, 3	0.34	0.81	0.52	Positive
Inflorescence Number					0.04	0.86	Negative
Inflorescence Size					1.38	0.33	Negative
<b>Number of Honeyeaters</b>	0.59	0.32	2, 3	0.57	2.18	0.26	Positive
Nectar Volume/Inflorescence					3.19	0.17	Negative
Sugar Concentration of Nectar					0.00	0.96	Positive
<b>Inflorescences Visited/Plant</b>	0.06	-0.57	2, 3	1.86	0.09	0.91	Positive
Inflorescence Number					0.01	0.92	Negative
Inflorescence Size					0.14	0.73	Negative
<b>Inflorescences Visited/Plant</b>	0.26	-0.23	2, 3	8.38	0.53	0.64	Positive
Nectar Volume/Inflorescence					0.84	0.43	Negative
Sugar Concentration of Nectar					0.00	0.96	Negative
<b>Foraging Time/Plant</b>	0.05	-0.59	2, 3	116.15	0.07	0.93	Positive
Inflorescence Number					0.05	0.84	Negative
Inflorescence Size					0.06	0.82	Negative
<b>Foraging Time/Plant</b>	0.23	-0.28	2, 3	595.93	0.44	0.67	Positive
Nectar Volume/Inflorescence					0.83	0.43	Negative
Sugar Concentration of Nectar					0.05	0.84	Negative



<b>November 2003 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.68	0.46	2, 3	0.46	3.12	0.19	Positive
Inflorescence Number					0.25	0.21	Positive
Inflorescence Size					4.93	0.11	Positive
<b>Number of Honeybees</b>	0.61	0.35	2, 3	0.42	2.37	0.24	Positive
Nectar Volume/Inflorescence					0.06	0.82	Negative
Sugar Concentration of Nectar					1.7	0.28	Positive
<b>Inflorescences Visited/Plant</b>	0.05	-0.59	2, 3	0.80	0.07	0.93	Positive
Inflorescence Number					0.12	0.75	Positive
Inflorescence Size					0.05	0.84	Positive
<b>Inflorescences Visited/Plant</b>	0.00	-0.65	2, 3	0.13	0.01	0.99	Positive
Nectar Volume/Inflorescence					0.01	0.93	Negative
Sugar Concentration of Nectar					0.02	0.90	Positive
<b>Foraging Time/Plant</b>	0.04	-0.6	2, 3	927.9	0.06	0.94	Positive
Inflorescence Number					0.10	0.78	Positive
Inflorescence Size					0.04	0.85	Positive
<b>Foraging Time/Plant</b>	0.02	-0.63	2, 3	478.4	0.03	0.97	Positive
Nectar Volume/Inflorescence					0.05	0.84	Negative
Sugar Concentration of Nectar					0.06	0.82	Positive
<b>November 2003 – Honeyeaters</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeyeaters</b>	0.38	-0.3	2, 3	0.04	0.92	0.49	Positive
Inflorescence Number					0.92	0.41	Positive
Inflorescence Size					0.54	0.52	Negative
<b>Number of Honeyeaters</b>	0.34	-0.11	2, 3	0.04	0.76	0.54	Positive
Nectar Volume/Inflorescence					1.32	0.33	Negative
Sugar Concentration of Nectar					0.59	0.50	Positive
<b>Inflorescences Visited/Plant</b>	0.09	-0.52	2, 3	3.03	0.15	0.87	Positive
Inflorescence Number					0.01	0.94	Negative
Inflorescence Size					0.30	0.63	Negative
<b>Inflorescences Visited/Plant</b>	0.30	-0.17	2, 3	10.11	0.64	0.59	Positive
Nectar Volume/Inflorescence					0.60	0.50	Negative
Sugar Concentration of Nectar					0.06	0.82	Positive
<b>Foraging Time/Plant</b>	0.09	-0.51	2, 3	704.61	0.15	0.87	Positive
Inflorescence Number					0.01	0.94	Negative
Inflorescence Size					0.30	0.62	Negative
<b>Foraging Time/Plant</b>	0.31	-0.15	2, 3	2370.69	0.67	0.57	Positive
Nectar Volume/Inflorescence					0.65	0.48	Negative
Sugar Concentration of Nectar					0.08	0.80	Positive

## (c) Illowra Lane

October 2002 - Honeybees	$R^2$	$R^2$ Adj.	df*	Mean Square	$F$ Ratio	$P$	Trend
<b>Number of Honeybees</b>	0.52	0.04	2, 2	2.71	1.08	0.48	Positive
Inflorescence Number					2.10	0.28	Positive
Inflorescence Size					0.06	0.83	Positive
<b>Number of Honeybees</b>	0.37	-0.27	2, 2	1.91	0.58	0.63	Positive
Nectar Volume/Inflorescence					0.34	0.62	Positive
Sugar Concentration of Nectar					1.14	0.40	Positive
<b>Inflorescences Visited/Plant</b>	0.55	0.11	2, 2	1.56	1.25	0.45	Positive
Inflorescence Number					2.45	0.26	Negative
Inflorescence Size					0.48	0.56	Negative
<b>Inflorescences Visited/Plant</b>	0.41	-0.18	2, 2	1.15	0.70	0.59	Positive
Nectar Volume/Inflorescence					1.09	0.41	Positive
Sugar Concentration of Nectar					0.05	0.84	Positive
<b>Foraging Time/Plant</b>	0.63	0.27	2, 2	4900.18	1.73	0.37	Positive
Inflorescence Number					3.13	0.22	Negative
Inflorescence Size					1.22	0.38	Negative
<b>Foraging Time/Plant</b>	0.36	-0.27	2, 2	2823.39	0.57	0.64	Positive
Nectar Volume/Inflorescence					0.59	0.52	Positive
Sugar Concentration of Nectar					0.01	0.93	Negative
October 2002 - Honeyeaters	$R^2$	$R^2$ Adj.	df*	Mean Square	$F$ Ratio	$P$	Trend
<b>Number of Honeyeaters</b>	0.61	0.22	2, 2	0.14	1.56	0.39	Positive
Inflorescence Number					3.00	0.23	Positive
Inflorescence Size					0.77	0.47	Positive
<b>Number of Honeyeaters</b>	0.49	-0.03	2, 2	0.12	0.95	0.51	Positive
Nectar Volume/Inflorescence					0.08	0.81	Negative
Sugar Concentration of Nectar					0.75	0.48	Positive
<b>Inflorescences Visited/Plant</b>	0.68	0.36	2, 2	12.51	2.10	0.32	Positive
Inflorescence Number					4.12	0.18	Positive
Inflorescence Size					0.13	0.75	Positive
<b>Inflorescences Visited/Plant</b>	0.24	-0.51	2, 2	4.50	0.32	0.76	Positive
Nectar Volume/Inflorescence					0.00	0.98	Neutral
Sugar Concentration of Nectar					0.36	0.61	Positive
<b>Foraging Time/Plant</b>	0.69	0.38	2, 2	2226.54	2.22	0.31	Positive
Inflorescence Number					4.31	0.17	Positive
Inflorescence Size					0.10	0.79	Positive
<b>Foraging Time/Plant</b>	0.23	-0.55	2, 2	728.26	0.29	0.77	Positive
Nectar Volume/Inflorescence					0.00	0.99	Neutral
Sugar Concentration of Nectar					0.34	0.62	Positive

<b>January 2003 - Honeybees</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeybees</b>	0.99	0.98	2, 2	16.49	85.55	<b>0.01</b>	Positive
Inflorescence Number					154.93	<b>0.01</b>	Positive
Inflorescence Size					168.64	<b>0.01</b>	Positive
<b>Number of Honeybees</b>	0.93	0.87	2, 2	15.60	14.31	0.07	Positive
Nectar Volume/Inflorescence					11.75	0.08	Positive
Sugar Concentration of Nectar					8.76	0.10	Negative
<b>Inflorescences Visited/Plant</b>	0.16	-0.79	2, 2	1.6	0.11	0.90	Positive
Inflorescence Number					0.23	0.68	Negative
Inflorescence Size					0.18	0.71	Negative
<b>Inflorescences Visited/Plant</b>	0.85	0.71	2, 2	13.30	5.87	0.15	Positive
Nectar Volume/Inflorescence					1.64	0.33	Positive
Sugar Concentration of Nectar					11.63	0.08	Positive
<b>Foraging Time/Plant</b>	0.27	-0.46	2, 2	3394.01	0.37	0.73	Positive
Inflorescence Number					0.61	0.52	Negative
Inflorescence Size					0.32	0.63	Negative
<b>Foraging Time/Plant</b>	0.96	0.92	2, 2	12087.4	23.42	<b>0.04</b>	Positive
Nectar Volume/Inflorescence					7.27	0.11	Positive
Sugar Concentration of Nectar					43.20	<b>0.02</b>	Positive
<b>January 2003 - Honeyeaters</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Number of Honeyeaters</b>	0.70	0.40	2, 2	0.34	2.31	0.30	Positive
Inflorescence Number					2.00	0.30	Positive
Inflorescence Size					3.85	0.19	Positive
<b>Number of Honeyeaters</b>	0.41	-0.17	2, 2	0.20	0.71	0.58	Positive
Nectar Volume/Inflorescence					0.40	0.59	Positive
Sugar Concentration of Nectar					0.62	0.51	Negative
<b>Inflorescences Visited/Plant</b>	0.71	0.41	2, 2	0.52	2.42	0.29	Positive
Inflorescence Number					2.08	0.29	Positive
Inflorescence Size					4.02	0.18	Positive
<b>Inflorescences Visited/Plant</b>	0.43	-0.15	2, 2	0.31	0.75	0.57	Positive
Nectar Volume/Inflorescence					0.43	0.58	Positive
Sugar Concentration of Nectar					0.64	0.51	Negative
<b>Foraging Time/Plant</b>	0.74	0.48	2, 2	211.28	2.81	0.26	Positive
Inflorescence Number					2.33	0.27	Positive
Inflorescence Size					4.61	0.17	Positive
<b>Foraging Time/Plant</b>	0.47	-0.17	2, 2	133.47	0.87	0.53	Positive
Nectar Volume/Inflorescence					0.52	0.55	Positive
Sugar Concentration of Nectar					0.73	0.48	Negative

\* Model degrees of freedom; Error degrees of freedom.

### A3.2 Analyses between Measures of Reproductive Success (Chapter 4), Floral Visitor Foraging Behaviour (Chapter 3), and Floral Traits (Chapter 2).

**Table A3.2 - Simple linear and multiple regressions between measures of reproductive success, honeybee and honeyeater foraging behaviour, floral traits, and nectar production for *Grevillea macleayana* plants.**

Seed number (recorded per month, 6 to 9 weeks after the relevant field study) was tested against three sets of independent variables: (1) nectar traits; (2) honeybee foraging behaviour; and (3) honeyeater foraging behaviour. The nectar traits used were: (1) mean inflorescence nectar volume ( $\mu\text{l}$ ) and (2) mean sugar concentration (%) of nectar per inflorescence. The measures of honeybee and honeyeater behaviour used were: (1) mean number per plant; (2) mean cumulative number of inflorescences visited per survey period; and (3) mean cumulative foraging time per survey period. Simple linear regressions tested for the significance of relationships between mean inflorescence pollen deposition and both inflorescence and pollen production (tested separately). Data collected on honeybee and honeyeater behaviour from Greenfields Beach in February 2002 and Chinamans Beach in March 2002 was not compared with subsequent seed production, because seed data was not available for April or May 2002. Significant  $P$  values ( $\alpha < 0.05$ ) are in bold type.

#### (a) Chinamans Beach

<b>October 2002</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.19	-0.63	2, 2	52.04	0.23	0.81	Positive
Nectar Volume					0.00	0.96	Positive
Sugar Concentration					0.44	0.58	Positive
<b>September/October 2002</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.98	0.95	3, 2	236.65	30.74	<b>0.03</b>	Positive
Number of Honeybees					22.16	<b>0.04</b>	Neutral
Inflorescences Visited/Plant					14.10	0.06	Neutral
Time per Plant					9.05	0.10	Negative
<b>Seed Number</b>	0.33	-0.68	2, 3	78.97	0.32	0.81	Positive
Number of Honeyeaters					0.07	0.82	Negative
Inflorescences Visited/Plant					0.03	0.88	Neutral
Time per Plant					0.02	0.89	Positive
<b>February 2003</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.82	0.29	3, 1	36.94	1.54	0.52	Positive
Number of Honeybees					0.25	0.70	Positive
Inflorescences Visited/Plant					0.02	0.9	Negative
Time per Plant					0.12	0.79	Positive
<b>Seed Number</b>	0.80	0.18	3, 1	35.76	1.30	0.56	Positive
Number of Honeyeaters					0.12	0.78	Positive
Number of Inflorescences Visited per Plant					0.13	0.78	Positive
Time per Plant					0.05	0.86	Negative

<b>September 2003</b>	<b><math>r^2</math></b>	<b>df*</b>	<b><math>P</math></b>	<b>Trend</b>
Day/Night 1- Pollen Deposition vs Inflorescence Number	0.03	1, 4	0.75	Positive
Day/Night 2- Pollen Deposition vs Inflorescence Number	0.35	1, 4	0.22	Positive
<b>December 2003</b>	<b><math>r^2</math></b>	<b>df*</b>	<b><math>P</math></b>	<b>Trend</b>
Day 1- Pollen Deposition vs Inflorescence Number	0.11	1, 4	0.52	Negative
Day 2- Pollen Deposition vs Inflorescence Number	0.45	1, 4	0.14	Positive

**(b) Greenfields Beach**

<b>October 2002</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.23	-0.53	2, 2	49.94	0.31	0.77	Positive
Nectar Volume					0.00	0.96	Positive
Sugar Concentration					0.51	0.55	Positive
<b>October/November 2002</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.19	-2.24	3, 1	5.51	0.08	0.96	Positive
Number of Honeybees					0.06	0.85	Negative
Inflorescences Visited/Plant					0.00	0.97	Negative
Time per Plant					0.00	0.97	Positive
<b>Seed Number</b>	0.17	-2.31	3, 1	4.93	0.07	0.97	Positive
Number of Honeyeaters					0.07	0.83	Negative
Inflorescences Visited/Plant					0.01	0.95	Negative
Time per Plant					0.01	0.93	Positive
<b>January 2003</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.61	0.34	2, 3	134.12	2.30	0.25	Positive
Inflorescence Number					2.59	0.21	Positive
Inflorescence Size					1.0	0.39	Positive
<b>Seed Number</b>	0.07	-0.56	2, 3	14.83	0.11	0.90	Positive
Nectar Volume					0.11	0.76	Positive
Sugar Concentration					0.01	0.91	Negative
<b>January 2003</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.14	-1.16	3, 2	20.09	0.11	0.95	Positive
Number of Honeybees					0.11	0.78	Negative
Inflorescences Visited/Plant					0.10	0.79	Positive
Time per Plant					0.18	0.71	Positive
<b>Seed Number</b>	0.45	-0.37	3, 2	67.00	0.55	0.69	Positive
Number of Honeyeaters					1.07	0.41	Negative
Inflorescences Visited/Plant					0.03	0.87	Positive
Time per Plant					0.01	0.93	Positive

<b>September 2003</b>	$r^2$		<b>df*</b>		<b>P</b>		<b>Trend</b>
Day/Night 1- Pollen Deposition vs Inflorescence Number	0.49		1, 4		0.12		Positive
<b>November 2003</b>	$R^2$	$R^2$ Adj.	<b>df*</b>	<b>Mean Square</b>	<b>F Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Seed Number</b>	0.41	0.01	2, 3	145.86	1.02	0.46	Positive
Nectar Volume					2.04	0.25	Negative
Sugar Concentration					1.41	0.32	Positive
<b>Seed Number</b>	0.26	-0.85	3, 2	62.27	0.23	0.87	Positive
Number of Honeybees					0.06	0.84	Negative
Inflorescences Visited/Plant					0.40	0.59	Negative
Time per Plant					0.50	0.55	Positive
<b>Seed Number</b>	0.90	0.74	3, 2	214.89	5.76	0.15	Positive
Number of Honeyeaters					4.13	0.18	Positive
Inflorescences Visited/Plant					3.19	0.22	Neutral
Time per Plant					3.20	0.22	Neutral
<b>January 2004</b>	$R^2$	$R^2$ Adj.	<b>df*</b>	<b>Mean Square</b>	<b>F Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Day 1 Pollen Deposition</b>	0.49	0.15	2, 3	485.69	1.43	0.37	Positive
Inflorescence Number					0.80	0.44	Negative
Pollen Production					1.13	0.37	Negative
<b>Day 1 Pollen Deposition</b>	0.19	-0.35	2, 3	47.10	0.35	0.73	Positive
Inflorescence Number					0.69	0.47	Negative
Pollen Production					0.17	0.71	Positive

## (c) Illowra Lane

<b>October 2002</b>	$R^2$	$R^2$ Adj.	<b>df*</b>	<b>Mean Square</b>	<b>F Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Seed Number</b>	0.11	-0.78	2, 2	75.53	0.12	0.89	Positive
Nectar Volume					0.04	0.85	Negative
Sugar Concentration					0.23	0.68	Negative
<b>Seed Number</b>	0.84	0.35	3, 1	41.70	1.73	0.50	Positive
Number of Honeybees					2.83	0.34	Positive
Inflorescences Visited/Plant					1.37	0.45	Negative
Time per Plant					1.87	0.40	Positive
<b>Seed Number</b>	0.99	0.95	3, 1	49.07	24.53	0.15	Positive
Number of Honeyeaters					33.87	0.11	Neutral
Inflorescences Visited/Plant					36.95	0.10	Neutral
Time per Plant					36.88	0.10	Neutral
<b>January 2003</b>	$R^2$	$R^2$ Adj.	<b>df*</b>	<b>Mean Square</b>	<b>F Ratio</b>	<b>P</b>	<b>Trend</b>
<b>Seed Number</b>	0.69	0.39	2, 2	767.88	2.27	0.31	Positive
Nectar Volume					4.37	0.17	Positive
Sugar Concentration					0.04	0.87	Positive

<b>January 2003</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>df*</b>	<b>Mean Square</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Seed Number</b>	0.82	0.26	3, 1	601.19	1.47	0.53	Positive
Number of Honeybees					3.91	0.30	Positive
Inflorescences Visited/Plant					0.95	0.51	Negative
Time per Plant					1.6	0.43	Positive
<b>September 2003</b>	<b><math>r^2</math></b>		<b>df*</b>		<b><math>P</math></b>		<b>Trend</b>
Day/Night 1- Pollen Deposition vs Inflorescence Number	0.32		1, 4		0.25		Positive
Day/Night 2- Pollen Deposition vs Inflorescence Number	0.36		1, 4		0.21		Negative

\* Model degrees of freedom; Error degrees of freedom

### A3.3 Analyses between Measures of Outcrossing Rates (Chapter 5), Reproductive Success (Chapter 4), Floral Visitor Foraging Behaviour (Chapter 3), and Floral Traits (Chapter 2).

**Table A3.3 - Simple linear and multiple regression analyses testing for the significance of relationships between family singlelocus and multilocus outcrossing rates and biparental inbreeding (tested separately) and measures of floral traits, honeybee and honeyeater foraging behaviour, and seed production, for *Grevillea macleayana* plants.**

The three floral traits used were: (1) mean inflorescence size (flowers per inflorescence recorded in January 2003); (2) nectar volume (total over two days in January 2003); and (3) nectar sugar concentration (mean over two days in January 2003). Simple linear regressions were used to test for the significance of relationships between family outcrossing rates and biparental inbreeding (tested separately) and inflorescence number (total between November 2002 and April 2003). Multiple regressions analyses were also used to test for the significance of relationships between outcrossing rates and biparental inbreeding (tested separately) and three measures of honeybee and honeyeater foraging behaviour, for the six plants included in these studies. The measures of honeybee and honeyeater activity used were: (1) mean number per plant; (2) mean cumulative number of inflorescences visited per survey period; and (3) mean cumulative foraging time per survey period. Simple linear regressions were used to test for the significance of relationships between outcrossing rates and biparental inbreeding (tested separately) and seed number (total between November 2002 and April 2003). Outcrossing rates were quantified from the seeds of eight *G. macleayana* plants at Greenfields Beach, collected between November 2002 and March 2003. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

Floral Traits	<i>R</i> <sup>2</sup>	<i>R</i> <sup>2</sup> Adj	df*	Mean Square	<i>F</i> Ratio	<i>P</i>	Trend
<b>Singlelocus Outcrossing Rate</b>	0.26	-0.86	3, 2	0.00	0.23	0.87	Positive
Inflorescence Size					0.44	0.57	Positive
Nectar Volume					0.43	0.59	Negative
Nectar Sugar Concentration					0.03	0.88	Positive
<b>Multilocus Outcrossing Rate</b>	0.25	-0.88	3, 2	0.00	0.22	0.88	Positive
Inflorescence Size					0.25	0.66	Positive
Nectar Volume					0.09	0.79	Negative
Nectar Sugar Concentration					0.25	0.67	Positive
<b>Biparental Inbreeding</b>	0.38	-0.54	3, 2	0.00	0.41	0.76	Positive
Inflorescence Size					0.06	0.82	Positive
Nectar Volume					0.04	0.86	Positive
Nectar Sugar Concentration					0.76	0.48	Positive



Inflorescence Number	$r^2$		df*		$P$		Trend
Singlelocus Outcrossing Rate	0.56		1, 6		<b>0.03</b>		Negative
Multilocus Outcrossing Rate	0.60		1, 6		<b>0.02</b>		Negative
Biparental Inbreeding	0.45		1, 6		0.07		Negative
Honeybee Activity	$R^2$	$R^2$ Adj	df*	Mean Square	$F$ Ratio	$P$	Trend
<b>Singlelocus Outcrossing Rate</b>	0.51	-0.22	3, 2	0.00	0.70	0.63	Positive
Number of Honeybees					0.00	0.98	Positive
Number of Inflorescences					0.13	0.76	Negative
Time per Plant					0.14	0.75	Negative
<b>Multilocus Outcrossing Rate</b>	0.47	-0.32	3, 2	0.01	0.60	0.68	Positive
Number of Honeybees					0.00	1.00	Neutral
Number of Inflorescences					0.13	0.75	Negative
Time per Plant					0.06	0.83	Negative
<b>Biparental Inbreeding</b>	0.37	-0.58	3, 2	0.00	0.39	0.78	Positive
Number of Honeybees					0.00	0.98	Negative
Number of Inflorescences					0.09	0.80	Negative
Time per Plant					0.01	0.95	Negative
Honeyeater Activity	$R^2$	$R^2$ Adj	df*	Mean Square	$F$ Ratio	$P$	Trend
<b>Singlelocus Outcrossing Rate</b>	0.28	-0.79	3, 2	0.00	0.26	0.85	Positive
Number of Honeyeaters					0.28	0.65	Positive
Number of Inflorescences					0.70	0.49	Neutral
Time per Plant					0.63	0.51	Positive
<b>Multilocus Outcrossing Rate</b>	0.23	-0.92	3, 2	0.00	0.20	0.839	Positive
Number of Honeyeaters					0.09	0.79	Positive
Number of Inflorescences					0.36	0.61	Negative
Time per Plant					0.32	0.63	Positive
<b>Biparental Inbreeding</b>	0.18	-1.05	3, 2	0.00	0.15	0.92	Positive
Number of Honeyeaters					0.00	0.99	Neutral
Number of Inflorescences					0.06	0.84	Negative
Time per Plant					0.05	0.85	Positive
Seed Number	$r^2$		df*		$P$		Trend
Singlelocus Outcrossing Rate	0.02		1, 6		0.77		Positive
Multilocus Outcrossing Rate	0.00		1, 6		0.94		Neutral
Biparental Inbreeding	0.01		1, 6		0.85		Positive

\* Model degrees of freedom; Error degrees of freedom.

### A3.4 Analyses between Non-reproductive Plant Traits and Environmental Variables (Chapter 6), Reproductive Success (Chapter 4), and Floral Traits (Chapter 2)

**Table A3.4 - Simple linear and multiple regression analyses testing for the significance of relationships between inflorescence and seed number (tested separately) and measures of plant size, distance to nearest conspecific, canopy cover, and leaf health, for *Grevillea macleayana* plants.**

Multiple regression analyses tested for significant relationships among *G. macleayana* plants in total inflorescence and total seed number, over two years, (tested independently) and the measures of: (1) plant height (cm); (2) plant area (m<sup>2</sup>); (3) distance to nearest conspecific (cm); and (4) mean percent canopy cover (Table a). Measurements were recorded on 19 *G. macleayana* plants at each site. Simple linear regressions tested for the significance of relationships between mean leaf photosynthetic yield and mean leaf moisture content and (1) monthly inflorescence production and (2) monthly seed number (recorded eight weeks after the study was conducted) (Table b). Leaf photosynthetic surveys were conducted in November 2002 and leaf moisture surveys were conducted in October and November 2003. Significant *P* values ( $\alpha < 0.05$ ) are in bold type.

(a)

<b>Inflorescence Production</b>							
<b>Study Site/ Variable</b>	<b><i>R</i><sup>2</sup></b>	<b><i>R</i><sup>2</sup> Adj.</b>	<b>MS</b>	<b>df (Model, Error)</b>	<b><i>F</i> Ratio</b>	<b><i>P</i></b>	<b>Trend</b>
<b>Chinamans Beach</b>	0.87	0.83	1917214	4, 14	22.98	< <b>0.01</b>	Positive
Height					3.09	0.10	Positive
Area					15.41	< <b>0.01</b>	Positive
Canopy Cover					0.37	0.56	Slight Negative
Distance to NC					2.86	0.11	Negative
<b>Greenfields Beach</b>	0.58	0.46	680269	4, 14	4.87	<b>0.01</b>	Positive
Height					3.07	0.10	Positive
Area					8.61	<b>0.01</b>	Positive
Canopy Cover					0.57	0.46	Negative
Distance to NC					3.08	0.10	Negative
<b>Illohra Lane</b>	0.71	0.63	476861	4, 14	8.53	< <b>0.01</b>	Positive
Height					10.51	<b>0.01</b>	Positive
Area					1.85	0.20	Positive
Canopy Cover					6.05	<b>0.03</b>	Negative
Distance to NC					0.01	0.91	Neutral

<b>Seed Production</b>							
<b>Study Site/ Variable</b>	<b><math>R^2</math></b>	<b><math>R^2</math> Adj.</b>	<b>MS</b>	<b>df (Model, Error)</b>	<b><math>F</math> Ratio</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Chinamans Beach</b>	0.76	0.70	33508.2	4, 14	11.34	< <b>0.01</b>	Positive
Height					8.18	<b>0.01</b>	Positive
Area					0.97	0.34	Positive
Canopy Cover					0.97	0.34	Negative
Distance to NC					4.24	0.06	Negative
<b>Greenfields Beach</b>	0.28	0.07	340466	4, 14	1.34	0.30	Positive
Height					0.46	0.51	Positive
Area					2.52	0.13	Positive
Canopy Cover					0.14	0.71	Negative
Distance to NC					1.49	0.24	Negative
<b>Illohra Lane</b>	0.77	0.70	32574.4	4, 14	11.47	< <b>0.01</b>	Positive
Height					8.68	<b>0.01</b>	Positive
Area					4.28	0.06	Positive
Canopy Cover					16.87	< <b>0.01</b>	Negative
Distance to NC					0.51	0.49	Positive

(b)

<b>Mean Leaf Photosynthetic Yield</b>				
<b>Study Site</b>	<b><math>r^2</math></b>	<b>df (Model, Error)</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Chinamans Beach</b>				
Inflorescence	0.12	1, 8	0.33	Positive
Seed	0.00	1, 8	0.91	Slight Negative
<b>Greenfields Beach</b>				
Inflorescence	0.02	1, 6	0.76	Positive
Seed	0.02	1, 6	0.69	Positive
<b>Mean Leaf Moisture Content</b>				
<b>Study Site</b>	<b><math>r^2</math></b>	<b>df (Model, Error)</b>	<b><math>P</math></b>	<b>Trend</b>
<b>Chinamans Beach*</b>				
Inflorescence	0.61	1, 8	<b>0.01</b>	Negative
Seed	0.23	1, 8	0.16	Negative
<b>Greenfields Beach</b>				
Inflorescence	0.13	1, 6	0.38	Negative
Seed	0.01	1, 6	0.84	Negative
<b>Illohra Lane</b>				
Inflorescence	0.37	1, 5	0.15	Negative
Seed	0.04	1, 5	0.65	Negative

\* Inflorescence production and leaf moisture data log (x + 1) transformed due to unequal variances.

## Appendix 4 - Paternity Analysis

### A4.1 Introduction

Paternity analysis uses multilocus genotype data to allocate the most genetically compatible father (pollen donor) to individual seeds (Schnabel and Hamrick, 1995). Ideally, the results of paternity analyses will: (1) identify the paternal seed success of individual plants within a population (i.e. the best and worst pollen donors); (2) allow an assessment of pollen flow between populations; and (3) generate more accurate measures of male reproductive success (Bernasconi, 2003). However, accurate paternity analysis requires that each individual plant within a population (and potentially surrounding populations) be genotyped, which is not always practical or feasible.

### A4.2 Methods

I used the paternity assignment program CERVUS (Version 2.0) (Marshall *et al.*, 1998) to calculate the statistical likelihood that a given parent plant within the population was the paternal parent of each seed, given the known maternal and seed genotypes. Sufficient variation had been identified using the six loci in this study (*Gm10*, *13*, *25*, *37*, and *Gi7* and *9*), plus an additional four, to assign paternity in a previous study, with an exclusion power of 0.91 (Ayre *et al.*, *unpublished*). Seed had previously been genotyped and outcrossing rates generated for families (Chapter 5).

When genotypes are available for a set of offspring, known maternal plants and potential paternal plants, CERVUS calculates a likelihood ratio between: (1) the likelihood that any given plant is the father and (2) the likelihood that any given plant is unrelated. This ratio represents the increased likelihood that a given plant, rather than a randomly selected plant, was able to donate the paternal alleles to the seed. The most-likely paternal plant is assigned to a seed when this ratio is large relative to the ratios of alternate parents. The likelihood ratio is expressed as an LOD score, which represents the logarithm of the likelihood ratio (Meagher, 1986; Marshall *et al.*, 1998).

Each candidate plant is considered in turn as the father of a seed, and an LOD score is generated. In order to discriminate between the most likely father and the next most likely father, the difference in LOD scores is calculated (a statistic called  $\Delta$ ). The

program compares the distribution of  $\Delta$  for cases where the most likely father was the true father, with that for cases where the most likely father was not. Assuming that a criterion is required for  $\Delta$ , giving 95% confidence, the program identifies the value of  $\Delta$ , such that true fathers obtain 95% of scores exceeding this value. When a plant fulfilling the 95% confidence criterion is assigned paternity of an offspring, the father-offspring relationship is described as a '95% confidence paternity'. The two highest levels of confidence are relaxed (80%) and strict (95%). For each level of confidence, the program shows the percentage of simulated paternity tests in which the  $\Delta$  score of the most-likely male parent exceeded the critical value of  $\Delta$  (i.e. the percentage of tests in which paternity was assigned). This statistic is known as the success rate (an estimate of the power of the loci to resolve paternity) (Marshall *et al.*, 1998).

#### A4.3 Results

Paternity was resolved for only 37 seeds (18.6%), at either the 80% or 95% confidence level (Table A3.1). Of the remaining 162 seeds (81.4%), a 'most-likely' paternal parent was assigned from the candidate plants for 122 seeds (61.3%) and several parents of equal LOD scores were assigned for 33 seeds (16.6%). Equal LOD scores are a result of identical genotypes among paternal plants (Meagher, 1986). No paternal parent could be assigned to seven seeds (3.5%). The power of the six loci to resolve paternity was low, and the exclusion power of the loci was 0.388.

Of the 26 detectably outcrossed seeds, paternity was assigned to seven (26.9%) with 80% confidence and 'most-likely' parents were nominated for eight (30.8%) (Table A3.2). Four seeds (15.40%) were also assigned several nominated paternal parents with equal LOD scores. Therefore, a single 'most-likely' parent could not be assigned. Paternity was not assigned to the remaining seven seeds (26.9%).

Of the 15 outcrossed seeds assigned paternity with either 80% confidence, or with 'most-likely' paternal parents, nine different paternal parent plants were nominated (Table A3.2). The plant most commonly nominated was Plant 2 (four seeds), followed by Plant VINY (three seeds) and Plant V2 (two seeds). The remaining six nominated paternal plants were each nominated for one outcrossed seed.

**Table A4.1 - Summary statistics from the paternity analysis of *Grevillea macleayana* seeds ( $n = 199$ ) from eight plants at Greenfields Beach.**

Confidence Level	Delta Criterion	Tests (Number of Seed)	Success Rate
Strict (95%)	1.81	13	6.53%
Relaxed (80%)	0.71	24	12.06%
Unresolved*	NA	155	77.89%
No nominated Parent	NA	7	3.52%

\* - Unresolved includes plants allocated a single 'most-likely' paternal plant and multiple 'most-likely' plants due to equal LOD scores.

**Table A4.2 - The nominated paternal parent for each of the detectably outcrossed *Grevillea macleayana* seeds ( $n = 26$ ), resulting from paternity analyses (using the CERVUS program).**

Paternity was assigned to seeds at three confidence levels: (1) 80% confidence criterion (relaxed); (2) 'most-likely' parent; or (3) as several parents with equal LOD scores. Seven seeds were not assigned a nominated paternal plant.

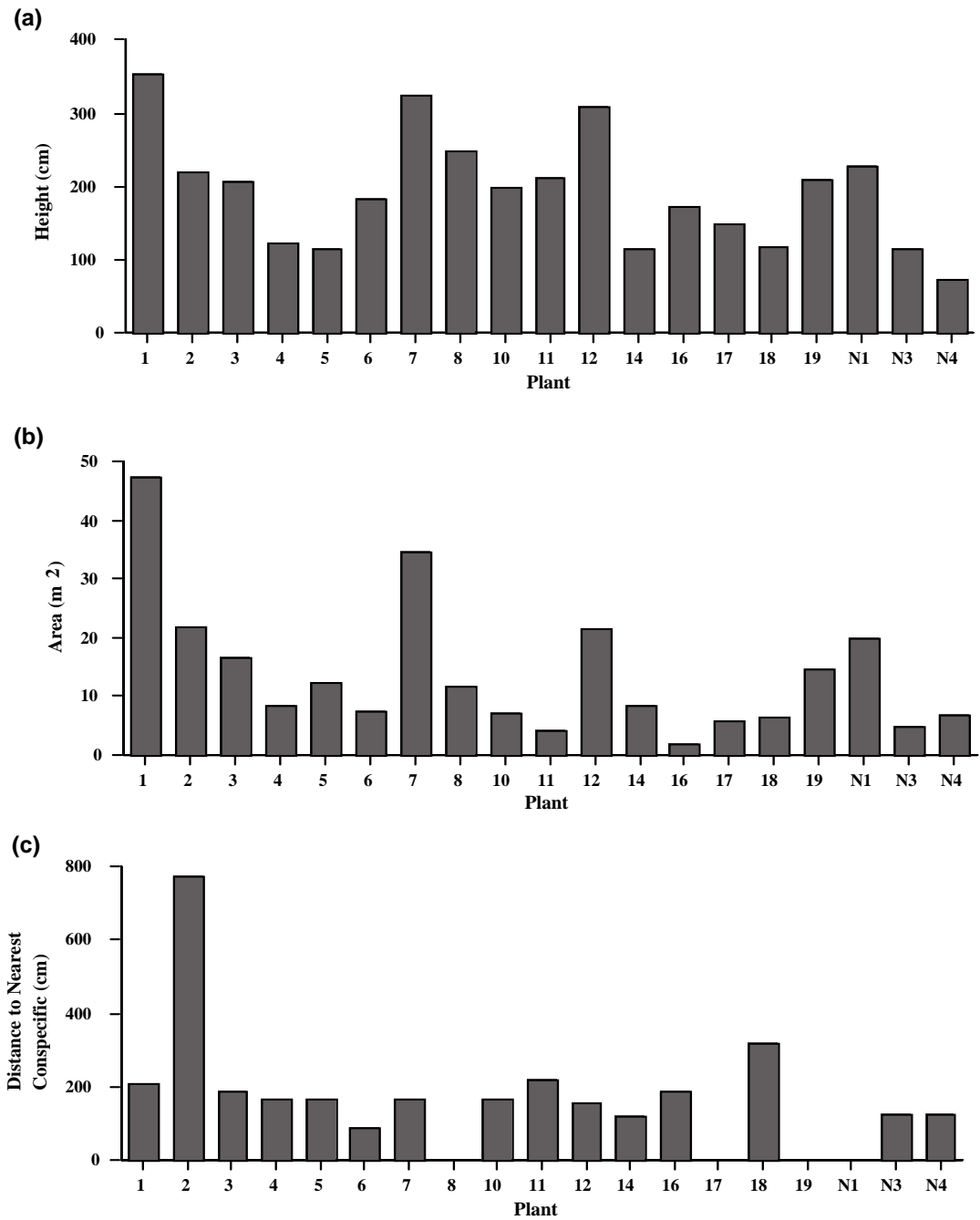
Seed ID	Maternal Plant	Nominated Paternal Plant	LOD Score	Delta	Confidence
S1.7	Plant 1	VINDR10	2.40E+00	6.91E-01	Most-Likely
S1.31	Plant 1	VINE	3.44E+00	7.11E-01	80%
2.5	Plant 2	V2	3.56E-01	3.56E-01	Most-likely
2.12	Plant 2	VINY	2.38E+00	4.09E-01	Most-likely
2.20	Plant 2	V2	5.04E-01	5.04E-01	Most-likely
2.24	Plant 2	Not nominated	NA	NA	NA
2.25	Plant 2	Not nominated	NA	NA	NA
3.12	Plant 3	Not nominated	NA	NA	NA
3.32	Plant 3	Not nominated	NA	NA	NA
3.39	Plant 3	VINY	1.95E+00	1.13E-03	Most-likely
5.2	Plant 5	P2	3.19E+00	9.62E-01	80%
5.3	Plant 5	Not nominated	NA	NA	NA
5.8	Plant 5	VINB	3.50E+00	1.36E+00	80%
5.11	Plant 5	Not nominated	NA	NA	NA
5.15	Plant 5	P2	3.19E+00	9.62E-01	Most-likely
5.23	Plant 5	P2	3.32E+00	9.62E-01	Most-likely
5.27	Plant 5	P2	3.19E+00	9.62E-01	Most-likely
7.4	Plant 7	VINT	3.36E+00	6.51E-01	Most-likely
7.6	Plant 7	Not nominated	NA	NA	NA
10.2	Plant 10	7 nominated parents with equal LOD scores	1.61E+00	0.00E+00	None
10.5	Plant 10	VINDR15	8.05E-01	6.00E-04	Most-likely
N7.10	Plant N7	7 nominated parents with equal LOD scores	1.49E+00	0.00E+00	None
N7.15	Plant N7	VINJ	2.80E+00	1.43E+00	80%
N7.18	Plant N7	2 nominated parents with equal LOD scores	2.63E+00	0.00E+00	None
N7.19	Plant N7	13 nominated parents with equal LOD scores	9.50E-01	0.00E+00	None
N7.21	Plant N7	VINY	3.27E+00	6.82E-01	Most-likely

#### A4.4 Discussion

I could only resolve paternity for 37 seeds (18.6%) overall, and only seven (27%) of the detectably outcrossed seed. These results imply that even though I used variable markers and had genotyped all the maternal plants in the population, the power of the six loci to resolve paternity was low, consistent with the results of a previous study (Roberts, 2001). However, the large proportion of self-fertilised, homozygous seed, the presence of common alleles among seeds, and the presence of alleles in progeny absent among the GB adults, makes it more unlikely that a single paternal plant would be nominated for any one seed (Meagher, 1986). Furthermore, adults from other nearby populations may have sired some seeds.

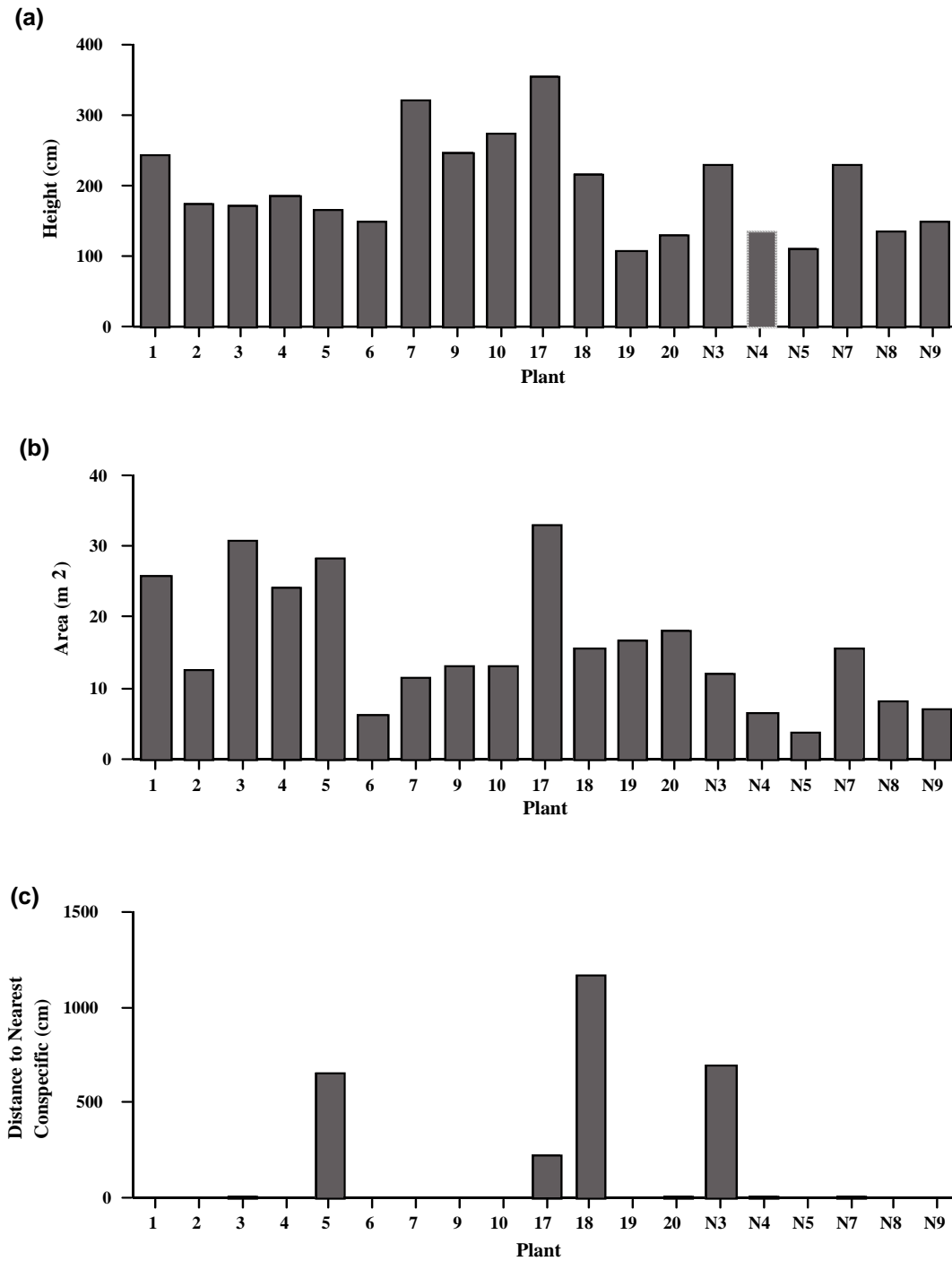
The proportion of total seed sampled that could not be assigned a father from the known population represents the minimum estimate of likely successful pollen gene flow into the population (Schnabel and Hamrick, 1995). Levin and Kerster (1968) proposed that gene flow in most species will average less than 1% among populations a few hundred metres apart. Furthermore, Hamrick (1982) proposed that in predominantly self-fertilised species with no mechanism for long distance seed dispersal, pollen flow between populations may be at levels approaching mutation rates. Seven detectably outcrossed seed (3.5%) were not allocated a father from the GB population. Therefore, this percentage may represent an estimate of pollen flow into the GB population. Of these seven seeds, four were outcrossed at loci *Gi7* with an allele that was very rare among the GB plants. Therefore, it is possible that at least these four seeds were germinated using pollen from outside the population. This proposed percentage of pollen flow is consistent with previous studies that found neighbouring populations of *G. macleayana* had moderate to high estimates of gene flow, potentially reflecting moderate pollen transfer (Hogbin *et al.*, 1998; England *et al.*, 2002). However, other studies have reported evidence of fine-scale genetic sub-division of populations, indicating low and restricted levels of pollen transfer (Ayre *et al.*, 1994; England *et al.*, 2001; Roberts, 2001).

## Appendix 5 - Plant Height, Area and Nearest Conspecific Figures

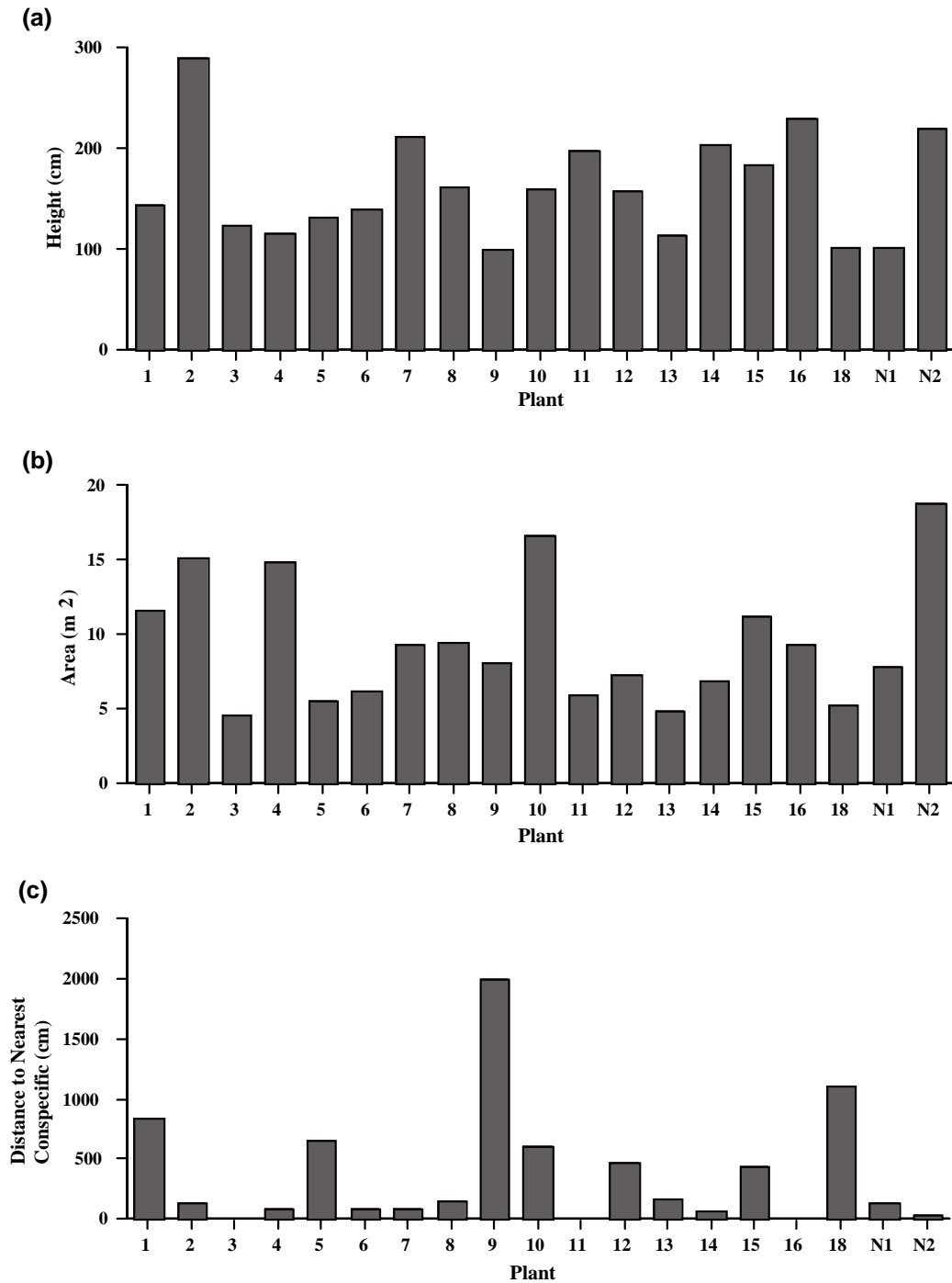


**Figure A5.1** - Variation among *Grevillea macleayana* plants in: (a) height (cm); (b) area (m<sup>2</sup>); and (c) distance to the nearest conspecific (cm). Variables were recorded for 19 *G. macleayana* plants at Chinamans Beach, between April and November 2003.





**Figure A5.2** - Variation among *Grevillea macleayana* plants in: (a) height (cm); (b) area (m<sup>2</sup>); and (c) distance to the nearest conspecific (cm). Variables were recorded for 19 *G. macleayana* plants at Greenfields Beach, between April and September 2003.



**Figure A5.3** - Variation among *Grevillea macleayana* plants in: (a) height (cm); (b) area (m<sup>2</sup>); and (c) distance to the nearest conspecific (cm). Variables were recorded for 19 *G. macleayana* plants at Illowra Lane, between July and November 2003.

## Appendix 6 - Regression and Correlation Analysis Summary

**Table A6.1 - A summary of the results of regression and correlation analyses.**

The table below outlines the most common trends (both significant and non-significant) detected from regression and correlation analyses between multiple measures of floral traits (i.e. inflorescence, nectar, and pollen production); honeybee and honeyeater foraging behaviour; reproductive success (i.e. seed production and pollen deposition); plant outcrossing and biparental inbreeding rates; and non-reproductive plant traits and environmental variables (i.e. plant size, distance to nearest conspecific, canopy cover and leaf health).

Research Question	Most Common Trend
<b>Chapter 2 - Floral Traits</b>	
Inflorescence number versus nectar production	Positive (eight of twelve)
Inflorescence number versus pollen production	No consistent trends
Inflorescence size versus inflorescence number	Negative (four of six)
Inflorescence size versus nectar production	Positive (ten of sixteen)
<b>Chapter 3 - Floral Visitor Foraging Behaviour</b>	
<b>Honeybees</b>	
Honeybee number versus inflorescence number & size	Positive (thirteen of sixteen)
Honeybee number versus nectar production	Positive (ten of sixteen)
Inflorescence number visited versus inflorescence number & size	Marginally negative (eight of fifteen)
Inflorescence number visited versus nectar production	Positive (ten of sixteen)
Foraging time versus inflorescence number & size	Positive (nine of fifteen)
Foraging time versus nectar production	Positive (ten of sixteen)
<b>Honeyeaters</b>	
Honeyeater number versus inflorescence number & size	Marginally positive (eight of thirteen)
Honeyeater number versus nectar production	Negative (eight of thirteen)
Inflorescence number visited versus inflorescence number & size	Positive (eight of fourteen)
Inflorescence number visited versus nectar production:	Negative (eight of thirteen)
Foraging time versus inflorescence number & size	Positive (nine of fifteen)
Foraging time versus nectar production	Negative (eight of thirteen)
<b>Chapter 4 – Reproductive Success</b>	
Seed number versus diurnal & nocturnal pollen deposition	Marginally negative (six of ten)
Seed number versus diurnal pollen deposition	Even (two positive & two negative)
Seed number versus nectar production	Positive (eight of twelve)
Seed number versus honeybee behaviour	Marginal positive (ten of twenty-one)
Seed number versus honeyeater behaviour	Marginal positive (seven of eighteen, but six neutral)
Pollen deposition versus pollen production	Even (one positive & one negative)
Pollen deposition versus inflorescence number	Positive (five of nine)

Research Question	Most Common Trend
<b>Chapter 5 - Outcrossing &amp; Biparental Inbreeding Rates</b>	
Outcrossing rates versus inflorescence size	Positive (two of two)
Outcrossing rates versus nectar production	Even (two negative & two positive)
Biparental inbreeding versus inflorescence size & nectar production	Positive (three of three)
Outcrossing rates & biparental inbreeding versus inflorescence production (three linear tests)	All three negative
Outcrossing rates versus honeybee behaviour	Negative (four of six, but one neutral)
Biparental inbreeding versus honeybee behaviour	Negative (three of three)
Outcrossing rates versus honeyeater behaviour	Positive (four positive, one negative & one neutral)
Biparental inbreeding versus honeyeater behaviour	No consistent trends
Outcrossing rates & biparental inbreeding versus seed production (three linear tests)	Positive (two of three)
<b>Chapter 6 – Non-reproductive Plant Traits &amp; Environmental Variables</b>	
Inflorescence production versus:	
Plant size (height & area)	Positive (six of six)
Canopy cover	Negative (three of three)
Distance to nearest conspecific	Negative (two of three)
Seed production versus:	
Plant size (height & area)	Positive (six of six)
Canopy cover	Negative (three of three)
Distance to nearest conspecific	Negative (two of three)
Leaf photosynthetic yield versus:	
Inflorescence production	Positive (two of two)
Seed production	Even (one positive & one negative)
Leaf moisture versus:	
Inflorescence production	Negative (three of three)
Seed production	Negative (three of three)