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The Ecological Effects of Pollen-stealing Insects on Plant Reproductive Success

by

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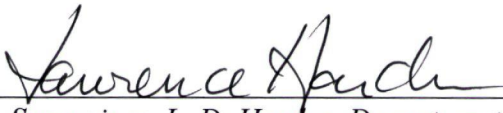
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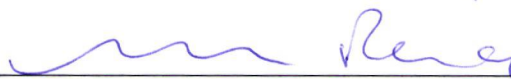
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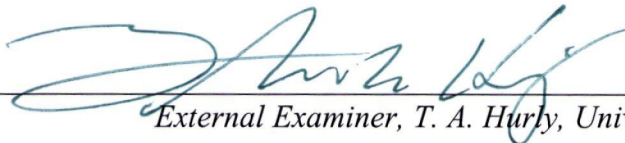
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## Abstract

Pollen is unique among floral rewards in functioning as a pollinator attractant and a carrier of gametes, which invokes potentially severe consequences for plants when floral visitors remove pollen, but do not pollinate in return. Such pollen theft was demonstrated by field and aviary experiments in which addition of honey bees increased pollen removal but decreased pollen receipt and seed production by bird-pollinated *Aloe maculata*. A literature survey revealed that most pollen thieves pollinate other plant species, suggesting that plant (not animal) characteristics largely determine whether pollen collectors thief or pollinate. A survey of flower-visitor interactions of ten *Aloe* species confirmed this conclusion and demonstrated that pollen theft was associated primarily with the extent of dichogamy and nectar inaccessibility to bees, and that pollen collectors deposited mainly low quality (probably self-) pollen for species with large inflorescences. Even though bees remove large quantities of pollen and greatly outnumber birds as visitors to aloe flowers, their contribution to seed set, compared to that from bird visitors, was found to be negligible in most aloes with dichogamous flowers and concealed nectar. These findings indicate that pollen theft affects plant reproduction more strongly than has been appreciated previously.

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## CHAPTER 1 – INTRODUCTION

### **The function of pollen**

Flowers of outcrossing plants produce finite numbers of male gametophytes, which are packaged as pollen and provisioned with high quality proteins and lipids to promote the growth of pollen tubes necessary to effect fertilisation (Roulston et al. 2000). Unlike most rich protein sources in nature, pollen is generally left exposed and undefended, as plants must rely on vectors to transport it to stigmas of conspecific plants. The high nutritional value and easy availability of pollen have prompted its incorporation into the diets of a variety of animals, from thrips to parrots (Kirk 1984, Diaz and Kitzberger 2006), many of which rely exclusively on pollen for protein. Indeed, pollen is in such high demand that it has become, along with nectar, one of the two most frequently offered and collected floral rewards for pollinators (Simpson and Neff 1983).

The diversity and abundance of pollen-collecting animals is a mixed blessing for plants. On one hand, obligate pollen-collectors (including all nonparasitic bees: Apoidea), are the primary pollinators for thousands of plant species. On the other hand, the variation in morphology and behaviour among pollen-collecting species means that many animals can collect pollen without pollinating in return (floral larceny: Inouye 1980). Because pollen functions as both a pollinator attractant and the carrier of male gametes, its loss to pollen thieves can affect pollination success both directly, by reducing the number of male gametes available for fertilisation, and indirectly, by altering pollinator-behaviour. Despite these intriguing and potentially serious consequences for plants, pollen theft has rarely been the subject of considered research, and little is known about the extent or severity of its effects.

## Objectives

In this thesis I address three main objectives: 1) to review patterns of occurrence and possible evolutionary and ecological consequences of pollen theft, 2) to examine the effects on pollination and seed production of adding pollen thieves to plant populations, and 3) to identify floral traits that determine whether pollen-collecting insects serve as pollinators or pollen thieves.

In Chapter 2, I summarise the existing documentation of pollen theft and identify essential features of its perpetrators and characteristics of the flowers it affects. In light of these associations, I discuss possible evolutionary responses of plants to pollen theft, including functional reinterpretations of floral traits that may mitigate pollen theft or its consequences, and the influence of pollen theft on sexual-system evolution in angiosperms. I also explore the theoretical ecological consequences of pollen theft and their probable frequency and importance. This chapter sets the conceptual context for the subsequent empirical chapters.

In Chapter 3, I assess the ecological effects of pollen theft by adding honey-bee (*Apis mellifera* L.) hives to populations of *Aloe maculata* (Medic.), which is naturally pollinated by both birds and insects. Most of this chapter considers a study of four field populations for which I compare pollen removal and receipt, fruit production and seed set in the presence or absence of supplemental honey bees. To evaluate the role of insect visitors further, I excluded birds (but not insects) from some plants and tested for pollen-limitation of seed set under increased pollen theft by supplementing natural pollination with hand pollination. This field study is complemented by an aviary experiment in which I compare the pollination success resulting from sunbird (Nectariniidae) visits alone to that when both sunbirds and honey bees forage on *A. maculata*.

In Chapter 4, I assess which floral traits govern whether pollen-collecting bees pollinate or steal pollen from ten *Aloe* species that range from exclusively insect-

pollinated to exclusively bird-pollinated. I explore traits identified in Chapter 2 as potential influences on the occurrence of pollen theft, namely temporal and spatial separation of pollen presentation and receptive stigmas, as well as nectar accessibility and self-compatibility.

Finally, in Chapter 5 I summarize and synthesize the results and conclusions from Chapters 2 through 4. This overview returns to questions and hypotheses raised in Chapter 2, and addresses them in light of findings from Chapters 3 and 4, highlighting the diversity of effects of and responses to pollen theft.

## CHAPTER 2 - THE ECOLOGICAL AND EVOLUTIONARY CONSEQUENCES OF POLLEN THEFT

### Introduction

Mutualistic interactions range in intensity from symbiosis (e.g., algal-fungal symbiosis in lichens) to weak participation by one or both actors (e.g., minor pollination effected by occasional floral visitors), and create opportunities for cheating by either the partners themselves, or third parties (Bronstein 2001, Yu 2001). Cheaters reap the benefits of mutualism without reciprocation. Such behaviour disrupts mutualistic interactions, with detrimental consequences for one or both partners (e.g., Ness and Bronstein 2004, Zettler et al. 2004). Consequently, cheating can modify the stability of mutualisms (Foster and Kokko 2006) and alter their (co)evolution.

More than 80% of angiosperm species engage in a prominent mutualism when they rely on animals to transfer their pollen from anthers to conspecific stigmas (Eriksson and Bremer 1992). To obtain this service, most plants offer rewards (typically nectar or pollen) that attract floral visitors and manipulate them into removing pollen from anthers and transporting it to stigmas (Simpson and Neff 1981). However, the animals that use floral rewards can vary greatly in their morphology and feeding behaviour, and many are capable of 'stealing' rewards without effecting pollination. Cheating on the pollination mutualism by animals, (floral larceny: Inouye 1980), and its effects on the ecology and evolution of plant reproduction have received increasing study during the past three decades (Irwin et al. 2001).

Floral larceny can affect plant reproduction indirectly and/or directly. Positive or negative indirect effects arise if theft of nectar or pollen alters the behaviour of primary pollinators (reviewed in Maloof and Inouye 2000, Irwin et al. 2001). Unlike nectar theft,

pollen theft can also reduce plant reproduction directly, because it involves the loss of male gametophytes, which can hamper siring success of individual plants and impose pollen limitation of seed set at the population level. The potential for chronic pollen theft to cause pollen limitation is especially concerning when the thieves are introduced species, such as honey bees (*Apis mellifera* L.), which may disrupt the pollination of native plants and threaten the long-term viability of their populations (e.g., Paton 1993, Vaughton 1996, Gross and Mackay 1998, Goulson 2003, do Carmo et al. 2004).

Despite the potentially significant impacts of pollen theft on plant reproduction and growing interest in the closely related topics of nectar larceny (Irwin et al. 2001), pollen limitation (Ashman et al. 2004), cheating on mutualisms (e.g., Bronstein 2001, Foster and Kokko 2006) and the effects of introduced species on native pollination systems (Traveset and Richardson 2006), the occurrence and consequences of pollen theft remain almost unstudied. Indeed, research on floral larceny has considered nectar theft disproportionately. A search of the ISI Web of Science for (“nectar rob\*” or “nectar theft\*” or “nectar thie\*”) in papers published from 1975 until October 2006 identified 191 citations, whereas a search with ‘nectar’ replaced by ‘pollen’ found 19 papers, of which only 14 addressed pollen theft from plants. Because pollen and nectar serve fundamentally different functions for plants, the two types of larceny can have very different repercussions for plant reproduction. Due to the potential importance of pollen theft in floral ecology and evolution, and its increasing relevance as a conservation issue, a synthesis of the existing work that identifies gaps in current understanding is timely.

In this review, I first propose a comprehensive definition of pollen theft, and then discuss pollen theft in the context of floral larceny, outlining similarities with and differences from nectar larceny. Next, I review the existing literature on pollen theft and identify weaknesses in current understanding of its ecological and evolutionary

importance. Finally, I discuss the evolutionary options for plants to escape pollen theft or mitigate its deleterious impacts on pollination.

### **When does pollen collection constitute theft?**

Inouye (1980) distinguished between floral theft, during which a floral resource (nectar or pollen) is stolen without damaging the flower, and robbery, which occurs when the stealing animal damages the flower to access the reward. While mindful of the mechanistic difference between these two categories, I have chosen, instead, to focus on the consequences of larceny for pollen transfer and fecundity. As robbery involves a specific instance of theft that also causes damage, I use ‘theft’ as the encompassing term (equivalent to Inouye’s “larceny”).

Pollen theft is best understood as one end of a pollen-collection continuum, rather than a distinct process, and as such is difficult to define precisely. For example, every bee is constructed of protein derived from pollen (Thorp 2000) that is diverted from dispersal to conspecific stigmas, and so represents lost mating opportunities for the producing plants. However, bees often act as important pollinators, so that the pollen taken by a pollinating bee represents payment for services rendered, rather than theft. The pollen-collecting continuum thus arises from the extent to which the pollen wage actually purchases pollen dispersal, and so depends on an animal’s efficacy as a pollinator.

Identification of pollen thieves is further complicated by the context-dependent nature of pollination interactions, whereby the net effect of a floral visitor often depends on the abundance of other visitors (Thomson and Goodell 2001). Thus, a visitor that generally reduces a plant’s reproductive success by stealing pollen may be beneficial in the absence of better visitors, as long as its pollen-collection results in more pollination than would occur otherwise. These perspectives are evident in the terms that have been



used to describe pollen theft, including pollen parasitism (Baker et al. 1971, Michener 1979, McDade and Kinsman 1980, Paciorek et al. 1995, Thomson and Goodell 2001), pollen eating or florivory (Olesen 1979, Weiss 1996), and minor, inefficient, or ‘ugly’ pollination (Baker et al. 1971, Thomson and Thomson 1992, Vaughton 1996, Lau and Galloway 2004).

In the context of the pollination-efficiency continuum, pollen theft is defined most simply as pollen removal by a flower-visitor that does not effect pollination (Inouye 1980). Although unambiguous, this definition is too restrictive, as pollen removal associated with occasional pollen transfer may still reduce plant fecundity. Therefore, I define pollen theft as deliberate pollen removal from flowers by animals that seldom transfer pollen to suitable conspecific stigmas, especially stigmas on other plants.

Accordingly, animals that remove pollen unintentionally (e.g., by knocking it off anthers while nectar feeding) and deposit little are inefficient pollinators, rather than pollen thieves. Pollen theft has ecological consequences for plants if it reduces seed production within a population, and evolutionary consequences if it reduces the relative fitness of individual plants in terms of siring ability and/or seed production.

As with inefficient pollination, pollen theft overlaps partially with florivory (reviewed by McCall and Irwin 2006). Florivory does not generally include pollen consumption by pollinators or floral damage by non-consumers, so pollen theft constitutes florivory only when other flower structures are actively consumed as well. Similarly, florivory constitutes pollen theft only when male structures are consumed preferentially, or when the effects of damage to male structures can be distinguished from those to female organs or other structures, such as petals.

Even with an explicit definition, pollen theft can be difficult to identify and conceptualize in practice. Whereas nectar theft is obvious when visitors ingest nectar without contacting reproductive structures (although see Maloof and Inouye 2000), all

pollen collectors contact at least the structures that present pollen. Like nectar theft, pollen theft is clear if visitors never contact stigmas. However, because I allow for limited pollination by thieves, some interactions previously described as inefficient pollination constitute pollen theft under our definition. To distinguish theft further from inefficiency in practice, I propose that although inefficient pollinators deposit little pollen relative to the amount they remove, increased exposure to inefficient pollinators in the absence of other visitors enhances pollen import. In contrast, for pollen thieves the discordance between pollen removal and deposition is so great that pollen import does not increase with exposure to thieves, so that pollen removal and import are uncorrelated (e.g., Thomson and Goodell 2001), or even negatively correlated.

### **Who are the pollen thieves?**

Pollen is a rich source of protein that is often poorly protected in plants, and is essential to the diets of diverse animals (reviewed in Roulston and Cane 2000). Pollen thieves take advantage of this easily available protein source, and deliberately remove pollen from flowers either by consuming it directly (e.g., *Trigona* bees, beetles, syrphid flies, thrips, snails), or collecting it to feed larval offspring (e.g., bees and masarid wasps).

We conducted a thorough literature search for studies that identified floral visitors as pollen thieves and/or documented pollen theft. The resulting 37 studies describe pollen theft from over 60 plant species in 35 families (Table 2.1). With one notable exception, all identified pollen thieves are invertebrates and, except for snails and mites, are flying insects that depend entirely on pollen for protein. The sole known vertebrate pollen thief is an Argentine parakeet (*Enicognathus ferrugineus*) that selectively consumes pollen and male flowers of a dioecious, wind-pollinated tree *Nothofagus pumilio* (Diaz and Kitzberger 2006). All cases of pollen theft documented here are species-level interactions, but individuals of an otherwise pollinating species may also act

as thieves. For example, pollen- versus nectar-foraging honey bees often differ in their pollination efficiency, and pollen-only foragers can act as pollen thieves while nectar foragers are efficient pollinators (Ish-Am and Eisikowitch 1993).

Most identified pollen thieves effectively pollinate other plant species; thus pollen theft results not simply from intrinsic characteristics that predispose an animal to theft, but more generally from a mismatch between the ecology and morphology of the animal and plant involved. Indeed, plants subject to theft may play an important role in plant communities by maintaining populations of ‘thieves’ that are important pollinators of species that flower at different times (Baker et al. 1971). Nevertheless, some animals have been documented as thieves far more frequently than others, such as the highly eusocial *Trigona* and *Apis* (Table 2.1). *Trigona* species are the only bees known to chew through anthers to access pollen, and so may be less constrained by floral morphology that encourages stigma contact by pollen collectors. Honey bees also display remarkable behavioural flexibility in their manipulation of flowers, which could predispose them to pollen theft (Westerkamp 1991). Both *Apis* and *Trigona* require pollen protein during much of the year to maintain their large perennial colonies, so they are necessarily generalist pollen feeders. However, even specialized pollen collectors can function as thieves (e.g., *Perdita* species: Barrows et al. 1976, Michener 1979). Given that bees are the most important group of flower visitors worldwide (Danforth et al. 2006), as well as the most frequently documented group of pollen thieves, pollen theft is likely much more widespread than indicated by the existing literature.

### **Pollen versus nectar theft**

#### *Direct effects on plant reproduction*

The most obvious and significant difference between pollen and nectar theft arises from their potential for direct effects on plant reproduction. Pollen plays a direct role in plant

inating as the carrier of male gametes, so its removal by pollen thieves can reduce siring opportunities directly (Krupnick and Weis 1999). Pollen theft can also cause pollen limitation at the population level by depleting the overall pool of pollen available to fertilize ovules (Harder and Wilson 1997, do Carmo et al. 2004). In contrast, nectar serves no direct role in plant reproduction or survival, so its loss through theft has no direct consequences for reproductive performance (Table 2.2).

#### *Indirect effects on plant reproduction*

Theft of floral rewards can affect plant fitness indirectly in two ways. First, when replacement of the stolen reward is possible but energetically costly, theft can decrease the resources available for seed development. Nectar replacement can cause resource limitation (Southwick 1984, Pyke 1991, Ordano and Ornelas 2005), although this effect is probably limited to species with unusually high costs of nectar production (Harder and Barrett 1992, Tindall 2007). In contrast, pollen cannot be replaced after it has been removed, so pollen theft does not impose a resource feedback on fecundity (Inouye 1980, Westerkamp 1996).

The second potential indirect effect of floral theft arises from its effect on the behaviour of pollinators, which to date has been considered only in the context of nectar theft. Decreased nectar standing crop due to theft can cause legitimate pollinators to leave inflorescences sooner than they would otherwise (reviewed in Maloof and Inouye 2000). Reduction in the number of flowers that pollinators visit per plant can diminish overall pollen import, but whether pollen export (i.e., successful dispersal) also suffers depends on the effects of fewer flower visits by individual pollinators on self-pollination between flowers (geitonogamy). Because this mode of self-pollination directly reduces the pollen on pollinators available for dispersal to other plants (Harder and Barrett 1995), processes that divert removed pollen from geitonogamy can enhance export.

Geitonogamy tends to increase with the number of flowers an animal visits on an inflorescence (e.g., Johnson and Harder 2005, Jersakova et al. 2006), so theft that promotes early visitor departure could reduce geitonogamy and enhance outcrossing. Thus, the overall reproductive consequences of nectar theft may depend largely on the extent of pollinator limitation it causes, being negative if nectar depletion causes pollinator limitation, but positive if cross-pollination is adequate and self-pollination is reduced (Maloof and Inouye 2000, Irwin et al. 2001, Irwin 2003).

The effects of pollen theft on pollinator behaviour are poorly understood, and the indirect consequences of theft for plant fitness probably depend on the extent to which pollinators use pollen to assess the reward status of flowers. Some nectarivorous pollinators use the presence of pollen to identify whether a flower has been visited recently and so may be depleted of nectar (Dobson and Bergstrom 2000, Lunau 2000), though in many cases nectarivores are unlikely to be affected by pollen theft. Pollen can also function as a floral attractant for pollen-collecting insects, who may evaluate its availability before landing on a flower (reviewed by Dobson and Bergstrom 2000, Lunau 2000). In either case, theft could result in pollinator limitation if pollinators avoid thieved flowers entirely. Pollinators may avoid flowers that have been visibly damaged during robbing (Renner 1983). Two studies of pollen robbery found reduced visitation to undamaged flowers on damaged plants compared to that on undamaged plants (Krupnick et al. 1999), but no difference in geitonogamy or outcrossing (Krupnick and Weis 1999), indicating that the net indirect effect of pollen robbery was negative.

For plants with concealed pollen, which requires pollen-collectors to visit flowers to assess reward abundance, pollen theft could reduce geitonogamy and pollen discounting in a similar manner to nectar theft. Several studies of buzz-pollination for species with pollen concealed in poricidal anthers have found that pollen-collecting bees spend less time on flowers with reduced pollen rewards (Buchmann and Cane 1989,

Harder 1990, Shelly and Villalobos 2000). Bees also visit fewer flowers per inflorescence visit on plants whose (hidden) pollen has been depleted (Gori 1989, Harder 1990, Shelly and Villalobos 2000). However, flowers with concealed pollen should also be less vulnerable to pollen theft (see Evolutionary Consequences below). Most instances of theft from such flowers involve robbery, which leaves visible signs of pollen depletion (Renner 1983, Snow and Roubik 1987, Gross 1993), or theft from non-anther floral structures such as stigmas and petals, which would not affect the pollen sought by pollinators (Snow and Roubik 1987, Gross and Mackay 1998, Raju and Rao 2006). Furthermore, note that the benefits of nectar theft in reducing geitonogamy require pollinators to acquire significant pollen loads despite spending less time on an inflorescence, which is much less likely if pollen theft has significantly reduced the pollen available to pollinators. Thus, although pollen theft could produce the same benefits for plants as nectar theft, the necessary conditions are much more restrictive and the potential negative impacts of pollen theft are much greater.

### **Ecological consequences of pollen theft**

Studies that use the terminology of floral larceny to identify pollen thieves rarely measure the effects of theft on seed production or pollen export and/or import, so most available information concerning the effects of pollen theft on plant reproduction comes from literature on inefficient pollination and floral herbivory. Of the 37 studies that identify pollen theft in Table 2.1, only 12 considered the reproductive consequences (Table 2.3). The lack of studies that conclusively test (or even attempt to measure) the effects of pollen theft on plant performance is the greatest deficiency of the existing literature on floral larceny.

An important step in establishing whether pollen theft affects male and/or female fitness is to determine whether stolen pollen might otherwise have been deposited on

stigmas. Harder (2000, Harder and Routley 2006) identified three possible pollen fates during removal: removal failure, which occurs when pollen remains in anthers after visits cease; removal loss, which results when pollen is dislodged from anthers, but falls without being carried away by the flower visitor (i.e., due to sloppy pollinators); and pollen that is removed by visitors and so has a chance of being involved in self-pollination or exported to conspecific stigmas. If stolen pollen would otherwise have been involved in removal loss or removal failure, pollen theft should not compromise plant fitness. Indeed, a negative relation between removal loss and potentially exportable pollen may underlie the evolution of pollination by pollen-collecting bees (Harder and Wilson 1997). However, if pollen is stolen from the pool that would have been available for self-pollination and export, without a compensating reduction in removal loss, theft reduces siring opportunities and potentially male fitness. Unfortunately, determining the consequences of pollen theft for pollen fates will often be difficult. One uncommon exception involves theft of pollen directly from stigmas (Gross 1993, Gross and Mackay 1998). Because this pollen was already deposited successfully, it is clearly being stolen from the exported pollen pool. Given that only 1% of the pollen removed from flowers of species with granular pollen typically reaches appropriate stigmas (reviewed in Harder 2000), direct theft from stigmatic pollen loads almost certainly reduces the siring success of the pollen donors.

### *Male success*

Even though reduced male success is the most direct potential consequence of pollen theft, very few studies measure it. At the population level, the extent of pollen limitation of seed production indicates the failure of the male component of pollination and provides some indication of average male success. However, pollen theft may affect the siring success of individual plants, even in the absence of pollen limitation in the

population as a whole. Definitive demonstration that pollen theft reduces siring success requires the ability to identify and track pollen from specific pollen donors, so that export can be correlated with the intensity of pollen theft experienced by a given plant. The only study to employ this technique to date found that thieves reduced pollen export only when pollinators were scarce (Lau and Galloway 2004). An indirect, but more tractable method to assess whether pollen theft reduces pollen export involves correlating the amount of pollen removed or carried by legitimate pollinators with the intensity of pollen theft (e.g., do Carmo et al. 2004). Reduced pollen loads in the presence of thieves suggest that at least some of the stolen pollen would otherwise have been picked up by pollinators and thus available for export.

#### *Female success*

Pollen limitation of seed production, caused by depletion of the pollen available for dispersal by primary pollinators at the population level, is the most obvious potential effect of pollen theft on the maternal success of plants. Recent reviews indicate that seed set in many plant species may commonly be restricted by pollen receipt that is insufficient in either quantity or quality (Burd 1994, Larson and Barrett 2000, Ashman et al. 2004, Knight et al. 2005). Severe pollen theft clearly has the potential to cause quantitative pollen limitation, but theft might also cause quality limitation if thieves preferentially collect or consume higher quality grains. Alternatively, theft could improve the quality of pollen receipt if it decreased geitonogamy, as discussed above. Although pollen theft is probably far more common than indicated by the current literature, whether it commonly contributes to (or causes) pollen limitation is unknown, and deserves further study.

Pollen limitation resulting from pollen theft is difficult to demonstrate empirically. The best method to do so is to manipulate thief abundance at the population



level and test for an expected negative correlation between thief abundance and seed set. No published studies have employed this technique, but Bertness and Shumway's (1992) study of the effect of herbivory on pollen limitation in two highly protogynous saltmarsh grasses suggests its utility. In this system, grasshoppers consumed many flowering plants before they reached male phase, resulting in pollen limitation of undamaged plants. Exclusion of grasshoppers from grass stands increased the number of male-phase plants and significantly reduced pollen limitation (Bertness and Shumway 1992).

When thief abundance cannot be manipulated, the effect of theft on female success should be assessed by relating temporal or spatial variation in average seed set to measures of both pollen dispersal (e.g., pollen loads on pollinators) and the intensity of theft. It is important to distinguish between cross- and self-pollen, as thieves may deposit self-pollen without effecting cross-pollination in self-incompatible species (Moco and Pinheiro 1999). The only study to examine explicitly the effect of pollen theft on a dioecious species is perhaps the best example of pollen theft and its negative impacts on seed set. Exotic, pollen-collecting honey bees visited only male plants of the dioecious tree *Clusia arrudae*, thus clearly acting as pollen thieves (do Carmo et al. 2004). Honey bees reduced the pollen picked up by legitimate pollinators (resin-collecting bees) by >99% and seed set of female plants correlated negatively with honey-bee abundance (do Carmo et al. 2004).

## **Evolutionary consequences of pollen theft**

### *Adaptation of floral traits*

When pollen theft decreases plant reproduction differentially among phenotypes, selection should favour traits that reduce theft or its impact. Three types of non-exclusive evolutionary responses could accomplish this: 1) tolerate theft, but mitigate its effects; 2) co-opt pollen thieves to become pollinators; and 3) escape via anti-pollen theft

mechanisms. A given floral adaptation may serve more than one of these functions. Most of these adaptations should evolve primarily through their consequences for male function, as pollen theft affects this sex role most directly. However, as discussed below, some adaptations could arise through selection on female fitness, especially in largely self-pollinating species.

### Tolerance

Many factors influence floral evolution, including pollinators, herbivores, and abiotic conditions (Strauss and Whittall 2006), which may constrain the evolutionary ability of flowers to either escape pollen thieves or use them as pollinators. Nevertheless, floral evolution may still mitigate the effects of pollen theft by increasing pollen production (more flowers or more pollen per flower), much as mast seeding is proposed to (over)compensate for losses to seed predators (Kelly and Sork 2002). However, consistently elevated pollen production could simply increase the abundance of pollen thieves. Furthermore, because many pollen collectors forage longer on highly rewarding flowers or plants (see Indirect effects on plant reproduction), increased pollen production could also attract more thieves and/or increase geitonogamy. Increased pollen production may therefore be effective only in combination with floral mechanisms that limit pollen removal by individual pollinators (Harder and Thomson 1989).

To the best of my knowledge, no studies have tested for evidence of compensatory pollen production while controlling for phylogeny. The literature on pollination by pollen-collecting bees might provide related evidence, because their activity should similarly select for increased pollen production (see Cruden 2000). Buzz pollination, which usually involves pollen-rewarding flowers, is sometimes associated with unusually high pollen production per flower (Buchmann 1983; L.D. Harder unpublished data). However, a study of six buzz-pollinated *Pyrolaceae* species did not find consistently

lower pollen production in the two species that also produce nectar (Knudsen and Olesen 1993).

### Turning thieves into pollinators

When pollen theft causes pollen limitation at the population level, selection through female fitness would not favour anti-theft mechanisms per se, as the benefit would not accrue to specific individuals. Instead it should favour traits that cause pollen collectors to serve as pollinators, rather than thieves. Most pollen collectors pollinate when their foraging brings them into contact with appropriate stigmas, but act as thieves when this does not occur. Two suites of floral traits primarily determine whether pollen collectors steal pollen or pollinate: the separation of pollen-presenting organs from receptive stigmas, and the attractive features of female or female-phase flowers.

In perfect (bisexual) flowers, male and female function can be separated in space (herkogamy: Webb and Lloyd 1986), or time (dichogamy: Lloyd and Webb 1986). As herkogamy increases, so does the proportion of visitors whose bodies are too small to contact stigmas as they gather pollen, whereas if anthers and stigma(s) occupy similar positions (i.e., minimal herkogamy), most visitors that contact anthers will also contact stigmas incidentally (Table 2.1). Similarly, as dichogamy increases, pollen collectors will be more likely to visit functionally male flowers only and avoid functionally female flowers from which the pollen has been removed (protandry), or that have not yet presented their pollen (protogyny: Table 2.1). For example, Ish-Am and Eisikowitch (1993) reported that nectar-collecting honey bees were the primary pollinators of avocado, but pollen-collecting honey bees acted as thieves because they rarely visited female-phase flowers. Of the 48 reported cases of pollen theft from hermaphroditic species that identified why a visitor was a thief (Table 2.1; each case representing one plant species and one thief species or group), 22 resulted from herkogamy and six resulted from dichogamy. Thus flowers with reduced herkogamy and/or dichogamy are

more likely to be pollinated than thieved by pollen-collectors. However, both traits serve important roles in reducing self-pollination and interference between the sex roles (Lloyd and Webb 1986, Webb and Lloyd 1986), which will tend to counteract selection imposed by pollen theft.

In general, the effect of dichogamy on pollen theft will depend on whether pollen-collectors visit functionally female flowers, which in turn depends largely on their ability distinguish them from functionally male flowers (e.g., Ashman et al. 2005). Some hermaphroditic species possess pollen-mimicking structures to attract pollen-collecting pollinators to female-phase flowers, including imitation stamens, and pollen-like style colouration or corolla patterns (Lunau 2000). Similarly, some species pollinated by pollen-collecting bees produce both conspicuous ‘feeding’ anthers (often with dysfunctional pollen) and inconspicuous ‘pollinating’ anthers (heteranthery: Jesson and Barrett 2003). Attractiveness of female flowers need not always rely on deception. In one unusual case, pollen-robbing solitary bees seem to be the most effective pollinators of *Proboscidea arenaria* flowers, despite never contacting stigmas while robbing flowers. After collecting pollen from unopened *P. arenaria* flowers, accessed by climbing into the bud via a hole pierced in the corolla, bees emerge covered in pollen and invariably fly to an open, female-phase flower to feed on nectar, entering them legitimately and usually contacting stigmas (Hurd and Linsley 1963).

Concealment of pollen within floral structures can also manipulate pollen collectors into pollinating, instead of thieving. If animals cannot detect the presence or absence of pollen before visiting flowers (e.g., in poricidal anthers: Buchmann 1983), pollen collectors may be deceived into visiting female-phase flowers and effecting pollination. Concealed pollen may also enable flowers to force insects to contact the stigma on their way into the flower. The complex keel flowers of papilionaceous legumes, which dispense hidden pollen in relatively controlled amounts (Harder and

Wilson 1994) and force most pollen collectors to contact stigmas, have been interpreted as an adaptation for the use of pollen-collecting bees as primary pollinators (Westerkamp 1997). In general, plants whose primary pollinators are pollen collectors may be less likely to suffer from pollen theft than those that reward with nectar, as these species already possess mechanisms to ensure that pollen collection promotes pollen deposition. For such plants, I expect that most pollen theft involves animals that bypass pollen-dispensing mechanisms, such as the piercing of poricidal anthers by *Trigona* bees (McDade and Kinsman 1980, Renner 1983, Young 1983, Gross 1993).

#### Anti-theft adaptations

In general, adaptations to escape pollen theft should evolve through selection on male function, as theft reduces individual siring opportunities directly, whereas it usually affects female function indirectly. However, pollen theft could promote the evolution of anti-theft traits under selection on female fitness when it reduces the attractiveness of flowers to primary pollinators, or in largely self-pollinating species.

*Timing of pollen presentation* – A difference in the peak foraging time of pollen thieves and pollinators could select on the timing of pollen presentation. As most pollen thieves are diurnal insects (Table 2.1), flowers that open only nocturnally are much less susceptible to pollen theft. For example, several studies have observed insects stealing pollen from bat-pollinated flowers during the morning (Baker and Harris 1957 and references therein, Baker et al. 1971), but this theft is inconsequential for plants, because all possible pollination occurred during the preceding night (Baker et al. 1971, Gribel et al. 1999). Early morning anthesis could allow diurnal flowers to reduce pollen theft if pollinators begin foraging before pollen-collecting insects, as is common for nectarivorous birds (e.g., Ramsey 1988a, Hansen et al. 2002, Timewell and MacNally 2004; but see McDade and Kinsmen 1980) and possibly some euglossine bees (Pansarin et al. 2006). However, prior access by pollinators is not beneficial when thieves steal

pollen directly from stigmas, as stolen pollen will not be replaced by pollinators (Gross and Mackay 1998).

*Cryptic pollen* – Pollen can act as a visual or olfactory floral attractant (Dobson and Bergstrom 2000, Lunau 2000, Pernal and Currie 2002), so pollen concealment could reduce pollen theft. Visually cryptic or odourless pollen may reduce the attractiveness of flowers to pollen collectors, limiting visitation by potentially thieving species. Perfect flowers of some species produce less-fertile, but more visible, decoy pollen to attract pollen-feeding pollinators, whereas the viable pollen is cryptic, presumably to reduce its consumption (Vogel 1978, Hrycan and Davis 2005). Similarly, Faden (1992) found that the scent of nectarless *Palisota hirsuta* flowers originates entirely from sterile pollen in the upper stamens, presumably to lure pollen consumers away from the scentless fertile pollen. Cryptic pollen should be especially advantageous in species pollinated by nectarivores, for which pollen is less likely to serve as a signal. Bird-pollinated cacti are more likely to have brown-red pollen than their insect-pollinated counterparts, whose pollen is generally bright yellow (Rose and Barthlott 1994). Rose and Barthlott proposed that the dark pollen was cryptic to bird pollinators to avoid being groomed off, but cryptic pollen could also lessen detection by pollen thieves, as many pollen-collecting insects show innate preferences for highly contrasting and/or yellow pollen (Lunau 2000).

*Triggered pollen release* – Consistent differences between pollinators and pollen thieves in physical size, strength, or ability may select for trigger mechanisms that must be tripped to expose pollen. Ramsey (1988a&b) suggested that the explosive opening of *Banksia menziensis* flowers, which can be triggered only by birds, evolved to limit pollen loss to pollen-thieving staphylinid beetles. Flowers of some species, such as those in the Lecythidaceae (Mori et al. 1980), Fabaceae (Yeo 1993, Westerkamp 1997), and Polygalaceae (Westerkamp 1999), have hood-like petals or other protective structures that must be pushed aside to access rewards, preventing access by smaller visitors. Buzz

pollination, which requires vibration of poricidal anthers to release pollen, typically occurs in bee-pollinated plants that provide only pollen as a pollinator reward (Buchmann 1983) and enables many plant species to restrict pollen access to legitimate pollinators (Harder and Barclay 1994). Nevertheless, some buzz-pollinated species suffer pollen theft, either from small bees that can buzz only individual anthers and so do not contact stigmas (Renner 1983), *Trigona* bees that simply pierce or eat the poricidal anthers to access pollen (Renner 1983, Snow and Roubik 1987, Gross 1993), or theft of pollen deposited on stigmas and thus no longer protected (Gross and Mackay 1998).

*Inedible or toxic pollen* – Pollen that is unattractive or detrimental to pollen thieves could eliminate pollen theft altogether, especially in plants not pollinated by pollen consumers. For example, pollen may be rendered inedible by physical packaging into pollinia, as in most orchids (Orchidaceae: Johnson and Edwards 2000) and milkweeds (Asclepiadoideae). Many of the most common pollen consumers, including bees, do not collect or consume pollinia (Thorp 2000), and to our knowledge no incidences of pollen theft have been reported from either milkweeds or orchids (Table 2.1). The structure of individual grains may also deter certain pollen feeders. Vaissière and Vinson (1994) found that long spines on pollen grains of cotton (*Gossypium hirsutum*) and okra (*Abelmoschus esculentus*) hindered or prevented collection by honey bees.

In addition to structural deterrents, pollen can contain a wide array of secondary compounds, including many that are repellent or toxic to certain animals (Detzel and Wink 1993, Roulston and Cane 2000, Pimentel de Carvalho and Message 2004, Cintra et al. 2005). For example, certain volatile compounds in the pollen odours of wind-pollinated plants likely evolved to deter pollen thieves (Dobson and Bergstrom 2000). The effectiveness of deterrence depends on the ability of pollen thieves to recognize toxic pollen. Several laboratory studies have shown that honey bees can distinguish between

concentrations of secondary compounds either in pollen (phenolics: Liu et al. 2006), or sugar solutions (amygdalin: London-Shafir et al. 2003), and feed preferentially on pollen containing lower concentrations of them (but see Liu et al. 2004). Furthermore, honey bees often avoid toxic pollen as long as other pollen sources are available (London-Shafir et al. 2003, Pimentel de Carvalho and Message 2004, Liu et al. 2006).

The evolution of toxic pollen as an anti-theft mechanism probably depends on its effects on primary pollinators. The general implications of pollen toxicity are poorly known, because toxicity has been tested almost exclusively on honey bees, which are commercially important but often non-native pollinators. Interestingly, Cintra et al. (2003) found that pollen of two Brazilian tree species, *Stryphnodendron adstringens* (Fabaceae) and *Dimorphandra mollis* (Caesalpiniaceae), was significantly less toxic to native bee pollinators than to introduced honey bees. These contrasting effects suggest that pollen toxicity could evolve to deter pollen thieves if the thieves were more affected by toxicity than the primary pollinators, especially if the thieves had access to more palatable options. The effect of toxic pollen on pollen thieves and native pollinators is a highly intriguing subject for future research.

### *Sexual-system evolution*

In addition to floral traits, pollen theft may influence selection on aspects of plant mating systems, including the occurrence and form of sexual dimorphism and mechanisms of self-pollination. The most extreme sexual dimorphism is dioecy, with distinct male and female plants. From a pollen-collector's perspective, pollenless female plants are akin to a distinct, non-rewarding species. As long as pollen-collecting insects can detect the presence of pollen (by sensing it directly or recognizing male flowers), they need only visit male plants, and consequently will never contact female flowers to deposit pollen (Ashman 2000). Severe and chronic pollen theft should therefore select against complete



sexual segregation between male and female plants. Indeed, the high frequency of dioecy on islands that lack native social, pollen-collecting bees, including New Zealand (Godley 1975), Hawai'i (Carlquist 1974, Sakai et al. 1995), Mauritius (Baker 1877, as cited in Baker and Cox 1984) and the Ogasawara Islands of Japan (Abe 2006), may partially reflect the consequences of relief from pollen theft.

When pollen theft selects against pollenless female plants or flowers, it could promote the evolution of alternate forms of sexual dimorphism, or mechanisms to entice pollen collectors to visit female flowers. For example, Sakai (2001) speculated that androdioecy may have evolved from dioecy in *Castilla elasticus* to attract pollen-feeding thrips pollinators to otherwise unrewarding female plants. Alternatively, pollen consumption that involves significant floral damage could help maintain female plants in gynodioecious species, although this process could also promote female plants in dioecious species (reviewed in Strauss and Whittall 2006). Pollen theft may also select for cryptic dioecy, in which female plants produce sterile pollen to attract pollen-collecting pollinators (Anderson and Symon 1988, Mayer and Charlesworth 1991, Kawagoe and Suzuki 2004), or male-mimicry, in which female plants mimic scent cues of male flowers, achieving pollination by deception (e.g., figs: Grison-Pige et al. 2001).

Finally, pollen theft could affect the evolution of reproductive assurance in self-compatible species. Delayed selfing, in which flowers self-pollinate autonomously after opportunities for cross-pollination have largely passed, is arguably the ideal strategy for reproductive assurance because, unlike prior-selfing or apomixis, it does not compromise opportunities for outcrossing (Lloyd 1992). However, delayed selfing requires limited pollen removal, so that self-pollen remains on the flower to pollinate unfertilized ovules. Obviously, pollen removal by thieves reduces the opportunity for delayed selfing (Vaughton 1996). Accordingly, selection for reproductive assurance in plants that

experience intense pollen theft may favour apomixis (Renner 1983), or autonomous self-pollination before pollen is exposed to floral visitors.

### **Concluding remarks**

Pollen theft is a more direct, and therefore undoubtedly more significant, influence on the ecology and evolution of pollination systems than nectar theft, but strangely has been overlooked despite growing interest in cheating on mutualisms (e.g., Bronstein 2001, Foster and Kokko 2006) and floral larceny (Irwin et al. 2001). Pollen theft may contribute to many aspects of pollination biology, including the widespread occurrence of pollen limitation, and provide a functional explanation for unusual floral adaptations, such as cryptic or toxic pollen. Throughout this review, I highlighted deficiencies in current knowledge of pollen theft and have provided many testable hypotheses about floral vulnerability and adaptations. Despite formidable methodological problems in studying pollen theft, including the difficulties of quantifying pollen (rather than gene) dispersal and the challenges of manipulating pollen theft at the population level, it is likely to receive much more future attention because of its impacts on many fundamental aspects of plant reproduction. In particular, the following questions must be addressed to elucidate the global importance of pollen theft.

#### *How widespread is pollen theft and which species are involved?*

The incidence of pollen theft in space and time, both among and within plant taxa, is poorly known. Pollen theft is likely underreported in studies that focus on effective pollinators, in which case the reports of pollen theft summarized in Table 2.1 grossly underestimate its occurrence. Quantification of temporal and spatial variation in pollen theft will enable assessment of its role in the selection of plant reproduction, whereas more complete documentation of its occurrence will reveal whether certain plant or animal groups (e.g., eusocial bees) are disproportionately involved.

*How often does pollen theft affect plant reproduction?*

Pollen theft can clearly impede pollination and seed production, but the frequency of this effect remains unknown. More complete tests of the effects of pollen theft on plant reproduction, especially experiments involving direct manipulation of thief abundance, are needed to assess its true ecological and evolutionary importance. Of particular urgency is the need to understand the ecological consequences of pollen theft by exotic eusocial bees for native plant species. The importance of pollen theft compared to other interactions that can limit reproductive potential, such as seed and seedling predation, is also unknown, though this in part reflects a general lack of studies that examine reproduction both before and after seed production.

*How does pollen theft affect the evolution of plant reproduction?*

Much remains to be understood about the evolutionary implications of pollen theft, and this provides an exciting topic for future research. Several of the plant adaptations that I propose mitigate pollen theft could be tested experimentally, including the effects of compensatory pollen production on pollination success and of toxic pollen on pollinating versus non-pollinating visitors. Correlative studies could also illuminate the influence of pollen theft on plant mating-system evolution. For example, has dioecy evolved more frequently in the absence of social pollen collectors, and is cryptic dioecy associated with the presence of pollen thieves? Finally, the unfortunate, but widespread, introduction of exotic species provides an excellent opportunity for testing hypotheses about the evolutionary implications of pollen theft, such as whether plants that evolve in the absence of certain pollen collectors are more vulnerable to theft, and whether altered incidences and/or intensities of pollen theft are responsible for differential survival of species with different sexual systems (e.g., Sjöström and Gross 2006). The answers to

these questions will provide new insight into the historic and current potential of non-pollinating visitors to influence floral evolution.

Table 2.1 Documented occurrences of pollen theft, including the plants and animals involved and factors that contribute to theft

| Pollen thief                | Plant species                | Pollinator       | Primary       | Sexual | Why animal acts as thief | Does thief | Reference      |
|-----------------------------|------------------------------|------------------|---------------|--------|--------------------------|------------|----------------|
|                             | Family                       |                  | floral reward | system |                          | pollinate? |                |
| Hymenoptera, Apoidea (bees) |                              |                  |               |        |                          |            |                |
|                             | <i>Apis mellifera</i>        |                  |               |        |                          |            |                |
|                             | <i>Parkia clappertoniana</i> | bats             | N             | H      | herkogamy                | U          | Baker & Harris |
|                             | Mimosaceae                   |                  |               | (hk)   |                          |            | 1957           |
|                             | <i>Callistemon rugulosus</i> | honey eaters     | N             | H      | herkogamy*               | Y          | Paton 1993     |
|                             | Myrtaceae                    |                  |               |        |                          |            |                |
|                             | <i>Salvia glutinosa</i>      | <i>Bombus</i>    | N             | H      | herkogamy                | U          | Westerkamp     |
|                             | Lamiaceae                    |                  |               |        | behaviour a)             |            | 1991           |
|                             | <i>Melastoma affine</i>      | large buzz-      | P             | H      | behaviour a): collects P | O          | Gross &        |
|                             | Melastomataceae              | pollinating bees |               | (bz)   | from stigma*             |            | Mackay 1998    |
|                             | <i>Correa reflexa</i>        | honey eaters     | N             | H      | dichogamy*               | Y          | Paton 1993     |

|                            |              |   |        |                         |           |                 |
|----------------------------|--------------|---|--------|-------------------------|-----------|-----------------|
| Rutaceae                   |              |   |        |                         |           |                 |
| <i>Persea americana</i>    | nectar-      | N | H      | dichogamy               | Y: nectar | Ish-Am &        |
| Lauraceae                  | collecting   |   | (♀♂)   |                         | foragers  | Eisikowitch     |
|                            | honey bees   |   |        |                         | only      | 1993            |
| <i>Grevillea barklyana</i> | birds        | N | H      | dichogamy*              | O         | Vaughton 1996   |
| Proteaceae                 |              |   | (♂♀)   | reduced delayed selfing |           |                 |
| <i>Clusia arrudae</i>      | resin-       | R | D      | dioecy*                 | N         | do Carmo et al. |
| Clusiaceae                 | collecting   |   |        |                         |           | 2004            |
|                            | bee          |   |        |                         |           |                 |
| also Refs 1*, 2*           |              |   |        |                         |           |                 |
| <i>Trigona</i> spp.†       |              |   |        |                         |           |                 |
| 34 unspecified spp.        | large buzz-  | P | H      | herkogamy               | N         | Renner 1983     |
| <i>Melastoma affine</i>    | pollinating  |   | (bz)   | behaviour b)            |           | Gross 1993      |
| Melastomataceae            | bees         |   |        |                         |           |                 |
| <i>Aphelandra</i>          | hummingbirds | N | H      | herkogamy               | U         | McDade &        |
| <i>golfodulcensis</i>      |              |   | (A.g.= | behaviour a)            |           | Kinsman 1980    |
| <i>Justica aurea</i>       |              |   | hk)    |                         |           |                 |

|                             |                     |    |      |                     |   |                |
|-----------------------------|---------------------|----|------|---------------------|---|----------------|
| Acanthaceae                 |                     |    |      |                     |   |                |
| <i>Cochlospermum</i>        | <i>Xylocopa</i> &   | P  | H    | herkogamy or        | U | Snow & Roubik  |
| <i>vitifolium</i>           | <i>Centris</i> bees |    | (bz) | behaviour a)        |   | 1987           |
| Cochlospermaceae            |                     |    |      |                     |   |                |
| <i>Medicago sativa</i>      | bees                | N? | H    | behaviour a)        | ? | Tezuka &       |
| <i>Astragalus sinicus</i>   |                     |    |      |                     |   | Maeta 1995     |
| Fabaceae                    |                     |    |      |                     |   |                |
| <i>Thunbergia</i>           | bees                | N  | H    | ? chew off anthers* | ? | Young 1983     |
| <i>grandiflora</i>          |                     |    |      |                     |   |                |
| Acanthaceae                 |                     |    |      |                     |   |                |
| <i>Bromelia antiacantha</i> | hummingbirds,       | N  | H    | ?                   | ? | Canela &       |
| Bromeliaceae                | maybe <i>Bombus</i> |    |      |                     |   | Sazima 2005    |
|                             |                     |    |      |                     |   | also Refs 2, 3 |
| Halictidae                  |                     |    |      |                     |   |                |
| <i>Datura</i> spp.          | hawkmoths           | N  | H    | herkogamy           | U | Thorp 2000     |
| Solanaceae                  |                     |    |      |                     |   |                |

|                                |                     |       |      |                   |           |  |
|--------------------------------|---------------------|-------|------|-------------------|-----------|--|
| 34 unspecified spp.            | large buzz-         | P     | H    | herkogamy         | some spp. | Renner 1983                            |
| Melastomataceae                | pollinating<br>bees |       | (bz) |                   |           |  |
| <i>Campanula<br/>americana</i> | <i>Bombus</i>       | P     | H    | dichogamy<br>(♂♀) | L         | Lau &<br>Galloway 2004                 |
| Campanulaceae                  |                     |       |      |                   |           |  |
| <i>Passiflora foetida</i>      | Colletidae          | N     | H    | ?                 | ?         | Garcia & Hoc<br>1998<br>also Refs 1, 2 |
| Passifloraceae                 |                     |       |      |                   |           |  |
| <i>Perdita</i> spp.            |                     |       |      |                   |           |  |
| <i>Mentzelia decapetala</i>    | sphingid moths      | N     | H    | herkogamy         | N         | Michener 1979                          |
| Loasaceae                      |                     |       |      |                   |           |  |
| <i>Opuntia phaeacantha</i>     | large bees          | N?    | H    | herkogamy         | N         | Barrows et al.<br>1976                 |
| Cactaceae                      |                     |       |      | behaviour a)      |           |  |
| <i>Proboscidea arenaria</i>    | same as thief       | N & P | H    | behaviour a)      | Y         | Hurd & Linsley<br>1963                 |
| Martyniaceae                   |                     |       |      |                   |           |  |
| <i>Bombus</i> spp.             |                     |       |      |                   |           |  |



|                          |                |         |        |                           |   |                  |
|--------------------------|----------------|---------|--------|---------------------------|---|------------------|
| <i>Tolmiea menziesii</i> | fungus gnats   | N       | H      | dichogamy                 | N | Goldblatt et al. |
| Saxifragaceae            |                |         | (♂♀)   | small P loads             |   | 2004             |
|                          |                |         |        |                           |   | also Ref 1       |
| mixed bees               |                |         |        |                           |   |                  |
| <i>Ceiba pentandra</i>   | bats           | N       | H      | herkogamy                 | N | Baker & Harris   |
| Bombacaceae              |                |         | (hk)   | timing                    |   | 1959             |
| <i>Ceiba acuminata</i>   | bats           | N       | H      | herkogamy                 | N | (1) Baker et al. |
| Bombacaceae              |                |         | (hk)   | timing                    |   | 1971             |
| <i>Swartzia apetala</i>  | buzz-          | P       | H      | herkogamy                 | L | (2) Moco &       |
| Fabaceae                 | pollinating    |         | (bz)   | behaviour c)              |   | Pinheiro 1999    |
|                          | solitary bees  |         |        |                           |   |                  |
| <i>Pongamia pinnata</i>  | larger bees    | N & P   | H      | behaviour b) too small    | Y | (3) Raju & Rao   |
| Fabaceae                 |                |         | (keel) | to depress keel petals    |   | 2006             |
| 8 spp. (6 genera)        | oil-collecting | oil, ~P | H      | behaviour b): rarely      | O | Sigrist &        |
| Malpighiaceae            | bees           |         | (♀♂)   | rupture stigmatic cuticle |   | Sazima 2004      |
|                          |                |         |        | as needed to deposit P    |   |                  |
| Coleoptera (beetles)     |                |         |        |                           |   |                  |

|                             |                      |       |      |                        |   |                  |
|-----------------------------|----------------------|-------|------|------------------------|---|------------------|
| <i>Isomeris arborea</i>     | likely <i>Bombus</i> | N & P | A    | larvae consume         | N | Krupnick &       |
| Capparaceae                 |                      |       |      | developing anthers     |   | Weis 1999,       |
|                             |                      |       |      |                        |   | Krupnick et al.  |
|                             |                      |       |      |                        |   | 1999             |
| <i>Banksia menziesii</i>    | honey eaters         | N     | H    | don't deposit enough P | N | Ramsey           |
| Proteaceae                  |                      |       | (♂♀) | to pollinate           |   | 1988a&b          |
| <i>Aconitum lycoctonum</i>  | long-tongued         | N     | H    | ?                      | ? | Utelli & Roy     |
| Ranunculaceae               | <i>Bombus</i>        |       | (♂♀) |                        |   | 2001             |
| <i>Trevoa quinquenervia</i> | flies, bees          | N & P | A    | ?                      | L | Medan &          |
| Rhamnaceae                  | & beetles            |       | (♂♀) |                        |   | D'Ambrogio       |
|                             |                      |       |      |                        |   | 1998             |
| Diptera (flies)             |                      |       |      |                        |   |                  |
| <i>Plantago</i> sp.         | wind                 | -     | H    | herkogamy?             | ? | Holloway 1976    |
| Plantaginaceae              |                      |       |      | small P loads          |   |                  |
| grasses                     |                      |       |      |                        |   |                  |
| Poaceae                     |                      |       |      |                        |   |                  |
| <i>Tolmiea menziesii</i>    | fungus gnat          | N     | H    | dichogamy              | N | Goldblatt et al. |

|                            |              |       |      |      |                           |   |                 |
|----------------------------|--------------|-------|------|------|---------------------------|---|-----------------|
| Saxifragaceae              |              |       |      | (♂♀) | small P loads             |   | 2004            |
| <i>Centropogon</i>         | hummingbirds | N     | H    |      | larvae eat anthers in bud | N | Weiss 1996      |
| <i>solanifolius</i> †      |              |       | (dc) |      | & leave before flower     |   |                 |
| Campanulaceae              |              |       |      |      | matures                   |   |                 |
| Other                      |              |       |      |      |                           |   |                 |
| mites & thrips             |              |       |      |      |                           |   |                 |
| <i>Hamelia patens</i>      | hummingbirds | N     | H    |      | Herkogamy                 | U | Paciorek et al. |
| Rubiaceae                  |              |       |      |      | behaviour c)              |   | 1995            |
| <i>Actinidia deliciosa</i> | bees         | P     | D    |      | ?                         | ? | Kirk 1987       |
| Actinidaceae               |              |       |      |      |                           |   |                 |
| <i>Echium plantagineum</i> | bees         | N & P | H    |      | eat P from stigmas        |   |                 |
| Boraginaceae               |              |       |      |      |                           |   | also Ref 3      |
| unknown insect†            |              |       |      |      |                           |   |                 |
| <i>Gelsemium</i>           | bees         | N     | H    |      | never observed            | ? | Leege & Wolfe   |
| <i>sempervirens</i>        |              |       | (ds) |      |                           |   | 2002            |
| Loganiaceae                |              |       |      |      |                           |   |                 |
| snails & slugs†            |              |       |      |      |                           |   |                 |

|  |              |   |      |   |   |                        |
|--|--------------|---|------|---|---|------------------------|
| <i>Streptocarpus</i> sp.                     | insects      |   | H    | ? presumably do not carry P   | N | Neijzing & Zeven 1976  |
| Gesneriaceae                                 |              |   |      |   |   |                        |
| <i>Primula elatior</i>                       | bees & flies | . | H    | ? presumably do not carry P   | ? | Oleson 1979            |
| Primulaceae                                  |              |   | (ds) |   |   |                        |
| <i>Enicognathus ferrugineus</i> (parakeet) † |              |   |      |   |   |                        |
| <i>Nothofagus pumilio</i>                    | wind         | - | D    | preferentially consumes ♂ flowers, damages ♀ flowers instead of pollinating | N | Diaz & Kitzberger 2006 |
| Nothofagaceae                                |              |   |      |   |   |                        |

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All information is that given in the referenced study. P=pollen. Sexual system: H=hermaphroditic, D=dioecious, A=andromonoecious. Additional information about floral design is included in parentheses below when given in the study: hk=herkogamous, bz=buzz-pollinated, dc=dichogamous (♂♀=protandrous, ♀♂=protogynous), ds=distylous, keel=keel flower. Primary floral reward (P is not listed as a reward unless collected by the plant's pollinators): N=nectar, R=resin, ~P=pollen collected occasionally. The reason that the pollen thief is designated as such includes: herkogamy (thief is too small to contact stigma while collecting/consuming P); dichogamy (thief visits primarily/only male-phase flowers); dioecy (thief visits only male plants); behaviour (thief's pollen-collecting behaviour is inappropriate for pollinating, because it a) enters or manipulates flowers in the 'wrong' position for stigma contact, b) is unable to trip a trigger required for pollination, or c) does not move between plants in self-incompatible

species), timing (thief visits flowers after opportunities for pollination are over). Incidence of thief pollination: Y=yes, N=no, O=occasionally (i.e., seldom or limited), U=unlikely and L=likely (i.e., study did not test).

\* Thief is not native where pollen theft occurs.

† Thief acts as a pollen robber, damaging the flower to access pollen.

Table 2.2 Differences between pollen and nectar and the consequences of their theft for plant fitness. Note that theft from flowers does not have positive direct consequences.

|                                  | Pollen   | Nectar   |
|----------------------------------|--|--|
| <i>Reward characteristics</i>    |  |  |
| Importance for plants            | attractant & reward,<br>carrier of male gametes  | attractant & reward  |
| Importance for visitors          | source of protein and lipids   | source of carbohydrates  |
| Replaceable?                     | no   | usually  |
| <i>Reward theft</i>              |  |  |
| Flowers involved                 | functionally male only   | all  |
| Potential effect on reproduction |  |  |
| negative indirect                | pollinator limitation  | pollinator limitation,<br>resource limitation from<br>nectar replacement |
| negative direct                  | lost siring opportunities,<br>pollen limitation  | none   |
| positive indirect                | reduced geitonogamy &<br>pollen discounting, (only<br>realized if sufficient<br>pollen remains for<br>dispersal after theft) | reduced geitonogamy &<br>pollen discounting                              |
| positive direct                  | none   | none   |

Must thief contact plant    yes – male always                      no

reproductive structures?

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Table 2.3 Summary of studies that assessed the effects of pollen theft on plant fitness

| Plant species<br>Family                       | Pollen<br>thief | Does thief<br>pollinate? | Consequence of pollen theft for plant reproduction  |   | Reference                 |
|---|-----------------|--------------------------|---|---|---------------------------|
|   |                 |                          | Male  | Female  |                           |
| <i>Clusia arrudae</i><br>Clusiaceae           | honey<br>bees*  | N                        | pollinator P loads reduced by<br>>99.9%   | frequency of theft negatively<br>correlated with seed set   | do Carmo et<br>al. 2004   |
| <i>Melastoma affine</i><br>Melastomataceae    | honey<br>bees*  | O                        | reduced siring success of<br>donors whose P is removed                                    | theft from stigma reduced fruit &<br>seed set when last visitor was a<br>thief                                | Gross &<br>Mackay<br>1998 |
| <i>Callistemon<br/>rugulosus</i><br>Myrtaceae | honey<br>bees*  | Y                        | —   | under high bee abundance fruit<br>set decreased in open plants, but<br>increased in absence of<br>pollinators | Paton 1993                |
| <i>Correa reflexa</i><br>Rutaceae             | honey<br>bees*  | Y                        | simulation of theft by anther<br>removal in aviary reduced P<br>loads on bird pollinators | as for male   | Paton 1993                |



|                            |          |           |                               |                                   |              |
|----------------------------|----------|-----------|-------------------------------|-----------------------------------|--------------|
| <i>Grevillea barklyana</i> | honey    | O         | —                             | theft reduced seed set below that | Vaughton     |
| Proteaceae                 | bees*    |           |                               | of unvisited flowers by           | 1996         |
|                            |          |           |                               | preventing delayed selfing        |              |
| <i>Campanula</i>           | Halictid | Y?        | decreased siring success when | —                                 | Lau &        |
| <i>americana</i>           | bees     |           | pollinators rare              |                                   | Galloway     |
| Campanulaceae              |          |           |                               |                                   | 2004         |
| <i>Ceiba pentandra</i>     | bees     | N         | none: theft occurs after      | as for male                       | Baker &      |
| Bombacaceae                |          |           | pollination finished          |                                   | Harris 1959  |
| <i>Ceiba acuminata</i>     | bees     | N         | none: theft occurs after      | as for male                       | Baker et al. |
| Bombacaceae                |          |           | pollination finished          |                                   | 1971         |
| <i>Proboscidea</i>         | solitary | Y but not | unlikely: after robbing buds, | as for male                       | Hurd &       |
| <i>arenaria</i>            | bee†     | while     | bees visit open flower for    |                                   | Linsley      |
| Martyniaceae               |          | robbing)  | nectar & pollinate            |                                   | 1963         |
| <i>Isomeris arborea</i>    | beetle   | N         | damage reduced P export,      | damage reduced P receipt,         | Krupnick &   |
| Capparaceae                |          |           | outcrossing rate unchanged    | frequency of geitonogamy          | Weis 1999    |
|                            |          |           |                               | unchanged                         |              |

|                           |           |   |                          |                                   |             |
|---------------------------|-----------|---|--------------------------|-----------------------------------|-------------|
| <i>Isomeris arborea</i>   | beetle    | N | reduced visitation       | reduced visitation                | Krupnick et |
| Capparaceae               |           |   |                          |                                   | al. 1999    |
| <i>Centropogon</i>        | flies     | N | theft reduced male phase | —                                 | Weiss 1996  |
| <i>solanifolius</i>       |           |   | duration                 |                                   |             |
| Campanulaceae             |           |   |                          |                                   |             |
| <i>Nothofagus pumilio</i> | parakeet† | N | —                        | theft reduced seed density 6-fold | Diaz &      |
| Nothofagaceae             |           |   |                          | compared to parrot-excluded       | Kitzberger  |
|                           |           |   |                          | branches                          | 2006        |

In all columns P=pollen.

\*Thief is not native where pollen theft occurs.

†Thief acts as a pollen robber, damaging the flower to access pollen.

## CHAPTER 3 - POLLEN THEFT REDUCES THE REPRODUCTIVE SUCCESS OF BIRD- AND INSECT-POLLINATED *ALOE MACULATA*

### Introduction

More than 80% of angiosperm species rely on animals to transfer their pollen from anthers to conspecific stigmas (Eriksson and Bremer 1992). Most plants obtain this service by offering floral rewards, typically nectar and/or pollen, to promote pollinator visitation. However, floral visitors vary widely in their morphology and behaviour, and many are capable of ‘stealing’ floral rewards without effecting significant pollination in return (Inouye 1980). Although the effects of nectar theft on plant reproduction have been considered extensively during recent decades (Irwin et al. 2001), the implications of pollen theft have been largely overlooked and seldom quantified, even though it potentially bears more serious fitness consequences for plants (Chapter 2)<sup>1</sup>.

Pollen is unique among floral rewards in functioning as both a carrier of gametes and an attractant and nutritious reward for pollinators. Pollen theft could therefore affect plant reproduction either directly, by reducing the pollen available for dispersal, or indirectly, by altering pollinator behaviour. The universally negative direct effects of pollen theft are intuitive to understand, but poorly studied. Pollen theft can directly reduce aspects of pollination associated with siring success, including the amounts of pollen carried by pollinators (Paton 1993, do Carmo et al. 2004) and exported to stigmas (Gross and Mackay 1998), although the magnitude of such effects can depend on the abundance of efficient pollinators (Lau and Galloway 2004). This removal of pollen from dispersal could additionally reduce average female success if insufficient pollen import limits seed production (do Carmo et al. 2004). With the exception of Lau and

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<sup>1</sup> ‘Theft’ refers to deliberate reward removal without significant pollination, whereas robbery refers to specific instances of theft that cause flower damage (see Chapter 2).

Galloway (2004), all of the studies that demonstrate negative consequences of pollen theft for plants examined pollen theft by non-native animals, which may be more prone to acting as thieves on plants with which they have not evolved (Chapter 2).

Pollen theft can also affect pollination indirectly, potentially offsetting its negative direct effects to some degree. If pollen-collecting pollinators leave less-rewarding plants earlier, pollen theft could reduce self-pollination between flowers on a plant (geitonogamy: Harder and Barrett 1995), leaving a higher proportion of the pollen they remove available for export to other plants. In contrast, theft would intensify pollinator limitation if pollinators use the presence or abundance of pollen to assess floral reward availability (Dobson and Bergstrom 2000, Lunau 2000) and thereby avoid pollen-thieved flowers. These indirect effects of pollen theft have been tested rarely, but Krupnick and Weiss (1999) found that pollen robbery indirectly reduced pollen export, but not self-pollination.

The impact of pollen theft on reproduction by animal-pollinated plants can be difficult to assess, and few studies have attempted to do so experimentally. As pollen thieves are usually small insects (Chapter 2), excluding them without affecting pollinators is often impossible. Thus, the few studies of how pollen theft affects plant fitness have generally relied on correlative approaches (e.g., do Carmo et al. 2004). Wind-pollinated plants are an exception to this difficulty and demonstrate the usefulness of an experimental approach; exclusion florivores that preferentially consume male flowers from dioecious and protogynous plants has increased seed set of female-phase flowers by increasing the number of male flowers and pollen availability (Bertness and Shumway 1992, Diaz and Kitzberger 2006 and references therein).

An alternative to excluding pollen thieves is to increase their numbers experimentally. Honey bees (*Apis mellifera* L.) are the most commonly documented pollen thieves (Chapter 2). Because foraging workers return to their hives each night,

many honey bees can be added and then removed for specific periods using commercial bee hives. I used this approach here to assess the population-level effects of pollen theft for a South African plant, *Aloe maculata* (Medic.). This species is pollinated primarily by sunbirds (Nectariniidae: M. Vaas, unpublished dissertation), but native honey bees commonly collect pollen from it without providing significant pollination (M. Vaas, unpublished dissertation). This study included two components. First, I compared pollination and seed production in four *A. maculata* populations under normal and increased densities of pollen thieves. Second, to compare the pollination effects of visits by birds alone with those by both birds and honey bees, I measured pollination in the aviary using potted *A. maculata* plants. This study represents the first attempt to manipulate thief abundance experimentally at the population level, and one of the few to test the effect of native pollen thieves on seed production.

## Methods

### *Study species and location*

*Aloe maculata* is a mid-sized succulent monocot, common throughout its South African range (Van Wyk and Smith 2003). Its branched inflorescences rarely grow taller than 1 m and typically produce one to four terminal racemes with 30 to 200 flowers each. Flowers are protandrous and herkogamous, typically bright orange, with long, narrow, fused corollas constricted at the base and opening. Flowers can open at any time of day and anthers dehisce one by one throughout the day and to a lesser extent during night (Chapter 4). *Aloe maculata* is visited frequently by nectar-feeding sunbirds and a variety of insects, predominantly pollen-collecting bees. The narrow corollas prevent most honey bees from accessing the nectar at the base of a flower, but smaller bees can easily crawl into flowers. Honey and other small bees (Halictidae and Apidae) sometimes

consume nectar that seeps through holes pecked in corollas by nectar-robbing, short-billed birds.

This study was conducted in KwaZulu Natal Province, South Africa, in four populations separated by 5 to 200 km: Hilton College private school grounds (Hilton; 29°29'S, 30°18'E), a private game reserve near Ixopo (Ixopo; 30°07'S, 30°09'E), Klipfontein ranch (Klipfontein; 28°50'S, 29°40'E), and Umgeni Valley Nature Reserve (Umgeni; 29°28'S, 30°17'E). All sites consisted of open grassland within larger natural areas, and contained *A. maculata* populations of differing sizes (Table 3.1).

### *Self-compatibility*

Self-compatibility was assessed by enclosing inflorescences in fine-mesh fabric to exclude all visitors and applying either self or outcross pollen as stigmas became receptive. To assess whether *A. maculata* can self-pollinate autonomously, I bagged inflorescences of five, 10 and 26 plants at Hilton, Klipfontein and Umgeni, respectively. Data from hand-pollinations were combined with those from an earlier, identical experiment at other *A. maculata* populations in the area (SD Johnson unpublished data), and seed and fruit set were compared to those from the autonomous self-pollination treatment. Plants in the autonomous treatment frequently produced swollen ovaries that looked like fruits, but contained no seeds. Such ovaries were counted as fruits for measures of fruit set but were not included in seed-set calculations.

### *Field experiment*

To test the effects of pollen theft at the population level, two commercial hives of 20 000 to 40 000 *Apis mellifera scutellata* (the local native subspecies) were added at each population for approximately half of the flowering period (9 to 14 days). Hives were added and removed before sunrise or after sunset so that all workers belonging to a hive were contained when it was moved. The order of the bee treatments ('hives present' and

‘natural’) was assigned randomly among sites to equalize the effects of preferential resource allocation to early or late (i.e., bottom or top) flowers within inflorescences (Corbet 1998). To distinguish between bee treatments, I tied a string around each study inflorescence between open and unopened flowers when the bee treatment changed. At Klipfontein, flowering lasted long enough to allow a second ‘natural’ treatment after hives were removed. At Umgeni, which experienced the hives-present treatment first, I marked 10 plants that were halfway through flowering at the beginning of the experiment to measure pollination success prior to hive-addition.

To assess the importance of bird pollinators and whether the effect of pollen theft differed in their absence, I applied one of three treatments to 55 to 82 randomly assigned *A. maculata* plants per population: 1) caged with rigid plastic mesh to exclude birds, but not insects; 2) unmanipulated and open to all visitors; and 3) pollen supplemented, to determine whether pollen theft caused or aggravated pollen limitation of seed set. Plants in the supplementation treatment were exposed to natural pollination and additional outcross pollen was applied by hand to as many flowers as possible (Table 3.1). Donor anthers were collected within 7 h of hand-pollinations from plants at least 10 m from the recipient and kept in a centrifuge tube while pollinations were being conducted. Individual flowers received pollen from two or more donors, applied with a toothpick to stigmas of all open flowers. Individual plants experienced the same pollination treatment during their entire flowering period, but could experience both bee treatments. Significantly higher seed production by pollen-supplemented versus unmanipulated plants is interpreted to indicate that seed set is limited by the quantity of pollen received (Bierzychudek 1981, Young and Young 1992).

The abundances of avian and insect flower visitors at each site were evaluated during both bee treatments. Preliminary observations indicated that bird activity peaked during midmorning (900-1100 h), whereas insect activity peaked during early to mid

afternoon (1400-1600 h), so I conducted morning and afternoon bird and insect surveys at these times. I estimated insect visitation by choosing 20 flowering *A. maculata* plants and counting the insects present on each one. Plants were observed only for as long as it took to count and record the insects on or flying around the inflorescence. Insects were identified to group (e.g., honey bee, small bee, wasp), and specimens were collected and sent for identification to C. Eardley at the South African Agricultural Research Council. To estimate bird abundance, I walked a fixed 30-min transect through the study population, with five stops of 5 min each plus 5 min for walking between stops. All birds seen or heard within 10 m of transects were counted, and identified if possible. Only records of species seen visiting *A. maculata* flowers were retained for abundance analyses. The number of flowering *A. maculata* plants on each transect was also counted, to estimate the pollinating birds/flowering plant.

To investigate factors that may influence whether honey bees act as pollen thieves or pollinators, I opportunistically observed their foraging behaviour on *A. maculata* flowers. During these observations I noted which resource was collected (pollen or nectar), dichogamy phase (male or female), and whether the bee contacted the stigma. I also observed bird foraging to determine whether birds visited young or old (i.e., male and female) flowers preferentially, and how often they moved between plants.

I evaluated the effects of bee and pollination treatments on pollen removal and receipt at each site. Two recently wilted flowers were collected from every caged and open plant during both bee treatments, and anthers and stigmas were stored in 70% ethanol until they could be processed. Freshly opened anthers were also collected from one flower on each of nine bagged plants to assess pollen production. I later counted the pollen grains remaining on anthers (using an Elzone particle counter as per Harder and Aizen 2004) and those on stigmas under a compound microscope (100x). Examination of hand-pollinated stigmas and pistils (Chapter 4) revealed that pollen tubes from outcross



and self pollen grains both reach the ovary and were visually indistinguishable. I therefore counted only pollen grains on the stigma after soaking stigmas in basic fuchsin stain for at least 4 h, and squashing them in glycerin.

On every caged, open and pollen supplemented plant I counted the fruits produced during each bee treatment and the seeds in three or four fruits per bee treatment, which were then averaged for each plant-bee treatment combination. Because it was impossible to distinguish swollen, seedless ovaries from true fruits that contained seeds, such ovaries were included in fruit set counts and their seed count (0) was included in seed set calculations. A large *A. maculata* plant can produce >1000 flowers, so I did not count the flowers exposed per bee treatment, but instead estimated the proportion of each raceme exposed to each bee treatment. These estimates were summed for every plant to produce the variable ‘flowering proportion’ (e.g., a plant with two racemes of which 0.5 and 0.35 were open during the with-hives treatment would have a ‘flowering proportion’ of 0.85 for this treatment). To estimate a single, complete measure of female fecundity, I calculated total seed production by multiplying the average seeds/fruit by the total number of fruits for each bee treatment experienced by a plant. All analyses of fruit production and total seed production included ‘flowering proportion’ as a factor to correct for potentially differing flower abundance among treatments.

Flower and seed predation varied among and within sites during this study. If birds or insects damaged more than 10% of flowers per raceme, I estimated the damaged proportion of each raceme and subtracted half of this amount (as roughly half of damaged flowers produced fruit) from the ‘flowering proportion’ during the relevant bee treatment. To account for seed predators I counted the number of cocoons, larvae and flies inside each fruit for which seeds were counted.

### *Aviary experiments*

Insects cannot be excluded from flowers in the field without also excluding birds, so I also tested the effect of honey bees on pollination of *A. maculata* in an aviary experiment, using two captive Amethyst Sunbirds (*Chalcomitra amethystina* Shaw, the most common avian visitor to *A. maculata* flowers), and a small nucleus hive of ca. 1000 honey bees. Arrays of 14 *A. maculata* inflorescences were arranged in two outdoor aviaries (3 x 1 x 2 m). One array was comprised of potted plants and the second of cut inflorescences with their stems in water. The latter inflorescences flowered normally and produced nectar for more than a week. Inflorescences were given a unique identifier so they could be distinguished during observations.

Pollination trials were conducted by adding a sunbird to an aloe array for 1 h during morning and 1 h during afternoon and allowing it to forage for nectar. During each trial I recorded which plants a bird visited, the number of probes to flowers and the duration of feeding. At the end of the hour, I caught the bird and collected pollen from its head and bill using fuschin-stained gel, which was then melted on a microscope slide to produce a permanent slide (Beattie 1971). Pollen on these slides was counted later under a light microscope (100x). At the end of a trial day I marked the pedicels of open flowers with permanent marker to distinguish between flowers of different trials. Plants were bagged between feeding trials to prevent visitation.

To test the effect of bees, I compared pollination success of flowers exposed to sunbird-only trials to that of flowers exposed while the nucleus bee hive was added to the aviary, 4 m from the cages containing the aloe arrays. Honey bees could fly freely through the mesh of the aviary cage and so had easy access to arrays. Honey bees took two days to adjust to hive relocation and begin foraging on study plants, so bee treatments were alternated in blocks: potted plants experienced two no-bee days, two bee days, and two more no-bee days; and cut inflorescences experienced two bee days

followed by two no-bee days. Flowers remained open for two days, so experiments were conducted every other day to ensure that each flower experienced only one bee treatment. For bee trials, plants were unbagged 1 h before a sunbird was added in the morning and left unbagged until the end of the afternoon trial. To assess pollen removal and deposition I collected anthers and stigmas from wilted flowers of the cut inflorescences as per the field experiment. Pollen removal, pollen receipt and seed production per flower was compared between flowers exposed to sunbird visitation only and those exposed to both sunbirds and honey bee visitation.

### *Statistical analysis*

With three exceptions the measured response variable did not comply with a normal distribution, so analyses involved generalized linear models (McCullagh and Nelder 1989: proc genmod SAS 9.1), which considered negative binomial distributions (unless otherwise noted) and a ln-link function. Bird abundance, the number of flowers probed by birds in the field and the number of honey bees at each site were analysed using a normal distribution. Tests of statistical hypotheses for analyses of independent observations involved likelihood-ratio ( $G$ ) tests, whereas repeated measures analyses involved score statistics ( $T$ ). For analyses of multiple bee and/or pollination treatments on individual plants, I used generalized estimating equations and an exchangeable correlation matrix (Liang and Zeger 1986) to account for correlated responses by flowers on individual plants.

Models initially considered all possible interactions between independent variables, but nonsignificant interactions and factors were dropped from the model using backward elimination ( $\alpha=0.05$ ). Interactions and factors that reflected the experimental design (i.e., pollination treatment  $\times$  bee treatment) were retained in the model, even if they were not statistically significant. Significant main effects or interactions were

explored further using multiple comparison tests. A priori orthogonal contrasts were tested with a Type I error rate of  $\alpha = 0.05$ , whereas the Dunn-Šidák procedure was used for all other comparisons (Kirk 1995). In addition to the overall models of fruit and seed set that incorporated all populations, separate analyses were conducted for Klipfontein and Umgeni that included the additional ‘natural’ bee treatment at each of these populations. All least-squares means and standard errors are back-transformed from the estimates derived from ln-transformed data.

## Results

### *Self-compatibility*

*Aloe maculata* appears to be self-incompatible, as no flowers hand-pollinated with self-pollen set fruit ( $n=78$  flowers on 12 plants), whereas 41 of the 90 cross-pollinated flowers set fruit ( $n=46$  plants), and produced an average of 36.2 seeds per fruit ( $n=37$  fruits on 32 plants). However, the self-incompatibility system may not be complete, as bagged plants produced a few fruits (mean=0.058 fruits/flower,  $n=4840$  flowers on 43 plants) and seeds (mean=0.69 seeds/fruit,  $n=42$  fruits on 15 plants) autonomously. Presumably this inconsistency reflects the much larger sample for bagged flowers.

### *Flower visitor abundance*

Of the birds observed visiting *A. maculata*, only three sunbird species probed the corolla mouth and so could act as pollinators: Amethyst Sunbirds, White-bellied Sunbirds (*Cinnyris talatala* Smith) and Malachite Sunbirds (*Nectarinia famosa* L). Sunbird abundance varied significantly among populations ( $G_3=7.82$ ,  $P<0.05$ ; Table 3.2), but not between bee treatments ( $G_1=0.50$ ,  $P>0.4$ ) or with the number of plants in flower ( $G_1=0.95$ ,  $P>0.3$ ). Sunbirds were relatively uncommon at Klipfontein and most common at Hilton (Table 3.2). Short-billed birds never visited *A. maculata* flowers legitimately.

Streaky-headed Seedeaters (*Crithagra gularis* Smith) sometimes robbed nectar by pecking holes in the base of flowers. This damage was especially common in the Hilton population.

The only insects observed collecting *A. maculata* pollen were honey bees and small (<2 cm long) halictid bees (Halictidae) and allodapine bees (small carpenter bees: Apidae). The number of honey bees foraging on *A. maculata* varied among populations ( $G_3=26.18$ ,  $P<0.0001$ ; Table 3.2), but not between bee treatments ( $G_3=2.43$ ,  $P>0.1$ ), and site and bee treatment did not interact significantly ( $G_3=6.65$ ,  $P>0.05$ ). Honey-bee abundance increased significantly during hive addition at Klipfontein (Table 3.2), and decreased when hives were removed ( $G_1=3.93$ ,  $P=0.1$ ); however, hive addition did not significantly increase honey-bee abundance during insect surveys in the other three populations (Table 3.2). No honey bees were recorded feeding at Hilton or Umgeni during insect surveys, but honey bees were seen foraging on these *A. maculata* populations at other times, though only when hives were present. The sole insect survey at Hilton when hives were present was conducted the day after hives were added, when bees may not yet have begun foraging in their new environment (as seen in the aviary experiment). The limited detection of honey bees during surveys may also reflect inappropriate survey times towards the end of the study. By August, when hives were added to the Ixopo, Umgeni and Hilton populations, midday temperatures regularly exceeded 35 °C. This temperature may have caused honey bees to shift their foraging activity to early morning or early evening (e.g., Schaffer et al. 1979), when they would not have been detected by my surveys.

Small-bee abundance also varied among populations ( $G_3=21.91$ ,  $P<0.001$ ) and between bee treatments ( $G_1=5.70$ ,  $P<0.05$ ), although a significant population x bee interaction indicated that this pattern was not consistent ( $G_3=8.72$ ,  $P<0.05$ ). Small bees were significantly more abundant on *A. maculata* when honey-bee hives were present at

Klipfontein, but their abundance did not differ significantly with bee treatments at the other sites (Table 3.2). Small bee abundance at Klipfontein decreased after hives were removed (Table 3.2) but did not differ significantly from that during either of the other bees treatments ( $P>0.4$ ). There was a dramatic increase in allodapine bee abundance at Umgeni at the end of the bee treatment. A total of 81 allodapine bees were seen during insect surveys the day before hives were removed, compared to one during the previous with-hives survey and three before hives were added. Allodapine abundance remained high through most of the no-hives treatments; a total of 66 and 76 were seen during the two no-hives survey days. However, when surveys are combined over bee treatments small bee abundance did not differ statistically between any of the bee treatments ( $P>0.1$ ; Table 3.2). When small- and honey-bee abundances are combined, overall bee abundance during surveys was higher with hives at Klipfontein ( $T_1=11.68$ ,  $P<0.001$ ), but did not differ significantly in the other populations ( $P>0.5$ ).

#### *Behaviour of flower visitors*

I observed visits by 57 honey bees, of which 55 collected pollen, two drank nectar from holes pecked in corollas by birds, and one drank nectar legitimately by pushing her head into flowers. Pollen-collecting honey bees usually landed on flowers with freshly dehiscent anthers and ignored those from which pollen had been removed (55 out of 57 observed visits; Chapter 4). Because *A. maculata* stigmas usually become receptive only as the final anther dehisces (Chapter 4), pollen-collecting bees mostly visited flowers in male phase. Bees did not contact stigmas during pollen-collection and such contact is unlikely unless the stigma is exerted from the corolla, as pollen-bearing anthers are generally exerted and the narrow corolla mouth prevents honey-bee entry. The flower visited by the sole legitimate nectar-drinking honey bee may have been receptive (five anthers dehiscent), and she probably contacted the stigma.

Most small bees gathered pollen, but pollen- versus nectar-collecting individuals were not counted. The pollen-collecting small bees that were observed ( $n = 25$  bees) visited only male-phase flowers. Being small, these bees collect pollen from one anther at a time and are unlikely to contact stigmas while doing so, although contact during nectar collection cannot be ruled out.

Sunbirds are the effective pollinators of *A. maculata*. Individuals of each sunbird species carried orange pollen on their chins that could have come only from *A. maculata*, and all observed sunbirds ( $n=20$ ) seemed to probe male- and female-phase flowers indiscriminately. Although birds could not be observed as closely as insects in the field, contact between birds' chins and anthers and/or stigmas was almost certain. The number of flowers probed per plant varied considerably from one (usually when a bird was chased by another bird) to  $>20$ , and birds sometimes revisited flowers that they had already probed. Sunbird species did not differ significantly in the number of flowers probed per plant ( $F_2=0.28$ ,  $P>0.7$ ), although White-bellied Sunbirds probed slightly more flowers per plant (mean  $\pm$  SE =  $10.7 \pm 3.9$ ,  $n = 3$  birds) than Amethyst or Malachite Sunbirds ( $7.6 \pm 1.3$ ,  $n = 19$  birds).

#### *Pollen removal and deposition*

The amount of pollen remaining on anthers of wilted flowers differed significantly among populations ( $T_3=10.25$ ,  $P<0.025$ ) and bee treatments ( $T_1=5.65$ ,  $P<0.025$ ), but was not affected by caging or its interactions with other factors ( $P>0.1$ ). *Aloe maculata* anthers produce an average of 18 300 pollen grains (USE=1250, LSE=1342). Pollen removal was highest at Umgeni and lowest at Klipfontein (Figure 3.1a). The effect of hive addition on that amount of pollen remaining at Umgeni differed significantly from that in the other populations (population  $\times$  bee treatment interaction,  $T_3=17.17$ ,  $P<0.001$ ). At Klipfontein, Hilton and Ixopo, hive addition almost halved the amount of pollen

remaining on anthers of caged and open flowers, compared to that under natural (i.e., no hives) conditions ( $T_1=8.3$ ,  $P<0.01$ ; Figure 3.1a). In contrast, at Umgeni significantly less pollen remained on anthers after exposure to natural bee densities than after hive addition ( $T_1=5.72$ ,  $P<0.05$ ; Figure 3.1a). At this site, the amount of pollen remaining before hives were added (mean=460 grains/anther, LSE=56, USE=64) was less than that when hives were present, but more than after hives were removed, and so did not differ statistically from either of the other bee treatments ( $P>0.3$ ).

This pattern of pollen availability among populations was reflected in my ability to find adequate pollen for hand pollinations. At Klipfontein, Hilton and Ixopo, pollen was available on non-study plants until 1100 h under natural conditions, but was completely depleted by 800 to 900 h when hives were present, obliging me to bag plants to act as pollen donors when hives were present. In contrast, at Umgeni pollen became even less available after hives were removed, probably due to the many allodapine bees that appeared at the end of the hives treatment.

Stigmas of caged plants received fewer pollen grains than those of open plants in all populations ( $T_1=8.26$ ,  $P<0.005$ ), and this effect did not differ between bee treatments (pollination x bee treatment;  $T_1=0.00002$ ,  $P>0.99$ ), or populations (population x pollination treatment;  $T_1=6.51$ ,  $P>0.05$ ). The effect of hive addition on pollen deposition differed significantly between Umgeni and the other populations (population x bee treatment interaction;  $T_3=12.85$ ,  $P<0.005$ ; Figure 3.1b and c). At Klipfontein, Ixopo and Hilton, stigmas received almost a third less pollen when hives were present than under natural conditions ( $T_1=9.45$ ,  $P<0.005$ ; Figure 3.1b), whereas at Umgeni the opposite was true (see below). Note that stigmas of bagged plants received an average of 88 grains (LSE=9.2, USE=10.3) from autonomous deposition, which exceeded deposition on stigmas of open plants during bee addition.



Stigmas of Umgeni plants received significantly less pollen than those in other populations ( $T_1=19.52$ ,  $P<0.001$ ), primarily because of low deposition after hives were removed (Figure 3.1b). Stigmas received somewhat more pollen before hives were present (mean=115 grains, LSE=13.1 grains, USE=14.8 grains) than with hives, but the difference was not statistically significant ( $T_1=2.71$ ,  $P=0.1$ ). Pollen deposition during the final no-hives treatment was significantly lower than when hives were present ( $T_1=5.74$ ,  $P<0.05$ ) or before hive addition ( $T_1=7.04$ ,  $P<0.025$ ), and was lower than during any treatment in any other population (Figure 3.1b). Overall, the addition of honey-bee hives increased pollen removal from anthers and reduced pollen deposition on stigmas for all populations, except Umgeni where the opposite was true (Figure 3.1c).

#### *Fruit and seed production*

The effects of bird exclusion and honey-bee addition on fruit production differed among populations (Table 3.3). Bird exclusion significantly reduced fruit production at Ixopo ( $T_1=8.11$ ,  $P<0.01$ ) and Umgeni ( $T_1=6.81$ ,  $P<0.01$ ), but not at Klipfontein or Hilton ( $P>0.1$ ). Honey-bee addition significantly reduced fruit production by caged and open plants at Hilton ( $T_1=8.36$ ,  $P<0.01$ ), Ixopo ( $T_1=9.82$ ,  $P<0.01$ ), and Klipfontein ( $T_1=4.41$ ,  $P<0.05$ ; Figure 3.2a). In contrast, fruit production by Umgeni plants was higher when hives were present than after they were removed ( $T_1=13.96$ ,  $P<0.001$ ).

Pollen supplementation increased fruit production overall, but this effect varied among populations and bee treatments (Table 3.3). Specifically, pollen-supplemented plants set more fruit than plants exposed to natural pollination only at Hilton ( $T_1=4.07$ ,  $P<0.05$ ) and Umgeni ( $T_1=7.23$ ,  $P<0.01$ ), indicating that fruit set in these populations was pollen limited. Over all populations, pollen supplementation increased fruit production significantly when hives were absent ( $T_1=11.89$ ,  $P<0.001$ ), but not when hives were present ( $T_1=2.11$ ,  $P>0.1$ ).

The effects of caging, pollen supplementation and honey-bee addition on the number of seeds per fruit (seed set) varied among populations (Table 3.3). Seed set of caged and open plants was lowest at Klipfontein and highest at Ixopo (Figure 3.2b). Bird-exclusion reduced seed set significantly at Ixopo ( $T_1=16.94$ ,  $P<0.001$ ), but not in the other populations ( $P>0.1$ ). Hive addition reduced seed set by caged and open plants at Ixopo ( $T_1 = 13.31$ ,  $P<0.001$ ) and Klipfontein ( $T_1 = 9.56$ ,  $P<0.01$ ), but not in the other populations ( $P>0.1$ ; Figure 3.2b). Pollen supplementation increased seed set at Klipfontein when hives were present ( $T_1 = 11.64$ ,  $P<0.001$ ), but not under natural conditions ( $T_1 = 2.79$ ,  $P>0.05$ ), indicating that seed set in this population became pollen limited when hives were added (Figure 3.2b).

The influences on total reproductive success (average seed set x fruit production for each plant during each bee treatment) are similar to, but more pronounced than, those apparent in the separate seed and fruit set analyses (Figure 3.3). Bird exclusion lowered total seed production significantly at Hilton ( $T_1=6.20$ ,  $P<0.05$ ) and Ixopo ( $T_1=12.73$ ,  $P<0.001$ ), but not in the other populations ( $P>0.1$ ; Figure 3.3), suggesting that either birds are more effective or insects are less effective pollinators in these populations than at Klipfontein or Umgeni. Hive addition significantly lowered total seed production of caged and open plants at Ixopo ( $T_1=12.67$ ,  $P<0.001$ ) and Klipfontein ( $T_1=8.55$ ,  $P<0.01$ ), but not at Hilton ( $T_1=0.39$ ,  $P<0.5$ ). Conversely, total seed production at Umgeni was higher when hives were present than after they were removed ( $T_1=4.11$ ,  $P<0.05$ ; Figure 3.3). Pollen supplementation increased total seed production at Klipfontein when hives were present ( $T_1=7.80$ ,  $P<0.01$ ), but not before ( $T_1=1.91$ ,  $P<0.1$ ). In contrast, pollen supplementation increased seed production under natural conditions at Hilton ( $T_1=5.46$ ,  $P<0.05$ ), but not when hives were present ( $T_1=0.95$ ,  $P>0.3$ ; Figure 3.3), which may reflect my greater supplemental pollination effort during the natural treatment at this site (Table 3.1). Pollen supplementation did not affect total seed production during either

bee treatment at Ixopo ( $P>0.05$ ), and increased seed production during both treatments at Umgeni, though the effect was stronger when hives were present ( $T_1=6.70$ ,  $P<0.01$ ) than after they were removed ( $T_1=3.87$ ,  $P<0.05$ ; Figure 3.3).

Additional analyses of fruit and seed production by open plants were conducted for Klipfontein and Umgeni to consider reproductive outcomes during the additional natural-pollination treatment for each population. At Klipfontein, where peak flowering lasted long enough for a second without-hives treatment, honey-bee addition did not affect fruit production ( $T_2=0.05$ ,  $P>0.9$ ), but significantly affected seed set ( $T_2=9.95$ ,  $P<0.01$ ). Specifically, seed set decreased after hives were added ( $T_1=8.80$ ,  $P<0.01$ ) and increased again after hives were removed, although not quite significantly ( $T_1=3.18$ ,  $P=0.07$ ), even though fewer plants were flowering by the end of this third treatment (Figure 3.4a). At Umgeni, both fruit production ( $T_1=19.71$ ,  $P<0.001$ ; Figure 3.4b) and seed production ( $T_1=10.97$ ,  $P<0.01$ ; Figure 3.4c) differed significantly among bee treatments, and both measures of reproductive success declined throughout the experiment.

The number of seed predators per fruit followed a Poisson distribution, indicating that they were distributed randomly among flowers. Seed predator abundance did not differ between caged and open plants ( $T_1=0.15$ ,  $P>0.7$ ), but was lower overall when hives were present ( $T_1=5.02$ ,  $P=0.025$ ). Thus, seed predators cannot account for lower seed production during hive treatments. Seed predation differed between sites ( $T_3=32.59$ ,  $P<0.0001$ ), primarily because of higher predation at Klipfontein and Hilton than at Ixopo and Umgeni ( $T_1=23.23$ ,  $P<0.001$ ).

#### *Aviary experiment*

Sunbird behaviour varied with plant characteristics, but not between birds or with the presence of bees, whereas the number of pollen-collecting bees was affected by hive

addition. The two sunbirds carried similar pollen loads and effected similar pollination ( $P>0.1$ ). Birds probed more flowers per visit to racemes with many flowers ( $G_1=6.35$ ,  $P=0.012$ ;  $n=83$  plant-trial observations). The presence of bees did not change either the duration of sunbird feeding bouts ( $G_1=0.57$ ,  $P>0.4$ ) or the number of flower visits per trial ( $G_1=0.003$ ,  $P>0.95$ ), even though three to 12 bees collected pollen during each bee trial but no bees visited plants during the no-hive trials.

The presence of honey bees in the aviary increased pollen removal, but reduced the amount of pollen carried by sunbirds, pollen deposition on stigmas, and seed set per fruit, compared to trials during which sunbirds foraged alone (Table 3.4). Because the aviary population is a closed system, reduced pollen deposition also indicates reduced average siring success (i.e., pollen export). The effect of bees on fruit set depended on the number of flowers open per raceme (bee  $\times$  ln(flowers) interaction,  $T_1=6.52$ ,  $P<0.01$ ; Figure 3.4). Bees did not affect fruit set by inflorescences with few open flowers, which was consistently very low, but significantly reduced fruit set by inflorescences with many open flowers.

## Discussion

### *Reproductive consequences of pollen theft*

This study involved the first experimental manipulation of pollen-thief abundance at the population level, and demonstrated clearly that pollen theft can significantly reduce the reproductive success of an entire plant population. Both the field and aviary experiments demonstrated that addition of pollen-collecting bees increased pollen removal from anthers, but decreased pollen deposition on stigmas. Thus, pollen theft reduced average pollen export within populations. In turn, the reduction in pollen receipt caused a decrease in fruit and seed production.

Seed and/or fruit set decreased with the addition of honey bee hives in three of the four populations, but the clearest evidence of the detrimental effect of pollen thieves on pollination was seen at Klipfontein. In this population seed production decreased with hive addition but rebounded after hive removal (Figure 3.4a). Correspondingly, in the presence of hives seed production by non-supplemented plants decreased significantly, whereas pollen supplemented plants achieved seed production similar to that prior to hive addition, indicating that the Klipfontein population became pollen limited when hives were present (Figure 3.3).

The reduction in pollen export and import caused by pollen thieves should correspondingly increase pollen limitation as detected by supplementation experiments, but this outcome did not occur consistently in this experiment. Deviations from this expectation can be explained largely by supplementation effort. At Klipfontein, where supplementation was applied most often (Table 3.1), hive addition caused pollen limitation (Figure 3.3), as expected. At Hilton, pollen limitation seemed to decrease when hives were present, even though natural pollen receipt and reproductive success declined significantly. This unexpected result may be due to the extra day of pollen supplementation during the no-hives treatment at this population. Although pollen supplementation was planned for a second day when hives were present, insufficient pollen remained on anthers of open plants, and bagged donor plants had finished flowering. The overall lack of response to pollen addition at Ixopo may also be due to insufficient pollen supplementation, as supplementation was applied only once during each bee treatment.

Insects may act as poor pollinators if they visit many flowers per plant and thus transfer primarily self-pollen among flowers of self-incompatible aloes. Preliminary data from *A. ferox* (Chapter 4) indicate that honey bees visit up to twice as many flowers per plant as birds. The equal growth of self- and cross-pollen tubes in *A. maculata* styles

(and other aloes: Chapter 4), but high fruiting failure of self-pollinated flowers suggests a late-acting (or ovarian) self-incompatibility system (LSI), which has been reported for *Gasteria*, a sister genus to *Aloe* (Naaborgh and Willemse 1991, Treutlein et al. 2003). Most forms of LSI involve the abortion of ovules penetrated by self-pollen tubes, even though fertilization does not occur (Seavey and Bawa 1986). If bees effect considerable self-pollination, abortion of such ovules (ovule discounting: Barrett et al. 1996) poses an alternative mechanism by which increased bee abundance could reduce seed production. However, if bees reduced seed production via ovule discounting, rather than pollen theft, pollen deposition should have been similar (if bees have no effect) or greater (given more potential flower visitors) at higher bee densities than under natural conditions. Instead, pollen deposition declined with seed set, so ovule discounting cannot explain the decreased seed production in this experiment.

*An exception proves the rule*

Several non-exclusive processes could explain the anomalous results from the Umgeni population, where seed production and pollen receipt were higher when honey-bee hives were present than after their removal. First, this population could have been pollinator limited, so that added honey bees increased overall pollination service, despite their relative inefficiency, and acted as inefficient or ‘ugly’ pollinators (detrimental when better pollinators are available but beneficial in their absence: Thomson and Thomson 1992). However, pollen receipt, fruit production and seed set were all highest before hive addition and declined significantly after hives were added (Figure 3.4), thus pollinator limitation cannot explain the low or declining seed production.

A second explanation for the continuously declining seed production at Umgeni is resource limitation, if flowers pollinated before the addition of honey bees used limited resources for fruit and seed production (Corbet 1998). However, this possibility is

inconsistent with the significant increase in seed production in response to pollen supplementation during both bee treatments (Figure 3.3).

The third and most likely explanation for the declining seed production involves the sudden appearance of many pollen-collecting allodapine bees near the end of the hives-present treatment. These bees stripped anthers of pollen by 800 h, visited primarily male-phase flowers, and did not contact stigmas, so acted as efficient pollen thieves. Insect surveys were too infrequent to identify the beginning and end of high allodapine abundance, but average abundance suggests that more were present during the natural bee-abundance treatment. Furthermore, less pollen remained on anthers and was deposited on stigmas during the natural bee-abundance treatment than the hives-present treatment, suggesting more intensive pollen theft after hive removal. Thus pollen-thief abundance probably increased during the three bee treatments, first with hive addition and then with the natural increase in small bee abundance, such that this population experienced the highest pollen theft during the final, hives-absent treatment. If so, the apparent exception to the negative effect of pollen-collecting honey bees on *A. maculata* reproduction can in fact be explained by pollen theft, if not by the experimental manipulation.

### *The role of bees*

During this study, honey bees and small bees provided the vast majority of insect visits to *A. maculata* flowers, but all may act as pollen thieves. If so, which insects effected pollen deposition and subsequent seed production by caged plants? Previous evidence that pollen thieves reduce pollination involved plants pollinated by visitors that collect nectar (Vaughton 1996, Gross and Mackay 1998, Lau and Galloway 2004) or resin (do Carmo et al. 2004). However, pollen- and nectar-collecting individuals of the same species can behave very differently, so nectar collectors could be effective

pollinators, whereas pollen collectors act as thieves (Ish-Am and Eisikowitch 1993). Honey bees occasionally collected nectar from *A. maculata*, and nectar-collecting bees may be more likely to visit female-phase flowers and act as pollinators. Small bees entered *A. maculata* flowers for nectar, although they may be too small to contact stigmas effectively. Pollen-collecting bees may also sometimes pollinate *A. maculata*, but at high bee densities this limited pollination is offset by the large reduction of pollen available for dispersal. More thorough observations of insect visitors and tests of pollination efficiency during single visits are needed to resolve this matter.

Honey bees, though generally regarded as excellent pollinators in agriculture, are the most commonly documented pollen thieves (Chapter 2). The frequent observations of pollen theft by honey bees is unlikely to reflect a research bias, as honey bees have rarely been documented as primary nectar thieves or robbers (I found only one study: Kalinganire et al. 2001), despite more research on nectar than pollen theft (Chapter 2). Rather, the frequency of pollen theft by honey bees suggests that as pollen-collectors these bees are especially detrimental for plants, which could occur for several non-exclusive reasons. The behavioural flexibility and generalist foraging of honey bees allow them to exploit unfamiliar floral resources (Westerkamp 1991). When honey bees act as pollen thieves, their large colonies and ability to communicate the location of food resources (references) make them particularly serious causes of reduced plant reproduction. Furthermore, honey bees have been introduced around the world (Huryn 1997, Goulson 2003), bringing them into contact with many plants not adapted to them. Indeed, previous demonstrations that pollen theft by honey bees adversely affects plant reproduction have all been conducted where honey bees are exotic species, such as Australia (Paton 1993, Gross and Mackay 1998) and Brazil (do Carmo et al. 2004).

Although the addition of two commercial hives may seem like an unnaturally high density of honey bees, it does not represent an unrealistic situation in South Africa. The



natural density of honey bee hives in neighbouring Botswana was estimated at 4.2 nests/km<sup>2</sup> (McNally and Schneider 1996), whereas in Europe, where honey bee colonies are often found in aggregations, hive densities can exceed 100 nests/km<sup>2</sup> (Oldroyd et al. 1995). More honey bees from a natural hive visited *A. vryheidensis* at a site 400 km north of this study (Johnson et al. 2006) than I observed during the hive treatments in this study, probably due to the much higher flowering density. Furthermore, honey-bee abundance in South Africa is undoubtedly higher today than historically, due to commercial bee keeping and forestry plantations, which produce abundant flowers during the dry winter months, enabling large colonies to persist through a usually limiting season. Furthermore, South African beekeepers often use aloe stands as pollen sources for increasing larval production in hives, and they may place more than 100 hives in a large stand (W. Ercket, Natal Beekeepers Association, personal communication). Thus, many aloe populations likely experience artificially increased bee densities.

### *Conclusions*

This study demonstrates that 1) pollen theft can decrease both the male and female components of pollination, 2) reduced pollen export and import can decrease siring success and seed production within plant populations, and 3) high densities of pollen-collecting bees can disrupt pollination even of plants that have evolved in their presence. Ecologically, these findings reveal the importance of monitoring the effects of commercial bee hives on native plants. Since relatively few honey bee hives can reduce the seed production of wild plants, there is a need to understand the collateral impacts on native plant populations when manipulating bee densities to enhance pollination of agricultural crops, and especially when using native plant populations as foraging resources for bees to enhance hive growth. Evolutionarily, the ubiquity of pollen-collecting bees suggests that pollen theft may commonly impose selection on floral traits,

both for plants interacting with the native pollinator fauna and for plants exposed to exotic pollinators.

Table 3.1 Characteristics of *Aloe maculata* study populations. Pollen supplementation effort is indicated by the number of days during which available flowers were supplemented.

| Population  | Numbers of<br>flowering and<br>nonflowering | Time since | Period of study | Bee<br>treatment<br>order | Days of pollen<br>supplementation |       |
|-------------|---|------------|-----------------|---------------------------|-----------------------------------|-------|
|             | plants                                      | last fire  |                 |                           | No hives                          | Hives |
| Hilton      | 365, 90                                     | 1 month    | Aug 12 – 30     | H – NH                    | 2                                 | 1     |
| Ixopo       | 120, unk.                                   | 2 month    | Jul 30 – Aug 27 | NH – H                    | 1                                 | 1     |
| Klipfontein | 545, 175                                    | 1 yr       | Jun 21 – Aug 1  | NH – H                    | 3                                 | 3     |
| Umgeni      | 100, 400                                    | 1 yr       | Jul 28 – Aug 18 | H – NH                    | 2                                 | 2     |

Table 3.2 Mean (lower SE, upper SE) abundance of flower visitors measured during surveys in four *Aloe maculata* populations under natural abundance of honey bees (N) and when honey-bee hives were present (H). Test statistics represent results from generalized models.

| Site                     | Survey days |   | Sunbirds<br>(birds/30 min<br>transect) | Honey bees (bees/20 plants) |               |                   | Small bees (bees/20 plants) |                      |                 |
|--------------------------|-------------|---|--|-----------------------------|---------------|-------------------|-----------------------------|----------------------|-----------------|
|                          | N           | H |  | N                           | H             | Test statistic    | N                           | H                    | Test statistic  |
| Hilton                   | 1           | 1 | 5.7<br>(1.0)                           | 0                           | 0             | $G_1=0$           | 7.2<br>(3.1, 5.5)           | 36.0<br>(12.8, 19.8) | $G_1=2.15$      |
| Ixopo                    | 2           | 1 | 3.2<br>(1.0)                           | 0.5<br>(2.7)                | 1.5<br>(1.9)  | $G_1=0.09$        | 13.0<br>(7.0, 15.2)         | 15.3<br>(6.4, 11.0)  | $G_1=0.03$      |
| Klipfontein <sup>†</sup> | 2           | 2 | 2.0<br>(0.80)                          | 4.8<br>(1.5)                | 12.3<br>(1.5) | $G_1=11.74^{***}$ | 0                           | 3.0<br>(1.2, 1.9)    | $G_1=8.38^{**}$ |
| Umgeni <sup>‡</sup>      | 2           | 2 | 4.25<br>(0.88)                         | 0                           | 0             | $G_1=0$           | 35.5<br>(14.7, 25.2)        | 20.5<br>(8.6, 14.7)  | $G_1=0.47$      |

\* $P<0.05$ , \*\*\*  $P<0.001$

<sup>†</sup> After hive removal, Klipfontein visitor abundances (1 survey day) were: 2.5 ( $\pm 0.77$ ) sunbirds, 1.5 ( $\pm 4.3$ ) honey bees, 1.5 ( $\pm 1.5$ ) small bees.

<sup>‡</sup> Before hive addition, Umgeni visitor abundances (1 survey day) were: 5.5 ( $\pm 1.76$ ) sunbirds, 0 honey bees, 2.0 (1.2, 3.1) allodapine bees.

Table 3.3 Score statistics ( $T$ ) for generalized models of the effects of pollination treatment (caged, open or pollen supplemented), bee treatment (natural or hives present) and population on the fruit production (fruits/plant/bee treatment), seed set (average seeds/fruit) and total reproductive success (total RS; fruit production x seed set per plant during each bee treatment) of *A. maculata*.

| Independent variable                     | df | Caged vs. Open |          |          | Open vs. Pollen supplemented |          |          |
|--|----|----------------|----------|----------|------------------------------|----------|----------|
|  |    | Fruit set      | Seed set | Total RS | Fruit set                    | Seed set | Total RS |
| Population <sup>1</sup>                  | 3  |                | 46.06*** | 24.58*** | 33.99***                     | 32.77*** | 20.21*** |
| Pollination treatment                    | 1  | 16.38***       | 5.65*    | 13.89*** | 11.66***                     | 5.90*    | 14.55*** |
| Bee treatment                            | 3  | 0.08           | 5.46*    | 2.09     | 2.60                         | 12.90*** | 10.33**  |
| Population x poll. treat.                | 1  | 14.52**        | 9.02*    | 11.44**  | 10.22*                       | 15.13**  | 14.20**  |
| Population x bee treat.                  | 3  | 25.66***       | 9.68*    | 9.13*    | 20.41***                     | 16.25*** | 17.29*** |
| Pollin. treat. x bee treat.              | 1  | 0.90           | 0.09     | 0.17     | 5.05*                        | 0.00     | 3.63     |
| Population x pollin. treat. x bee treat. | 3  | 4.68           | 3.48     | 6.45     | 0.45                         | 11.80**  | 10.06*   |

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$

<sup>1</sup>The analysis of fruit set for caged versus open plants did not converge on parameter estimates for this factor.

Table 3.4 Comparison of *Aloe maculata* pollination during the aviary experiment in the presence or absence of honey bees. Means (and lower and upper SE) are back transformed from ln-transformed data.

| Variable            | No bees              | With bees          | repeated | Test statistic    |
|---------------------|----------------------|--------------------|----------|-------------------|
|                     |                      |                    | subject  |                   |
| Pollen remaining    | 5832<br>(553, 612)   | 3185<br>(387, 441) | plant    | $T_1 = 7.35^{**}$ |
| Pollen deposited    | 251<br>(29.1, 33.0)  | 39<br>(9.7, 12.1)  | plant    | $T_1 = 4.56^*$    |
| Sunbird pollen load | 9118<br>(1654, 2021) | 3080<br>(644, 815) | none     | $G_1 = 9.03^{**}$ |
| Seeds per fruit     | 26.8<br>(3.3, 3.7)   | 20.0<br>(1.6, 1.8) | plant    | $T_1 = 4.73^*$    |

\*  $P < 0.05$ , \*\*  $P < 0.01$

Figure 3.1 Mean ( $\pm$ SE) pollen transfer under natural conditions (●) and with two added honey-bee hives (○), including: a) pollen remaining on anthers of wilted flowers, b) pollen receipt, and c) the relation between receipt and removal for each study population (HC – Hilton, IX – Ixopo, KF – Klipfontein, UG – Umgeni). Based on analyses of ln-transformed data.



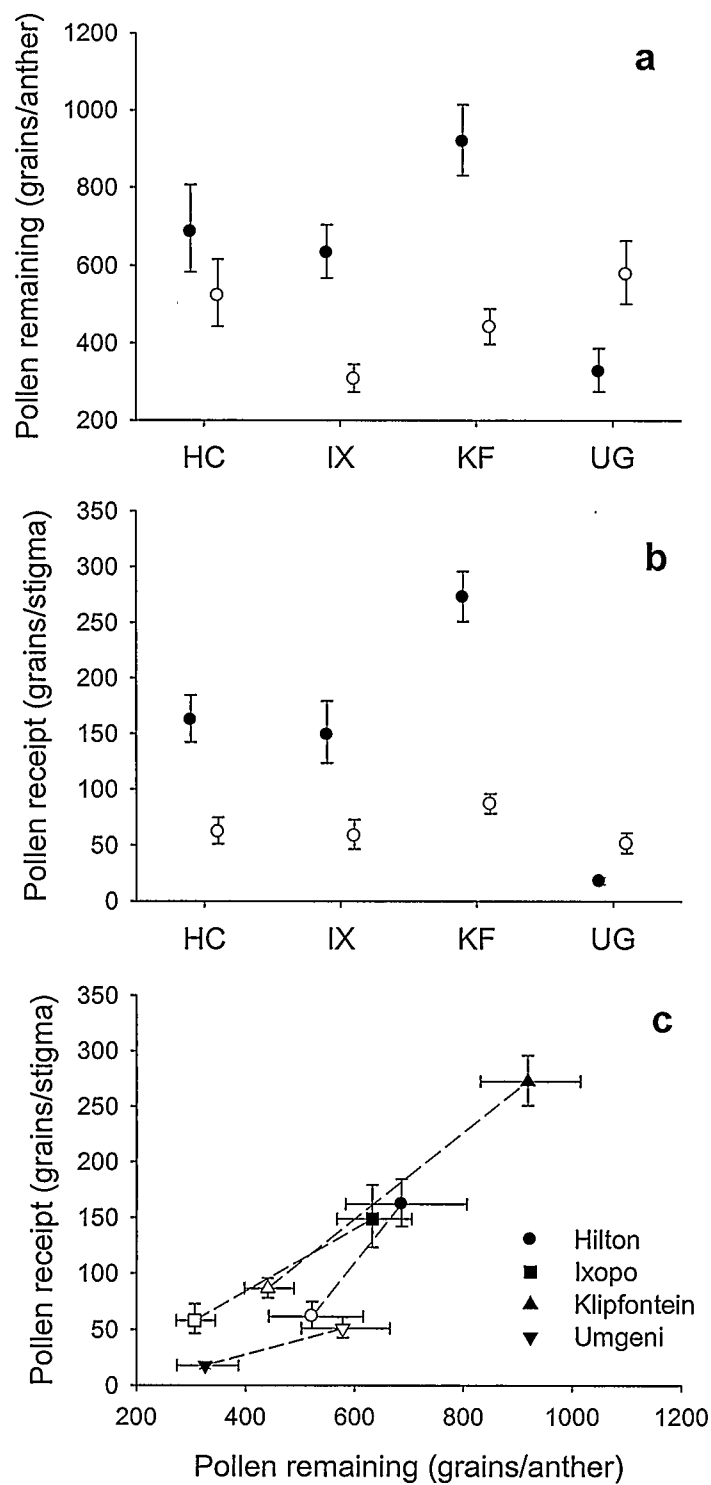


Figure 3.2 The effects of honey-bee addition on mean ( $\pm$  SE) fruit production (a) and seed set (b) by *A. maculata* plants subject to caging ( $\blacksquare$ ), open pollination ( $\circ$ ), and pollen supplementation ( $\blacktriangledown$ ) in four populations. Note that bee-treatments are not presented in chronological order for Hilton and Umgeni. Based on analyses of ln-transformed data.

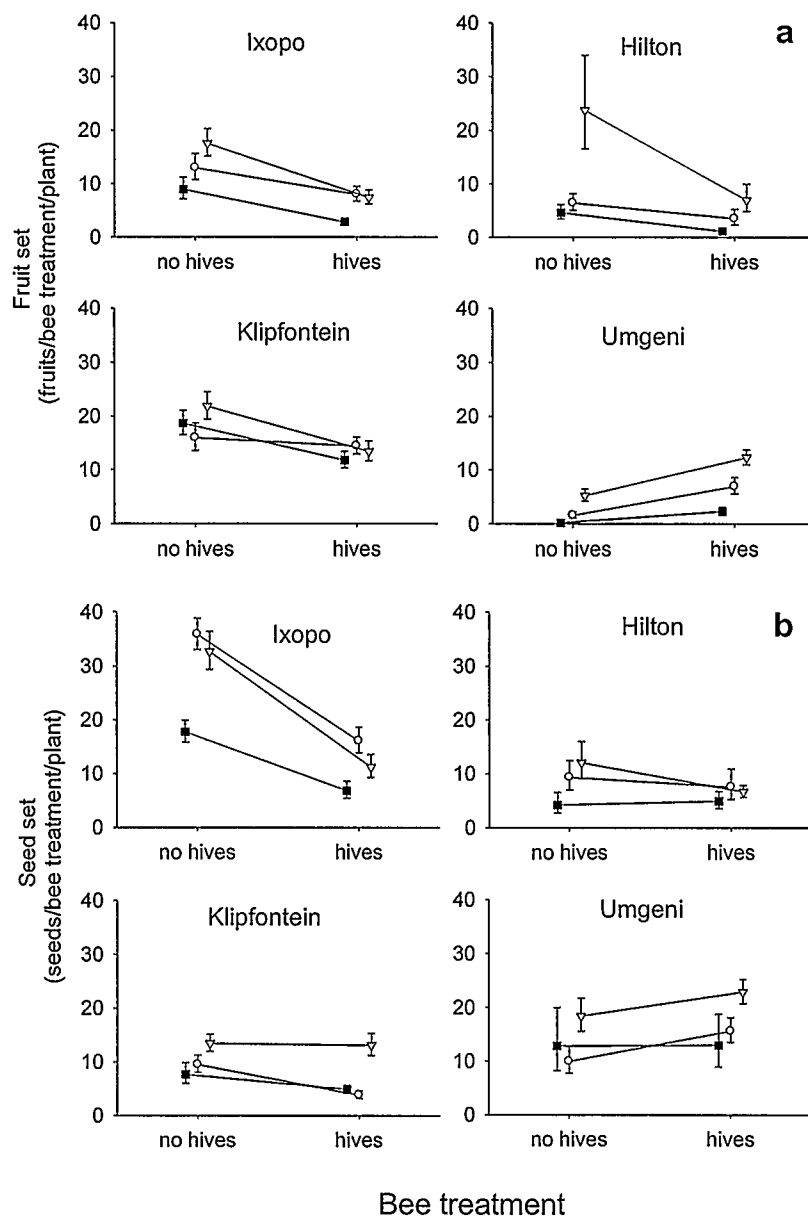


Figure 3.3 Overall effects of honey-bee addition on *A. maculata* reproductive success; mean ( $\pm$  SE) seed production by plants subject to caging (■), open pollination (○), and pollen supplementation (▼) in four populations (note the different y ordinates for Ixopo). Note that bee-treatments are not presented in chronological order for Hilton and Umgeni. Based on analyses of ln-transformed data.

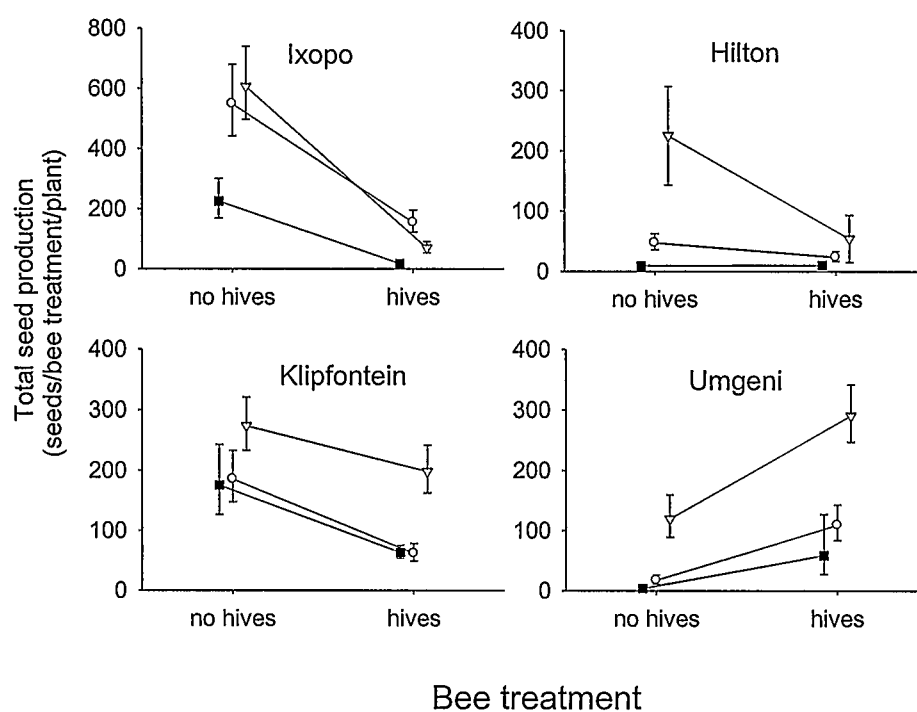


Figure 3.4 The effects of honey-bee addition on open-pollinated plants, including an additional natural (no hives) treatment at Klipfontein (a) and Umgeni (b and c). Fruit and seed production (mean  $\pm$  SE) are based on analyses of ln-transformed data. Treatments are presented in chronological order.

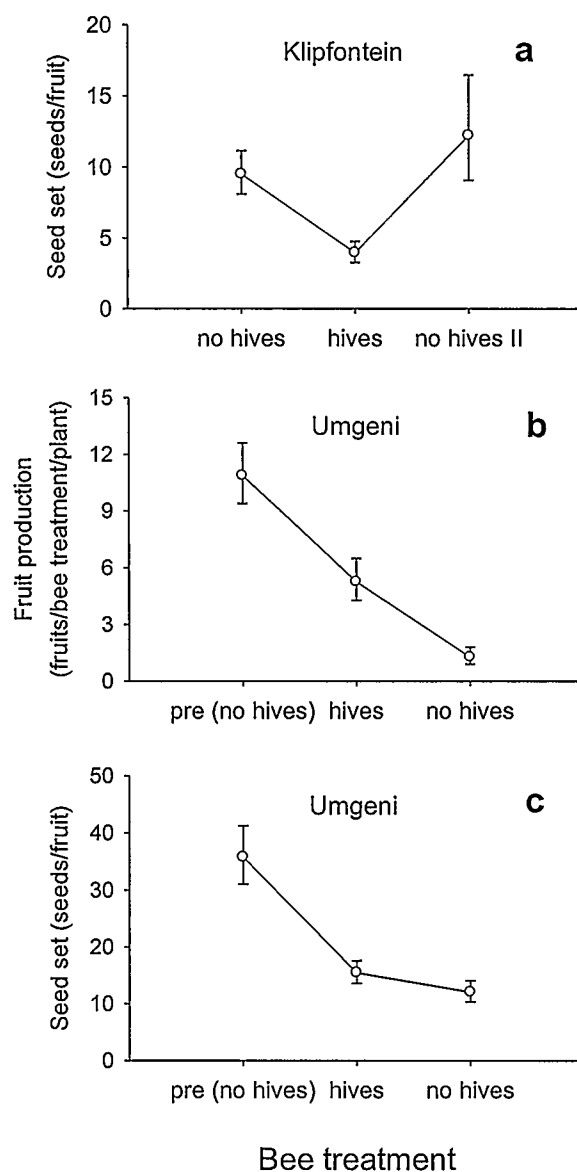
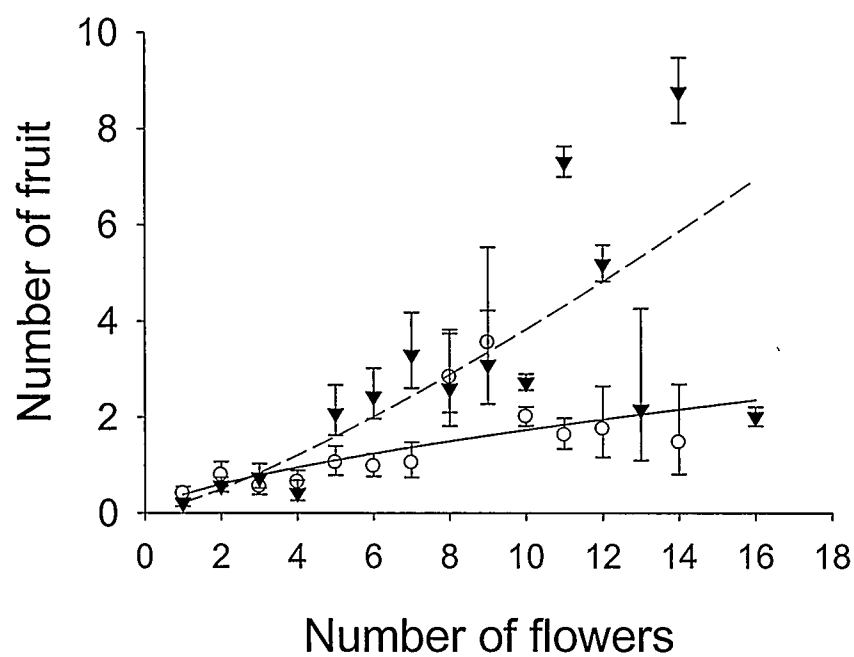


Figure 3.5 The interacting effects of the presence ( $\circ$ ) or absence ( $\blacktriangledown$ ) of honey bees and the number of open flowers on *A. maculata* inflorescences on mean ( $\pm$  SE) fruit production during the aviary experiment. Based on analysis of ln-transformed observations.



## CHAPTER 4 – FLORAL TRAITS THAT INFLUENCE PLANT VULNERABILITY TO POLLEN THEFT

### Introduction

Flowers of outcrossing plants produce finite numbers of male gametophytes, packaged as pollen, and rely on vectors (biotic or abiotic) to transport this pollen to stigmas of conspecific plants to reproduce sexually. Plants that rely on animal vectors usually promote visitation by producing floral rewards, which are removed and generally consumed by visitors (Simpson and Neff 1981). The most common floral rewards are nectar and pollen, thus pollen is unique in serving as both the carrier of gametes and a consumable pollinator attractant. Although flowers may have evolved to attract the most effective pollinators (Stebbins 1970), most plant species attract a spectrum of reward-consuming visitors that vary in their effectiveness as pollen vectors. Highly efficient pollinators are but one end of this visitor spectrum, whereas the other end is represented by floral larcenists that consume nectar and/or pollen, but rarely pollinate in return (Inouye 1980, Chapter 2).

Floral larceny is not uncommon among flowering plants, and its effect on plant reproduction varies. Nectar theft sometimes enhances outcrossing by reducing geitonogamy (reviewed in Maloof and Inouye 2000), but its net effect on plant reproduction is probably negative (Irwin et al. 2001). Although considerably less attention has been paid to pollen theft, studies that measure its effect on plant reproduction report a neutral or negative effect (Chapter 2, 3). Indeed, pollen theft should present a more serious problem for plants, as it reduces not only the rewards offered to legitimate pollinators, but also the number of male gametophytes available for reproduction. Pollen theft has been shown to reduce both siring success (Lau and

Galloway 2004) and seed production (Vaughton 1996, Gross and Mackay 1998, do Carmo et al. 2004, Chapter 3).

Most pollen thieves serve as pollinators of other plant species (Chapter 2), which suggests that the outcome of pollen collection (theft or pollination) depends primarily on plant rather than animal characteristics. Strong spatial or temporal separation between male function (pollen presentation) and female function (stigma receptivity) are two mechanisms that may turn pollen-collectors into pollen thieves. For example, the most extreme separation of sexual function occurs in dioecious species, in which individual plants perform a single sex role. Pollen collectors need only ever visit rewarding (i.e., male) plants, and so clearly act as pollen thieves (do Carmo et al. 2004). However, male and female function need not be separated among different plants to promote pollen theft. Segregation of pollen presentation and receptive stigmas in either time (dichogamy) or space (herkogamy) is common among hermaphroditic species (Lloyd and Webb 1986, Webb and Lloyd 1986). Both traits play a beneficial role in reducing interference between male and female function (Barrett 2002), but may reduce the likelihood that pollen collectors visit female-phase flowers (dichogamy) or contact stigmas (herkogamy).

Pollen theft could also result from animal characteristics, if animals contact receptive stigmas without effecting pollination. A clear example is pollen theft from stigmatic surfaces, whereby bees remove pollen that has been deposited by legitimate pollinators (Gross and Mackay 1998). Incidental stigma contact may not result in pollination if animals carry insufficient pollen loads, or if they deposit only poor quality pollen, such as that from other plant species or self-pollen for self-incompatible species (Aizen and Harder 2007).

Here I explore four non-exclusive hypotheses for why pollen-collecting insects fail to pollinate some hermaphroditic flowers: 1) insects visit only male-phase flowers as female-phase flowers do not present pollen (in the case of aloes, because protandrous

flowers have already been stripped of their pollen; dichogamy hypothesis); 2) pollen-collecting insects visit receptive flowers, but do not contact stigmas (herkogamy hypothesis); 3) pollen-collecting insects contact stigmas, but do not deposit pollen (no deposition hypothesis); and 4) insects deposit only poor quality pollen (pollen quality hypothesis; Table 4.11). Hypotheses 1-3 address situations where pollen-collecting insects act as thieves, whereas in Hypothesis 4 pollen-collecting insects deposit pollen, and so behave as ineffective pollinators rather than pollen thieves. To address these hypotheses I studied ten *Aloe* species (Asphodelaceae) representing the spectrum of floral forms within this genus. Although most *Aloe* species share a basic tubular floral morphology, they vary greatly in their relations with pollen-collecting insects: some aloes exhibit floral characteristics that suggest they may be insect-pollinated, whereas others are entirely bird-pollinated, despite being visited frequently by insects for pollen (Stokes and Yeaton 1995). To distinguish between the four theft hypotheses, I 1) established the degree to which each aloe species is visited and pollinated by insects, 2) measured floral phenology to assess the temporal and spatial overlap between male and female function, 3) observed insect visitors to characterise their foraging, and 4) compared the amount of pollen deposited by insect visitors alone to that deposited by all visitors combined. This study demonstrates that several floral traits are associated with pollen theft, but the combination of important traits varies among plant species.

## Methods

### *Study species and location*

Aloes are succulent, perennial monocots with flowers grouped into terminal inflorescences that can be branched to form several racemes. The protandrous flowers last more than 24 h and open roughly sequentially from the bottom of the inflorescence to the top.



Most *Aloe* species, including the ten that I studied, can be categorised into four basic floral types: 1) loose racemes of long, narrow flowers (*A. arborescens* Mill., *A. boylei* Baker, *A. maculata* All.; Figure 4.1A, E), 2) dense racemes of narrow, medium-length flowers with highly exerted anthers and stigmas (*A. ferox* Mill., *A. marlothii* A. Berger; Figure 4.1B, C), 3) short, tubular flowers with constricted corolla mouths (*A. dominella* Reynolds, *A. inconspicua* Plowes, *A. kraussii* Baker, *A. tenuior* Haw.; Figure 4.1F, G), and 4) densely flowered racemes of short, campanulate flowers (*A. vryheidensis* Groenew.; Figure 4.1D). The first two floral forms comprise putatively bird-pollinated species (Table 4.6), and are by far the most common among South African aloes. Short, constricted flowers are fairly common among the grass aloes, a subsection of small aloes notable for flowering during summer, whereas most aloes flower during winter (Reynolds 1950, Van Wyk and Smith 2003). Short, open flowers are rare in the genus, occurring in only four of 125 South African species, all of which occur in the section *Anguialoe* (Reynolds 1950, Van Wyk and Smith 2003).

Species were studied at single sites in KwaZulu Natal, South Africa, except for *A. arborescens*, *A. ferox* and *A. maculata*, which were studied at two, two and four sites respectively (Appendix A). Data for *A. maculata* were collected from plants at three sites described in Chapter 3 (Klipfontein, Hilton and Ixopo) during natural bee abundance treatment, and at an additional site (Hesketh Conservation Area). The Umgeni population described in Chapter 3 is not included, owing to the extraordinarily low seed and apparent resource limitation and/or high pollen theft during the no-hives treatment, which may bias estimates of self-compatibility and pollination success.

The extent of data differs among species, with less data for *A. dominella* and *A. tenuior*. I found only one *A. dominella* population of about 30 plants, which was ravaged by florivorous beetles (Scarabaeidae: Hopliini) that had damaged all plants in the stand to varying degrees. Insufficient healthy inflorescences were available to conduct

insect-exclusion or self-compatibility experiments, but I did collect the least damaged inflorescences to measure the phenology of undamaged flowers. This species is included despite incomplete data, because it is one of only three aloes with scented flowers known in southern Africa (the two others are *A. modesta* Reynolds; van der Riet 1977, and *A. rupestris*; personal observations), and therefore provides an interesting addition to a comparison of bird- versus insect-pollinated species. I was unable to study *A. tenuior* in the wild, as it grows hundreds of kilometres from the other study sites. However, I have included morphological data from plants in the University of KwaZulu Natal Botanical Garden in Pietermaritzburg, as this species belongs to an *Aloe* series suggested to represent a basal lineage from which other species evolved (Holland 1978, Treutlein et al. 2003).

#### *Floral morphology and intra-floral phenology*

Flower growth and sexual development were examined for five to 20 flowers on three to 13 plants of all species, except *A. ferox* and *A. marlothii* (data for these species are obtained from Hoffman 1988 and Reynolds 1950 when possible, but time constraints prevented measurements during this study). Measurements were made in the field whenever possible, but due to time constraints and site inaccessibility I measured cut inflorescences for *A. boylei*, *A. dominella* and *A. kraussii*. Cut inflorescences of such mid-sized *Aloe* species continue to flower and produce nectar normally as long as they are kept in water, and can even produce normal fruits and seeds (Chapter 3). For *A. vryheidensis* I conducted a less detailed assessment of floral phenology using high-resolution photographs, from which I could identify the openness of anthers and exposure of stigmatic papillae and whether pollen was visible on the stigma (Figure 4.1D). The volume and concentration of nectar standing crop was measured from flowers of various ages using calibrated microcapillary tubes and a handheld refractometer, respectively.

For all species, except *A. ferox*, *A. marlothii* and *A. vryheidensis*, I marked all open flowers on an inflorescence and took a series of measurements every 2 h during daylight. Anthesis and flower growth slowed greatly between evening and early morning. I inserted a microcapillary tube marked at 1-mm intervals into the corolla to measure: corolla depth from the top of the ovary to the point where petal tips splayed (to assess nectar accessibility), minimum and maximum exertion of open anthers (corolla mouth to the tip of the longest open anther and base of the shortest open anther, respectively), and stigma exertion. I recorded negative exertion when anthers or stigmas were included within the corolla tube. Corolla width was measured with the same marked microcapillary tube at the corolla mouth (where the petals began to splay) to evaluate which insects would be too wide to enter the corolla in search of nectar. During each visit I noted the number of dehiscent anthers and whether open anthers still had pollen.

Numerical scores were assigned to stigma appearance based on colour changes in the style and stigma and the expansion and moistness of the stigmatic papillae:

- 0 – papillae not or half expanded, stigma white
- 1 – papillae expanded, stigma white
- 2 – papillae expanded or maximally expanded, stigma white but turning translucent
- 3 – papillae maximally expanded, stigma translucent and often moist
- 4 – stigma drying, style turning brown
- 5 – papillae closing or closed, stigma dry and brown, flower closing or closed.

Stigma receptivity was determined by cross-pollinating virgin flowers during the five stages of stigma development. Styles were collected 24 h after pollination and kept in 70% ethanol until they could be examined for pollen tubes. Stigmas were rinsed with tap water, softened in 0.8 M NaOH for 6-12 h, rinsed again and stained using 0.1%

aniline blue for 24 h. Stained stigmas were mounted in glycerine and viewed at 100X using a UV light microscope (Carl Zeiss Axio Imager, blue fp reflector) to count the pollen grains attached to the stigma, the pollen tubes in the distal third of the style and the pollen tubes that reached the base of the style. Because of the small inflorescence and population size of *A. inconspicua*, too few flowers were available to assess stigma receptivity adequately using hand pollinations. However, outcrossed flowers in the self-compatibility experiment were all pollinated during perceived maximum stigma receptivity (stigma score 2-3), so seed set by these flowers can be compared to those by open-pollinated flowers to assess receptivity at this stage.

#### *Flower visitors*

To evaluate the abundance and effectiveness of floral visitors, wild plants of each species, except *A. tenuior* and *A. dominella*, were observed during at least three different days for a minimum of 4 h. *Aloe dominella* plants were observed for 2 h, but seemed too damaged and perhaps too few to attract a normal visitor complement. *Aloe tenuior* was observed for 5 h in the University of KwaZulu-Natal Botanical Garden. The abundance of avian and insect visitors at each site was evaluated using morning and afternoon transect surveys or patch observations (for *A. kraussii*, *A. inconspicua*). Bird transects were walked for 30 min through the study population, with five observation stops of 5 min each plus 5 min for walking between stops. Patch observations were conducted by observing a predefined patch of flowering aloes from a hidden location (often a vehicle) for 10 to 30 min. All birds seen or heard within 10 m of the transect or seen in the patch were counted, and identified if possible by sight or song. Foraging birds could not be observed closely enough to determine the state of the individual flowers they visited, but I recorded whether birds fed on upper, lower or all flowers on a raceme. Flower phase (hereafter referred to as male or female) could be judged by position on the raceme. For

species with vertical racemes lower flowers are older and therefore female, whereas upper flowers are male. For species with loose racemes (e.g., *A. kraussii*) flower orientation changes from pendulous to erect as flowers wilt, so older female flowers are held above younger male flowers.

Insect visitation was estimated by counting the insects present on 20 randomly chosen, flowering aloes, watched for 10 s each. Insects were identified to group (e.g., honey bee, small bee, wasp). During each flower visit I recorded a measure of flower stage (number of freshly opened anthers and/or stigma exsertion), whether the visitor collected nectar and/or pollen, and whether it contacted the stigma tip. In some cases insects were collected to determine their pollen load and sent to C. Eardley at the South African Agricultural Research Council for identification.

### *Self-compatibility*

Self-compatibility was assessed for five species: *A. arborescens*, *A. boylei*, *A. inconspicua*, *A. kraussii*, and *A. maculata*. I enclosed racemes in fine-mesh fabric to exclude all visitors and applied one of three treatments as flowers became receptive: 1) hand-pollination with outcross pollen from plants >10 m away, 2) hand-pollination with self-pollen (Figure 4.1A), and 3) unpollinated to test for autonomous pollination (i.e., without visitation). I counted the fruits per raceme and the seeds per fruit for 3–4 fruits per raceme. Plants in the autonomous treatment frequently produced swollen ovaries that looked like fruits, but contained no seeds. Such ovaries were counted as fruits for measures of fruit set and their seed count (0) was included in seed-set calculations. Female reproductive success was measured as seeds per fruit (mean seed set x proportion of flowers that set fruit) or total seeds per raceme for *A. arborescens* (mean seed set x total fruits/raceme). For each of these species, except *A. inconspicua*, stigmas and styles from bagged plants or those hand-pollinated with self-pollen were

viewed using UV microscopy to determine whether self-pollen tubes could be distinguished from outcross tubes. Self-compatibility was determined by seed set alone for *A. inconspicua*, due to the small number of flowers available, and obtained from Hoffman (1988) for *A. ferox*.

### *Pollination system*

The contribution of insect visitors to aloe pollination was assessed by excluding birds from inflorescences (*A. arborescens*, *A. ferox*, *A. marlothii*, *A. vryheidensis*), or plants (*A. boylei*, *A. inconspicua*, *A. kraussii*, *A. maculata*) with rigid plastic-mesh cages that allowed insects to pass freely (Figure 4.1B). Seed production was calculated as for the self-compatibility experiment and compared to seed production by unmanipulated plants or racemes that were open to all visitors. To distinguish between the pollen-quality hypothesis and the pollen-theft hypotheses (Table 4.1), I compared pollen deposition on stigmas of caged and open plants for four of the putatively bird-pollinated species (*A. arborescens*, *A. boylei*, *A. ferox* and *A. maculata*). Stigmas were collected from wilted flowers (i.e., after all possible deposition had occurred) at one site per species, except for *A. maculata* for which stigmas from three sites were analysed (Klipfontein, Hilton and Ixopo). Stigmas were stored in 70% ethanol and later rinsed and stained with basic fuchsin before I counted pollen grains under a light microscope.

### *Statistical analysis*

I used general linear models (Kutner et al. 2005: proc mixed SAS 9.1) to compare floral morphology among species and generalized linear models (McCullagh and Nelder 1989: proc genmod SAS 9.1) to analyze reproductive outcomes. The latter analyses considered negative-binomial distributions and a ln-link function. Models initially considered all possible interactions between independent variables, but nonsignificant interactions and factors were dropped from models using backward elimination ( $\alpha=0.05$ ). Factors that

reflected the experimental design (i.e., site and pollination treatment) were retained in the model, even if they were not statistically significant. Significant main effects or interactions were explored further using multiple comparison tests. A priori, orthogonal contrasts were tested with a Type I error rate of  $\alpha=0.05$ , whereas the Dunn-Šidák procedure was used for all other comparisons (Kirk 1995), and resulting estimates of least-squares means and standard errors are back-transformed.

Plants were sampled repeatedly during all experiments, so that different observations for individual plants may not be independent. For flower phenology, I averaged observations for each plant prior to analysis. For other variables, I used a variance-covariance model of compound symmetry to account for correlated responses, using the methods of either Kenward and Roger (1997: general linear models), or Liang and Zeger (1986: generalized linear models).

The effect of bird exclusion was explored first with a model that included all species, after which the effect on each species was further explored in separate models. For species studied at more than one site (*A. arborescens*, *A. ferox* and *A. maculata*) ‘site’ was included in the species-specific models as a factor.

## Results

### *Floral morphology*

The three floral types used to group species were supported by statistical analyses of flower length and width. Flowers of *A. arborescens*, *A. maculata* and *A. boylei* had similar widths and were significantly longer than flowers of the other species (Table 4.2). *Aloe boylei* flowers appear much larger than those of *A. maculata* or *A. arborescens*, but their large ovaries result in a slightly shorter inner corolla length. Although *A. ferox* and *A. marlothii* had similar corolla widths to the species with long, tubular flowers, their corolla mouths were congested with exerted anther filaments and styles (Figure 4.1C),

precluding access by insects, so the functional width in terms of accessibility to insect pollinators was zero. *Aloe vryheidensis* was similar to the species with short, tubular flowers with respect to flower length, but clearly differed from all other species in its wider corolla opening (Table 4.2). *Aloe inconspicua* had the smallest flowers of the species studied, which were also notable for their unusual white-green colour. Even bagged *A. inconspicua* flowers produced too little nectar to measure its concentration.

The amount of pollen produced per anther varied significantly among species ( $F_4=4.09$ ,  $P=0.01$ ). The only short-tubed species in the comparison, *A. kraussii*, produced the fewest pollen grains per anther, but this was only significantly different from pollen production by *A. boylei*, which had the highest per anther production (Table 4.2).

#### *Flower visitors*

##### Birds

Bird visitors to *Aloe* species ranged from sunbirds only (*A. boylei* and *A. maculata*) to short-billed birds only (*A. vryheidensis*) to a mixture of sunbirds and short-billed birds (*A. ferox* and *A. marlothii*; Table 4.3). *Aloe inconspicua* was never visited by birds, even though sunbirds and short-billed species that visit aloes were abundant at the site. Too few observations were made of *A. dominella* and *A. tenuior* to be certain that they are not avian-visited.

The diversity of sunbird (Nectariniidae) visitors varied among aloe species. Amethyst (*Chalcomitra amethystina* Shaw) and Malachite sunbirds (*Nectarinia famosa* L) visited all species visited by sunbirds. White-bellied sunbirds (*N. talatala* Smith) were regular visitors to *A. maculata* (Figure 4.1E), and Grey and Olive sunbirds (*N. veroxii* Smith and *N. olivacea* Smith) were seen visiting *A. arborescens* and *A. ferox*. Aloe pollen was visible on sunbirds foraging at *A. ferox* and *A. maculata*, and aviary



pollination experiments confirmed that significant quantities of pollen was transferred from anthers to receptive stigmas by Malachite sunbirds for *A. kraussii* (mean=79.2  $\pm$  SE=9.5 grains/stigma), and Amethyst sunbirds for *A. maculata* (251 grains/stigma, lower SE=29.1, upper SE=33.0; Chapter 3). The Collared sunbird (*Hedydipna collaris* Vieillot), which is the only sunbird in the region with a short (<17 mm) bill (Hockey et al. 2005), visited *A. ferox* and sometimes *A. arborescens* legitimately, but otherwise tore *A. arborescens* flowers open to access nectar, which it had difficulty reaching through the long, fused corolla. During limited bird observations, sunbirds visited 2.9 flowers per plant on *A. kraussii* ( $\pm$  SE=0.25,  $n=14$  birds), 9.3 flowers per plant on *A. arborescens* ( $\pm$  2.2,  $n=18$  birds), and 7.6 flowers per plant on *A. ferox* ( $\pm$  2.6,  $n=3$  birds).

A variety of short-billed birds, including Dark-capped Bulbuls (*Pycnonotus tricolor* Hartlaub), Fork-tailed Drongos (*Dicrurus adsimilis* Bechstein), Cape White-eyes (*Zosterops pallidus* Swainson) and several weaver species (Ploceidae) visited *A. ferox*, *A. marlothii* and *A. vryheidensis* regularly. These birds often carried visible loads of more than 100 000 pollen grains (data collected while banding birds in *A. ferox* and *A. vryheidensis* stands; Figure 4.1C). Short-billed birds visited  $6.7 \pm 4.6$  flowers per *A. ferox* plant ( $n=7$  birds) and  $13.4 \pm 6.1$  flowers per *A. vryheidensis* plant ( $n=8$  birds). White-eyes and bulbuls occasionally visited *A. arborescens*, sometimes trying to access nectar via the corolla mouth and other times pecking at the base of flowers. The number of flowers visits often depended on bird interactions, as birds visited the fewest flowers per plant when they were chased by, or left to chase another bird.

Streaky-headed Seed eaters (*Crithagra gularis* Smith) pecked holes in the base of *A. maculata* corollas to drink the nectar that seeped out. In doing so, these birds sometimes damaged the ovary and/or style and never contacted the anthers or stigma. This species also picked *A. marlothii* flowers and unlike other short-billed bird visitors never carried visible pollen loads.

### Insects

Bees (Apoidea) were the most common insect visitors to aloe flowers, whereas other insects were rare and generally unlikely to act as pollinators. Flies and butterflies sometimes consumed nectar that had seeped out of flower corollas (*A. ferox*) or holes made by nectar robbers (*A. maculata*, *A. dominella*, *A. kraussii*), but did not appear to contact anthers or stigmas and were not observed carrying pollen. Ants commonly visited all *Aloe* species studied, except *A. dominella*, *A. tenuior*, and *A. vryheidensis*, entering corollas to access nectar, but never contacting reproductive structures. Wasps occasionally fed on nectar of *A. arborescens*, *A. kraussii* and *A. ferox*. The *A. dominella* population was heavily visited by monkey beetles, which forced open young, sometimes unopened, flowers to access nectar. This activity seemed to damage flowers and despite the abundance of these beetles, none of the plants in this population set seeds, suggesting that the beetles behave as florivores or nectar robbers, rather than pollinators. Four similar beetles were seen on *A. kraussii*, either consuming flowers or forcing them open to consume nectar.

Honey bees (*Apis mellifera* L) were the most common insect visitors to all of the primarily bird-pollinated species, except *A. maculata* and *A. vryheidensis*, which were visited even more often by small (halictid or allodapine) bees (Table 4.3). Honey bees were also common visitors to *A. kraussii*, and often collected nectar and pollen from this species (Table 4.3). Honey bees visited an average ( $\pm$  SE) of  $16 \pm 10.1$  flowers per plant on *A. ferox* ( $n=4$  bees), and  $2.4 \pm 0.24$  flowers on *A. kraussii* plants ( $n=8$  bees). Although many honey bees foraged on plants a few feet from *A. inconspicua*, none visited this aloe during more than 50 h of observations. The only visitors to *A. tenuior* were pollen-collecting allodapine bees, but as this species was watched only in a garden outside its natural range, its natural visitors may be quite different.

Reward collection by insects varied among aloe species according to floral form. *Aloe vryheidensis* and *A. marlothii* were the only aloes that insects visited exclusively for pollen (Table 4.4). Bees avoid consuming the readily available, but phenolic-rich, nectar of *A. vryheidensis* (Johnson et al. 2006), and seemed unable to access nectar of *A. marlothii*, though preliminary analyses suggest that *A. marlothii* nectar may also have a high phenolic concentration (S.D. Johnson, unpublished data). Flowers of species with long corollas were also visited mostly for pollen (Table 4.4). Bees consumed nectar that had seeped out of the corolla mouth (*A. ferox*) or holes made by nectar-robbing birds (*A. maculata*, *A. arborescens*), so the lack of nectar collection probably reflects difficulty accessing it, as bees were usually excluded by the narrow corolla tubes (*A. arborescens*, *A. maculata*) or filaments that congested the corolla mouth (*A. ferox*, *A. marlothii*). An exception to the lack of nectar collection by insects for bird-pollinated species is *A. boylei*, which was visited regularly by *Amegilla* sp. (Apidae), which probed for nectar during every visit. These bees moved too quickly to observe distinct pollen-collecting behaviour, but two captured individuals carried 990 and 2750 grains of *A. boylei* pollen, most of which was packed in their scopae. Aloes with short, tubular corollas were visited most often by nectar-collecting insects, which could access nectar legitimately through the corolla mouth. Some insects collected only pollen from *A. kraussii*, but otherwise pollen collection seemed secondary to nectar collection for this species, occurring either passively or while insects probed for nectar.

The likelihood of insect visitors contacting stigmas also varied among aloe species and floral forms (Table 4.4). The exerted and splayed anthers and stigmas on densely flowered *A. vryheidensis* inflorescences allowed honey bees to contact stigmas readily while collecting pollen (Figure 4.1D, Table 4.4). Due to their larger size, honey bees made better contact with stigmas than did small bees. Although racemes of *A. marlothii* and *A. ferox* are also densely covered in flowers, anthers remain tightly

clustered when exerted (Figure 4.1C) and bees generally flew between flowers instead of walking. Thus, for these species bees could and mostly did avoid female-phase flowers from which all pollen had been removed (Table 4.4). However, bee visitors to all three of these species usually carried abundant aloe pollen, despite frequent grooming, so brief stigma contact could have resulted in pollen deposition. Pollen-collecting bees avoided flowers from which all pollen had been removed, but may have visited receptive flowers if stigmas became receptive before all anthers opened or all pollen had been removed. Nectar-collecting bees visited male and female flowers indiscriminately and pushed their heads into corollas while probing (e.g., Figure 4.1F, G), so they likely contacted even enclosed stigmas of all species.

*Aloe inconspicua* is notable as the only exclusively insect-pollinated species: this is the first report of an insect-pollinated aloe. The most common visitors were *Amegilla* sp., which always probed for nectar ( $n=31$  bees, >350 visits; Table 4.4), and generally visited every open flower on an inflorescence, often beginning at the bottom (i.e., female) flowers. *Amegilla* usually landed on flowers to probe, closely contacting the exerted anthers and stigmas (Figure 4.1G), but sometimes they probed while hovering, which may have resulted in less contact with floral organs. A few other small, unidentified bees (7 bees, 14 visits) and a small, ground-dwelling fly (5 flies, 37 visits) occasionally visited *A. inconspicua* flowers for nectar. Two single-visit experiments showed that *Amegilla* deposited pollen (45 grains) and both *Amegilla* and the fly effected pollination (11 and 4 seeds/fruit respectively). *Amegilla* usually carried aloe pollen in their scopae. One *Amegilla* visited flowers on a hand-held, cut inflorescence, and vibrated each flower while probing for nectar. Although the bee did not appear to be collecting pollen with her legs, all pollen from fresh anthers had been removed when she left. Evening observations were conducted on three separate nights, beginning at sunset and continuing

until no more potential visitors were flying (ca. 2200h). Many moths were seen, but none visited *A. inconspicua*.

#### *Self-compatibility*

Examination of pollen tubes from *A. arborescens*, *A. boylei*, *A. dominella*, *A. kraussii*, *A. maculata*, and *A. tenuior* revealed that self-pollen germinated successfully on the stigmatic surface and that self-pollen tubes were indistinguishable from outcross pollen tubes, growing normally and often reaching the base of the style. However, seed set by hand-pollinated flowers showed that all aloes studied were strongly self-incompatible (Table 4.5). Self-pollinated flowers set fewer than 5% as many seeds as cross-pollinated flowers, and this difference was highly significant for most species (Table 4.5).

#### *Pollination system*

The effect of bird exclusion varied significantly among aloe species (pollination treatment x species interaction  $T_7=43.11$ ,  $P<0.001$ ), but not among populations within species ( $P>0.25$ ). Bird exclusion reduced seed production for *Aloe* species with long, tubular flowers by 69 – 98%, and for *A. vryheidensis* by 45% (Table 4.6). This result demonstrates that bees acted primarily as pollen thieves for species with long, tubular flowers, but as inefficient pollinators for *A. vryheidensis*. In contrast, bird exclusion did not affect seed production significantly by the two species with short, tubular flowers, *A. kraussii* and *A. inconspicua* (Table 4.6), indicating that bees are effective pollinators of these species. Whereas *A. inconspicua* is entirely insect-pollinated, Malachite sunbirds were frequent visitors to *A. kraussii* in the field, and deposited large pollen loads on *A. kraussii* stigmas during aviary experiments (up to 350 grains during 1 h of foraging; unpublished data).

Predation of flowers, fruits and seeds differed among aloe species and populations. *Aloe inconspicua* and *A. vryheidensis* experienced some flower predation by

locusts, whereas the *A. ferox* population at Ixopo was subject to flower predation by rodents. Holes were chewed in four of 21 cages at Ixopo and the racemes inside were stripped of most or all of their flowers. Although rodents were not seen, fallen flowers collected on the broad leaves below the inflorescence along with rodent feces, which were also found on the mesh of two cages at the edge of the holes. These flowers were chewed to expose the base of the flower where nectar collects, whereas anthers were untouched, suggesting that rodents sought nectar, rather than pollen. Although rodents probably move large quantities of pollen from exerted anthers to exerted stigmas as they move over inflorescences, they destroyed most open flowers while feeding. This damage to cages largely accounts for the smaller sample for caged versus open inflorescences for *A. ferox*. *Aloe boylei* experienced intense fruit and seed predation by insects, as did *A. maculata* in two of four populations (Klipfontein and Hilton), so measured seed production may be unusually low for these species.

#### *Pollen receipt*

Bird-exclusion affected pollen receipt differently among the four putatively bird-pollinated *Aloe* species (Table 4.7). Pollen receipt did not differ significantly between caged and open inflorescences of *A. arborescens* and *A. ferox* (Table 4.7), even though caged plants produced significantly fewer seeds. Exclusion of birds reduced pollen receipt by *A. maculata* flowers (Table 4.7), but this reduction was not as great as the reduction seen in seed set. Bird-exclusion significantly reduced pollen receipt by *A. boylei* stigmas. Insects frequently visited caged inflorescences and pollen removal did not differ noticeably in amount or timing from that of open plants, so pollen deposition probably did not result from increased autonomous deposition due to insufficient removal.

### *Stigma receptivity*

I define the initiation of stigma receptivity as the earliest stigma stage at which pollen adhered to and germinated on stigmas, such that pollen tubes could be seen in the distal third of collected styles. *Aloe maculata* stigmas became receptive at stigma stage 2, whereas *A. arborescens*, *A. boylei*, *A. kraussii* became receptive at stage 1. Stigma appearance was not a reliable predictor of receptivity for *A. dominella*, as pollen germinated on stigmas at all stages as long as at least two anthers had opened, and stigmas remained receptive even as flowers were closing and drying out. I did not test stigma receptivity directly for *A. inconspicua*, but during hand-pollinations for other experiments I found that pollen did not stick to stigmas until they reached stage 1. Initial receptivity was therefore assumed to occur during stage 2, which is consistent with earliest receptivity of other species, barring *A. dominella*. *Aloe inconspicua* flowers in the self-compatibility experiment were hand-pollinated once, when the stigma appeared to be maximally receptive (very exposed papillae, tip changed from white to translucent, moist surface; stage 3). These fruits had slightly higher mean seed set (mean=17.5 seeds/fruit, LSE=2.2, USE=2.5) than fruits of naturally pollinated flowers that were visited multiple times during stigma receptivity (12.1 seeds/fruit, LSE=0.7, USE=0.8), indicating that stigmas were indeed receptive at stage 3.

### *Intra-floral phenology*

The phenology of the sex organs of *Aloe* species with tubular flowers followed a similar pattern (e.g., Figure 4.2). When the first anther opened the stigma was generally enclosed within the corolla. As the flower matured, anthers elongated and opened one by one, and eventually wilted and receded, so that six fresh anthers were never open at once. As illustrated in Figure 4.2, anthers grew to different lengths before becoming receptive. The style grew gradually, so that the stigma was usually level with the corolla mouth

when it became receptive and eventually surpassed the anthers. By mid- to late female phase all anthers had wilted and receded, at which point they were ignored by pollen-collecting insects. *Aloe vryheidensis* flowers were widely open and all anthers and stigma exerted before anthers began to dehisce.

Phenological variation among species involved a) the number of anthers open when the stigma first became receptive, b) the period between the dehiscence of the sixth anther and initial stigma receptivity and c) the separation between pollen-bearing anthers and stigmas at initial and maximum receptivity (Figure 4.2, Table 4.8). The first two variables determine the temporal separation of male and female function (dichogamy), whereas the third represents the spatial separation (herkogamy). Pollen removal from caged and open plants occurred quickly; anthers that dehisced during daylight were usually stripped of their pollen within 4 h, although this varied among populations and days, depending on visitor abundance and weather. Anthers that opened between dusk and dawn retained their pollen into morning, when insects became active.

### Dichogamy

Stigmas of primarily bird-pollinated species became receptive only at the end of the pollen-bearing (male) phase, generally after at least five anthers had dehisced (Table 4.8). *Aloe maculata* showed the greatest mean dichogamy, as initial receptivity often occurred several hours after the last anther had dehisced. Late receptivity should increase the likelihood that pollen is stripped from anthers before flowers become receptive, and this was observed for *A. maculata*, but not *A. arborescens* (Table 4.8). In contrast, stigmas of *A. vryheidensis* received pollen after as few as three anthers had dehisced and anthers retained some pollen when stigmas appeared to be fully receptive (stage 2 and 3). Thus male and female function probably overlap temporally for this species, more than in species with long, tubular flowers.



Except for *A. inconspicua*, the stigmas of insect-pollinated species became receptive relatively early during a flower's life, before all anthers had opened and thus before all pollen was removed. The enclosed stigmas were sometimes difficult to observe in the small *A. inconspicua* flowers, which could have biased the estimate of initial receptivity, as flowers were not included in the analysis unless the stigma was seen before it became receptive. However, of the six flowers for which stigma state could be assessed before all anthers had dehisced, only one was receptive before the last anther dehisced, which suggests that stigmas of this species become receptive later than those of the other insect-pollinated species (i.e. greater dichogamy).

The average number of anthers dehisced at first receptivity did not differ significantly among species overall ( $T_5=6.29$ ,  $P<0.2$ ; analysis does not include *A. vryheidensis* or *A. dominella* as occurrence of first receptivity is unknown for these species), presumably due to the small sample size (Table 4.8).

### Herkogamy

Insect-pollinated and insect-thieved species did not differ obviously for any of the three herkogamy measures (Table 4.8). Stigmas and anthers were closely associated during initial and maximum receptivity for all species. This limited average herkogamy resulted partially from negative and positive values cancelling each other, but nonetheless indicates that receptive stigmas must grow past anthers, as so would occupy similar positions at some time. Receptive stigmas of all species were level with pollen-bearing anthers during fewer than half of the observations (Table 4.8). *Aloe vryheidensis* stigmas were level with anthers throughout pollen presentation. Although stigmas of this species could be separated laterally from splayed anthers of the same flower, they were never more than 2 mm from open anthers of other flowers in these dense inflorescences.

The frequency of autonomous self-pollination by flowers kept indoors provides additional, circumstantial evidence for the differing spatial and temporal separation of male and female functions among *Aloe* species. *Aloe kraussii* flowers used in an aviary experiment had to be emasculated to avoid autonomous pollen deposition, as receptive stigmas were invariably covered with pollen otherwise. Similarly, autonomous pollination occurred in all but two of 25 *A. dominella* flowers that were observed until all anthers dehisced. In contrast, emasculation was not necessary for *A. maculata* plants used for aviary experiments, as stigmas rarely received pollen autonomously.

## Discussion

At the beginning of this Chapter, I proposed four hypotheses for why insects might act as pollen thieves, rather than as pollinators, and lines of evidence that would support or contradict each one (Table 4.1). Observations collected to test these hypotheses, which are summarised in Table 4.9, indicate that the ten *Aloe* species that I studied represent the full range of interactions with flower-visiting insects, from exclusively insect-pollinated (*A. inconspicua*), to almost exclusively bird-pollinated and therefore primarily thieved by pollen-collecting insects (*A. ferox*). Although bird-pollination cannot be ruled out for *A. dominella* and *A. tenuior* in the absence of bird-exclusion experiments and adequate pollinator observations, they resemble insect-pollinated *A. kraussii* and *A. inconspicua* in floral morphology and intra-floral phenology, suggesting that they are at least partially insect-pollinated, whether or not they are also visited by birds.

The only pollen-theft hypothesis that can be reasonably excluded for all ten species involves the role of herkogamy (Table 4.9). All *Aloe* species exhibited strong herkogamy late during flowers' lives, when stigmas were strongly exerted and anthers had wilted entirely. However, stigmas were not greatly separated from anthers during either initial or maximum receptivity, so pollen-collecting insects could contact receptive

stigmas of all species during some stage of flowering. This lack of relation between herkogamy and pollen theft for aloes does not imply that herkogamy is generally unimportant in determining whether insects act as pollinators or pollen thieves. In fact, many reports of pollen theft cite spatial separation between anthers and stigmas as the primary reason for pollen-collectors serving as thieves (Chapter 3). Intuitively, this influence is most likely when there is a mismatch in visitor and flower size, either because flowers are too large (e.g., *Ceiba acuminata*; Baker et al. 1971) or pollen collectors are too small (e.g., *Trigona* bees; McDade and Kinsman 1980). In contrast, the limited herkogamy displayed by the aloes that I studied was insufficient to preclude pollen deposition by pollen-collectors. This suggests that herkogamy in aloes evolves primarily to enhance flower-pollinator interactions, rather than to avoid pollen theft.

Dichogamy influenced the role of pollen-collecting insects for these aloes, although not without exception. Among species with tubular-flowers, whether insects served as pollinators, rather than as thieves, varied negatively with the temporal separation of male and female phases; primarily bird-pollinated species generally exhibited greater dichogamy than those pollinated by insects. However, insects deposited many pollen grains on *A. arborescens* stigmas and were the only pollinators of *A. inconspicua*, despite strong protandry in flowers of both species. Strong dichogamy does not prevent insect pollination of *A. inconspicua*, because insects visit female flowers for nectar. Indeed, dichogamy is a common feature of bee-pollinated plants that are visited for nectar, especially those with vertical inflorescences on which bees move predictably from lower, female-phase to upper, male-phase flowers, as it reduces sexual interference and geitonogamy (Harder et al. 2004, Jordan and Harder 2006). However, this explanation seems insufficient for the higher insect-mediated pollen deposition for *A. arborescens*, which insects rarely visited for nectar.

When flowers are strongly dichogamous but only weakly herkogamous, pollen-collectors may deposit pollen on young, nonreceptive stigmas. If this pollen adheres to immature stigmas and is sufficiently long-lived, it could germinate when stigmas become receptive. Although pollen did not adhere to very young stigmas of some species due to the lack of stigma exudates, pollen germinated on stigmas of *A. dominella* and *A. ferox* (Hoffman 1988) before exudate was observed, so it must adhere without exudates. The role of dichogamy in preventing pollination by pollen-collectors therefore depends partially on pollen longevity, whereby longer-lived pollen could mitigate the delay between pollen presentation and stigma receptivity and so enable pollen collectors to act as pollinators. This prediction contrasts with theoretical results of Thomson and Thomson (1992), who found that ‘ugly’ pollinators, which removed much pollen but deposited little, were better pollen exporters when pollen was short-lived, because “low viability puts a premium on pollen removal” (pg. 13, Thomson and Thomson 1992). However, their simulations considered adichogamous plants, so pollen longevity determined how long pollen could ‘wait’ before removal from anthers, rather than the period during which stigmas could receive it.

Nectar accessibility to insect foragers partially determines whether insects visit female-phase flowers, and therefore influences whether insects act as pollinators or pollen thieves. The two species pollinated most successfully by insects, *A. kraussii* and *A. inconspicua*, have short corollas and were visited regularly by nectar-feeding bees. If individual bees collect pollen and nectar simultaneously, as was the case for *Amegilla* on *A. inconspicua*, they will visit female- and male-phase flowers and so likely act as pollinators. In contrast, if individual bees collect only one resource at a time, such as the honey bees that visited *A. kraussii*, conspecific pollen- and nectar-collectors may act as pollen thieves and pollinators, respectively (e.g., Ish-Am and Eisikowitch 1993).

However, the onset of stigma receptivity before all anthers dehisce, as seen in *A. kraussii*, increases the chance that even strict pollen collectors deposit pollen.

*Aloe boylei* is an intriguing case, as bird exclusion reduced pollen receipt, despite regular visits to female-phase flowers by nectar-collecting bees. Pollen-collecting bees may have avoided female-phase flowers of this strongly protandrous species, but flowers of all ages produced ample nectar. Furthermore, nectar-collecting bees pushed their heads into corolla mouths and so likely contacted stigmas, which are inserted by only 2 mm, so appeared to act as potential pollinators. The two *Amegilla* that I collected carried ample *A. boylei* pollen (although twice as much pollen of other species), but much of this was contained in the scopae on their hind legs and so may have been less available to stigmas. In contrast, sunbirds carried many *A. boylei* pollen grains on their chins, an area which is difficult to groom and likely contacts stigmas while birds probe for nectar (Hargreaves et al. 2004). Thus, although dichogamy may have caused limited pollination by pollen collectors, the lack of pollen deposition by nectar-collecting insects may have arisen because they carried insufficient pollen loads (Hypothesis 3; Table 4.9).

Alternatively, the low abundance of insect pollinators may explain the low insect-mediated pollen deposition for *A. boylei*, despite visitation to female-phase flowers. Mount Gilboa was the highest site studied (1700 m.a.s.l.; Appendix A), and the weather there tends to be either cold and foggy or very windy. Inclement weather creates difficult flying conditions for small insects, which may explain increased frequency of bird-pollinated plants at high elevations observed in Mexico (Cruden 1972, Kromer et al. 2006). Although insect-pollinated species are abundant on Mt. Gilboa (e.g., Johnson 2000, Alexandersson and Johnson 2002, Johnson et al. 2002), only 14 bees were seen visiting *A. boylei* during 6 h of observation, whereas >100 bees were seen on the other bird-pollinated species during equivalent observation periods (Table 4.3). The ability of

birds to fly in adverse weather and perhaps the better location of pollen on their bodies likely increased their relative effectiveness as pollinators.

Evidence from several species suggests that insects may deposit lower-quality pollen as well as lesser quantities than do birds. Flowers on caged and open inflorescences imported equivalent numbers of pollen grains for *A. arborescens* and *A. ferox* (Table 4.7), but caged plants produced significantly fewer seeds (Table 4.6). Assuming that apparently equal insect visitation translated into equivalent pollen removal (i.e., deposition is not affected by the amount of pollen remaining in anthers), equal pollen receipt eliminates the three hypotheses for pollen theft, which propose that insects do not deposit pollen (Table 4.1). Given that both *A. ferox* and *A. arborescens* are self-incompatible and produce thousands of flowers per plant, the low fruit production by caged inflorescences suggests that insects primarily caused self-pollination. This conclusion is consistent with observations that honey bees visited twice as many flowers per visit to *A. ferox* plants as birds. Low-quality pollination by insects may also have been a factor for *A. maculata* and *A. vryheidensis*. Bird-exclusion reduced pollen receipt by *A. maculata* flowers, but this reduction was not as great as the reduction in seed set. Although pollen receipt was not measured for *A. vryheidensis*, pollen-collecting insects carried abundant pollen on their bodies (not just in their scopae) and regularly contacted stigmas (Figure 4.1D), but nonetheless effected less pollination than birds, despite being more frequent visitors.

This survey of *Aloe* pollination revealed that a combination of factors determine whether pollen-collecting insects serve as pollen thieves, or as pollinators of varying quality, and that the relative importance of these factors can differ among species with similar floral morphology and visitor assemblages. As predicted, plant characteristics, in this case dichogamy and nectar accessibility, largely governed whether insects visited female-phase flowers, and therefore whether they acted as pollinators or pollen thieves.

However, insect behaviour may also be important in determining whether they act as pollen thieves. Insects deposited lower quality, probably self-pollen, on flowers of several species, and preliminary evidence suggests they move less frequently between plants than do birds. Because low-quality, insect-mediated pollen deposition was a particular problem for species with large inflorescences, plant characteristics may again ultimately determine patterns of pollen robbery and inefficient pollination, though at the level of inflorescence, instead of floral design.

Table 4.1 Hypotheses for why insects act as pollen thieves (1-3) or inefficient pollinators (4) for certain *Aloe* species and resulting predictions. Yes/No indicate the expected findings if a given hypothesis is true, whereas blanks mean the prediction could be true or false for that hypothesis.

| Hypotheses                           | Floral phenology |               | Insect behaviour |           |                     | Pollen      |
|--------------------------------------|------------------|---------------|------------------|-----------|---------------------|-------------|
|                                      | Dichogamy        | Herkogamy     | Collect pollen   | No stigma | Less inter-plant    | deposition  |
|                                      | insect < bird    | insect < bird | only             | contact   | movement than birds | cage < open |
| 1) Do not visit female-phase flowers | Y                |               | Y                |           |                     | Y           |
| 2) Do not contact receptive stigmas  | N                | Y             | M                | Y         |                     | Y           |
| 3) Do not deposit pollen             | N                | N             |                  | N         |                     | Y           |
| 4) Deposit only poor-quality pollen  | N                | N             |                  | N         | Y                   | N           |



Table 4.2 Floral and inflorescence characteristics of ten *Aloe* species. Note that racemes/plant reflect plants used for this study, not species means. Superscript letters denote significant differences between species ( $\alpha=0.05$ ). Nectar concentration (conc.) is %sucrose by weight. Flower colour: O-Orange, Y-Yellow, W-White, G-Green.

|                       | Flower |       | Corolla                  |                         | Nectar     |            | Pollen per               | Flowers | Racemes   |
|-----------------------|--------|-------|--------------------------|-------------------------|------------|------------|--------------------------|---------|-----------|
|                       |        |       | depth                    | width                   | vol.       | conc.      | anther                   | per     | per plant |
|                       |        |       | (mm)                     | (mm)                    | ( $\mu$ L) | (%sucrose) | (1000 grains)            | raceme  | median    |
| <i>Aloe</i> species   | colour | scent | mean (SE)                | mean (SE)               | mean       | mean       | mean (SE)                | median  | (max)     |
| Long, tubular         |        |       |                          |                         |            |            |                          |         |           |
| <i>A. arborescens</i> | O      | N     | 32.1 (0.25) <sup>a</sup> | 3.7 (0.07) <sup>b</sup> |            | 15.3*      | 18.0 (2.1) <sup>ab</sup> | 168     | 5 (16)    |
| <i>A. boylei</i>      | O      | N     | 30.3 (0.71) <sup>a</sup> | 3.8 (0.44) <sup>b</sup> | 19.4       | 22.1       | 26.7 (2.7) <sup>a</sup>  | 40      | 1         |
| <i>A. maculata</i>    | O      | N     | 31.0 (0.78) <sup>a</sup> | 4.3 (0.16) <sup>b</sup> | 32.2*      | 16.6*      | 16.7 (2.4) <sup>ab</sup> | 34      | 3 (7)     |
| <i>A. marlothii</i>   | YO     | N     | 23.5 <sup>†</sup>        |                         | 46.6*      | 8.3*       |                          | 300     | 6 (13)    |
| <i>A. ferox</i>       | O      | N     | 27 <sup>†</sup>          |                         | 81.3*      | 8.6*       | 15.8 (4.8) <sup>ab</sup> | 400     | 5 (12)    |
| Short, tubular        |        |       |                          |                         |            |            |                          |         |           |
| <i>A. dominella</i>   | Y      | Y     | 14.3 (0.17) <sup>b</sup> | 2.6 (0.10) <sup>c</sup> | 4.5        |            |                          | 45      | 1         |
| <i>A. kraussii</i>    | Y      | N     | 10.7 (0.19) <sup>c</sup> | 1.6 (0.08) <sup>c</sup> | 2.2        | 19.1       | 12.6 (2.2) <sup>b</sup>  | 67      | 1 (2)     |

|                        |    |   |                          |                          |       |      |     |       |
|------------------------|----|---|--------------------------|--------------------------|-------|------|-----|-------|
| <i>A. inconspicua</i>  | WG | N | 7.8 (0.63) <sup>c</sup>  | 1.8 (0.09) <sup>c</sup>  | trace |      | 23  | 1     |
| <i>A. tenuior</i>      | O  | N | 14.1 (0.75) <sup>b</sup> | 2.2 (0.15) <sup>c</sup>  | 1.2   | 29.6 | 56  |       |
| Short, open            |    |   |                          |                          |       |      |     |       |
| <i>A. vryheidensis</i> | Y  | N | 13.7 (0.37) <sup>b</sup> | 11.3 (0.35) <sup>a</sup> | 41.1  | 11.6 | 500 | 2 (4) |

\*unpublished data from S. Nicholson

†Reynolds 1950

Table 4.3 Number of visitors seen feeding on nine *Aloe* species during insect and bird surveys and pollinator observations, excluding nectar-robbing birds. Small bees include halictid (Halictidae) and allodapine (Apidae) bees, whereas large bees include large carpenter bees (*Xylocopa* spp.: Apidae) and megachilid bees (Megachilidae). Bold numbers indicate that the pollinator group carried pollen of the corresponding *Aloe* species. Hours of observation (hr) are given for each species (excluding nocturnal observations for *A. inconspicua*).

| <i>Aloe</i> species    | Total number of visitors seen feeding on <i>Aloe</i> |            |            |      |                 |            |          |                 |
|------------------------|--|------------|------------|------|-----------------|------------|----------|-----------------|
|                        | Birds  |            |            | Bees |                 |            |          |                 |
|                        | hr   | Sunbird    | Short-bill | hr   | <i>Apis</i>     | Small      | Large    | <i>Amegilla</i> |
| Long, tubular          |  |            |            |      |                 |            |          |                 |
| <i>A. arborescens</i>  | 5  | 38         | 2          | 5    | <b>153</b>      | <b>36</b>  | <b>1</b> | 0               |
| <i>A. boylei</i>       | 6  | <b>20*</b> | 0          | 6    | 8 <sup>†</sup>  | 1          | 0        | <b>5</b>        |
| <i>A. maculata</i>     | 17   | <b>54</b>  | 0          | 14   | 83 <sup>†</sup> | <b>174</b> | 2        | 0               |
| <i>A. marlothii</i>    | 4  | 2          | <b>47</b>  | 4    | <b>80</b>       | 29         | 0        | 0               |
| <i>A. ferox</i>        | 7  | <b>44</b>  | <b>60</b>  | 6    | <b>392</b>      | <b>140</b> | 0        | 0               |
| Short, tubular         |  |            |            |      |                 |            |          |                 |
| <i>A. kraussii</i>     | 6  | <b>42</b>  | 0          | 4    | 29 <sup>†</sup> | 0          | <b>1</b> | <b>8</b>        |
| <i>A. inconspicua</i>  | 25   | 0          | 0          | 25   | 0               | 0          | 0        | <b>69</b>       |
| <i>A. tenuior</i>      | 5  | 0          | 0          | 5    | 0               | <b>10</b>  | 0        | 0               |
| Short, open            |  |            |            |      |                 |            |          |                 |
| <i>A. vryheidensis</i> | 5  | 1          | <b>92</b>  | 2    | <b>61</b>       | <b>133</b> | <b>2</b> | 0               |

\*pollen collected from Malachite sunbirds (Hargreaves et al. 2004)

<sup>†</sup>pollen only in corbiculae

Table 4.4 Observations of bee visitors to aloes, including; number of nectar- (N) vs. pollen-collecting (P) flower visits, the number of visits to female- (F) vs. male-phase (M) flowers, number of visits during which stigma was or was not contacted. Sample sizes vary depending on the ease of observing the phenomenon while following a bee. 'Both' indicates bees did not seem to discriminate amongst male- and female-phase flowers, whereas 'all' indicates bees visited all open flowers on a plant. Stigma contact is noted as 'likely' if it could not be observed directly (e.g., stigma enclosed), but is likely based on visitor behaviour.

| <i>Aloe</i> species   | Bee type<br>(n)      | N:P<br>visits | F:M<br>flowers | Stigma<br>contact Y:N |
|-----------------------|----------------------|---------------|----------------|-----------------------|
| Long tubular flowers  |                      |               |                |                       |
| <i>A. arborescens</i> | honey bee (7)        | 8 : 23        | 14 : 22        | 7(brief): 10          |
| <i>A. boylei</i>      | honey bee (3)        | 3 : 4         | .              | 0 : 1                 |
|                       | small (1)            | 0 : 1         | 0 : 1          | 0 : 1                 |
|                       | <i>Amegilla</i> (4)  | 12*           | both           | likely                |
| <i>A. maculata</i>    | honey bee (30)       | 2 : 55        | all M          | rare                  |
|                       | allodapine (14)      | 0 : 14        | all M          | .                     |
| <i>A. ferox</i>       | honey bee (4)        | 3 : 107       | 2 : 104        | 1 : 20                |
| <i>A. marlothii</i>   | honey bee (4)        | 0 : 10        | 0 : 10         | .                     |
| Short, tubular        |                      |               |                |                       |
| <i>A. inconspicua</i> | <i>Amegilla</i> (31) | 244 : 9       | all            | usually               |
| <i>A. kraussii</i>    | honey bee (8)        | 3 : 30        | 2 : 36         | 16 : 2                |
|                       | megachilid (3)       | 12 : ?        | both           | Y                     |
|                       | <i>Amegilla</i> (4)  | 6 : 0         | both           | likely                |
| <i>A. tenuior</i>     | allodapine           | 72 : 11       | 10 : 19        | 4 : 4                 |

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|                                       |                      |       |       |         |
|---------------------------------------|----------------------|-------|-------|---------|
| Short, wide<br><i>A. vryheidensis</i> | honey bee (61)       | all P | all M | usually |
|                                       | small (133)          | all P | all M | M       |
|                                       | <i>Xylocopa</i> spp. | all P |       | Y       |
| (3)                                   |                      |       |       |         |

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\*bees moved too quickly to observe distinct pollen-collecting behaviour, but carried *A. boylei* pollen in their scopae.

Table 4.5 Differences in the mean ( $\pm$  SE) seed production per flower by bagged flowers subject to autonomous self-pollination, or hand pollination with outcross pollen or self-pollen. Values are means (LSE, USE), and the flowers/treatment given for species for which it was recorded. RS= measure of reproductive success, 1=seeds per raceme, 2=seeds per flower.

| Floral form           | Number of plants             |                | Selfed vs. Outcross  |                      |                    |                    |
|-----------------------|------------------------------|----------------|----------------------|----------------------|--------------------|--------------------|
| <i>Aloe</i> species   | (flowers)                    | RS             | Autonomous           | Selfed               | Outcross           | $T_1$              |
| Long, tubular         |                              |                |                      |                      |                    |                    |
| <i>A. arborescens</i> | 6                            | 1              | 0                    | 8.0<br>(4.1, 8.2)    | 795<br>(189, 247)  | 37.05 <sup>†</sup> |
| <i>A. boylei</i>      | 6                            | 2              | 0                    | 1.3<br>(0.54, 0.90)  | 23.1<br>(6.3, 8.7) | 10.88**            |
| <i>A. maculata</i>    | 43, 12, 46<br>(4840, 78, 90) | 2 <sup>†</sup> | 0.02<br>(0.01, 0.06) | 0                    | 30.1<br>(5.8, 7.2) | 39.49***           |
| Short, tubular        |                              |                |                      |                      |                    |                    |
| <i>A. inconspicua</i> | 6<br>(25)                    | 2              | 0                    | 0.33<br>(0.19, 0.46) | 15.0<br>(2.3, 2.7) | 3.91 <sup>††</sup> |
| <i>A. kraussii</i>    | 7, 7, 10<br>(154, 133, 586)  | 2              | 0.11<br>(0.05, 0.1)  | 0.21<br>(0.12, 0.29) | 8.2<br>(1.3, 1.6)  | 5.79*              |

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|                        |               |                |   |              |            |          |
|------------------------|---------------|----------------|---|--------------|------------|----------|
| Short, wide            |               |                |   |              |            |          |
| <i>A. vryheidensis</i> | 1, 1, 4       | 2 <sup>†</sup> | 0 | 0.74         | 24.3       | 30.22*** |
|                        | (40, 40, 308) |                |   | (0.32, 0.58) | (1.2, 1.2) |          |

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\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

<sup>†</sup> $P = 0.053$ , <sup>††</sup> $P = 0.061$

<sup>†</sup>=no repeated measure used

Table 4.6 Effect of bird exclusion on mean ( $\pm$  SE) seed production per raceme (R) or flower (F) by eight *Aloe* species. Unit of replication is either plant (P) or raceme (R). Sample size ( $n$ ) refers to the number of plants per treatment (different sample sizes are listed as CG, CT).

| Floral form            |   | RS        |        | Open         | Caged        | $T_1$    |
|------------------------|---|-----------|--------|--------------|--------------|----------|
| <i>Aloe</i> species    |   | replicate | type   | mean         | mean         |          |
|                        |   |           | $n$    | (LSE, USE)   | (LSE, USE)   |          |
| Long, tubular          |   |           |        |              |              |          |
| <i>A. arborescens</i>  | R | R         | 34     | 3219         | 977          | 16.39*** |
|                        |   |           |        | (370, 418)   | (198, 248)   |          |
| <i>A. boylei</i>       | P | F         | 12     | 10.7         | 2.9          | 7.96**   |
|                        |   |           |        | (2.5, 3.2)   | (0.75, 1.0)  |          |
| <i>A. maculata</i>     | P | F         | 96, 98 | 237          | 42           | 27.29*** |
|                        |   |           |        | (42, 51)     | (7.5, 9.1)   |          |
| <i>A. ferox</i>        | R | R         | 44, 63 | 4115         | 194          | 19.61*** |
|                        |   |           |        | (697, 839)   | (72, 114)    |          |
| <i>A. marlothii</i>    | R | R         | 8, 21  | 6351         | 75           | 8.96**   |
|                        |   |           |        | (651, 726)   | (33, 60)     |          |
| Short, tubular         |   |           |        |              |              |          |
| <i>A. inconspicua</i>  | P | F         | 5      | 3.4          | 5.1          | 2.36     |
|                        |   |           |        | (0.67, 0.83) | (0.65, 0.74) |          |
| <i>A. kraussii</i>     | P | F         | 15     | 2.8          | 2.5          | 0.29     |
|                        |   |           |        | (0.37, 0.42) | (0.31, 0.35) |          |
| Short, wide            |   |           |        |              |              |          |
| <i>A. vryheidensis</i> | R | F         | 10     | 5.5          | 3.0          | 5.89*    |



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|           |           |
|-----------|-----------|
| 0.95, 1.1 | 0.89, 1.3 |
|-----------|-----------|

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\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

Table 4.7 Mean ( $\pm$  SE) pollen receipt by caged and open-pollinated flowers of four *Aloe* species with long, tubular flowers. Unit of replication is either plant (P) or raceme (R), and sample size ( $n$ ) refers to the number of plants per treatment (different sample sizes are listed as CG, CT).

| Species               | Replicate | Number    |  | Open                | Caged                | $T_1$    |
|-----------------------|-----------|-----------|--|---------------------|----------------------|----------|
|                       |           | of plants |  |                     |                      |          |
| <i>A. arborescens</i> | R         | 17        |  | 77.7<br>(8.5, 9.5)  | 82.0<br>(12.1, 14.1) | 0.07     |
| <i>A. boylei</i>      | P         | 12        |  | 208<br>(25.3, 28.8) | 67.5<br>(11.0, 13.2) | 15.34*** |
| <i>A. maculata</i>    | P         | 47, 52    |  | 213<br>(18.3, 20.0) | 157<br>(19.7, 22.6)  | 3.92*    |
| <i>A. ferox</i>       | R         | 13, 19    |  | 103<br>(10.2, 11.3) | 139<br>(19.4, 22.5)  | 2.56     |

\* $P < 0.05$ , \*\*\* $P < 0.001$

Table 4.8 Temporal and spatial separation of male and female function for flowers of eight *Aloe* species. Initial receptivity is the earliest possible stigma stage (see text) during which pollen adhered to the stigma and/or germinated successfully, whereas maximum receptivity was determined with hand pollinations (blank if maximum pollen deposition and germination did not correspond to stigma score). Sample size ( $n$ ) is the number of plants, and plant was treated as the unit of repeated measurement for all variables except those marked with '\*'. Negative stigma-anther separation indicates that the anthers exceeded the stigma. 'Proportion' refers to the proportion of flowers or flower x time observations for which the statement was true.

|                        |          | Stigma first receptive |                   |             |               | Maximum       | Throughout        |
|------------------------|----------|------------------------|-------------------|-------------|---------------|---------------|-------------------|
|                        |          |                        |                   |             |               | receptivity   | receptivity       |
|                        |          | Open                   | Time after 6th    | All pollen  | Stigma-anther | Stigma-anther | Stigma level with |
|                        |          | anthers                | anther dehiscence | removed     | separation    | separation    | unwilted anthers* |
|                        |          |                        | (h)               | proportion* | (mm)          | (mm)          | proportion        |
| <i>Aloe species</i>    | <i>n</i> | mean (SE)              | mean (SE)         | (n flowers) | mean (SE)     | mean (SE)     | (no. obs)         |
| Long, tubular          |          |                        |                   |             |               |               |                   |
| <i>A. arborescens</i>  | 3        | 5.7 (0.22)             | 0.79 (0.31)       | 0.20 (10)   | -0.91 (0.052) | 0.42 (0.27)   | 0.48 (95)         |
| <i>A. boylei</i>       | 5        | 5.2 (0.35)             | 0.86 (0.53)       |             | -0.47 (0.50)  |               | 0.31 (45)         |
| <i>A. maculata</i>     | 6        | 6.0 (0.07)             | 5.8 (1.2)         | 0.81 (37)   | -1.91 (0.84)  | -1.1 (1.1)    | 0.32 (111)        |
| Short, tubular         |          |                        |                   |             |               |               |                   |
| <i>A. dominella</i>    | 4        | 2+                     | 0                 | 0           | -1.7 (0.89)   |               | 0.42 (224)        |
| <i>A. kraussii</i>     | 13       | 3.4 (1.1)              | 0                 | 0           | -0.5 (0.42)   |               | 0.27 (124)        |
| <i>A. inconspicua</i>  | 4        | 5.4 (0.26)             | 2.0 (1.4)         | 0.5 (10)    | -1.0 (0.68)   | -0.15 (0.21)  | 0.43 (122)        |
| <i>A. tenuior</i>      | 4        | 3.9 (0.79)             | 0.3 (0.3)         | 0           | -4.3 (1.4)    | -2.0 (0.99)   | 0.36 (150)        |
| Short, open            |          |                        |                   |             |               |               |                   |
| <i>A. vryheidensis</i> | 2        |                        | 0                 | 0           | 0             |               | 0                 |

Table 4.9 Summary of study results, showing whether predictions were true (Y) or false (N) for each of the bird-pollinated (i.e., pollen-thieved) *Aloe* species, and therefore which of the four proposed hypotheses for pollen-theft are supported for that species (see Table 4.1). Insect-stigma contact is recorded as ‘(N)’ if it was not observed but insects deposited considerable pollen.

| <i>Aloe</i> species    | Floral phenology |               |               | Insect behaviour |           |                                | Supported hypotheses |
|------------------------|------------------|---------------|---------------|------------------|-----------|--------------------------------|----------------------|
|                        | Deposition       | Dichogamy     | Herkogamy     | Collect          | No stigma | Less inter-plant movement than |                      |
|                        | cage < open      | insect < bird | insect < bird | pollen only      | contact   | birds                          |                      |
| <i>A. arborescens</i>  | N                | Y             | N             | Y                | (N)       |                                | 4 (1)                |
| <i>A. boylei</i>       | Y                | Y             | N             | N                | N         |                                | 1 or 3               |
| <i>A. ferox</i>        | N                | N*            | N*            | Y                | (N)       | Y                              | 4                    |
| <i>A. maculata</i>     | Y                | Y             | N             | Y                |           |                                | 1, 2, 3              |
| <i>A. marlothii</i>    |                  |               |               | Y                |           |                                | insufficient data    |
| <i>A. vryheidensis</i> |                  | N             | N             | Y                | N         |                                | 4                    |

\* Hoffman 1988

Figure 4.1 Representative *Aloe* species and their visitors. (A) Hand-pollination of female-phase flowers of *A. arborescens* (scale bar 20 mm). (B) *A. ferox* with one raceme caged to exclude birds (scale 50 cm). (C) Black-capped Bulbul carrying visible pollen on its forehead, feeding on *A. ferox* (scale 20 mm). (D) Honey bees collecting pollen from *A. vryheidensis*. Arrow shows stigma at stage 3 with pollen (scale 10 mm). (E) White-bellied Sunbird on *A. maculata* (scale 20 mm). (F) Megachilid bee probing for nectar from *A. kraussii*, pollen clearly visible on bee's abdomen. Note exerted stigma with pollen in flower to right of bee (scale 10 mm). (G) *Amegilla* probing *A. inconspicua*, whose leaves are almost indistinguishable from surrounding grass. Note pollen in scopae (scale 10 mm). Photos C, D, E: SD Johnson; F, G: GT Langston.



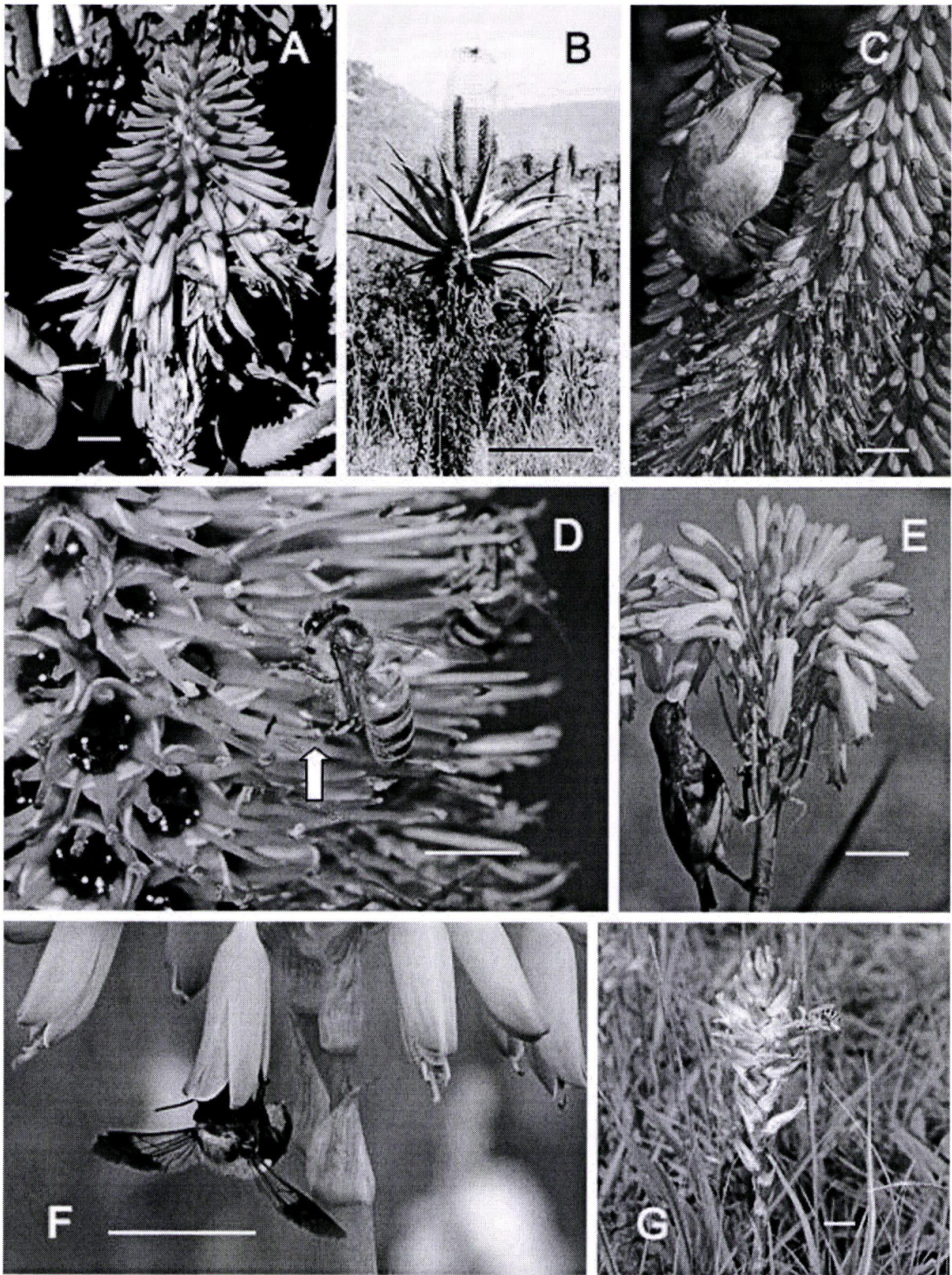
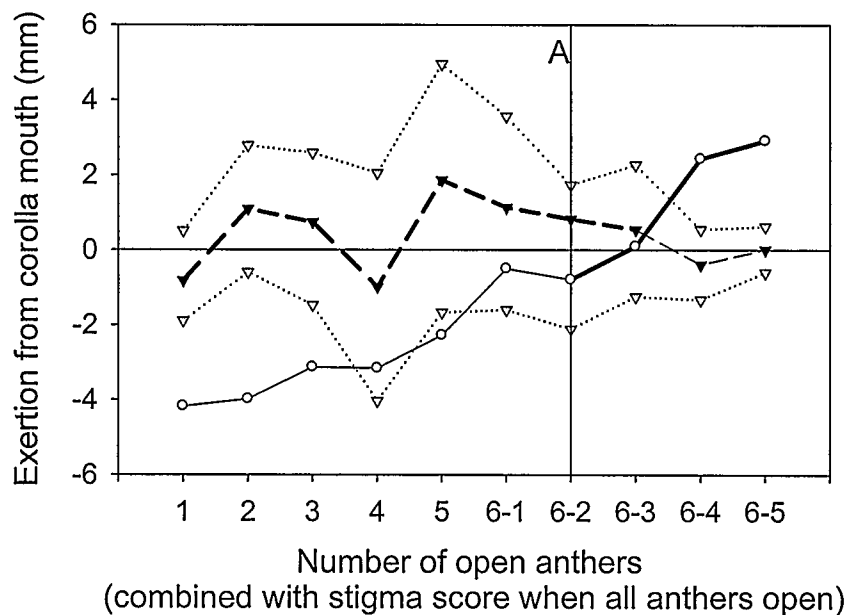


Figure 4.2 Mean anther and stigma positions for *Aloe maculata* during a flower's life, as measured by the progression of anther dehiscence (1 to 6 dehisced anthers), and subsequent stigma receptivity (see text). Open circles (delineated with solid line) represent mean stigma exsertion, black triangles (delineated with dashed line) represent mean anther exsertion, and lower and upper grey triangles (delineated with dotted lines) represent mean minimum and maximum anther exsertion, respectively. Entirely retracted anthers were assigned an exsertion of -1. Thick lines for mean anther and stigma exsertion represent the (mean) period when anthers bear pollen and the stigma is receptive, respectively. Line A marks the average occurrence of initial stigma receptivity.





## CHAPTER 5 – CONCLUDING DISCUSSION

The relation between flowers and their visitors varies from highly specialised, coevolved mutualisms to cheating, in which either the plant or visitor benefits at the expense of the other (reviewed by Bronstein et al. 2006). The evolutionary and ecological implications of cheating by plants (rewardlessness and pollination by deceit: Bronstein 2001) and by animals that steal nectar have received considerable attention (Irwin et al. 2001). In contrast, pollen theft has been largely ignored, even though it is more widespread than floral deception and, because of its direct implications for gamete transfer, has greater potential to influence plant ecology and evolution implications than does nectar theft.

This dissertation introduced several new perspectives on the incidence of pollen theft and its consequences for plant reproduction. The review of current literature on pollen theft in Chapter 2 identified generalisations about the animals involved and the plant and animal characteristics that result in pollen theft, rather than pollination. Pollen theft seems to be dictated primarily by floral traits that prevent or discourage pollination by pollen-collecting insects (Table 2.1). The importance of floral traits in mediating pollen theft suggests functional reinterpretations of plant characteristics, such as cryptic pollen, toxic pollen and reduced separation of male and female function, as mechanisms that may evolve to deter pollen thieves or co-opt them into pollinating. The experimental manipulations described in Chapter 3 demonstrated that pollen theft reduces both male and female components of pollination and reproductive success, even for hermaphroditic plants (Figures 3.1, 3.3). Pollen theft can therefore reduce both individual and population fitness, and so can have both evolutionary and ecological consequences for plant populations. Finally, exploration of differences in floral traits for ten *Aloe* species and their consequences for interactions with pollen-collecting bees in Chapter 4 revealed that a species' susceptibility to pollen theft depends largely on nectar accessibility to insects and temporal separation between pollen presentation and stigma receptivity. Based on

these new results, I now consider the evidence for each suite of floral adaptations to pollen theft proposed in Chapter 2, the potential evolutionary conflicts between these and other adaptive traits, and the broader implications of pollen theft for plant ecology

### **Floral adaptation to pollen theft in *Aloe***

In Chapter 2, I proposed three possible evolutionary responses to pollen theft: tolerance of pollen loss to thieves, co-opting pollen collectors into pollinating, and anti-theft adaptations. I now return to these hypotheses and assess whether such responses are evident in *Aloe*.

#### *Tolerance*

The reproductive consequences of pollen consumption by pollen-collecting bees could select for increased pollen production, as is evident among plants that offer pollen as a reward to pollinators (Buchmann 1983; L.D. Harder unpublished data). Pollen production varied significantly among the *Aloe* species that I studied, and the only insect-pollinated species measured (*A. kraussii*) produced the fewest pollen grains per anther. It is impossible to determine whether this pattern reflects compensatory pollen production without data from additional insect-pollinated aloes and pollen:ovule ratios; however, both *A. ferox* and *A. vryheidensis* seem to mitigate the effects of pollen thieves by swamping pollen-collectors with pollen via high flower production. Both species grow in dense populations of hundreds of individuals and produce several thousand flowers per plant, of which several hundred may be open simultaneously at any time during flowering. The high pollen production per plant combined with synchronous flowering in large populations results in a seemingly undepletable pollen resource, though the amount of pollen per ovule is unknown. Despite frequent visits by pollen-collecting bees (Table 4.3), which acted exclusively as pollen thieves of *A. ferox* and as inefficient pollinators of *A. vryheidensis*, both species maintained high seed production. Although

high pollen production undoubtedly attracts pollen collectors, sufficiently high production may outweigh these additional thieves. This hypothesis leads to the prediction that the consequences of pollen theft for seed production by species that tolerate theft may be greater when these species occur in smaller (e.g., fragmented or remnant) populations, including disturbed ecosystems.

#### *Anti-theft adaptations*

I found no evidence of any of the proposed anti-theft adaptations in the aloes that I studied. All aloes share a relatively simple floral structure that does not restrict access to pollen, and produce bright orange-yellow pollen, so that pollen is neither selectively available nor cryptic. Even though aloes produce diverse secondary compounds to reduce both herbivory (Gutterman and Chauser-Volfson 2000, Chauser-Volfson and Gutterman 2004) and nectar theft (Johnson et al. 2006), insects seek and collect the pollen of all species, indicating that it is neither toxic nor inedible. More *Aloe* pollen is typically available on anthers during early morning before insects become active, but this results primarily from low visitation during late evening and night, rather than adaptively timed pollen release, as anthers of all species dehisced throughout the day. This genus of more than 300 species includes one probable exception to diurnal pollination: *Aloe suzannae*, native to Madagascar, produces erect inflorescences up to 3 m tall, whose white flowers open during evening and produce a musky scent (Reynolds 1966), traits commonly associated with bat-pollination (Faegri and van der Pijl 1966). Sadly, like many Malagasy plants, this extraordinary species is now extinct in the wild, so the opportunity to study its pollination system, and therefore whether nocturnal flowering affects pollen theft in aloes, may have passed.

*Co-opting pollen thieves into pollinating*

Some *Aloe* species are less vulnerable to pollen theft than others. In so far as vulnerability depends on floral characteristics, these species reveal potential evolutionary resolutions of pollen theft, if theft imposes continuous and sufficiently strong selection on plants. Unfortunately, the phylogeny of *Aloe* is not yet well enough resolved to illuminate whether insect species evolved from bird-pollinated ancestors or vice versa. The most current phylogenetic analysis (Treutlein et al. 2003) did not consider species from the sections that include *A. inconspicua* (Graminialoe) or *A. vryheidensis* (Angiualoe) and included only one or two species per section, even though sections often include species with diverse floral morphologies (e.g., *A. kraussii*, *A. dominella* and *A. boylei* all belong to the section Leptoaloe: Reynolds 1950). However, the climbing aloes (subsection Prolongatae: Reynolds 1950), which include *A. tenuior* and other species with similar, small flowers, appear to be a basal clade, suggesting that bird pollination may have evolved originally from insect pollination (Treutlein et al. 2003). Conversely, the placement of the Leptoaloe within an otherwise largely bird-pollinated branch (Treutlein et al. 2003) suggests that insect-pollination may also have re-evolved from bird-pollinated species more recently.

*Aloe inconspicua*, is unique among aloes whose pollination systems have been investigated, as the sole exclusively insect-pollinated species, although the similar inflorescences of other 'grass aloes' (Van Wyk and Smith 2003) suggest that it is not the only insect-pollinated species in the genus. *Aloe inconspicua* was not visited by insects that collected only pollen, but this may reflect the behaviour of its primary pollinators, *Amegilla*, which were never seen collecting pollen only from any of the aloes studied (Table 4.3), rather than floral traits.

*Aloe vryheidensis* and *A. kraussii* were the only species pollinated by both birds and insects, though the reduced seed set of caged inflorescences (Table 4.6) indicates that

insects are inefficient pollinators of *A. vryheidensis*. *Aloe vryheidensis* and likely *A. kraussii* were successfully pollinated by pollen-collecting bees due to the minimal dichogamy and herkogamy of their flowers, lending support to the hypothesis that these traits promote pollen theft. *Aloe kraussii* is unique among the species studied, as it seems to be adapted for pollination by both insects and specialised birds. Unlike other sunbird-pollinated aloes, *A. kraussii* has a short corolla, which enables relatively short-tongued bees to access its nectar, encouraging insect visitation to female-phase flowers. However, unlike *A. vryheidensis*, *A. kraussii* corollas have an extremely narrow mouth, ensuring precise contact between floral organs (anthers and stigmas) and narrow sunbird bills. Surprisingly, pollen sticks to smooth, hard sunbird bills well enough to be transported to stigmas on other plants (aviary experiment, unpublished data). Thus the generalised pollination system of this species may actually reflect adaptation to the two most common, but very different, visitors to aloes: sunbirds and bees.

### **Resolution of the conflict between avoidance of both pollen theft and self-pollination**

Herkogamy and dichogamy are floral mechanisms that reduce the incidence of self-pollination (Lloyd and Webb 1986, Webb and Lloyd 1986, Harder et al. 2000).

However, I have proposed that pollen theft may limit the evolution of these traits to facilitate contact of stigmas and anthers by pollen-collecting flower visitors. This apparent conflict between avoidance of pollen theft and self-pollination may pose an evolutionary ‘dilemma’ for plants, which is resolved differently among the aloes that I studied.

The only species that seems to avoid both self-pollination and pollen theft is also the only exclusively insect-pollinated species, *A. inconspicua*. Bees visit female- and male-phase flowers equally because they invariably probe for nectar while collecting pollen, thus flowers can afford to be strongly protandrous without risking pollen theft by

pollen collectors that ignore female-phase flowers. Furthermore, *A. inconspicua* plants expose few flowers simultaneously and *Amegilla* bees tend to visit bottom (i.e., female-phase) flowers first, which should minimise self-pollination between flowers (geitonogamy: Harder and Barrett 1995, Harder et al. 2004). Surprisingly, *A. inconspicua* was the only dichogamous insect-pollinated species, even though insects foraged for nectar on other species and so visited pollenless female flowers (Table 4.4).

All *Aloe* species studied, other than *A. inconspicua*, seem vulnerable to either extensive self-pollination, pollen theft, or both. All other species with small, tubular flowers have minimal dichogamy and herkogamy, which facilitate pollen deposition by pollen collectors and self-pollination within flowers. Species with long, tubular flowers generally have greater dichogamy, reducing the possibility of sexual interference within flowers, but are vulnerable to pollen theft by pollen collectors that ignore pollen-depleted, female-phase flowers. *Aloe vryheidensis* seems to mitigate pollen theft through reduced dichogamy and herkogamy, but probably experiences intense geitonogamous pollination by all visitors, which probe multiple flowers per plant visit and do not move consistently from female- to male-phase flowers. Finally, species with large inflorescences of tubular flowers (*A. ferox*, *A. marlothii*) suffer the highest pollen theft, as insects visited frequently but effected little pollination (Chapter 4), but they also seem especially vulnerable to geitonogamous self-pollination, as they display hundreds of flowers simultaneously.

## **Ecological consequences of pollen theft**

### *Male reproductive success*

Male reproductive success by species with granular pollen is inherently difficult to measure, as pollen is difficult to track (Snow and Lewis 1993) and to associate with specific donor plants once it has been dispersed. Use of genetic markers overcomes some

of these problems, but these techniques are tractable only in small experimental populations and they provide information that is affected by processes that occur between pollination and the genetic assay. Experimental manipulations at a population level provide a method for overcoming this difficulty. For the experiments described in Chapter 3, honey-bee hives were added to clearly defined aloe populations, so that pollen flow between study plants and plants not affected by the manipulation was unlikely. In such closed systems, average pollen receipt represents average siring success (Harder and Routley 2006), thus the population effects of pollen theft on male and female pollination success can be understood simply by studying pollen deposition on stigmas. High pollen removal coupled with low pollen receipt under natural pollination indicates inefficient pollination, which at its most extreme constitutes pollen theft, whereas the same pattern in the absence of effective pollinators indicates pollen theft definitively. This method holds promise for future studies of pollen theft, and is certainly less damaging or time consuming than alternative methods such as insecticides (Ramsey 1988a) or insect repellents (Nicodemo and Nogueira Couto 2004). However, studies of pollen theft will also continue to rely heavily on correlative data between the abundance of thieves and pollination success, as most thieves cannot be manipulated as easily as honey bees.

The results from Chapter 3 reveal that pollen theft can reduce average male pollination success (pollen export) and subsequently average female pollination success (pollen receipt). This reduction, need not affect the average reproduction of a population if pollen receipt does not limit seed set. However, it is clear that pollen thieves can reduce pollen receipt sufficiently to reduce seed production (Figure 3.2), which could have serious implications for plant populations if pollen thief abundance were to remain high.

*Honey bees: globally important pollinators or invasive pollen thieves?*

The effects of honey bees on ecosystems have been topics of intense debate recently, especially regarding areas where honey bees are not native (Huryn 1997, Hansen et al. 2002, Valido et al. 2002). Honey bees are the most commonly documented pollen thief (Chapter 2), and can disrupt plant reproduction, even for species that have evolved in environments with honey bees (Chapter 3). However, my results from Umgeni (Chapter 3), in which natural abundance of pollen-collecting allodapine bees outweighed the effect of adding two commercial honey-bee hives, suggest that unmanaged populations of native bees can also cause severe pollen theft. Furthermore, honey bees maximise their foraging efficiency by exploiting a plant population only as long as it provides resources that can be collected efficiently, given the alternative foraging options (Seeley 1995). This behaviour is suggested by the densities of foraging bees observed during the hive-addition experiment. The Klipfontein population of *A. maculata* was separated from other populations of flowering plants by at least 2 km in every direction. Hive addition at this site greatly increased the number of foraging bees, presumably because they had few foraging options. However, at the smaller Hilton and Umgeni populations, which are much closer to residential areas with flowering garden plants, it was more difficult to increase foraging bee density by hive addition.

Honey bees constitute a potential disturbance for plant communities, but their detrimental impact should be kept in perspective, especially when compared to disturbances such as habitat loss and fragmentation. Indeed, in disturbed environments honey bees sometimes maintain pollination of native plants that have lost their native pollinators due to more serious human impacts (e.g., Dick et al. 2003).



**Is pollen theft worthy of further study?**

Pollen theft is not a unique interaction, but rather one end of a continuum of interactions that result from the incongruent goals of flowers (pollen transfer) and their visitors (foraging). This diversity of interactions is both reflected and largely governed by floral design, which dictates whether and when floral rewards become available and which visitors can access them. Although efforts to understand the mechanisms of pollen theft can draw greatly from the existing knowledge of the role of inefficient pollinators (e.g., Thomson and Thomson 1992), much remains to be understood about the effects of pollen theft on plant evolution and ecology. Is pollen theft a ubiquitous selective influence, as might be expected from the cosmopolitan distribution of its main perpetrators, pollen-collecting bees? Do pollen thieves, especially those whose populations are manipulated or introduced by humans, pose serious ecological threats to the long-term viability of plant populations? How serious is the apparent conflict between reducing pollen theft and reducing self-pollination? The research described in this thesis highlights the diverse implications of pollen theft on plants, which should stimulate further study.

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Appendix A Locations and elevation (to the nearest 50 m) of study sites for the ten *Aloe* species considered in this study. NR=Nature Reserve

| Species                | Site                           | Location         | Elevation<br>(m.a.s.l.) |
|------------------------|--------------------------------|------------------|-------------------------|
| <i>A. arborescens</i>  | Hilton College (Hilton)        | 29°29'S, 30°18'E | 900                     |
|                        | Umungu Lodge, Ixopo (Ixopo)    | 30°07'S, 30°09'E | 750                     |
| <i>A. boylei</i>       | Mt. Gilboa                     | 29°19'S, 30°17'E | 1700                    |
| <i>A. dominella</i>    | Road to Middlerus              | 29°06'S, 30°02'E | 1550                    |
| <i>A. ferox</i>        | Hilton                         |                  |                         |
|                        | Ixopo                          | 30°07'S, 30°09'E | 750                     |
| <i>A. inconspicua</i>  | Escort                         | 28°53'S, 29°58'E | 1000                    |
| <i>A. kraussii</i>     | Midmar Dam NR                  | 29°32'S, 30°10'E | 1100                    |
| <i>A. maculata</i>     | Hilton                         | 29°29'S, 30°18'E | 850                     |
|                        | Ixopo                          | 30°08'S, 30°10'E | 750                     |
|                        | Klipfontein Farm (Klipfontein) | 28°53'S 29°41'E  | 1150                    |
|                        | Umgeni Valley NR               | 29°28'S, 30°14'E | 850                     |
| <i>A. marlothii</i>    | Klipfontein                    |                  |                         |
| <i>A. tenuior</i>      | University of KwaZulu Natal    | 29°39'S, 30°24'E | 750                     |
| <i>A. vryheidensis</i> | Igwala gwala NR (private)      | 27°24'S, 31°14'E | 1200                    |