CHAPTER 1

INTRODUCTION

Photograph: newly opened *Trachymene incisa* umbel

For each pollinator bee is in fact a little cupid

that creates hot romances between plants thought to be stupid.

Each bee can be considered a matchmaking agency

that arranges introductions for a nominal nectar fee.

From “Cupid” by Paul D. Haemig, http://www.ecology.info/cupid.html

POLLINATION ECOLOGY

Mutualistic interactions are among the most widespread interactions in nature, but also the least well understood compared to other interactions such as predation (Ollerton 2006). Mutualisms are relationships between species that result in reciprocal benefits. Biotic pollination, the transfer of pollen by animals from the anther to the stigma of flowers, is a mostly mutualistic interaction (see Renner 2006 for exceptions), with pollinators usually receiving pollen and/or nectar from plants in return for their pollinating service. Animal pollinators have been important since the angiosperms evolved, with evidence indicating that early members of the angiosperms were insect pollinated (Crane *et al.* 1995). Over 91% of the 240 000 angiosperm species worldwide are animal-pollinated (Buchmann and Nabhan 1996), distributed throughout approximately 93% of angiosperm genera (Renner and Ricklefs 1995). With vertebrates pollinating only 0.51% of species, insects are responsible for pollinating over 90% of angiosperm species (Buchmann and Nabhan 1996). The four largest orders of insects, Coleoptera, Hymenoptera, Lepidoptera and Diptera, include species that are known to pollinate flowering plants (Proctor *et al.* 1996). The total number of pollinator species is unknown, although estimates vary between 130 000 and

300 000 (Shepherd *et al.* 2003). Pollination is therefore a dominant interaction involving several hundred thousands of species, spanning most terrestrial habitats.

Pollination ecology addresses the mechanisms through which plants donate and receive pollen, including floral phenology, floral adaptation, pollinator behaviour and plant breeding (Wyatt 1983; Morgan and Schoen 1997). Pollination ecology provides examples of coevolution and evolutionary conflict, and was used by Charles Darwin and other early evolutionary biologists to test ideas of evolutionary theory (Pellmyr 2002). Pollination is a fundamental aspect of plant reproduction, and is therefore important for the disciplines of plant life history and demography, sustainable agriculture and horticulture. On larger scales, pollination is an essential ecosystem service and is central to understanding interaction networks and food webs, ecosystem function, conservation and restoration biology.

Many ecological interactions are dynamic, varying in the number and interdependence of participants and the strength of the outcome of the interaction. A fundamental aspect of any ecological interaction is the degree to which it is specialised or generalised (Waser 2006). Pollination is no exception and with so many species involved, there is a range of specialised (plants pollinated by one or very few pollinators) and generalised (plants pollinated by several pollinators) systems (although see Appendix 1 for other definitions). Variation in the interacting partners forms a major part of the origin, maintenance and stability of an interaction. In terms of pollination, variation in pollinators is critical for plant mating (ecological context) and long term dependence and adaptation of the plant to its pollinating partners (evolutionary context).

VARIATION IN POLLINATORS

Flowers can receive visits from one to many pollinators. Variation in pollinators can affect the number and quality of seed production by plants and thus have flow on effects for plant demography and population structure. Figure 1-1 is a flow diagram of these links between pollinators and plant reproduction. Firstly, pollinator assemblages can vary in several natural ways. Plants and their pollinators rarely completely overlap in range (Thompson 1994; Waser *et al.* 1996) which results in different assemblages across the range of a plant. Community composition varies among habitats, which support different assemblages of pollinators and relationships between a plant species and a pollinator species may change among communities (Kearns and Inouye 1997). Widespread plant species are likely to experience varying climatic conditions throughout their range, which may favour different assemblages of floral visitors. Similarly, plants with long or multiple flowering periods may overlap flowering with different plants and pollinators during the course of the flowering period (reviewed by Minckley and Roulston 2006).

Disturbance is another source of variation to pollinator assemblages. For example, fragmentation of subtropical dry forests caused declines in native flower visitors (Aizen and Feinsinger 1994). The introduction of non-native pollinators, such as honey bees (*Apis mellifera*) and bumble bees (*Bombus terrestris*) in Australia, is also a source of disturbance to pollinator assemblages. Several studies have shown that these introduced pollinators differ in foraging behaviour to native pollinators, can alter the foraging patterns of native pollinators, and can displace or compete with native floral visitors (Gross and Mackay 1998; Hingston and McQuillan 1998; Hingston and McQuillan 1999; Horskins and Turner 1999; Gross 2001). Therefore, the foraging behaviour of pollinators will be dependent on the presence of other competing pollinators (Hingston and McQuillan 1998).

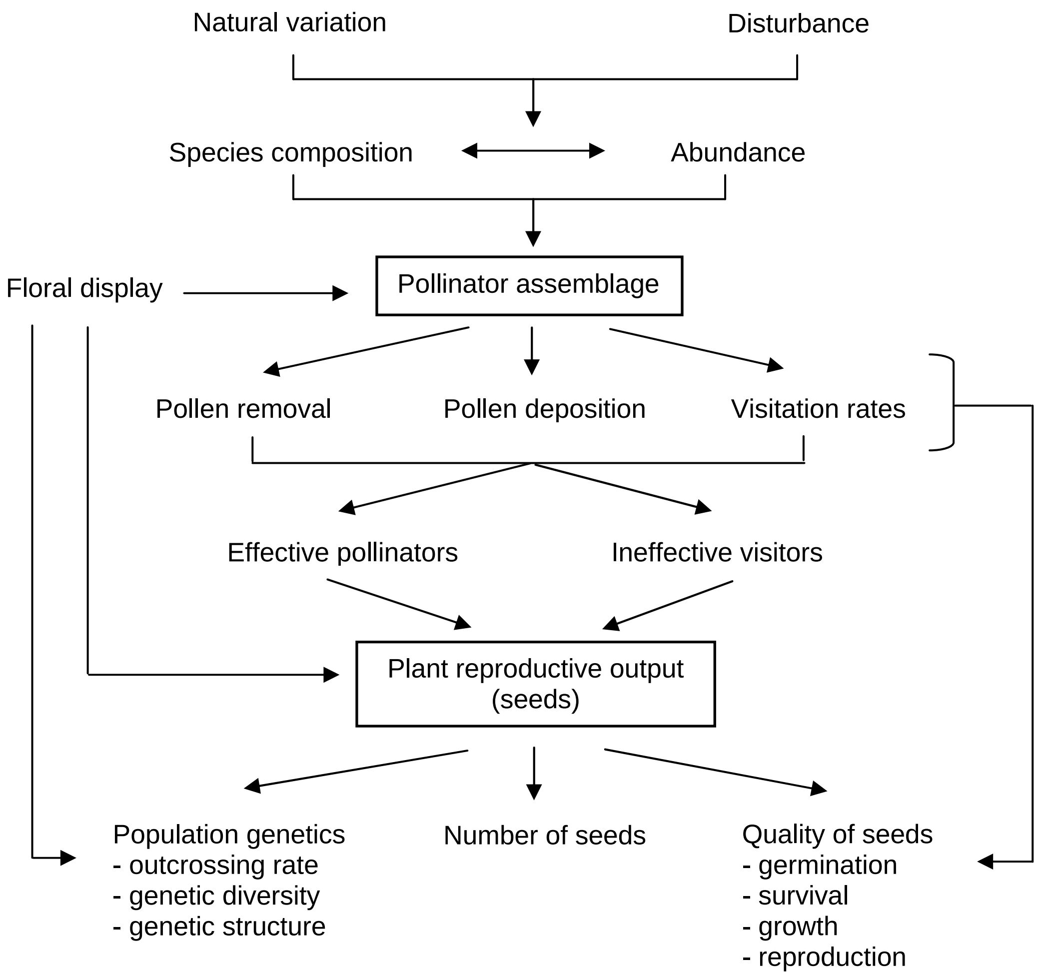

Fig. 1-1 Flow diagram of the links between variation in pollinators and plant reproduction.

Natural and induced variation in the pollinator assemblage leads to spatial and temporal variation in the species composition and abundance of each species. The pollinator assemblage also has properties that determine success at pollination. These are the ability of pollinators to collect pollen from anthers and deposit it onto conspecific stigmas, and the rate at which they do this. Not all floral visitors are effective pollinators and not all pollinators are equally effective at pollination (Herrera 1987; Wilson and Thomson 1991; Herrera 2000). The behaviour of pollinators individually and as a group will determine the reproductive output of the plant species they pollinate.

Plant reproductive output can be measured in terms of the quantity and quality of seeds. The number of seeds produced is related to the reproductive effort (number of ovules produced) and the floral display (number and arrangement of flowers). The floral display plays an important role in attracting pollinators. Pollinators determine the type of mating, self- and/or cross-pollination, that occurs between flowers and influence the quality of offspring in terms of germination, survival, growth and subsequent reproduction (Herrera 2000). At the population level, pollinator behaviour affects plant population genetics through the outcrossing rate and different patterns of pollen (gene) flow.

A greater understanding of pollination requires a clearer idea of the range of specialisation and generalisation of plants and pollinators, the temporal and spatial variation in these relationships, and the factors that promote and constrain the evolution of niche breadth (Waser *et al.* 1996; Waser 2006).

GENERAL AIMS OF THIS THESIS

The role of variation in pollination is fundamental for understanding ecological dynamics of plant populations and is a major factor in the evolution and maintenance of generalised and specialised pollination systems. My study centred on the role of variation in pollination by addressing the following questions:

1. How variable are pollinators in a generalist pollination system? Specifically, to what degree do insect visitation rates and assemblage composition vary spatially among populations and temporally among flowering seasons?

2. How does variation in pollinators affect plant reproductive success (seed production, seed mass and seedling emergence) and the potential for inbreeding?

In order to answer these questions, a study system which exhibits generalised plant-pollinator interactions, the ability to record this variation in floral visitors on relevant spatial and temporal scales, the ability to record differences in reproductive success and the ability to conduct experimental pollination treatments is needed. I chose to answer these questions with *Trachymene incisa* subsp. *incisa* (Apiaceae), which exhibits these and other features that make it ideal to study variation in a plant-pollinator system.

TRACHYMENE INCISA* SUBSP. *INCISA

There are approximately 56 species of *Trachymene* Rudge, 39 occur in Australia and 38 are endemic (Hart and Henwood 2006). *Trachymene incisa* Rudge subsp. *incisa* is a common and widespread herbaceous species occurring on sandy soils and rock crevices from Fraser Island, Queensland, south to Ulladulla and inland to the Nandewar Range near Narrabri, NSW (Powell 1992; Hart and Henwood 2006). This large latitudinal range provides the potential for significant variation in pollinator assemblage composition among populations. This widespread species is distinguished from the restricted *T. incisa* subsp. *corrugata* L.A.S.Johnson using mericarp (seed) characteristics (Hart and Henwood 2006). *Trachymene incisa* subsp. *incisa* has been abbreviated to *T. incisa* throughout this thesis for brevity.

Trachymene incisa is an erect or ascending perennial herb 60-80 cm high (Wardle 2003; Hart and Henwood 2006; Fig. 1-2). It grows on infertile sandy soils and in rock crevices in dry eucalypt woodland or scrub (Benson and McDougall 1993). Population sizes range from less than 20 individuals to more than 10 000 (Wardle 2003). Plants have a thick rootstock with some lateral branching, although plants are not connected by underground branching and are not clonal (Watson 2000).

Fig. 1-2 *Trachymene incisa* subsp. *incisa* in flower, growing on sandy soils at the Tomago Sandbeds.

Plants flower between September and May, with a peak in flowering during summer between December and February (Powell 1992; Y. C. Davila, personal observation). There are approximately 72 flowers per inflorescence, arranged in simple umbels approximately 2 cm in diameter (Davila 1999; Davila and Wardle 2002). Umbels are borne in dichasial cymes (Hart and Henwood 2006), where the secondary umbels branch off the stalk of the terminal primary umbel and the tertiary umbels branch off the stalks of the secondary umbels and so forth, usually up to four orders. There are on average four inflorescence stalks per plant (Davila and Wardle 2002). The umbels attract a large suite of insect visitors, with morphospecies from 20 families from three orders (Hymenoptera, Diptera and Lepidoptera) observed visiting umbels at one population (Agnes Banks) in 1999 (Davila and Wardle 2002). With such a large visitor assemblage (Fig. 1-3), significant variation is possible and expected on both spatial and temporal scales.

Each flower has five stamens and two equally sized carpels (bilocular) each of which are connected to an elongated style at female anthesis (Hart and Henwood 2006). The flowers are protandrous and open centripetally within the umbel (Davila and Wardle 2002). During the male phase, the anthers dehisce sequentially within the flower while the styles remained curled and stigmas are non-receptive. Once the anthers are spent, the stamens fall off and the flower enters a quiescent phase. The female phase begins once all the flowers in the umbel have entered the quiescent phase (Davila and Wardle 2002). Therefore, whole umbels function as either male (pollen presenting, ~16 d) or female (pollen receptive, ~4 d) at any one time (Fig. 1-4). This phenological pattern, termed complete or synchronous protandry at the umbel level, prevents self-pollination within the umbel (Davila 1999; Davila and Wardle 2002).

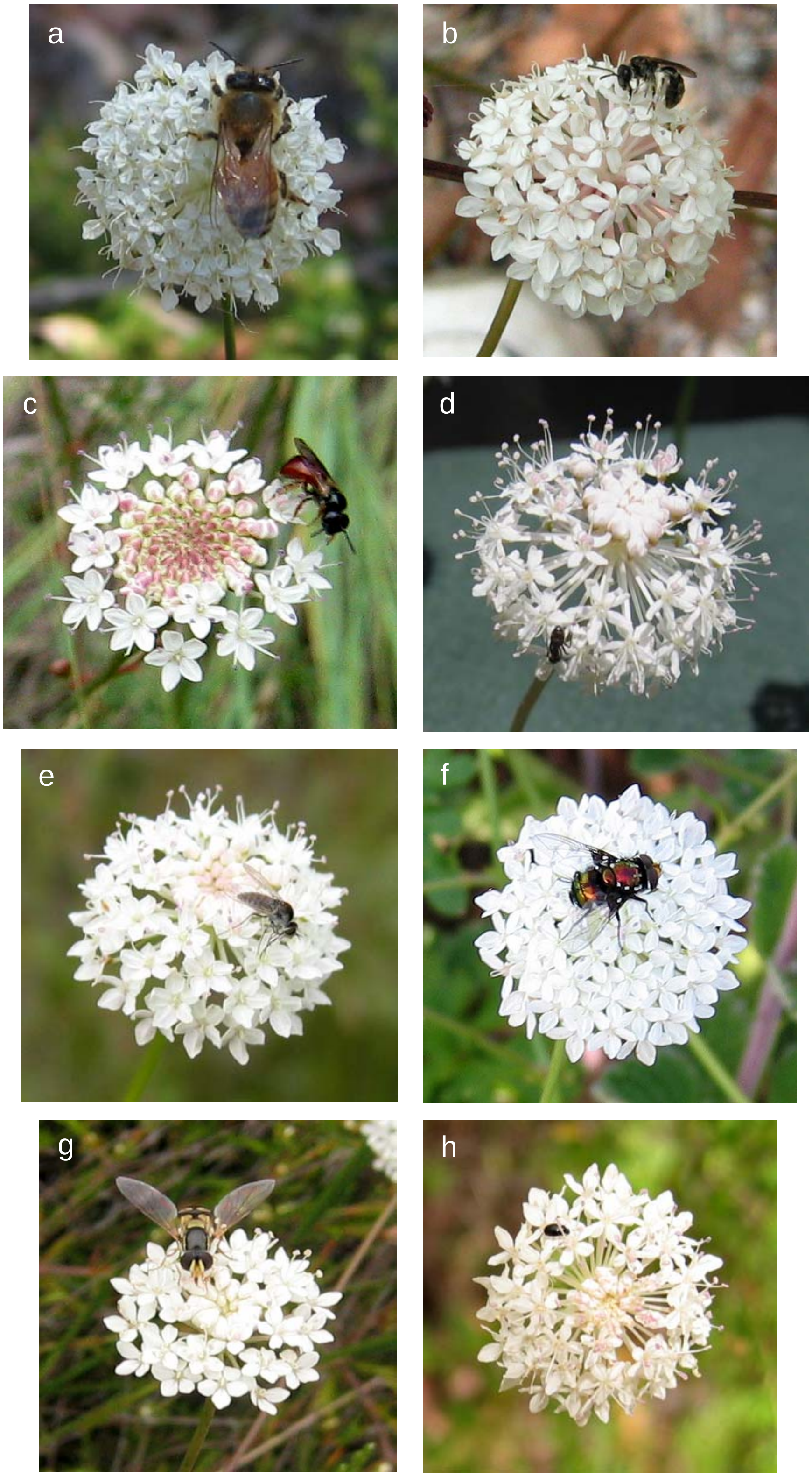

Fig. 1-3 Diversity of floral visitors to umbels of *Trachymene incisa*: (**a**) introduced honey bee *Apis mellifera* (Apidae); (**b**) native bee *Lasioglossum* sp. (Halictidae); (**c**) native bee *Lasioglossum* sp.; (**d**) ant (Formicidae); (**e**) bee fly (Bombyliidae); (**f**) fly (Muscidae); (**g**) hover fly (Syrphidae); (**h**) beetle (Coleoptera). Umbel diameter is approximately 2 cm.

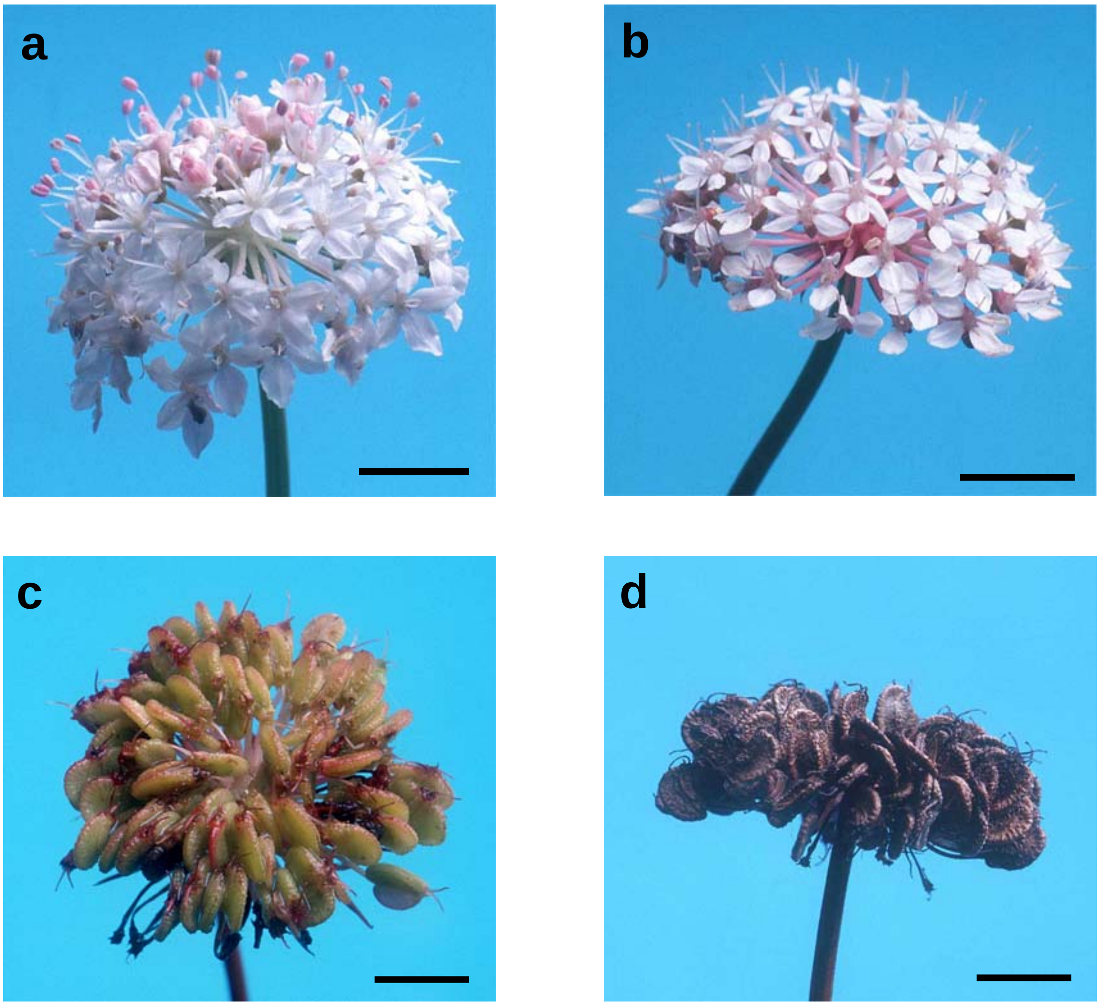

Fig. 1-4 *Trachymene incisa* subsp. *incisa* umbels from the Agnes Banks Woodland: (**a**) male phase umbel with outermost flowers in quiescent phase, intermediate flowers with dehiscing anthers and innermost flowers in bud; (**b**) female phase umbel with all flowers with elongated styles and receptive stigmas; (**c**) developing fruit (view from above); (**d**) mature seeds prior to dispersal (view from the side). Scale bar = 5 mm.

However, male and female phase umbels are usually present on the same plant at the same time, as each umbel order flowers, allowing the possibility for geitonogamous pollination. This multicycle protandry (Schlessman and Graceffa 2002) provides the possibility for a mixed mating system, where the degree of selfing is determined by the amount of pollinator movements between male and female umbels on the same plant. Nectar is produced during the male and female phases, but pollen is only available during the male phase (Davila and Wardle 2002). This fundamental difference in floral rewards between umbel phases may also affect pollinator visitation and effectiveness.

The mature fruit of *T. incisa* may consist of one or two mericarps (Hart and Henwood 2006; Fig. 1-4). The diaspore or seed is a single mericarp. *Trachymene incisa* is self compatible, although seed set resulting from self-pollination is less than seed set resulting from cross-pollination (Davila and Wardle 2002). Pollinators are needed for seed production, therefore, variation in pollinator visitation rates and behaviour will play a critical role in determining reproductive output and the amount of inbreeding experienced in *T. incisa*.

Trachymene has traditionally been placed within the Apiaceae, however, recent molecular studies have questioned this positioning (Chandler and Plunkett 2004). Since the phylogenetic relationships have not yet been resolved, I have followed Hart and Henwood's (2006) conservative placement of *Trachymene* within the Apiaceae.

The Apiaceae are characterised by a high degree of floral uniformity. The plants have been termed "promiscuous" because of their open and simple floral systems that present no restrictions to floral rewards and the large diversity of insect visitors that apparently pollinate them indiscriminately (Bell 1971). In a study examining generalisation across 962 plant species, Olesen (2000) found that *Anthriscus aemula* from the Apiaceae was the most generalised, being visited by 189 insect species.

Practically, experimental crosses in the field and glasshouse are easy to conduct on *T. incisa*, the seeds readily germinate in the field, glasshouse, growth cabinet and laboratory and plants can be kept in cultivation in the greenhouse (Davila and Wardle 2002; Wardle 2003; Appendix 2). These features, in addition to the widespread distribution and generalised pollination system, make *T. incisa* an ideal species to consider one of the central issues in pollination ecology, the spatial and temporal variation in plant-pollinator interactions.

SPECIFIC AIMS AND THESIS STRUCTURE

To investigate the degree and effects of spatial and temporal variation in pollinators in a generalist plant-pollinator system, I conducted mensurative and manipulative experiments using *Trachymene incisa* and its pollinator assemblage.

Chapter 2 explores spatial variation in insect visitation rates and assemblage composition of *T. incisa*, and the relationship with reproductive success. The objectives of this chapter were to:

- Quantify the variation in insect visitation rates, throughout the day and across three populations of *T. incisa* that occur in floristically similar habitats that are geographically separated
- Identify the main insect groups visiting umbels at different times of the day and at different populations, and their contribution to the overall assemblage in terms of visitation
- Determine how the pollinator assemblage relates to the floral display and reproductive output across the three populations

Chapter 3 investigates whether there is more spatial than temporal variation in the floral visitors of *T. incisa*, which is important in maintaining generalisation at different scales. Some data from the previous chapter and data collected in a subsequent year were used to:

- Determine the degree of variation in visitation rates of insects, taxonomic composition of the visitor assemblage, and resulting reproductive output of *T. incisa* across three populations and two years
- Examine whether there is a correlation between insect visitation rates and percentage seed set, considering the role of ants and the absence of honey bees in the system

Chapter 4 explores the pollination ecology and the role of fire in altering pollination of *T. incisa* in the Agnes Banks Woodland, a unique endangered ecological community which hosts one of the largest populations of *T. incisa*. Some data from Chapters 2 and 3, in addition to a third year of data collected in the Woodland, were used to:

- Investigate the variation in diurnal and nocturnal floral insect visitors and the resulting reproductive success of *T. incisa* in the Woodland over three flowering seasons after fire
- Report how fire affects the onset of flowering and pollination services to *T. incisa* in the Woodland

The expression of synchronous protandry at the umbel level in *T. incisa* provides a special case where pollinators must visit both male and female umbels during a foraging trip for successful pollination to occur. Chapter 5 addresses the potential for pollinator preferences in this generalist system given that male and female phase umbels offer different floral rewards. More specifically, I investigated the following questions:

- Do insects visit male and female inflorescences equally overall?
- Do insects visit male and female umbels equally during a foraging trip?
- Do insects spend equal amounts of time foraging on male and female umbels?

Chapter 6 addresses the effects of synchronous protandry on pollinator foraging behaviour, the potential for and effects of inbreeding in *T. incisa*. I used experimental hand-pollinations, analysis of inbreeding depression on seedling performance and observations of insect foraging within and between plants of *T. incisa* to:

- Determine whether geitonogamous self-pollination and biparental inbreeding (matings between relates) results in negative effects in terms of seedling emergence, short-term survival and growth
- Investigate the potential for geitonogamous pollination through insect visitation patterns

Chapter 7 is a discussion of two conceptual frameworks, defining generalisation in pollination systems and an assemblage approach to investigating the consequences of variation in pollinators on plant reproductive success, in light of the major results of this thesis and further directions in the study of generalised plant-pollinator systems.

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CHAPTER 2

VARIATION IN NATIVE POLLINATORS IN THE ABSENCE OF HONEY BEES: IMPLICATIONS FOR REPRODUCTIVE SUCCESS OF A GENERALIST-POLLINATED HERB *TRACHYMENE INCISA* (APIACEAE)

A similar version of this chapter has been submitted to the *Australian Journal of Botany*: Davila YC and Wardle GM (in review) Variation in native pollinators in the absence of honey bees: implications for reproductive success of a generalist-pollinated herb *Trachymene incisa* (Apiaceae).

Photographs: left – hover fly, Syrphidae; right – developing *Trachymene incisa* fruit

INTRODUCTION

Insects pollinate approximately 90% of flowering plant species worldwide (Buchmann and Nabhan 1996). Pollinators are important in determining the mating opportunities of plants. The geographic ranges of given plants and pollinators rarely correspond closely (Waser *et al.* 1996), resulting in the taxonomic composition of pollinator assemblages varying among populations (Lindsey 1984; Herrera 1988) and across the geographic range of widespread plant species (Eckert 2002). Plants with large geographic ranges are often found in a variety of habitats, latitudes or altitudes, exposing them to different pollinators. Introduced pollinators are also a source of variation to pollinator assemblages. The composition of pollinator assemblages can affect foraging behaviour of pollinators, as a group as well as individually, through their interactions (Gross and Mackay 1998). Pollinator assemblages can also vary dramatically at different temporal scales (Herrera 1988; Herrera 2000; Price *et al.* 2005).

Most plant species are generalists in terms of pollination (Waser *et al.* 1996; Olesen 2000; but see Johnson and Steiner 2000 for an alternative view), relying on multiple pollinator species. Generalist-pollinated plants are therefore thought to be buffered against shifts or decreases in pollinator abundance. However, not all pollinators are equally effective at depositing pollen and effecting seed set (e.g. Herrera 1987; Wilson and Thomson 1991; Herrera 2000; Mayfield *et al.* 2001). Given the inequality of pollinators and significant spatial and temporal variation in pollinators, this suggests that the reproductive output of generalist-pollinated plants can be affected by variation in pollinators. Few studies have addressed how much variation in pollinators occurs spatially and temporally (e.g. Herrera 2005; Price *et al.* 2005) or how variation in pollinator assemblages affects reproduction in generalist-pollinated plants (e.g. Herrera 1988; Herrera

2000). Pollinator declines and disruption to plant-pollinator interactions have been reported worldwide (Kearns *et al.* 1998; Kremen and Ricketts 2000), and we need to understand the consequences of variation in pollinator assemblages for plant reproduction if we are to attempt to conserve or restore depleted pollination systems.

Here I investigated the degree of variation in pollinators among populations of a generalist-pollinated plant, and the consequences of this variation in terms of plant reproductive success. I studied the Australian herb, *Trachymene incisa* subsp. *incisa* Rudge, Apiaceae (*T. incisa* hereafter), which has a large geographic range along the east coast of Australia. The native pollinators are unknown, although the Apiaceae in general are assumed to be generalist pollinated due to their relatively unspecialised flowers (Bell 1971). The introduced European honey bee *Apis mellifera*, considered to be a super-generalist, has been the most abundant visitor to *T. incisa* at two populations in recent years (Davila 1999; Davila and Wardle 2002; Y. C. Davila and G. M. Wardle, personal observation). The absence of honey bees in the assemblage during this study provided the first opportunity to observe native insects foraging on *T. incisa*, and gives us an idea of the assemblage composition before honey bees were introduced to Australia in 1822 (New 1997).

The objectives of the study were to: (i) quantify the variation in insect visitation rates, throughout the day and across three populations that are floristically similar but geographically separated; (ii) identify the main insect groups visiting umbels at different times and populations, and their contribution to the overall assemblage in terms of visitation; and (iii) determine how the pollinator assemblage relates to the floral display and reproductive output across the three populations.

MATERIALS AND METHODS

Study species

Trachymene incisa subsp. *incisa* Rudge (Apiaceae) is a common perennial herb endemic to eastern Australia, with a large latitudinal range from South-east Queensland to the south coast of New South Wales (Powell 1992). It grows on infertile sandy soils and in crevices of granite outcrops in dry eucalypt woodland or scrub (Benson and McDougall 1993). Population sizes range from less than 20 individuals to more than 10000 (Wardle 2003).

There are approximately 72 flowers per inflorescence, arranged in simple umbels (Davila and Wardle 2002). Both flowers and umbels are completely protandrous, with the male and female umbel phases separated by a quiescent phase lasting one day (Davila and Wardle 2002). Therefore, whole umbels function as either male (pollen presenting, ~16 d) or female (pollen receptive, ~4 d) at any one time which prevents self-pollination within the umbel (Davila 1999; Davila and Wardle 2002). However, male and female phase umbels are usually present on the same plant at the same time, allowing the possibility for geitonogamous pollination. Umbels are arranged in a hierarchy, where the secondary umbels branch off the stalk of the terminal primary umbel and the tertiary umbels branch off the stalks of the secondary umbels and so forth, usually up to four orders. There are on average four inflorescence stalks per plant, which grow to a height of approximately 60 cm (Davila and Wardle 2002). *Trachymene incisa* is self compatible and self-fertilised seeds are viable (Davila and Wardle 2002). The umbels attract a large suite of insect visitors from the orders Hymenoptera (Fig. 2-1), Diptera, Lepidoptera and Coleoptera, although the introduced European honey bee has been the most abundant visitor in recent years (Davila 1999; Davila and Wardle 2002).

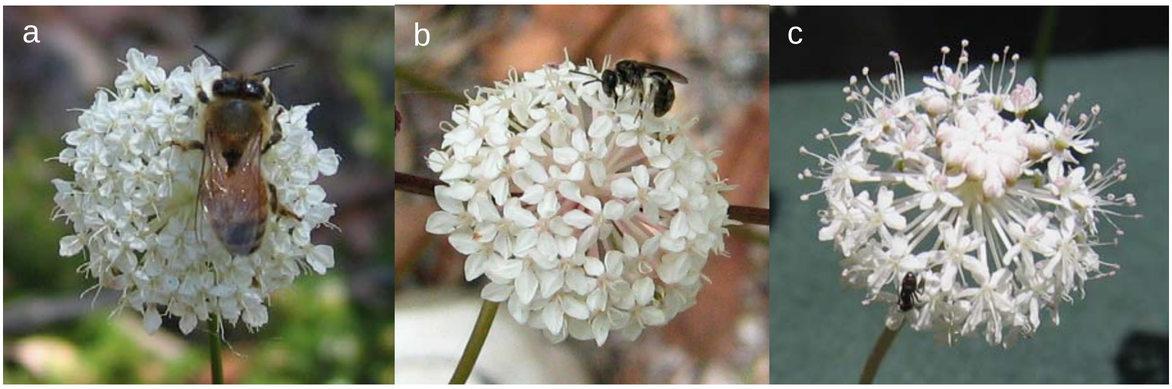

Fig. 2-1 Insect visitors to *Trachymene incisa* (**a**) introduced European honey bee *Apis mellifera*; (**b**) native bee *Lasioglossum* sp.; (**c**) Formicidae. Umbel diameter is approximately 2 cm.

The flowering period usually begins in late spring with a peak in flowering (highest density of flowering umbels) for approximately one month in summer (Powell 1992; Davila and Wardle 2002; Y. C. Davila, personal observation). Following this peak, many umbels set seed while a small proportion of plants continue to flower through to the end of autumn. Although *T. incisa* is common, it has been listed as an important component of at least three endangered ecological communities in New South Wales (New South Wales Scientific Committee 2000a; 2000b; 2000c).

Study populations

Three populations of *T. incisa* were studied in New South Wales, Australia, during the flowering season of 2003. At Myall Lakes National Park, located on the east coast approximately 200 km north of Sydney, *T. incisa* grows in dry heath and dry heath forests on freely drained sands (Myerscough and Carolin 1986). The study area was located in dry heath along the side of an old mining road (32°30′S, 152°21′E). Peak flowering was observed in January.

The Tomago Sandbeds are located on the central coast of New South Wales, approximately 70 km south of Myall Lakes National Park. *Trachymene incisa* occurs within the dry open-forest, low woodland and in adjacent fire breaks. The study site was located in a cleared area adjacent to shrubland and an access road (32°47′S, 151°51′E). Peak flowering at Tomago was observed in February. Myall Lakes National Park and the Tomago Sandbeds share similar climate, geomorphology, soils and vegetation (Fox *et al.* 1996; Ross *et al.* 2002).

The Agnes Banks Woodland is a unique low woodland community occurring on wind blown sand dunes, located in the Agnes Banks Nature Reserve, 55 km west of Sydney (Benson 1992). The Woodland is listed as an endangered ecological community with *T. incisa* as an identifying species (New South Wales Scientific Committee 2000a). The study site was located 250 m from the entry to the reserve (33°39′S, 150°41′E), and was exposed to fire in December 2002. *Trachymene incisa* began resprouting approximately one month later, flowering began in March 2003 and peak flowering was observed in May. Benson (1981) noted that 47% of the Agnes Banks flora is also found at Myall Lakes, including some of the dominant tree, shrub and undergrowth species. Therefore, all three sites are floristically similar but geographically separated.

Observation patch set-up

At each population, six observation patches consisting of 3-6 plants in an area of approximately 2 m^2 were located and tagged. Each umbel in a patch was given a unique number and its individual plant recorded. At the beginning of each day the flowering umbels were checked and labels changed if umbels had finished flowering, and newly flowering umbels were labelled. The number of umbels flowering per patch did not differ among days ($F_{6, 45} = 0.55$, $P = 0.77$). However, Tomago (mean ± SE, 12.4 ± 0.4) had significantly more umbels per patch than Myall Lakes (8.9 ± 0.6) and Agnes Banks (8.5 ± 0.4) ($F_{2,6} = 44.3$, $P < 0.0003$; Tukey-Kramer post-hoc tests significant at $P = 0.01$).

Insect visitor observations

Observations of insect visitors were made at each patch for 10 min, five times per day at 0730-0830, 1000-1100, 1300-1400, 1600-1700 and 1830-1930 h (“time periods”). Patches were observed in pairs by two observers and the order of paired patches and observers randomised at each time period. During the observation period, the morphospecies of insects (as a surrogate for species because identification was difficult in the field) and which umbels they visited were recorded. The observations were made over three consecutive sunny days during peak flowering for each population. A total of 43 h 50 min of insect observations were made across the three populations. Due to differences in the number of umbels per patch among populations (see above), visitation rate was calculated as the number of visits (landings by insects) made per umbel per 10 min for each patch.

Floral display

Two transects were established in each population, with five 2 m × 1 m quadrats marked out every 10 m (n = 10 quadrats). The total number of plants, number of plants with flowering umbels, and the number of flowering umbels per 2 m^2 were recorded during peak flowering as a measure of floral display.

Reproductive output

Twenty plants were chosen at random towards the end of the flowering period from Myall Lakes and Agnes Banks, and the number of umbels produced per plant and the number of umbels producing seeds were recorded as a measure of reproductive output. Reproductive output was recorded on 20 plants that had completed flowering at Tomago during peak flowering, because the plants were to be cleared soon afterwards.

I collected seeding umbels, which were pollinated during peak flowering, prior to dispersal from each population. These umbels were used for calculation of percentage seed set, seed mass and seedling emergence. Percentage seed set, calculated for n = 7-8 umbels, is the percentage of ovules per umbel that developed into healthy seeds (Davila and Wardle 2002). The equation for calculating percentage seed set (equivalent to seed:ovule ratio) is:

$$\%\text{ seed set} = \frac{(\text{number of seeds per umbel} \times 100)}{(\text{number of flowers per umbel} \times \text{number of ovules per flower})}$$

There are two ovules per flower. Individual seed mass was recorded from n = 11-15 seeds from six umbels per population.

To evaluate seedling emergence, five healthy seeds from n = 11-13 umbels per population were sown into seedling trays containing moist river sand. Two replicates of the trays were setup in November 2003 and placed in one of two environments: growth cabinet and glass house. The growth cabinet was programmed with temperatures, light and day length to resemble conditions at Agnes Banks in April, which is the time when most seeds germinate in this population. The glass house environment was included to compare a semi-natural environment (natural day length, sunlight and outdoor temperature) to the growth cabinet environment. Trays in these treatments were uniformly watered and fertilised. Final percentage emergence (shoot visible emerging above soil level) was determined after 90 days. A third treatment was initially attempted to investigate seedling emergence simultaneously in the field. However, due to drought conditions experienced during the experimental period no seeds germinated.

Data analyses

The null hypothesis that visitation rate (number of visits per umbel per 10 min) does not vary among populations nor time periods, was analysed using repeated measures analysis of variance (ANOVA, SuperANOVA v1.1, Abacus Concepts 1989-1990). Population was treated as the fixed between-plots factor, since the three populations were chosen specifically for this study (Bennington and Thayne 1994). These populations vary minimally in latitude compared to the entire range of the species, are large enough to adequately record plant-pollinator interactions, and occur in habitats that are floristically similar and have been compared in previous studies (e.g. Benson 1981; Fox *et al.* 1996; Ross *et al.* 2002). Patch is nested in population and time period was the fixed within-plots factor, which is crossed with population. Each day was treated as a separate set of patches, with some buds opening or female phase umbels senescing each day. Two replicate patches from Myall Lakes were excluded from the analysis due to missing data at one time period, so the data were analysed as an unbalanced repeated measures ANOVA with no missing cells (Quinn and Keough 2002). Therefore the sample sizes are 16, 18 and 18 patches for Myall Lakes, Tomago and Agnes Banks respectively. Data were square root transformed to improve normality and homogeneity of variances. I assumed that multi-sample sphericity was not met and used the Greenhouse-Geisser adjustment to avoid risk of Type I error (Quinn and Keough 2002).

A two-way crossed analysis of similarities (ANOSIM, Primer v5, Clarke and Gorley 2001) was used to test the null hypotheses that there were no differences in assemblage composition among populations and time periods. The visitation rate was used as a surrogate for abundance for constructing the similarity matrix. A subset of data (only observations made by Y. C. Davila at each population) was analysed to ensure consistency across populations when identifying morphospecies. Data were square root transformed (which downweights the effect of single species and allows intermediate abundance species to contribute more to the overall assemblage pattern, Clarke and Warwick 2001) and Bray-Curtis similarities used to construct the similarity matrix.

Differences among populations in terms of floral display (density of plants, proportion of plants flowering and density of flowering umbels) and reproductive output (number of umbels per plant, proportion of umbels setting seed per plant and percentage seed set) were analysed with a one-way analysis of variance ($\alpha = 0.05$). Seed mass was analysed with a two-factor nested analysis of variance, with umbel nested within population ($\alpha = 0.05$). Seedling emergence was analysed with a two-factor crossed analysis of variance with population and environment (growth cabinet or glasshouse) as fixed factors ($\alpha = 0.05$). Prior to analyses, a Cochran's test (for balanced data) or Bartlett's test (for unbalanced data) was performed to test for homogeneity of variances. Data with heterogeneous variances were transformed (details in Results) to achieve homogeneity of variances. Untransformed means ± standard errors (SE) are reported.

RESULTS

Insect visitation rates

Few insect visits were recorded during the 0730-0830 h time period at Tomago and Agnes Banks, and the 1830-1930 h time period at Myall Lakes and Agnes Banks (Fig. 2-2, Table 2-1). The majority of visits to *T. incisa* occurred between 1000 and 1700 h at all populations, with a total of 15 visits recorded at Myall Lakes during the 0730-0830 time period and most of the visits during the 1830-1930 h time period at Tomago made by ants (which are not considered to be effective pollinators, discussed below). Therefore I restricted the repeated measures ANOVA to the three observation periods recorded between 1000 and 1700 h.

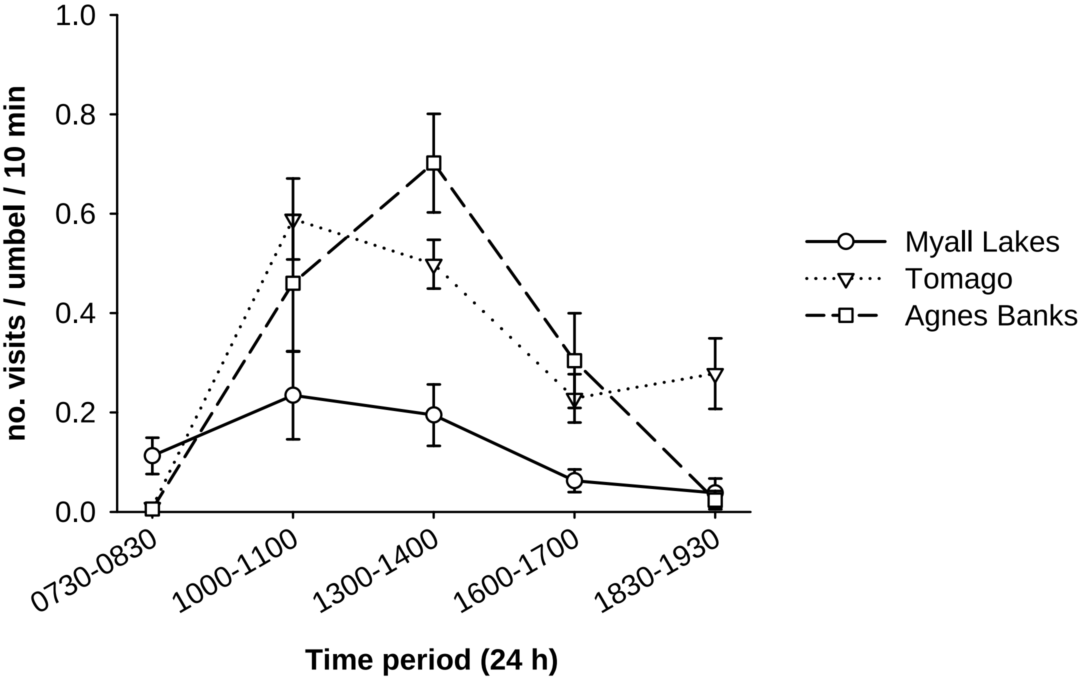

Fig. 2-2 Mean ± SE rate of insect visitation (number of visits per umbel per 10 min) to *Trachymene incisa* for three populations at five time periods. Populations varied significantly ($F_{2,49}$ = 13.680, P = 0.0001) and time periods between 1000 h and 1700 h varied significantly ($F_{2,4}$ = 14.734, P = 0.0001).

Table 2-1 Percentage of insect visits to *Trachymene incisa* recorded at each population for each time period, as a proportion of the total number of visits for that population. Percentage calculated as the number of visits recorded during each time period, as a proportion of all visits for that population, pooled across patches and days.

Time period (h)	Myall Lakes NP	Tomago Sandbeds	Agnes Banks NR
0730-0830	14.7	0.5	0.4
1000-1100	36.3	35.2	33.8
1300-1400	33.3	32.4	44.6
1600-1700	9.8	14.6	20
1830-1930	5.9	17.3	1.3
Total # visits	102	364	240

The analysis revealed a significant population and time period effect, with no interaction between population and time period (Fig. 2-2, Table 2-2). Visitation rates at Myall Lakes were consistently lower than at the other populations. At Tomago, visitation rates were similar between the 1000-1100 h and 1300-1400 h time periods. At Agnes Banks, visitation peaked during the 1300-1400 h time period, although visitation was highly variable during the day. All populations showed a decrease in visitation from midday (1300-1400 h) to afternoon (1600-1700 h). Tukey-Kramer post-hoc tests revealed that overall Myall Lakes received significantly fewer visits than Tomago and Agnes Banks ($P = 0.05$).

Table 2-2 Results of a repeated measures ANOVA on square root transformed visitation data (number of visits per umbel per 10 min) from three populations and three time periods. Population is the fixed between-plots factor, patches are nested in population, and time period is the fixed within-plots factor. The degrees of freedom for Time and Time × Population are multiplied by the Greenhouse-Geisser epsilon (G-G = 0.939) before calculating *F* probabilities.

Source	df	MS	*F*	*P*
Population	2	1.880	13.680	0.0001
Patch(Population)	49	0.137		
Time	2	1.091	14.734	0.0001
Time × Population	4	0.145	2.031	0.101
Time × Patch(Population)	98	0.072		

Taxonomic composition of insect visitor assemblage

The taxonomic composition of the insect assemblages varied significantly among populations (Global $R = 0.254$, $P = 0.001$), and pair-wise tests revealed that all populations differ from each other ($P < 0.01$).

Nine morphospecies were identified at Myall Lakes although most of the visits were made by the native bee *Hylaeus (Prosopisteron) woyensis* (Colletidae, 55.3% of visits) and ants (Formicidae, 22.6% of visits), including *Ochetellus glaber* and *Monomorium* species (Fig. 2-3a). These bee and ant species were also present at the other populations. Seven morphospecies were identified at Tomago, with most visits from ants (49.4% of visits), *H. (P.) woyensis* (23.4%) and halictid bees from the genus *Lasioglossum* (18.7%), which include species of *L. (Austrevylaeus)* and *L. (Ctenonomia).*

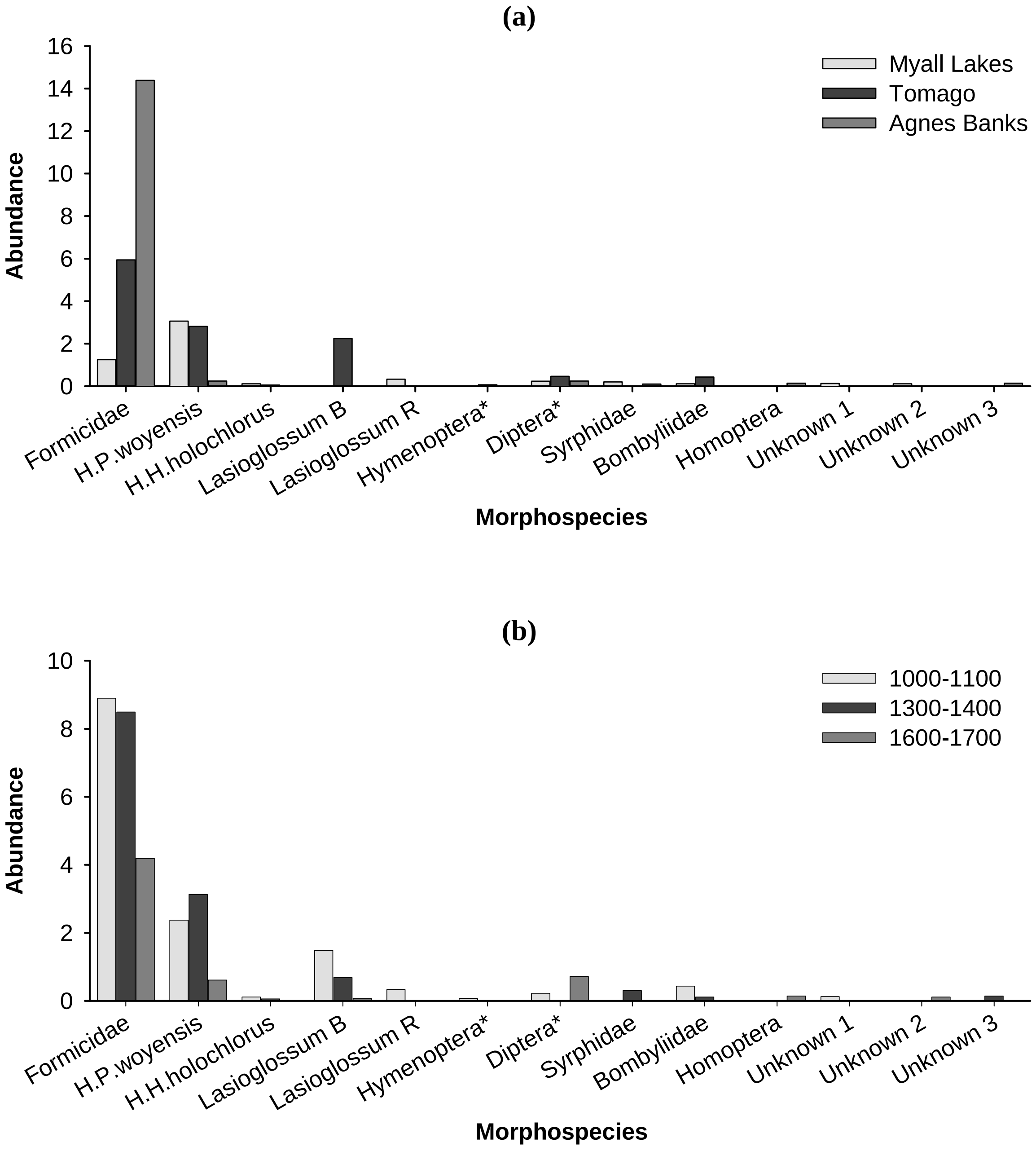

Fig. 2-3 Insect assemblage composition visiting *Trachymene incisa*: (**a**) per population, pooled across patches, days and times (Global $R = 0.254$, $P = 0.001$); (**b**) per time period, pooled across patches, days and populations (Global $R = 0.021$, $P = 0.282$). Abundance is measured as the pooled visitation rate per umbel per 10 min. Native bees include *Hylaeus (Prosopisteron) woyensis* (Colletidae), *Homalictus (Homalictus) holochlorus* (Halictidae) and *Lasioglossum* species (Halictidae). *Lasioglossum* B refers to bees that appear completely black, *Lasioglossum* R refers to bees with a red abdomen. Hymenoptera* refers to species of Hymenoptera not including ants (Formicidae) and the native bees. Diptera* refers to species of flies not including hover flies (Syrphidae) and bee flies (Bombyliidae).

Of the three populations, flies were most prevalent at Tomago, with 7.5% of the assemblage comprised of bee flies (Bombyliidae) and other Diptera. The assemblage at Agnes Banks was dominated by ants, with over 94% of visits made by Formicidae, although six morphospecies were identified visiting umbels at this population.

In contrast, the insect assemblage composition did not vary significantly among time periods (Global $R = 0.021$, $P = 0.282$). The 1000-1100 and 1300-1400 h time periods were very similar in assemblage composition (Fig. 2-3b), with ants dominating the assemblages (63.3% and 65.7% of all visits respectively), followed by the native bees *H. (P.) woyensis* (16.9% and 24.2% respectively) and *Lasioglossum* species (10.6% and 5.3% respectively). These three morphospecies accounted for over 90% of all visits at these two time periods. The 1600-1700 h time period was also dominated by ants (71.6% of visits), with other Diptera comprising 12.3% of visits and the native bee *H. (P.) woyensis* comprising 10.5% of visits.

Floral display

There was no significant difference in the density of plants among populations ($F_{2,27} = 0.442$, $P > 0.6$). The mean number of plants per 2 m^2 was 12.4 ± 4.0, 7.9 ± 2.7 and 10.9 ± 3.5 at Myall Lakes, Tomago and Agnes Banks respectively. There were also no differences among populations in the density of flowering umbels (data were square root transformed, $F_{2,27} = 2.414$, $P > 0.1$), although there were, on average, more umbels per 2 m^2 at Tomago (5.9 ± 2.4) than at Myall Lakes (1.9 ± 0.9) and Agnes Banks (1.7 ± 0.9).

There was no significant difference in the proportion of plants with flowering umbels among populations measured at peak flowering (data were $\log_{10}$ transformed, $F_{2,25} = 1.949$, $P > 0.1$; two quadrats, one each from Myall Lakes and Agnes Banks, were excluded in the ANOVA because these had no plants). Tomago had the highest proportion of plants flowering (30.0 ± 8.4 %), followed by Myall Lakes (15.1 ± 8.5 %). Agnes Banks recorded a very low proportion of plants flowering (4.3 ± 2.0 %).

Reproductive output

The mean number of umbels produced per plant was significantly different across populations ($F_{2, 57} = 4.268$, $P < 0.02$). Tukey-Kramer post-hoc tests revealed that Myall Lakes produced significantly more umbels per plant (8.0 ± 1.1) than Tomago (4.3 ± 0.6), and Agnes Banks was intermediate (6.1 ± 0.9) at $P = 0.05$.

In contrast, plants at Tomago had a significantly higher fruiting success, with a mean of 76.8 ± 6.1% of umbels per plant producing seeds, compared to 39.7 ± 4.9% at Myall Lakes and 33.0 ± 4.7% at Agnes Banks ($F_{2, 57} = 19.93$, $P < 0.001$; Tukey-Kramer post-hoc tests $P = 0.01$). Plants at Tomago also had significantly higher seed set per umbel ($F_{2, 20} = 5.98$, $P < 0.01$; Tukey-Kramer post-hoc tests significant at $P = 0.05$), with 46.5 ± 7.0% (n = 8) of ovules developing into seed, compared to 22.0 ± 3.7% (n = 7) at Myall Lakes and 24.7 ± 4.9% (n = 8) at Agnes Banks.

There were significant differences among umbels nested within populations in terms of mean seed mass ($F_{15, 248} = 44.3$, $P < 0.0001$). However, overall Tomago had higher mean seed mass (0.95 ± 0.02 mg) compared to Myall Lakes (0.69 ± 0.03 mg) and Agnes Banks (0.61 ± 0.03 mg). The ranges for mean individual seed mass were 0.813-1.280 mg at Tomago, 0.453-1.045 mg at Myall Lakes and 0.373-0.920 mg at Agnes Banks.

The final percentage emergence for seeds varied significantly among populations ($F_{2, 64} = 19.9$, $P < 0.0001$), with fewer seedlings from Agnes Banks emerging compared to seedlings from Myall Lakes and Tomago (Tukey-Kramer post-hoc test significant at $P = 0.01$, Fig. 2-4). Only 26.2 ± 0.1% of seeds from Agnes Banks emerged (mean across both environments), compared to 73.6 ± 0.1% of seeds at both Myall Lakes and Tomago. There was no environment effect ($F_{1, 64} = 3.87$, $P > 0.05$) or interaction ($F_{2, 64} = 1.89$, $P > 0.1$).

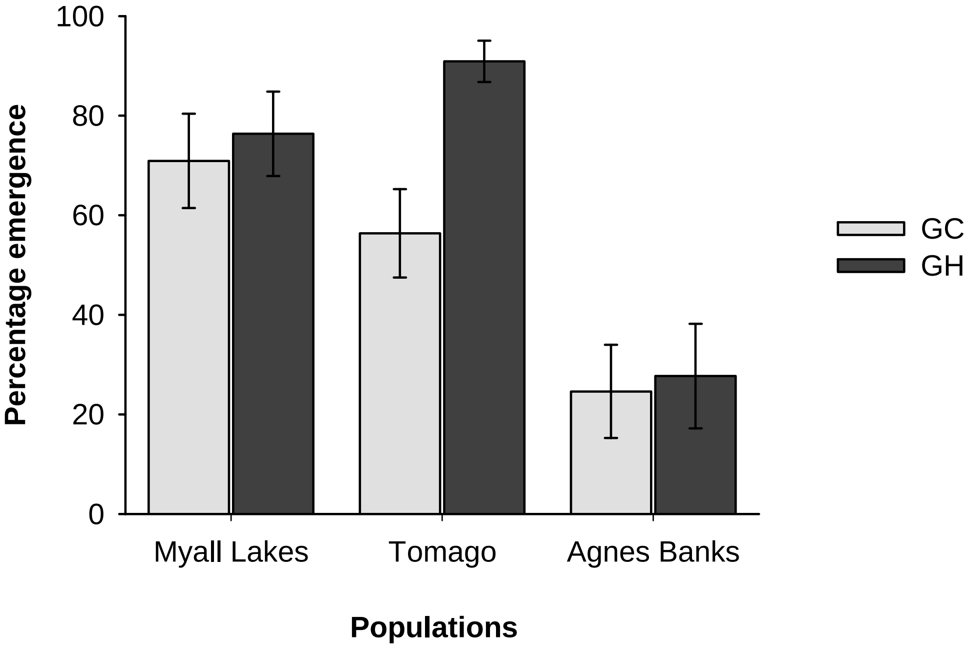

Fig. 2-4 Mean ± SE percentage emergence of seedlings after 90 days from three populations of *Trachymene incisa*, placed in two environments: growth cabinet (GC) and glasshouse (GH). Populations varied significantly ($F_{2, 64} = 19.9$, $P < 0.0001$), environments did not vary ($F_{1, 64} = 3.87$, $P > 0.05$). Sample sizes are n = 13 umbels for Myall Lakes, n = 11 umbels for Tomago and Agnes Banks. There were five seeds per umbel per environment tested.

DISCUSSION

This study revealed significant variation in pollinators among the three populations of *Trachymene incisa* in terms of visitation rates and the taxonomic composition of the assemblages. Insect visitation rates varied significantly throughout the day, highlighting that sampling of pollinators at one time will result in an inaccurate estimate. Whilst there were no significant differences among populations in terms of floral display (density of umbels), reproductive output in terms of the proportion of seeding umbels per plant, seed set per umbel, seed mass and final seedling emergence did vary across populations.

Pollinators are essential for the reproductive cycles of most flowering plants. Variation in pollinators, in terms of visitation rates, assemblage composition and behaviour, can have large effects on the reproductive output of plants. Low pollinator visitation rates as well as resource limitation can result in low seed production. The types of pollinators and their behaviour can affect self-pollination rates and the deposition of compatible pollen, and influence seed production and offspring viability.

Correlation between insect visitation, assemblage composition and reproductive output

Some studies have shown a positive correlation between insect visitation rate and plant reproductive output (e.g. Herrera 2000; Steffan-Dewenter *et al.* 2001). *Trachymene incisa* plants at Tomago followed this pattern with relatively high visitation rates and a higher proportion of umbels producing seeds, high seed set per umbel, high seed mass and viable offspring with high emergence rates. Plants at Myall Lakes received significantly fewer insect visits than plants at Tomago, and correspondingly, there was a significant decrease in the proportion of umbels that developed seeds, seed set per umbel and seed mass, compared to Tomago. In contrast, *T. incisa* plants at Agnes Banks which had similar visitation rates to plants at Tomago, had a significantly lower reproductive output compared to Tomago. The proportion of seeding umbels, seed set per umbel and final emergence at Agnes Banks was most similar to Myall Lakes. This indicates that high insect visitation rates alone do not result in high plant reproductive output.

The taxonomic composition of the pollinator assemblage also plays an important role in seed production and viability. Plants at Agnes Banks had a high insect visitation rate, however, most of the visits were from ants (Fig. 2-3a). Ants are unlikely to be pollinators because they forage only for nectar, which is located at the base of the *T. incisa* flowers, and rarely contact the anthers and stigmas (Y. C. Davila, personal observation). In other plant species, pollen function (viability, germination, pollen tube length) and seed production is disrupted by contact with ants (Beattie *et al.* 1984; Galen and Butchart 2003). Ants may also disrupt foraging of other pollinators (Ashman and King 2005) or deplete nectar resources for legitimate pollinators. In terms of *T. incisa*, ants are likely to be depleting nectar without performing any pollination service (acting as nectar thieves), which explains the low reproductive output of plants at Agnes Banks despite the high insect visitation rates.

In contrast to Agnes Banks, plants at Myall Lakes received low insect visitation rates. However, the native bee *H. (P.) woyensis* accounted for 55.3% of visits and was observed visiting both male and female umbels. Bees are well-known to be major pollinators of flowering plants, foraging on pollen and nectar. High seedling emergence suggests that although pollination levels may be low at Myall Lakes resulting in low seed numbers, good quality pollen was transferred by bees.

Plants at Tomago Sandbeds received a high visitation rate, with 49.6% of visits by native bees and flies. Despite low numbers of umbels per plant, plants at Tomago had a high proportion of umbels producing seeds, seed set was high, seed mass was greater and a larger proportion of seeds germinated, compared to other populations.

Plants at Agnes Banks are capable of higher reproductive output resembling that of Tomago. For example, plants at Agnes Banks produced mean seed set of 44.6 ± 7.5%, seed mass of 1.05 ± 0.11 mg, and seedling emergence of 63.7 ± 5.2% in 1999 (Davila and Wardle 2002). During that flowering season, floral density was high and the most abundant insect visitor was the introduced European honey bee. This further highlights how differences in the composition of the pollinator assemblage can affect reproductive output of plants.

If variation in pollinator composition can have measurable effects on populations of generalist-pollinated plants, which only a few studies have investigated (Galen 1996; Herrera 2000), then there is the potential for large-scale changes in all plant-pollinator interactions. In the case of *T. incisa*, being a generalist in terms of pollination did not prevent low reproductive output in populations receiving high visitation rates or high quality pollinators such as bees.

Determining pollinators from non-pollinating flower visitors

The Apiaceae exhibit exposed pollen and nectar, making it relatively easy for many insects to harvest these floral resources (Proctor *et al.* 1996). For these reasons it was once thought that each visitor to the inflorescence was a potential and probable pollinator (Bell 1971). However, with more recent studies of the Apiaceae that show not all visitors are pollinators (e.g. Grace and Nelson 1981; Lindsey 1984; Zych 2002; Evans *et al.* 2003), this viewpoint is changing.

Trachymene incisa appears to be a generalist-pollinated plant, with several types of insects visiting umbels throughout the day and at different populations, and no single species on which it is dependent for pollination. However, not all insects that visit *T. incisa* are effective pollinators, which is evident from the low reproductive output following high visitation by ants at the Agnes Banks population. This is in agreement with other studies of the Apiaceae, where several insects were observed visiting umbels and only a few were considered to contribute to pollination (Grace and Nelson 1981; Lindsey 1984; Zych 2002; Evans *et al.* 2003).

The most important insects for pollination of *T. incisa* appear to be native bees, and to a lesser extent, flies including bee flies (Bombyliidae) and hover flies (Syrphidae). The Apiaceae worldwide are visited by a large variety of insects, particularly flies (Diptera), as well as beetles, honey bees and some of the more unspecialised bees and wasps (Bell 1971; Proctor *et al.* 1996). Other studies of Apiaceae in Australia (Pickering 2001) and worldwide (Bell and Lindsey 1977; Grace and Nelson 1981; Lindsey 1984; Lamborn and Ollerton 2000; Larson *et al.* 2001; Zych 2002) confirm a high proportion of insect visitors to be flies. The Hymenoptera are not always well represented in the insect assemblages of many Apiaceae, although they often carry significant amounts of pollen and therefore are considered important pollinators (Bell and Lindsey 1977; Grace and Nelson 1981; Lindsey 1984; Zych 2002).

Insect species are not identical in their role of pollination, because the behaviour of various groups of insects and the individual species within those groups is quite diverse (Herrera 1988). To determine legitimate pollinators from non-pollinating floral visitors requires evidence that the visitor can pollinate by picking up pollen and depositing pollen onto stigmas; evidence that a visitor pollinates by showing exclusion of all visitors causes lack of seed set and visitor access results in seed set; and evidence that a particular visitor contributes to pollination (Pellmyr 2002). Having determined the visitor assemblage for three populations, I attempted to determine which of the most abundant visitors were effective at depositing pollen by exposing previously bagged virgin umbels to these visitors (Kearns and Inouye 1993) and introducing insects into cages with virgin umbels. This was difficult considering the relatively low visitation rates experienced and that female umbels are out-numbered by male umbels in the field. Unfortunately, these attempts also coincided with harsh drought conditions experienced throughout NSW (Appendix 3) and the umbels dried out after treatment and failed to mature any seeds. Further study is needed to confidently determine the pollinators of *T. incisa*.

Determination of pollinators requires consideration of spatial and temporal variation in order to understand the coevolution of plants and their pollinators and pollination networks. The pollinator assemblage of *T. incisa* varied significantly among populations on a relatively small scale; Myall Lakes and Tomago are located 50 km apart, compared to the entire geographic range of *T. incisa* which covers approximately 1200 km from the northern to southern range edges. Therefore, determining the pollinators from a single population would be unreliable and would not characterise the extent of generalisation of the species (Herrera 2005). Similarly, determining the pollinators from a single point in time is unreliable because pollinators varied throughout the day for *T. incisa*, and can vary seasonally, yearly and over decades (Herrera 1988; Herrera 2000; Cane *et al.* 2005; Price *et al.* 2005). Interestingly, a higher degree of spatial variation compared to temporal variation in pollinators has been reported (Herrera 2005), and therefore the assumption that different localities are similar enough to permit temporal changes to be investigated (a “space-for-time” substitution) is not warranted. For example, in order to monitor the success of pollination services in restoration projects the restored site is compared directly to nearby reference sites. However, the spatial variation among reference sites is likely to be as great as the variation between restored and reference sites, given that pollination interactions can vary significantly on small spatial scales.

Other sources of variation in pollinators

The fire at Agnes Banks caused a shift in flowering time from peak flowering in February (summer) to May (late autumn), when flowering is usually in its final weeks, many seeds have dispersed and seedlings germinated (Davila and Wardle 2002; Wardle 2003). Interestingly, *T. incisa* was able to produce seeds in the Agnes Banks population when the abundance of diurnal flying insects was very low, probably due to direct fire-caused mortality and/or post-fire vegetation that is poor in nectar and pollen (Ne'eman *et al.* 2000). Other studies have shown that flowers can produce high fruit levels and suffer weak pollen limitation when pollinator visitation is infrequent, and this was attributed to the long duration of flowers (e.g. Herrera *et al.* 2001). Flowers that are open more than 12 hours per day are also exposed to potential visitation by a variety of nocturnal insects (Young 2002; and papers cited therein). It is possible that nocturnal insects play an important role in pollination of *T. incisa*, considering that the white flowers remain open during the night as well. There have been no published studies on the nocturnal pollination of Apiaceae to date.

The introduced European honey bee, which was the most abundant visitor in recent years at Myall Lakes and Agnes Banks (Y. C. Davila and G. M. Wardle, personal observation), was absent from the assemblages of Agnes Banks and Tomago, and made only two visits at Myall Lakes during this study. However, honey bees were observed to forage on other native plants, such as *Platysace* (Apiaceae) and *Persoonia* (Proteaceae), flowering at the same time as *T. incisa* at Myall Lakes and Tomago. The absence of honey bees provided a unique opportunity to record native pollinators and also indicates a detectable change in pollinators across years. Continued monitoring of insect visitation at these populations will determine whether honey bees return to the assemblage, how often they are present in the assemblage and the long term effects of the presence and absence of honey bees on *T. incisa* reproduction.

This study has established that different populations of *T. incisa*, growing in similar habitats that are geographically separated, experience variation in pollinator visitation rates, assemblage composition, seed production and seedling emergence. In addition, high insect visitation rates do not guarantee high plant reproductive success. Variation in pollinators throughout the flowering season and across years is also likely to be an important source of variation in reproduction and fitness in *T. incisa*, as it is a perennial plant that experiences one to several reproductive episodes during its life time. This raises the question of whether there is more spatial than temporal variation in pollinators of *T. incisa*, which is central to maintaining generalised plant-pollinator interactions.

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CHAPTER 3

MAINTAINING GENERALISED POLLINATION SYSTEMS: IS THERE MORE SPATIAL THAN TEMPORAL VARIATION IN THE FLORAL VISITOR ASSEMBLAGE OF *TRACHYMENE INCISA* (APIACEAE)?

Photograph: *Trachymene incisa* at the Tomago Sandbeds, NSW

INTRODUCTION

Ecological interactions between species comprise many individual encounters, each of which may accumulate to an overall relationship that varies in intensity over time and among populations. Each population of a species may interact with overlapping but still unique combinations of other species, as a result of the combined effects of the physical environment, genetic and demographic population structure and most importantly the composition of the community for each population. Therefore, these differences among populations and the degree of specialisation between interacting species create a geographic mosaic of interactions, which is the raw material for the dynamics of coevolution and evolution of interactions in general (Thompson 1994). This mosaic may result in a species coevolving with many other species across its range. In terms of pollination, spatial variation in pollinators increases the opportunity for plants to interact with many pollinators over their range. Temporal variation in pollinators reduces the opportunity for specialisation of plants on a particular pollinator.

Spatial variation in plant-pollinator interactions has been well documented, with pollinators varying across populations (Lindsey 1984; Herrera 1988) and throughout the range of the plants they pollinate (Eckert 2002). In fact, Herrera (2005) suggests that variation in pollinators among populations of the same plant species appears to be the rule in nature. Pollinators also vary temporally during the day (Herrera 2000a; Wiggam and Ferguson 2005), within a season (Herrera 1988; Price *et al.* 2005; Wiggam and Ferguson 2005), and across seasons or years (Herrera 1988; Fishbein and Venable 1996; Price *et al.* 2005). Given the coevolutionary nature of plant-pollinator interactions and the occurrence of variation in pollinators, what properties of plant species would lead to more spatial or temporal variation in pollinators?

Specialised plants and specialised pollinators are common in some environments, however, very few plants and their pollinators are involved in a reciprocally specialised interaction (reviewed by Minckley and Roulston 2006). Most plants and pollinators are involved in asymmetric generalised interactions, where plants may interact with both generalist and specialist pollinators and pollinators may interact with simple or complex/specialised flowers (Jordano *et al.* 2006; Minckley and Roulston 2006; Vázquez and Aizen 2006). Pollinators can also vary in abundance within the pollinator assemblage both spatially and temporally. In contrast, plants involved in specialised interactions, those pollinated by a narrow range of pollinators that harvest resources from very few plants (Minckley and Roulston 2006), are less likely to experience variation in pollinator types due to the close association between the plant and its pollinators.

Widespread plant species will have populations exposed to a variety of climates and habitats; it follows that different pollinator types are supported in these varying climates and habitats. Community composition varies among habitats and close relationships between a plant species and a pollinator species may change among communities (Kearns and Inouye 1997). In addition, plants and their pollinators rarely completely overlap in range (Thompson 1994; Waser *et al.* 1996) resulting in different assemblages across the range of a plant. In contrast, plant species with restricted distributions across fewer habitat types are less likely to experience fluctuation in pollinators.

Do widespread generalist-pollinated plant species exhibit more spatial than temporal variation in the pollinators? Most pollination studies have been restricted to a single population over 1-3 seasons (reviewed by Waser and Ollerton 2006), or sample many sites over small spatial scales compared to the geographic range of the plant over several seasons (e.g. Fenster and Dudash 2001; Price *et al.* 2005). Very few studies have simultaneously investigated spatial (distinct populations and/or regions) and temporal (among years or seasons) variation in pollinators, although the results suggest more spatial variation than temporal variation (e.g. Gómez and Zamora 1999; Herrera *et al.* 2002a; Cane *et al.* 2005; Herrera 2005). Spatial variation coupled with temporal stability of local plant-pollinator interactions can lead to populations specialising or coevolving with different species (Thompson 1994). In contrast, temporal variation in pollinators can maintain generalisation at the population level. Both spatial and temporal variation can maintain generalisation at the species level. Furthermore, very few studies have investigated whether significant differences in pollinator visitation and assemblage result in differences in plant reproductive output and mating (e.g. Galen 1996; Herrera 2000a; Eckert 2002), which has implications for demography and population persistence.

I examined the degree of variation in visitation rates of insects, taxonomic composition of the insect assemblage and resulting reproductive output of *Trachymene incisa* (Apiaceae) across three distinct populations (spanning 210 km) and two consecutive years. Significant spatial variation in pollinators is expected, particularly assemblage composition, given that *T. incisa* is a widespread perennial herb that is visited by a large suite of insects (Chapter 2). In contrast, less variation in pollinators between years is expected, because factors affecting pollinators (e.g. flowering, climate) are likely to be more similar between consecutive years at each population than between populations. A positive correlation between flying insect visitation rates and percentage seed set is also expected. Determining the degree of spatial and temporal variation in pollinators and the resulting reproductive output is the first step towards understanding how generalisation is maintained in this widespread species.

MATERIALS AND METHODS

Study species

Trachymene incisa Rudge subsp. *incisa* (Apiaceae) is a common perennial herb endemic to eastern Australia, with a large latitudinal range from South-east Queensland to the south coast of New South Wales (Powell 1992). It grows in crevices of granite outcrops and on infertile sandy soils in dry eucalypt woodland or scrub (Benson and McDougall 1993).

The flowers are completely protandrous and arranged into simple umbels (Davila and Wardle 2002). The male and female umbel phases are separated by a quiescent phase lasting one day such that whole umbels function as either male (pollen presenting, ~16 d) or female (pollen receptive, ~4 d) at any one time which prevents self-pollination within the umbel (Davila 1999; Davila and Wardle 2002). However, male and female phase umbels are usually present on the same plant at the same time, allowing the possibility for geitonogamous pollination. *Trachymene incisa* is self compatible and self-fertilised seeds are viable (Davila and Wardle 2002). The umbels attract a large suite of insect visitors, including the introduced European honey bee as well as native bees and flies (Davila 1999; Davila and Wardle 2002). The flowering period usually begins in late spring and continues through to late autumn, with peak flowering during summer (Powell 1992; Davila and Wardle 2002; Y. C. Davila personal observation).

Study populations

Three populations of *T. incisa* were studied in New South Wales, Australia over two consecutive flowering seasons during 2003 and 2004. At Myall Lakes National Park, located on the east coast approximately 200 km north of Sydney, *T. incisa* grows in dry heath and dry heath forests on freely drained sands (Myerscough and Carolin 1986).

During peak flowering in January 2003, the study area was located in dry heath along the side of an old mining road (32°30′S, 152°21′E). The following season, peak flowering was observed in December 2003 (included in the 2004 data set) and the study area was located in low open woodland adjacent to Boomeri camp site (32°31′S, 152°19′E). These two sites are located approximately 5 km apart. The mean temperature range for January is 17.7-27.4°C (Nelson Bay weather station, Bureau of Meteorology, 18 year average) and the mean annual rainfall is 1347.4 mm (Nelson Bay weather station, Bureau of Meteorology, 104 year average).

The Tomago Sandbeds are located on the central coast of New South Wales, approximately 70 km south of Myall Lakes National Park. *Trachymene incisa* occurs within the dry open-forest, low woodland and in adjacent fire breaks. The study site was located in a cleared area adjacent to shrubland and an access road (32°47′S, 151°51′E). Peak flowering at Tomago was observed in February in 2003 and in January 2004. Myall Lakes National Park and the Tomago Sandbeds share similar climate, geomorphology, soils and vegetation (Fox *et al.* 1996; Ross *et al.* 2002). The mean temperature range for January is 18.0-27.6°C (Williamtown RAAF weather station, Bureau of Meteorology, 54 year average) and the mean annual rainfall is 1120.4 mm (Williamtown RAAF weather station, Bureau of Meteorology, 56 year average).

The Agnes Banks Woodland is a unique low woodland community occurring on wind blown sand dunes, located in the Agnes Banks Nature Reserve, 55 km west of Sydney (Benson 1992). The Woodland is listed as an Endangered Ecological Community with *T. incisa* as an identifying species (New South Wales Scientific Committee 2000). The study site was located approximately 200 m from the entrance to the reserve (33°39′S, 150°41′E), and was exposed to fire in December 2002. During the first season, *T. incisa* began flowering in March 2003 and peak flowering was observed in May 2003. The following season, peak flowering was observed in January 2004, which is the expected month for peak flowering (Y. C. Davila, personal observation). Benson (1981) noted that 47% of the Agnes Banks flora is also found at Myall Lakes. Therefore, all three sites are floristically similar but geographically separated. The mean temperature range for January is 18.0-27.6°C and for May is 7.9-20.3°C (Richmond RAAF weather station, Bureau of Meteorology, 55 year average) and the mean annual rainfall is 810.3 mm (Richmond RAAF weather station, Bureau of Meteorology, 58 year average).

Observation patch set-up

Six observation patches consisting of 3-6 plants in an area of approximately 2 m^2 were located and tagged at each population, as described in Chapter 2. A different set of plants was used each year because I was interested in the population level response to pollinators, and not all plants tagged in the first season flowered in the second season. I labelled each flowering umbel in a patch with a sticker and unique number on its inflorescence stalk. Visits to male and female umbels were noted, however, visitation by insects to male and female umbels is not biased to either gender at two of the study populations (Chapter 5). Therefore, visits to male and female umbels were pooled for this study. At the beginning of each day I labelled newly flowering umbels and changed labels if umbels had finished flowering or changed sexual phase.

Insect visitor observations

Insect visitors were observed at each patch for 10 min, three time periods per day in the morning (0930-1100 h), midday (1230-1400 h) and afternoon (1530-1700 h). Observations were made at sunrise (0700-0800 h) and sunset (1830-1930 h) in 2003, and at night (2100-2200 h) in 2004, however, insects were either absent or in very low numbers, indicating that pollination does not occur or is very limited during those times (Chapter 2; Appendix 4). Patches were observed in pairs by two observers and the order of paired patches and observers randomized at each time period. During the observation period, the morphospecies of insects (as a surrogate for species because identification was difficult in the field) and all umbels they visited were recorded. Native bees from the genus *Lasioglossum* were separated into two morphospecies based on the colour of their abdomen: black or brown (B), and red (R). Observations were made over three consecutive days during peak flowering for each population. Visitation rate was calculated as the number of visits (landings by insects) made per umbel per 10 min for each patch.

Floral display

Two transects were established in each population, with five 2 m × 1 m quadrats marked out every 10 m (n = 10 quadrats). The total number of plants, number of plants with flowering umbels, and the number of flowering umbels per 2 m^2 were recorded during peak flowering as a measure of floral display. At the end of the flowering season of each population, 19-20 plants were chosen at random and reproductive effort was recorded in terms of the number of umbels produced per plant.

Reproductive output

The same 19-20 plants used to record reproductive effort were used to record reproductive output, in terms of the proportion of umbels producing seeds. Umbels flowering during peak flowering (including umbels from the observation patches) were bagged during their post-receptive phase and collected when seeds were mature. Percentage seed set was calculated for n = 8-15 umbels pollinated during peak flowering and is calculated as the percentage of ovules per umbel that developed into healthy seeds (Davila and Wardle 2002).

Data analyses

The null hypotheses that visitation rate (number of visits per umbel per 10 min) does not vary among populations, among years or throughout the day, was analysed using Repeated Measures Analysis of Variance (ANOVA, SuperANOVA v1.1, Abacus Concepts 1989-1990). Whether a factor is treated as fixed or random is critical for correct tests in ANOVA models and is often up for some debate (Bennington and Thayne 1994). Fixed factors are those where the levels were specifically chosen, multiple tests are used to determine which pairs of means are different from each other and if the experiment is to be repeated, the same levels would be used (Bennington and Thayne 1994). The Myall Lakes, Tomago Sandbeds and Agnes Banks populations vary minimally in latitude, are large enough to adequately record plant-pollinator interactions, occur in habitats that are floristically similar and have been compared in previous studies (e.g. Benson 1981; Fox *et al.* 1996; Ross *et al.* 2002). Therefore, the purpose of the among population comparison was to document differences in visitation rates among these three populations only, and to limit interpretations to how these chosen populations vary. These points provide justification of the decision to treat population as a fixed factor. Many studies would be interested in generalising about differences among populations and would, therefore, include population as a random factor in the analyses. The experimental design would then consist of a sample of populations chosen at random from all populations possible, irrespective of any other reasons to locate the study in a particular place. This was not desirable in this study as I wanted to revisit the same populations each year, rather than choose three new populations each year. A further limitation is that I needed to ensure that there were adequate plants in flower across the study years and therefore some populations were not suitable. In fact, other populations across a wider latitudinal gradient were sampled, but due to low visitation rates (Appendix 5) could not be included in the analyses. The choice of these three populations can be considered a more conservation test of spatial variation as they share attributes of substrate, floristics and latitude, and therefore are less expected to vary than populations at the extremes of the geographical range of the species, or across the different substrates (sand versus rock crevices) that *T. incisa* inhabits.

Therefore, population is the fixed between-plots factor and patch (plots) is nested in population. Year was a random between-plots factor. The years in which this study was conducted were not fixed or specifically chosen for the study and therefore these years could represent other years in general. Time period was the fixed within-plots factor. Each day was considered to be independent and treated as a separate set of patches, because each day some buds opened or female phase umbels senesced, provide a different set of flowering umbels each day. Two replicate patches from data collected from Myall Lakes in January 2003 were excluded from the analysis due to missing data at one time period, so the data were analysed as an unbalanced repeated measures ANOVA with no missing cells (Quinn and Keough 2002). Data were square root transformed to improve normality and homogeneity of variances. I assumed that multi-sample sphericity was not met and used the Greenhouse-Geisser adjustment to avoid risk of Type I error (Quinn and Keough 2002).

A series of one-way analyses of similarities (ANOSIM, Primer v5, Clarke and Gorley 2001) were used to test the null hypotheses that there were no differences in assemblage composition among populations, years and time periods. The visitation rate was used as a surrogate for abundance for constructing the similarity matrix. A subset of data (only observations made by Y. C. Davila at each population over two years) was analysed to ensure consistency across populations and years when identifying morphospecies. Data were square root transformed (which down weights the effect of single species and allows intermediate abundance species to contribute more to the overall assemblage pattern, Clarke and Warwick 2001) and Bray-Curtis similarities used to construct the similarity matrix.

Differences among populations and years in terms of floral display (density of plants, proportion of plants flowering, density of flowering umbels and number of umbels per plant) and reproductive output (proportion of umbels setting seed per plant and seed set) were analysed with two-way analysis of variance ($\alpha = 0.05$), with population as a fixed factor and year as a random factor. Prior to analyses, a Cochran's test (for balanced data) or Bartlett's test (for unbalanced data) was performed to test for homogeneity of variances. Data with heterogeneous variances were transformed (details in Results) to achieve homogeneity of variances. Means ± standard errors (SE) are reported and populations are abbreviated as follows: ML – Myall Lakes, TS – Tomago Sandbeds, AB – Agnes Banks.

RESULTS

Insect visitation rates

Insect visitation rates were highly variable among the three populations and two years, resulting in a significant population by year interaction (Table 3-1, Fig. 3-1). The Tomago population experienced a decrease in visitation rate between consecutive years, whereas Myall Lakes experienced an increase in visitation rate between years. There was no difference in visitation rate between years at Agnes Banks.

Table 3-1 Results of a repeated measures ANOVA on square-root transformed visitation data (number of visits per umbel per 10 min) from three populations over two years at three time periods. Population is fixed, Year is random, Patches are nested in Populations, and Time is the fixed within-plots factor. The degrees of freedom for effects involving Time are multiplied by the Greenhouse-Geisser epsilon (0.952) before calculating *F* probabilities.

Source	df	MS	*F*	*P*
Population	2	0.504	0.115	0.8972
Year	1	0.019	0.153	0.6967
Population × Year	2	4.394	36.005	0.0001
Patch(Population)	100	0.122		
Time	2	1.977	24.918	0.0001
Time × Population	4	0.347	1.042	0.4845
Time × Year	2	0.542	6.832	0.0016
Time × Population × Year	4	0.333	4.195	0.0033
Time × Patch(Population)	200	0.079		

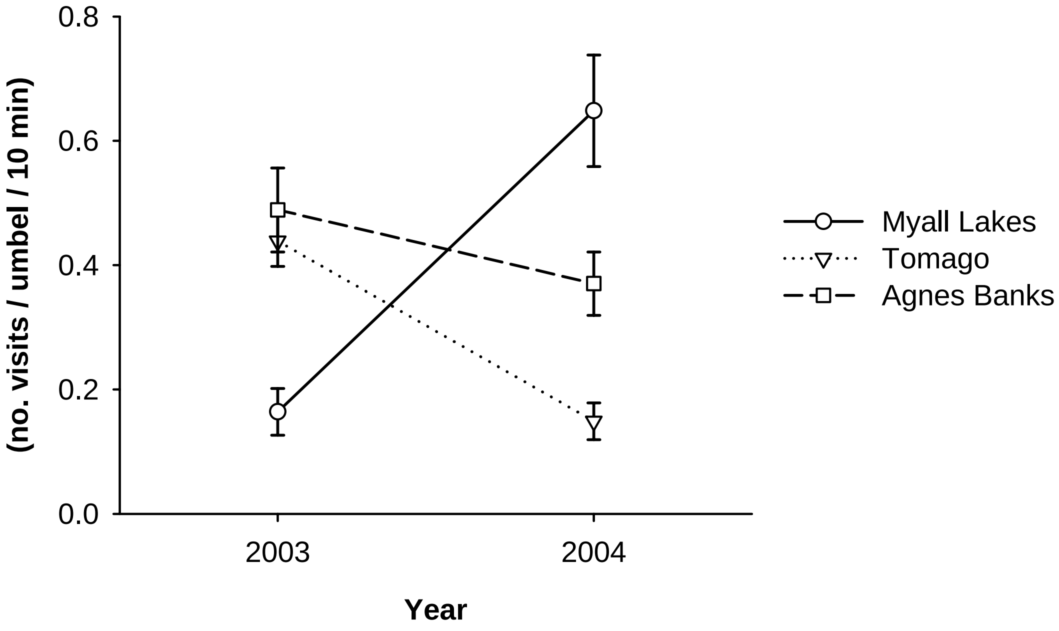

Fig. 3-1 Mean ± SE rate of insect visitation (number of visits per umbel per 10 min) to *Trachymene incisa* for three populations over two years. This population × year interaction was highly significant ($F_{2,\,100} = 36.005$, $P = 0.0001$).

While there were no overall differences among populations or years, separate analyses of variance revealed that in each year the visitation rate varied significantly among the three populations (Table 3-2, Fig. 3-1). In 2003, Myall Lakes received significantly fewer visits than Tomago and Agnes Banks (Tukey-Kramer post-hoc tests $P = 0.01$). However, in 2004 Tomago received significantly fewer visits than Myall Lakes and Agnes Banks ($P = 0.01$).

Insect visitation rate also varied throughout the day, resulting in a significant time period × population × year interaction (Table 3-1). Midday peaks were observed at Agnes Banks in 2003 and Myall Lakes in 2004. Consistently low visitation rates were recorded throughout the day at Myall Lakes in 2003 and Tomago in 2004. The highest visitation rates during the morning time period were recorded at Tomago in 2003, at midday visitation was highest at Myall Lakes in 2004 and in the afternoon at Agnes Banks in 2004

(Fig. 3-2). Overall visitation rates varied significantly throughout the day (Table 3-1), with a peak in visitation recorded at midday.

Table 3-2 Summary of repeated measures ANOVAs and Tukey-Kramer post-hoc tests on insect visitation rate to *Trachymene incisa* for 2003 and 2004 separately, across three populations and three time periods. Population is fixed, Patches are nested in Populations, and Time is the fixed within-plots factor. The degrees of freedom for effects involving Time are multiplied by the Greenhouse-Geisser epsilon (0.939 for 2003; 0.897 for 2004) before calculating *F* probabilities.

Year	ANOVA					Post-hoc	
	Source	df	MS	*F*	*P*	Result	*P*
2003	Population	2, 49	1.880	13.68	0.001	TS, AB > ML	0.01
	Time	2, 98	1.091	14.73	0.0001		
	Population × Time	4, 98	0.145	2.03	0.101		
2004	Population	2, 51	3.020	28.16	0.0001	ML, AB > TS	0.01
	Time	2, 102	1.472	16.97	0.0001		
	Population × Time	4, 102	0.548	6.32	0.0003		

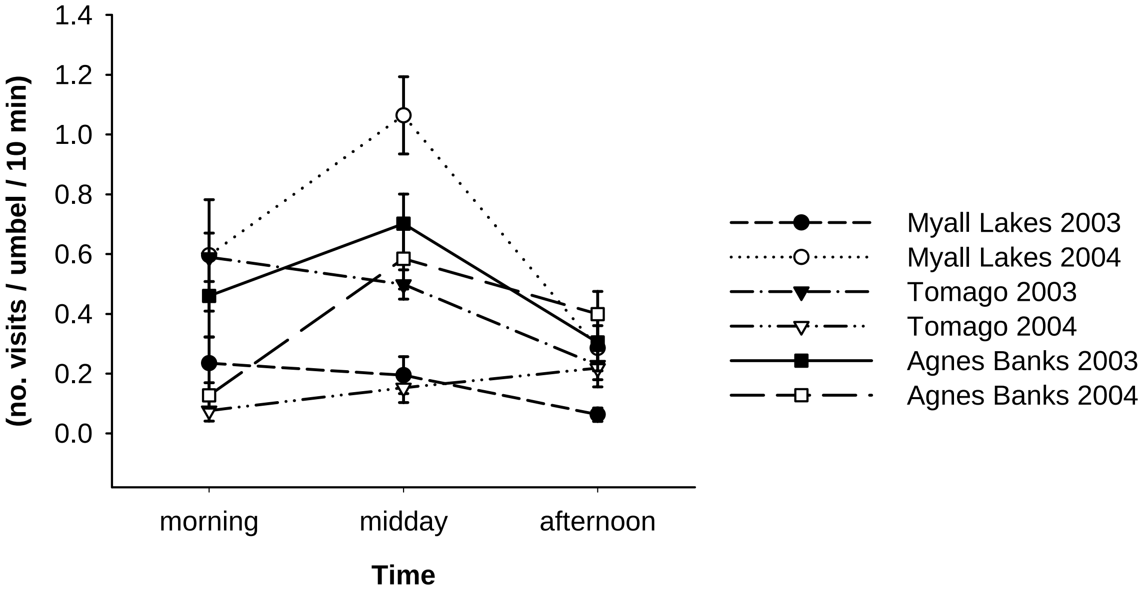

Fig. 3-2 Mean ± SE rate of insect visitation (number of visits per umbel per 10 min) to *Trachymene incisa* for three populations over two years at three time periods. This time × population × year interaction was highly significant ($F_{4,\,200} = 4.195$, $P = 0.0033$).

Taxonomic composition of insect visitor assemblage

The composition of the visiting insects to *T. incisa* varied significantly among the three populations (pooled across years, Global R = 0.121, $P = 0.001$), with pair-wise tests revealing all populations were significantly different from each other (ML v TS: R = 0.087, $P = 0.002$; ML v AB: R = 0.207, $P = 0.001$; TS v AB: R = 0.078, $P = 0.004$). Native bees (*Lasioglossum* sp. and *Hylaeus (Prosopisteron) woyensis*) dominated the assemblage at Myall Lakes (Table 3-3). In contrast, ants (Formicidae) made the majority of the visits at Agnes Banks, with bee flies (Bombyliidae) the second-most abundant visitor. Ants were the most abundant morphospecies at Tomago, with native bees (*H. (P.) woyensis* and *Lasioglossum* sp. B) the most abundant flying insects.

Table 3-3 Composition of the insect assemblage of *Trachymene incisa* and percentage contribution to visits across three populations and two years. Bold numbers indicate the two highest abundances for each population and year. Adjusted number of visits per umbel per 9 h is the number of visits corrected for the number of umbels observed per patch, summed over 9 h of observation per population each year. *Lasioglossum* sp. B includes *L. (Austrevylaeus)* and *L. (Ctenonomia)*. YCD = Y. C. Davila (author).

Common name (Order)	Morphospecies (Family)	Myall Lakes		Tomago		Agnes Banks	
		2003	2004	2003	2004	2003	2004
Native bees (Hymenoptera)	*Hylaeus (Prosopisteron) woyensis* (Colletidae)	**56.40**	4.04	**23.39**	4.23	1.59	0.56
	Lasioglossum sp. B (Halictidae)	-	**39.10**	18.68	11.44	-	3.88
	Lasioglossum sp. R (Halictidae)	6.14	**25.89**	-	2.86	-	16.48
	Homalictus (Homalictus) holochlorus (Halictidae)	2.05	-	0.46	3.15	-	**21.15**
Ants (Hymenoptera)	Ants (Formicidae)	**23.03**	3.70	**49.38**	**40.07**	**94.29**	13.32
Flies (Diptera)	Bee flies (Bombyliidae)	2.05	9.21	3.60	10.72	-	**39.63**
	Hover flies (Syrphidae)	3.68	6.15	-	6.07	0.66	1.13
	Other flies	4.35	2.39	3.89	**16.23**	1.59	1.41
Butterflies (Lepidoptera)	*Zizina labradus labradus* (Lycaenidae)	-	9.11	-	-	-	-
Other insects	Other insects	2.30	0.41	0.60	5.23	**1.87**	2.44
	Adjusted number of visits per umbel per 9 h	5.43	18.90	12.03	3.18	15.26	11.81
	Total number of visits recorded by YCD	55	241	156	48	141	154

Overall the insect assemblage was significantly different between years (Global R = 0.185, *P* = 0.001), with the assemblage differing significantly between years at all populations (ML: R = 0.251, *P* = 0.001; TS: R = 0.670, *P* = 0.001; AB: R = 0.485, *P* = 0.001). Differences between years at Myall Lakes are apparent with the addition of *Lasioglossum* sp. B and the butterfly *Zizina labradus labradus* to the assemblage in 2004, which together contributed almost half of the visits. At Tomago, the species diversity was similar between years although the abundance of *H. (P.) woyensis* decreases whereas flies (all Diptera) increase in abundance. Agnes Banks showed the most drastic change in assemblage with over 94% of visits made by ants in 2003 compared to 13.3% in 2004. In addition, bee flies and native bees (*Homalictus (Homalictus) holochlorus* and *Lasioglossum* sp R.) made over 77% of visits in 2004.

There were no significant differences in assemblage among the three time periods surveyed, pooled across populations and years (Global R = 0.015, *P* = 0.143). At all time periods, ants were the most abundant visitor, contributing 45.6% of visits in the morning, 29.0% of visits at midday and 46.4% of visits in the afternoon. After ants, native bees (*Lasioglossum* sp. B) were the most abundant morphospecies in the morning and midday (17.2% and 20.9% respectively), whereas bee flies contributed 23.4% of visits in the afternoon.

Insect visitation and composition without ants

Although ants made up a large proportion of the visits, they are unlikely to be pollinators because they forage for nectar at the base of the flowers and rarely contact the reproductive structures (Chapter 2). Therefore, I re-analysed the visitation data without the contribution of ants to determine whether visitation rate of flying insects varied among populations, among years or throughout the day.

ANOVA results were similar to the original analysis, with a significant time period × population × year interaction ($F_{4,\,200} = 5.047$, $P = 0.0008$, Greenhouse-Geisser epsilon = 0.9597), time period × year interaction ($F_{2,\,200} = 7.600$, $P = 0.0008$) and overall time period effect ($F_{2,\,200} = 19.815$, $P = 0.0001$). However, due to low visitation rates by flying insects at Agnes Banks in 2003, the visitation rate is significantly lower in 2003 compared to 2004 (overall year effect, $F_{1,\,100} = 23.882$, $P = 0.0001$). There was also a significant population × year interaction ($F_{2,\,100} = 41.412$, $P = 0.0001$), due to increases in visitation between 2003 and 2004 at Agnes Banks and Myall Lakes, and a decrease in visitation between years at Tomago (Fig. 3-3). In 2003, the flying insect visitation rate at Tomago was significantly greater than at Myall Lakes and Agnes Banks (Tukey-Kramer post-hoc tests $P = 0.01$). In 2004, the flying insect visitation rate differed significantly among all three populations ($P = 0.05$).

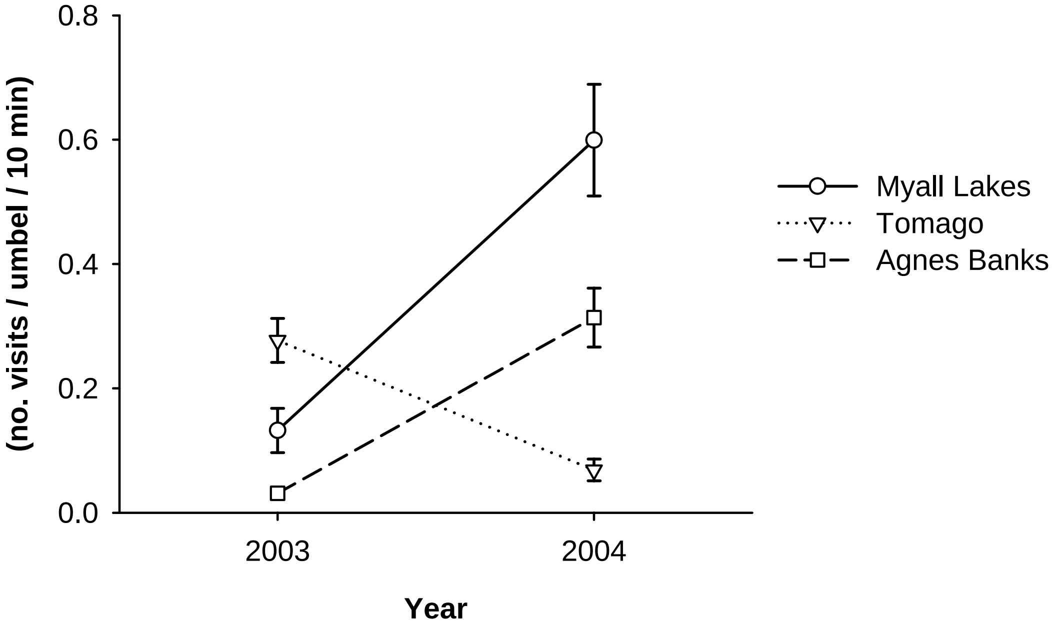

Fig. 3-3 Mean ± SE rate of non-ant insect visitation (number of visits by insects not including ants per umbel per 10 min) to *Trachymene incisa* for three populations over two years. This population × year interaction was highly significant ($F_{2,\,100} = 41.412$, $P = 0.0001$).

The composition of visiting insects varied significantly among the three populations (Global R = 0.127, $P = 0.001$). Pair-wise tests revealed that the assemblage at Agnes Banks, with a high proportion of bee fly visits (Table 3-3), differed from Myall Lakes (R = 0.149, $P = 0.001$) and Tomago (R = 0.201, $P = 0.001$). However, Myall Lakes and Tomago were similar in composition (R = 0.036, $P = 0.07$), with native bees (*H. (P.) woyensis* and *Lasioglossum* sp. B) in common (Table 3-3). Overall the assemblages varied between years (Global R = 0.214, $P = 0.001$), and differences between years were experienced at all populations (ML: R = 0.383, $P = 0.001$; TS: R = 0.350, $P = 0.001$; AB: R = 0.571, $P = 0.001$).

Floral display

The density of plants was on average greater at Tomago in 2004 than in 2003 and compared to the other populations (Fig. 3-4a). This was due to two quadrats that each had over 170 plants, while the remaining quadrats had much lower densities of plants. This large variation resulted in no differences in plant density between years (data fourth-root transformed, $F_{1,54} = 0.009$, $P = 0.924$) or among populations ($F_{2,2} = 0.806$, $P = 0.554$), and there was no significant interaction ($F_{2,54} = 2.921$, $P = 0.062$).

Flowering varied significantly among populations and years. There was a significant population × year interaction in terms of proportion of plants flowering (data fourth root transformed, $F_{2,48} = 4.076$, $P = 0.023$), driven by an increase in flowering plants from 4.3% to 55.4% at Agnes Banks and no significant change at Myall Lakes and Tomago over two years (Fig. 3-4b). There were no overall differences among populations ($F_{2,2} = 0.088$, $P = 0.919$), although proportionally more plants flowered during 2004 compared to 2003 ($F_{1,48} = 10.479$, $P = 0.002$).

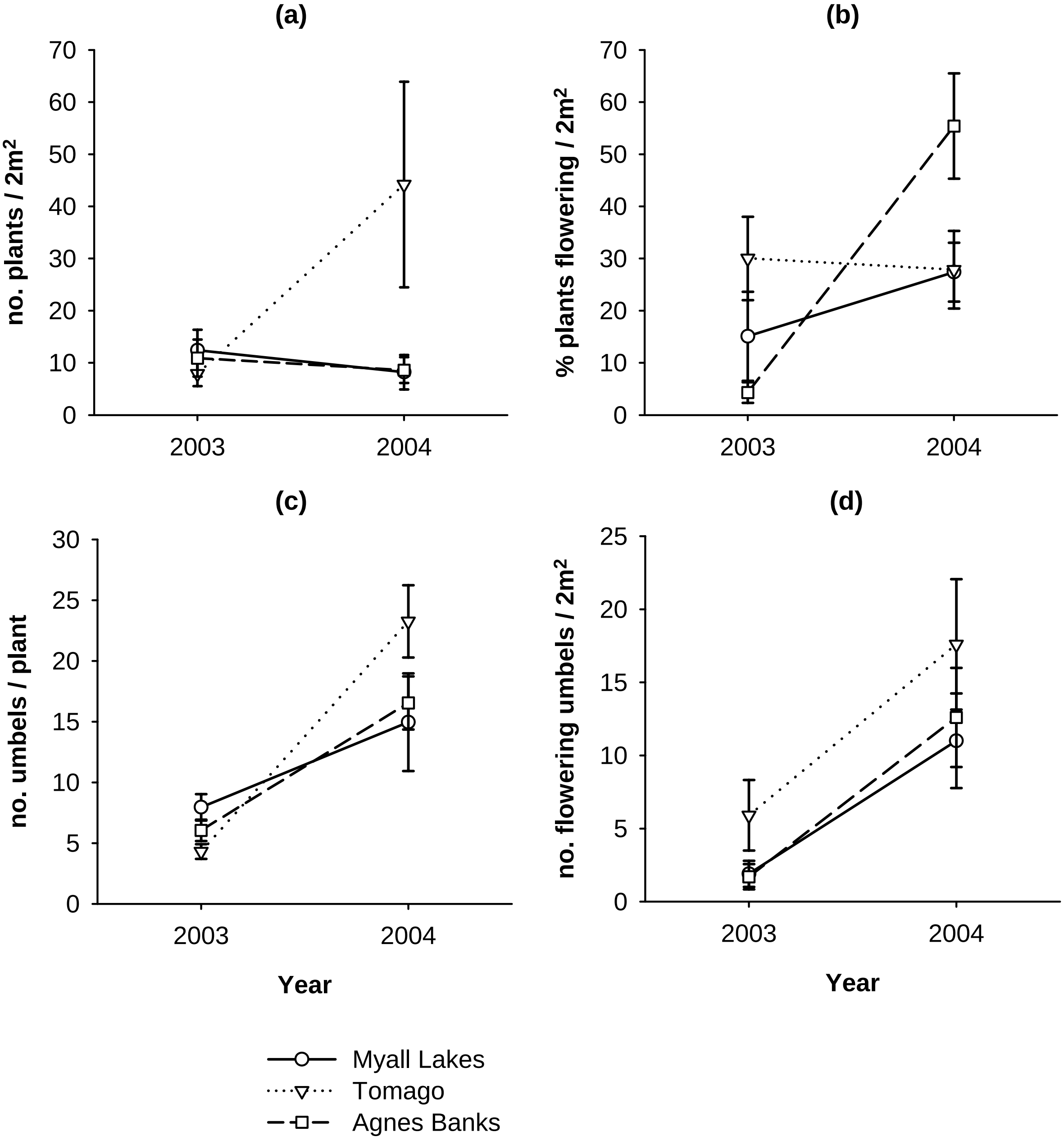

Fig. 3-4 Floral display of *Trachymene incisa* across three populations and two years: (**a**) plant density per 2 m^2 (not significant); (**b**) percentage of plants flowering per 2 m^2 during peak flowering (population × year interaction $F_{2,48} = 4.076$, $P = 0.023$); (**c**) number of umbels per plant (population × year interaction $F_{2,113} = 8.647$, $P = 0.003$); (**d**) flowering umbel density per 2 m^2 during peak flowering (year effect $F_{1,54} = 9.799$, $P = 0.003$). Mean ± SE presented.

Between 2003 and 2004 there was an increase in umbel production at both Agnes Banks and Tomago with no change in umbel production at Myall Lakes (Fig. 3-4c), indicated by a significant population × year interaction (data ln transformed, $F_{2, 113} = 8.647$, $P = 0.003$). In addition, there was an overall difference in umbels per plant between years ($F_{1, 113} = 62.167$, $P = 0.0001$), but no differences among populations ($F_{2, 2} = 0.020$, $P = 0.980$).

With a general increase in umbel production and proportion of flowering plants, the density of flowering umbels at peak flowering also increased significantly between 2003 and 2004 (data fourth root transformed, $F_{1, 54} = 9.799$, $P = 0.003$), with an average of 3.2 ± 0.9 umbels per 2 m^2 in 2003 and 13.7 ± 2.1 umbels per 2 m^2 in 2004. There were no overall differences in density of flowering umbels among populations ($F_{2, 2} = 1.839$, $P = 0.352$) and no interaction between population and year ($F_{2, 54} = 0.864$, $P = 0.427$; Fig. 3-4d).

Reproductive output

There was a decrease in the percentage of umbels per plant producing seeds at Tomago over two years (Fig. 3-5a), with no change at Agnes Banks and Myall Lakes, resulting in a significant population × year interaction ($F_{2, 113} = 10.193$, $P = 0.0001$). However, the actual number of seeding umbels per plant increased significantly between years at Tomago (df = 24, $t = 3.053$, $P = 0.005$; Fig. 3-5b) and Agnes Banks (df = 25, $t = 4.357$, $P = 0.0002$). There were no differences in absolute number of seeding umbels per plant at Myall Lakes (df = 22, $t = 1.491$, $P = 0.150$) between years.

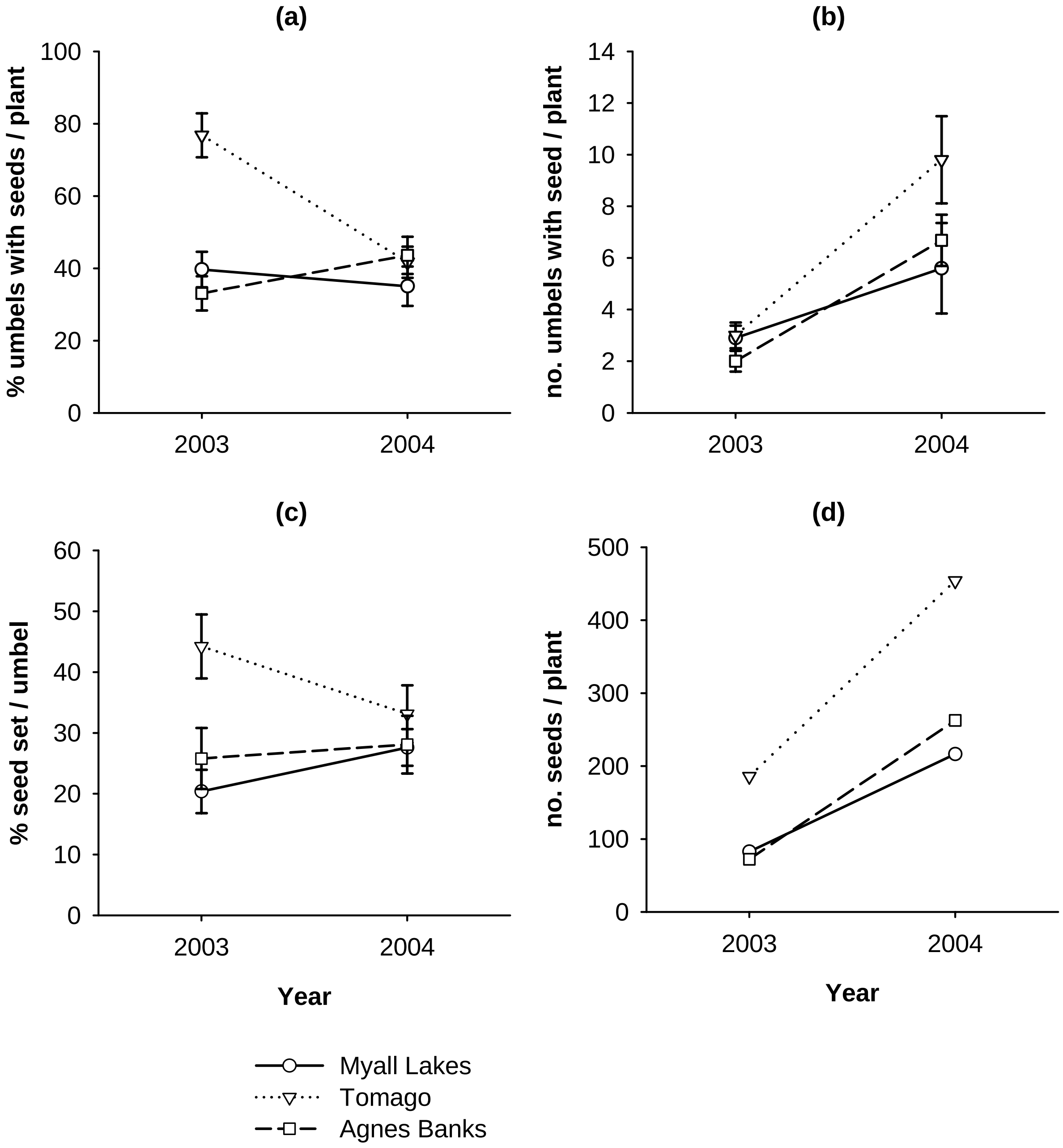

Fig. 3-5 Reproductive output of *Trachymene incisa* across three populations and two years: (**a**) percentage of umbels per plant producing seeds (population × year interaction $F_{2,\,113} = 10.193$, $P = 0.0001$); (**b**) number of umbels per plant producing seeds (year effect for Tomago $P = 0.005$ and Agnes Banks $P = 0.0002$); (**c**) percentage seed set per umbel (not significant); (**d**) estimate of the number of seeds produced per plant. Mean ± SE presented.

The percentage of umbels producing seeds per plant decreased significantly between 2003 and 2004 ($F_{1,\,113}$ = 5.374, P = 0.022), but there were no differences among populations ($F_{2,\,2}$ = 1.140, P = 0.467). However, the absolute number of seeding umbels increased between 2003 and 2004 (df = 82, t = 2.974, P = 0.004).

There were no differences in percentage seed set per umbel (Fig. 3-5c) among populations ($F_{2,\,2}$ = 2.729, P = 0.268) or years ($F_{1,\,75}$ = 0.018, P = 0.893), and there was no interaction ($F_{2,\,75}$ = 1.948, P = 0.150). An estimate of the number of seeds produced per plant (mean percentage seed set × mean number of flowers per umbel (70) × number of ovules per flower (2) × mean number of umbels producing seeds per plant) shows that all populations increased mean seed production between 2003 and 2004 (Fig. 3-5d).

DISCUSSION

Variation among populations and temporal stability in plant-pollinator interactions can lead to specialisation and coevolution between plants and pollinators. In contrast, temporal variation in plant-pollinator interactions at the population level can reduce the opportunity for specialisation between interacting partners, thereby maintaining generalised pollination systems. This raises the question, is there more spatial than temporal variation in floral visitors to *T. incisa*, which is a widespread species with the opportunity for local specialisation across the range? There is both significant spatial and temporal variation in insect visitation rates and the taxonomic composition of the visiting insect assemblage of *T. incisa*. The three populations were expected to differ in pollinators given that *T. incisa* is generalist-pollinated and the populations were discrete. However, significant differences in visitation rates and considerable changes to the assemblage composition between consecutive years were also recorded at each population. The degree of spatial and temporal variation depends on the presence of ants in the analyses. Interestingly, percentage seed set was not significantly different between years at each population, despite significant variation in flying insect visitation rates and assemblage composition.

Spatial versus temporal variation in floral visitors

Insect visitation rates were variable among the three populations within each year (Table 3-1, Fig. 3-1). This suggests that these populations are not equally serviced. Overall there were no differences among populations or years, suggesting that there are equal amounts of spatial and temporal variation when looking at the visitor assemblage of *T. incisa*.

The presence of ants in the analysis altered the differences between years at Agnes Banks (compare Fig. 3-1 and Fig. 3-3), with no difference in visitation rates when ants were included and an increase between years when ants were excluded. The exclusion of ants also led to a significant difference in overall visitation rates between years, while there remained no overall differences among populations. This suggests more temporal than spatial variation in flying insect visitation rates to *T. incisa*.

Similar patterns were revealed through the analyses of similarities on assemblage composition. When ants were included in the analyses, there were significant differences in assemblage composition among all populations and between years at all populations. When ants were removed from the analyses, yearly differences were recorded at each population. However, Myall Lakes and Tomago had similar assemblage composition, and these were different to Agnes Banks. These results indicate more temporal than spatial variation in flying visitor assemblage composition.

Contrary to expectations, the floral visitor assemblage of *T. incisa* does not show more spatial than temporal variation. The few studies investigating both spatial and temporal variation in pollinator assemblages indicate a pattern for more spatial than temporal variation (Gómez and Zamora 1999; Herrera *et al.* 2002a; Cane *et al.* 2005; Herrera 2005). These studies were performed on intermediate to large spatial scales, where populations were disjunct and covered several to hundreds of kilometres, and are congruent with the distances between the populations in my study. Studies on these larger scales are needed to determine whether populations are serviced by different assemblages of pollinators, because on smaller scales populations may be sharing a pollinator assemblage when pollinators can travel the distances between them. For example, Price *et al.* (2005) found no spatial variation (but high temporal variation) in visitors among sites of *Ipomopsis aggregata* located up to 575 m apart, a distance that most floral visitors are able to traverse. However, differences in assemblages at these small spatial scales do occur. For example, Minckley *et al.* (1999) found that bee assemblages of the widespread *Larrea tridentata* (Zygophyllaceae) from sites 1-5 km apart varied as much as repeat samples made at single sites, and Herrera (2005) showed that individual plants in the same population had quite variable degrees of generalisation which was comparable in magnitude to variation among populations. The Myall Lakes and Tomago populations of *T. incisa* in my study had similar assemblage composition (without ants) despite being located 70 km apart. These two populations are located in habitats with similar climate, geomorphology, soils and vegetation (Ross *et al.* 2002), which are likely to support similar invertebrate communities. Surveying populations throughout the rest of the range of *T. incisa* is likely to reveal greater differences in spatial variation among populations in visitor assemblage and reveal a more complete assessment of the degree of generalisation on pollinators (Appendix 5).

Temporal variation in the visitor assemblage between consecutive years was observed. Herrera (2005) and Cane *et al.* (2005) found a high degree of similarity between consecutive years in the pollinator assemblage of generalist-pollinated plants. I also sampled over two consecutive years, but found the visitor assemblages to vary between years at all populations. These differences are most likely driven by fire events at Agnes Banks (December 2002) and Tomago (October 2003), and the effects of drought during the course of the study. Fire-related decreases in insect abundance can explain the low visitation rates by flying insects during the post-fire flowering seasons at Agnes Banks in 2003 and Tomago in 2004. *Trachymene incisa* grows in fire-prone habitats, with the three populations in this study being exposed to fire at least once since 1998 (G. M. Wardle, personal communication; Y. C. Davila, personal observation). *Trachymene incisa* also responds quickly and positively to fire by resprouting and flowering (Chapter 4). The recovery of pollinator communities after fire will depend on the extent of the fire and ability to return to the burnt area from adjacent areas. Two years of sampling, while typical of most pollination studies, is only a snap-shot in the perennial life span of *T. incisa*. Longer term studies are needed to determine the extent of temporal variation in pollinators of *T. incisa*, and the role of fire in maintaining temporal variation.

Role of ants and honey bees in the visitor assemblage

Functionally, ants are not likely to be contributing to pollination directly, because they forage for nectar at the base of flowers and rarely contact reproductive structures (Chapter 2). In this case, ants are considered to be floral thieves that enter flowers legitimately to collect nectar but transfer little pollen (Irwin *et al.* 2001). Floral larceny can have a negative effect on female reproductive success of plants (Irwin *et al.* 2001). Therefore, visitor assemblages with a large proportion of ants may function differently and less effectively as pollinators, compared to assemblages with a small proportion of ants. The high proportion of ants in the assemblage of all populations of *T. incisa* suggests they have the capability to interfere with pollination by other insects, and the role of ants is an important area for future study.

Interestingly, the removal of ants from the analysis of assemblage differences resulted in no differences between Myall Lakes and Tomago, with both assemblages dominated by native bees. This highlights that the removal of one species with different abundance between populations can change whether populations are determined to be significantly different or not. In addition, removing ants from the analysis of visitation rates resulted in an increase in visitation rate between years at Agnes Banks (Fig. 3-3) and an overall increase in visitation between years, whereas including ant visitation shows no difference between years at Agnes Banks (Fig. 3-1) and no overall difference between years. This supports the general consensus that the visitor assemblage and pollinator assemblage are not equivalent. One of the major questions in plant reproductive ecology is what factors affect plant fitness? Recent research has been directed at understanding the contributions of non-pollinating floral visitors to plant reproduction, such as nectar robbers and thieves (Irwin *et al.* 2001; Galen and Butchart 2003; Price *et al.* 2005) and floral herbivores (Krupnick and Weis 1999; Krupnick *et al.* 1999; Herrera 2000b; Herrera *et al.* 2002b). The inclusion and subsequent exclusion of non-pollinating ants from my analyses helps identify the qualitative differences between the visitor and pollinator assemblages of *T. incisa*.

The absence of honey bees from the assemblage remains a point of interest, because they have been observed collecting pollen and nectar from *T. incisa* in previous years, and examination of corbiculae pollen loads indicates honey bees used *T. incisa* as a reliable pollen source (Davila 1999). Honey bees were observed foraging on other plant species at Myall Lakes and Tomago in 2003 and at all three populations in 2004. However, honey bees were not observed visiting *T. incisa* during this study. The current absence of honey bees from the visitor assemblage means there is more pollen and nectar available for other floral visitors. While ants were probably the major consumers of nectar at all populations, native bees would collect much of the pollen at Myall Lakes and Tomago, comprising 68.0% and 38.2% of visits respectively. In contrast, native bees made only 19.3% of visits at Agnes Banks, compared to bee flies which made 59.0% of visits and can also consume pollen. Introduced honey bees have been shown to interfere with native bee foraging (Gross and Mackay 1998), and it would be interesting to see if visitation by native bees, flies and ants can be retained if honey bees return to the assemblage at all populations.

No correlation between visitation rates and reproductive output

Although insect visitation rates varied among the three populations within years, reproductive output did not follow the same pattern (compare Figs. 3-1, 3-3 and 3-5). Floral display increased between 2003 and 2004 (Figs. 3-4c and 3-4d), which increased both the attraction of the population and chance for increased reproductive output. The number of umbels producing seeds followed this pattern with a significant increase between years at Tomago and Agnes Banks, and a non-significant increase at Myall Lakes (Fig. 3-5b). In contrast, percentage seed set did not differ significantly across populations and years (Fig. 3-5c). This indicates that reproductive output is a function of reproductive effort, where plants that produce more seeds will do so by producing more umbels rather than increasing the ratio of seeds to ovules (percentage seed set) within an umbel. This is shown by the estimate of the number of seeds produced per plant (Fig. 3-5d) following a similar pattern as the number of umbels per plant (Fig. 3-4c).

Seed production was not correlated with insect visitation rates, such that populations with low visitation rates were still capable of producing similar percentage seed set per umbel as populations with higher visitation rates. This result suggests that populations are not pollen limited. This was found to be the case at Agnes Banks in 2000 where supplemental hand-pollination did not increase percentage seed set per umbel (Davila and Wardle 2002). Each flower has two stigmas, each of which leads to a single ovule. Theoretically, only one pollen grain per stigma is required to fertilise the ovule, and therefore only approximately 150 pollen grains are needed to fertilise all ovules in an umbel (based on a mean of 72 flowers per umbel, Davila and Wardle 2002). The pollen:ovule ratio of 1902:1 indicates pollen is unlikely to be limited in the system (Davila and Wardle 2002). *Trachymene incisa* is self-compatible but suffers weak to intermediate levels of inbreeding depression as a result of self-fertilisation and biparental inbreeding (Chapter 6). Therefore, the quality of pollen transferred by pollinators appears to be of more importance than quantity of pollen. Seedling performance was not investigated in this study due to time limitations. However, emergence of seeds produced in 2003 varied across the three populations (Chapter 2), indicating that variation in assemblage composition can affect seedling performance and has the potential to affect population dynamics in *T. incisa*.

Maintenance of generalised plant-pollinator systems

Trachymene incisa is a generalist in terms of pollination; temporal variation in the assemblages of populations among years maintains generalisation at the population level, and spatial variation among populations maintains generalisation at the species level. A striking pattern is that plants from Tomago in 2003, Myall Lakes in 2004 and Agnes Banks in 2004 produced similar estimated numbers of seeds per plant (Fig. 3-5d), and all of these populations had different assemblage compositions (Table 3-3). This shows that *T. incisa* can utilise different flowering strategies and insect visitors to produce similar reproductive output across its range. This strategy of generalisation is proposed to evolve and be maintained when the abundance of the best pollinators varies strongly over time (Waser *et al.* 1996). Therefore, identifying the scales at which pollinators vary is important in both an evolutionary context, for the evolution and maintenance of specialised and generalised systems, and ecological context, by affecting plant reproductive output and plant population dynamics (Price *et al.* 2005; Gómez and Zamora 2006).

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RESILIENCE OF POLLINATION IN AN ENDANGERED ECOLOGICAL COMMUNITY AFTER FIRE: DIFFERENT POLLINATOR ASSEMBLAGES ACHIEVE SIMILAR SEED PRODUCTION FOR *TRACHYMENE INCISA* (APIACEAE), A DOMINANT HERBACEOUS PLANT IN THE AGNES BANKS WOODLAND, NSW

Photographs: top – Agnes Banks Woodland February 2003; bottom – Agnes Banks Woodland January 2004

INTRODUCTION

There are an estimated 216 000 animal pollinated flowering plant species worldwide (Buchmann and Nabhan 1996), and almost 300,000 obligate or facultative flower-visiting animal species worldwide (reviewed by Kearns *et al.* 1998). Most plants and their pollinators are involved in generalised interactions, where plants are pollinated by more than one pollinator, and these pollinators interact with more than one plant species (Waser *et al.* 1996; Olesen 2000). Plant-pollinator interactions can be used to evaluate the success of ecological restoration efforts (Kearns *et al.* 1998; Forup and Memmott 2005), and pollinators have been suggested as bioindicators of ecosystem health (Kevan 1999). Given the importance of the pollination mutualism to many plants, animals and ecosystem function, it is timely that there has been a recent shift in conservation effort away from single-species and towards habitats and ecosystems (Kearns and Inouye 1997), and a recognition that restoration is likely to be successful only when the functional processes and interactions within the ecosystem are also re-established (Neal 1998).

In the state of New South Wales (NSW), Australia, an endangered ecological community is defined as a community that is likely to become extinct or is in immediate danger of extinction as determined by the Scientific Committee, and is protected under the Threatened Species Conservation Act 1995. As of January 2006, there were 78 endangered ecological communities listed in NSW (NSW Department of Environment and Conservation 2006). The Agnes Banks Woodland was listed as an endangered ecological community in 2000 (New South Wales Scientific Committee 2000a). The Woodland has been extensively cleared and only 4% of its historical extent or 80 ha remain, which is located in western Sydney (New South Wales Scientific Committee 2000a). It is a unique low woodland community dominated by *Eucalyptus sclerophylla* (Myrtaceae), *Angophora* *bakeri* (Myrtaceae) and *Banksia serrata* (Proteaceae), with a diverse understorey of sclerophyllous shrubs (Benson 1981; de Barse 2001). The vegetative structure and composition have been studied in detail (Benson 1981; de Barse 2001). In addition, a plan of management has been prepared for the Agnes Banks Nature Reserve, which holds approximately half of the remaining area of the Woodland (New South Wales National Parks and Wildlife Service 1999). However, these conservation strategies do not consider the role of insects, including the pollinators of nine threatened plant species, in the Woodland. The fate of many plants may depend on preserving their mutualistic relationships with pollinators and with the organisms that affect both plant and pollinator (Kearns and Inouye 1997). Fire has played an important role in determining spatial and temporal variation in plant species distribution in the Woodland (de Barse 2001). There have been at least six fires between 1974 and 2006 in sections of the Woodland (New South Wales National Parks and Wildlife Service 1999; de Barse 2001; Y. C. Davila, personal observation). Few studies worldwide have investigated the response of plant-pollinator interactions to fire (Ne'eman *et al.* 2000; Potts *et al.* 2001; Potts *et al.* 2003).

Trachymene incisa subsp. *incisa* (Apiaceae) is a common perennial herb listed as an identifying species of the Agnes Banks Woodland (New South Wales Scientific Committee 2000a). *Trachymene incisa* is one of the first species in the Agnes Banks Woodland to resprout after fire (Y. C. Davila, personal observations) and flowers from late spring to late autumn, with a peak in flowering during summer (Powell 1992; Davila and Wardle 2002; Y. C. Davila, personal observation). As a generalist-pollinated herb, *T. incisa* provides nectar and pollen for several insect groups such as bees, flies, beetles, butterflies and moths within the Agnes Banks Woodland (Davila and Wardle 2002; Chapters 2 and 3).

I investigated the variation in diurnal and nocturnal floral insect visitors of *T. incisa* and the resulting reproductive success in the Agnes Banks Woodland over three flowering seasons after fire. Investigation of the pollinator assemblage of *T. incisa* will enhance our understanding of the role of generalist pollinators in servicing an endangered community and the interactions between common plants, insects and fire in a community under threat.

MATERIALS AND METHODS

Trachymene incisa

There are 39 species of *Trachymene* (Apiaceae) in Australia, 38 of which are endemic (Hart and Henwood 2006). *Trachymene incisa* subsp. *incisa* Rudge is a common perennial herb endemic to eastern Australia, with a large latitudinal range from South-east Queensland to the south coast of New South Wales (Powell 1992). It grows on infertile sandy soils and in crevices of granite outcrops in dry eucalypt woodland or scrub (Benson and McDougall 1993). Established plants may be killed by fire (Fox 1988) or can resprout from the taproot (Benson 1981; Wardle 2003).

There are approximately 72 flowers per inflorescence, arranged in simple umbels (Davila and Wardle 2002). Both flowers and umbels are completely protandrous, with the male and female umbel phases separated by a quiescent phase lasting one day (Davila and Wardle 2002). Therefore, whole umbels function as either male (pollen presenting, ~16 d) or female (pollen receptive, ~4 d) at any one time which prevents self-pollination within the umbel (Davila 1999; Davila and Wardle 2002). However, male and female phase umbels are usually present on the same plant at the same time, allowing the possibility for geitonogamous pollination. Umbels are arranged in a hierarchy, where the secondary umbels branch off the stalk of the terminal primary umbel and the tertiary umbels branch off the stalks of the secondary umbels and so forth, usually up to four orders. There are on average four inflorescence stalks per plant, which grow to a height of approximately 60 cm (Davila and Wardle 2002). *Trachymene incisa* is self compatible and self-fertilised seeds are viable (Davila and Wardle 2002). The umbels attract a large suite of insect visitors including native bees and flies, although the introduced European honey bee was the most abundant visitor in recent years (Davila 1999; Davila and Wardle 2002).

The flowering period usually begins in late spring with a peak in flowering (highest density of flowering umbels) around January-February (Powell 1992; Davila and Wardle 2002; Y. C. Davila, personal observation). Following this peak, many umbels set seed while a small proportion of plants continue to flower through to the end of autumn. Seed dispersal occurs between March and October, with no obvious peak (Gill 2000). Although *T. incisa* is common, it has been listed as an important component of three endangered ecological communities in New South Wales (New South Wales Scientific Committee 2000a; 2000b; 2000c).

Agnes Banks Woodland

The Agnes Banks Woodland is a unique low woodland community occurring on wind blown sand dunes, partially located in the Agnes Banks Nature Reserve, 55 km west of Sydney (Benson 1992). The study area is located 250 m from the entry to the reserve (33°39′ S, 150°41′ E), and was exposed to fire in late December 2002. A different section of the Woodland, which was not part of the study area, was also exposed to fire in early January 2003. At the time of these fires, *T. incisa* was flowering in low numbers and the fire scorched the understorey vegetation. *Trachymene incisa* began resprouting approximately one month later, flowering began in March 2003 and peak flowering was observed in May in the study area. In 2004 and 2005, peak flowering was observed in January and most flowering had ceased by May. The extent of the December 2002 and January 2003 fires in parts of the Agnes Banks Woodland meant that comparisons between burnt and unburnt areas were unable to be made. These fires were not planned but did provide a unique opportunity to investigate the recovery of pollination after fire in this endangered ecological community.

Several other plants were observed flowering in the study area during peak flowering of *T. incisa* such as *Platysace ericoides* (Apiaceae), *Pimelea linifolia* (Thymelaeaceae), *Acacia ulicifolia* (Mimosaceae), *Persoonia nutans* (Proteaceae), *Haemodorum planifolium* (Haemodoraceae), *Stylidium graminifolium* (Stylidiaceae), *Bossiaea heterophylla* (Fabaceae), *Baeckea diosmofolia* and *Kunzea capitata* (Myrtaceae). This list is not exhaustive, as other plants were likely to be flowering in other parts of the Woodland, adjacent Castlereagh Woodlands and in surrounding properties at the same time. Insects caught visiting *T. incisa* carry pollen from other flowering plants (Y. C. Davila, unpublished data), suggesting the plant species may share pollinators with co-flowering plants.

Observation patch set-up

Six observation patches consisting of 3-6 plants in an area of approximately 2 m^2 were located and tagged. A different set of plants was used each year because I was interested in the population level response to pollinators, and not all plants that were tagged flowered every year. I labelled each flowering umbel in a patch with a sticker and unique number on its inflorescence stalk. At the beginning of each day, newly flowering umbels and umbels that had finished flowering or changed sexual phase were labelled appropriately. Visitation to male and female umbels was not biased to either phase for native bees and flies at Agnes

Banks in 2003, although preferences for male phase umbels occurred in 2004 (Chapter 5). However, these results were not conclusive and therefore, visits to male and female umbels were pooled for this study.

Insect visitor observations

Insect visitors were observed at each patch for 10 min, three time periods per day in the morning (0930-1100 h), midday (1230-1400 h) and afternoon (1530-1700 h). Sampling took place over three days during peak flowering between 2003 and 2005. Observations were also made at sunrise (0700-0800 h) and sunset (1830-1930 h) in 2003, and at night (2100-2200 h) in 2004. Patches were observed in pairs by two observers and the order of paired patches and observers randomised at each time period. During the observation period, the morphospecies of insects was recorded (as a surrogate for species because identification was difficult in the field), which umbels they visited (and the temperature in the shade. Native bees from the genus *Lasioglossum* were separated into two morphospecies based on the colour of their abdomen: black or brown (B), and red (R). Visitation rate was calculated as the number of visits (landings by insects) made per umbel per 10 min for each patch.

Floral display

Two 50 m transects were established in 2003, each with five 2 m × 1 m quadrats marked out every 10 m (n = 10 quadrats). The total number of *T. incisa* plants, number of plants with flowering umbels, and the number of flowering umbels per 2 m^2 were recorded each year during peak flowering as a measure of floral display. At the end of the flowering season each year 19-20 plants were chosen at random and the number of umbels produced per plant (reproductive effort) was recorded.

Reproductive output

The same 19-20 plants used to record reproductive effort were used to record reproductive output. Umbels flowering during peak flowering (including umbels from the observation patches) were bagged during their post-receptive phase and collected when seeds were mature. The absolute number and percentage of umbels producing seeds per plant were recorded. Percentage seed set was calculated for $n = 15$ umbels pollinated during peak flowering each year and is calculated as the percentage of ovules per umbel that developed into healthy seeds (Davila and Wardle 2002; Chapter 2).

Data analyses

The null hypotheses that visitation rate (number of visits per umbel per 10 min) does not vary among years or throughout the day, were analysed using Repeated Measures Analysis of Variance (ANOVA, SuperANOVA v1.1, Abacus Concepts 1989-1990). Year is a between-plots factor, patches (plots) are nested in year, and time period is the within-plots factor. Each day was considered to be independent and treated as a separate set of patches, with some buds opening or female phase umbels senescing each day. Three replicate patches from 2005 were excluded from the analysis due to missing data in the morning time period, so the data were analysed as an unbalanced repeated measures ANOVA with no missing cells (Quinn and Keough 2002). Data were square root transformed to improve normality and homogeneity of variances. I assumed that multi-sample sphericity was not met and used the Greenhouse-Geisser adjustment to avoid risk of Type I error (Quinn and Keough 2002). Differences in temperature during observations among years and time periods were analysed with analysis of variance ($\alpha = 0.05$), and simple linear regression analysis was used to explore any relationship between insect visitation rate and temperature.

A crossed analysis of similarities (ANOSIM, Primer v5, Clarke and Gorley 2001) was used to test the null hypotheses that there were no differences in assemblage composition among years and time periods. The visitation rate was used as a surrogate for abundance for constructing the similarity matrix. A subset of data (only observations made by Y. C. Davila over the three years) was analysed to ensure consistency when identifying morphospecies. Data were square root transformed (which downweights the effect of single species and allows intermediate abundance species to contribute more to the overall assemblage pattern, Clarke and Warwick 2001) and Bray-Curtis similarities used to construct the similarity matrix.

Differences among years in terms of floral display (density of plants, percentage of plants flowering, density of flowering umbels, number of umbels per plant and number of flowers per umbel) and reproductive output (number of seeding umbels per plant, percentage of umbels setting seed per plant and percentage seed set) were analysed with analysis of variance ($\alpha = 0.05$). Prior to analyses, a Cochran's test (for balanced data) or Bartlett's test (for unbalanced data) was performed to test for homogeneity of variances. Data with heterogeneous variances were transformed (details in Results) to achieve homogeneity of variances. Means ± standard errors (SE) are reported.

RESULTS

Insect visitation rates and temperature

Insect visitation rates varied through the day. Very few insects were observed during the sunrise (1 ant) and sunset (2 ants, 1 moth) periods in 2003 and night (1 ant, 3 flies) time period in 2004. Therefore, sampling in subsequent years (2004-2005) and all analyses were restricted to the morning, midday and afternoon time periods.

In all years insect visitation rate increased from morning to midday, this trend being more pronounced in 2004 and 2005. While visitation rate remained constant between the midday and afternoon time periods in 2004 and 2005, there was a decrease in visitation rate in the afternoon in 2003. These patterns resulted in a significant time period × year interaction (Table 4-1, Fig. 4-1). Overall, the visitation rate varied significantly throughout the day (Table 4-1), with a peak in visitation rate at midday. There were no differences in overall visitation rate among years.

Mean temperature during observations varied across time periods and years. There was a significant time period × year interaction ($F_{4,\,150} = 45.897$, $P = 0.0001$; Fig. 4-2), due to lower temperatures in 2004 in the morning but higher temperatures at midday and afternoon, compared to 2005, and a decrease in afternoon temperatures in 2003. Overall, mean temperatures were significantly different across years ($F_{2,\,150} = 322.2$, $P = 0.0001$; Tukey-Kramer post-hoc test $P = 0.01$). There was no relationship between insect visitation rates and temperature ($r^2 = 0.011$, $F_{1,\,157} = 1.789$, $P = 0.183$).

Table 4-1 Results of a repeated measures ANOVA on square-root transformed insect visitation data (number of visits per umbel per 10 min) to *Trachymene incisa* in the Agnes Banks Woodland from three consecutive years and three time periods. Year is the between-plots factor, patches are nested in year, and time period is the within-plots factor. The degrees of freedom for Time and Time × Year are multiplied by the Greenhouse-Geisser epsilon (G-G = 0.944) before calculating *F* probabilities.

Source	df	MS	*F*	*P*
Year	2	0.111	0.844	0.436
Patch(Year)	48	0.132		
Time	2	1.438	17.678	0.0001
Time × Year	4	0.299	3.680	0.009
Time × Patch(Year)	96	0.081		

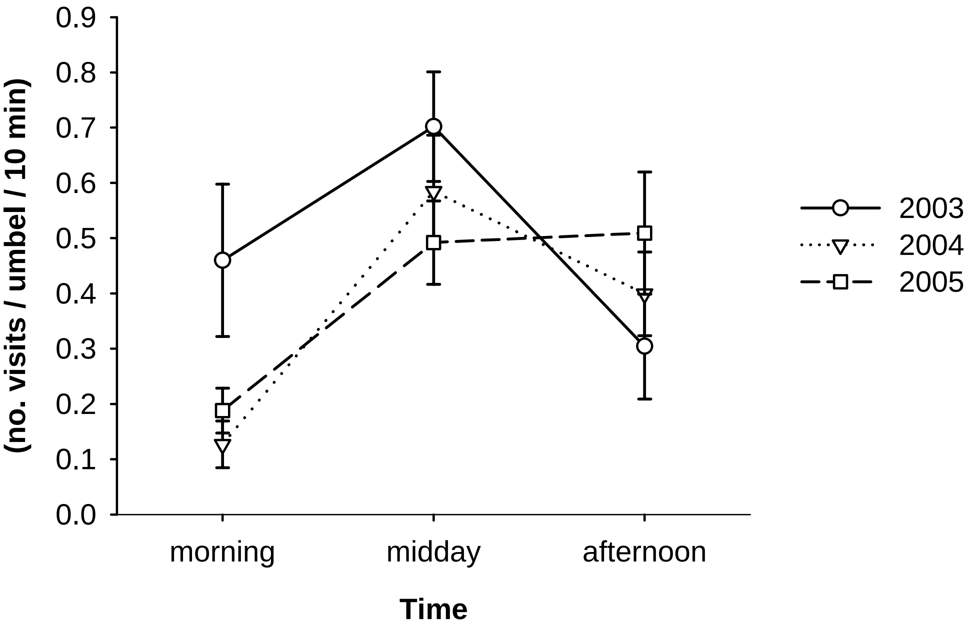

Fig. 4-1 Mean ± SE rate of insect visitation (number of visits per umbel per 10 min) to *Trachymene incisa* for three years after fire at three time periods. Sample sizes: 2003 and 2004 n = 18 patches, 2005 n = 15 patches. This time × year interaction was highly significant ($F_{4, 96} = 3.680$, $P = 0.009$).

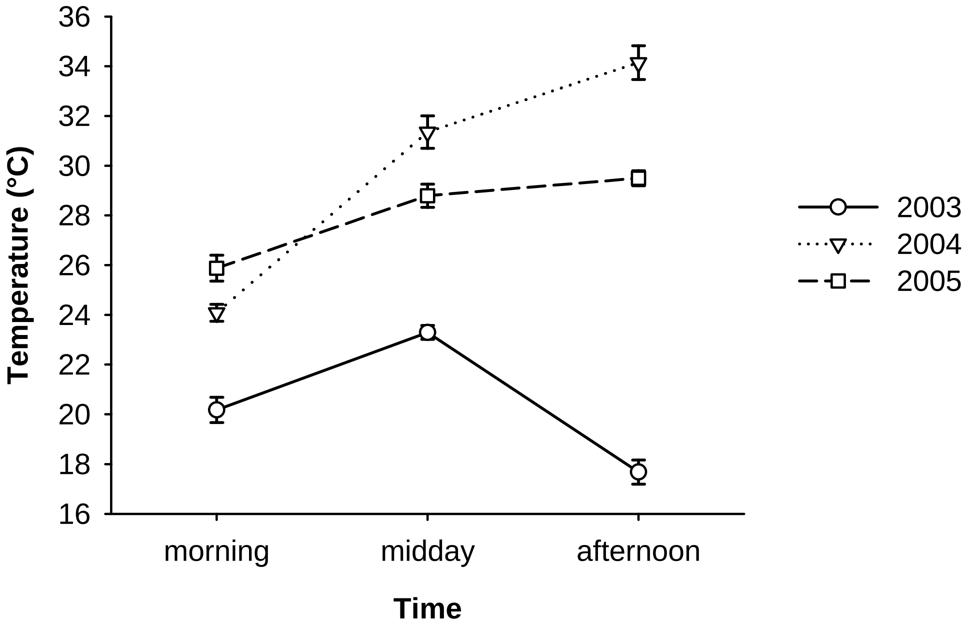

Fig. 4-2 Mean ± SE temperature (°C) during observations of insect visitation to *Trachymene incisa* in the Agnes Banks Woodland over three years and at three time periods. This time × year interaction was highly significant ($F_{4,\,150}$ = 45.897, P = 0.0001).

Taxonomic composition of the insect visitor assemblage

There were significant differences in assemblage composition among consecutive years (Global R = 0.464, P = 0.001; Fig. 4-3a). Pairwise tests reveal all years are significantly different from each other (2003 vs. 2004: R = 0.590, P = 0.001; 2004 vs. 2005: R = 0.187, P = 0.009; 2003 vs. 2005: R = 0.609, P = 0.001). The major differences among years is a shift from ants (Formicidae) being the most abundant visitor in 2003 (over 94% of visits) to bee flies (Bombyliidae) being the most abundant visitor in 2004 and 2005 (39.6% and 58.8% of visits respectively). In 2004 four native bee species collectively contributed 42.1% of all visits (Fig. 4-3a). In contrast only one native bee, *Hylaeus (Prosopisteron) woyensis* (Colletidae), was observed in 2003 contributing to 1.6% of visits and in 2005 two bee species, *Lasioglossum* sp. R and *Homalictus (Homalictus) holochlorus* (Halictidae), contributed 3.1% of visits.

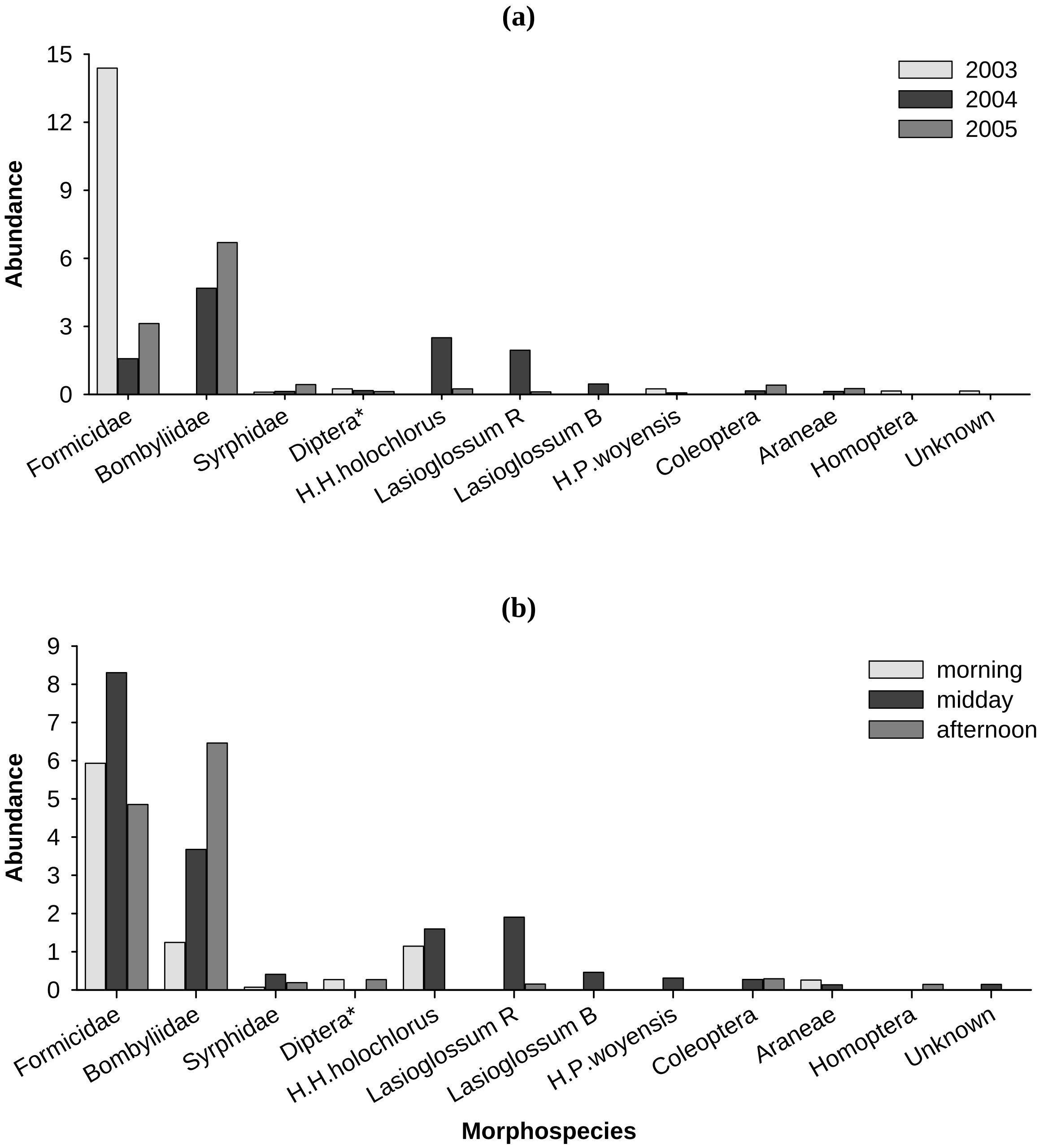

Fig. 4-3 Insect assemblage composition visiting *Trachymene incisa* in the Agnes Banks Woodland: (**a**) each year, pooled across patches, days and time periods (Global $R = 0.464$, $P = 0.001$); (**b**) each time period, pooled across patches, days and years (Global $R = 0.168$, $P = 0.001$). Abundance is measured as the pooled visitation rate per umbel per 10 min. Diptera* refers to species of flies not including bee flies (Bombyliidae) and hover flies (Syrphidae). Native bees include *Homalictus (Homalictus) holochlorus* (Halictidae), *Lasioglossum* species (Halictidae) and *Hylaeus (Prosopisteron) woyensis* (Colletidae). *Lasioglossum* R refers to bees with a red abdomen, *Lasioglossum* B refers to bees with brown/black abdomen.

Introduced honey bees, *Apis mellifera*, were absent from the assemblage in all years, although they were observed visiting other flowering plants in the Woodland during observations.

Significant differences in assemblage composition were detected between different time periods (ANOSIM Global $R = 0.168$, $P = 0.001$; Fig. 4-3b). Pairwise tests reveal all time periods are significantly different from each other (morning vs. midday: R = 0.123, $P = 0.013$; midday vs. afternoon: R = 0.167, $P = 0.008$; morning vs. afternoon: R = 0.226, $P = 0.005$). Ants were the most abundant visitor in the morning and at midday, contributing 66.5% and 48.3% of visits, respectively. Bee flies were the most abundant visitor in the afternoon contributing 52.3% of visits. Native bees *H. (H.) holochlorus*, *Lasioglossum* species and *H. (P.) woyensis* peaked in visitation during midday.

Floral display

There were no differences in plant density over the three years ($F_{2,27} = 0.574$, $P = 0.570$; Fig. 4-4a), although there was a trend for plant density to decrease over time since fire. The percentage of plants flowering per quadrat (Fig. 4-4b) was significantly higher in 2004 and 2005 compared to 2003 ($F_{2,25} = 11.135$, $P = 0.0003$; Tukey-Kramer post-hoc test significant at $P = 0.01$). Plant reproductive effort, in terms of the number of umbels produced per plant, was significantly greater in 2004 than in other years (data ln transformed prior to analysis, $F_{2,56} = 10.873$, $P = 0.0001$; Tukey-Kramer post-hoc test, $P = 0.05$; Fig. 4-4c). The density of flowering umbels varied significantly among years ($F_{2,27} = 5.001$, $P = 0.014$; Fig. 4-4d), with a higher density of flowering umbels during peak flowering in 2004 compared to 2003 (Tukey-Kramer post-hoc test, $P = 0.05$). Overall, floral display was lower in 2003 compared to 2004 and 2005.

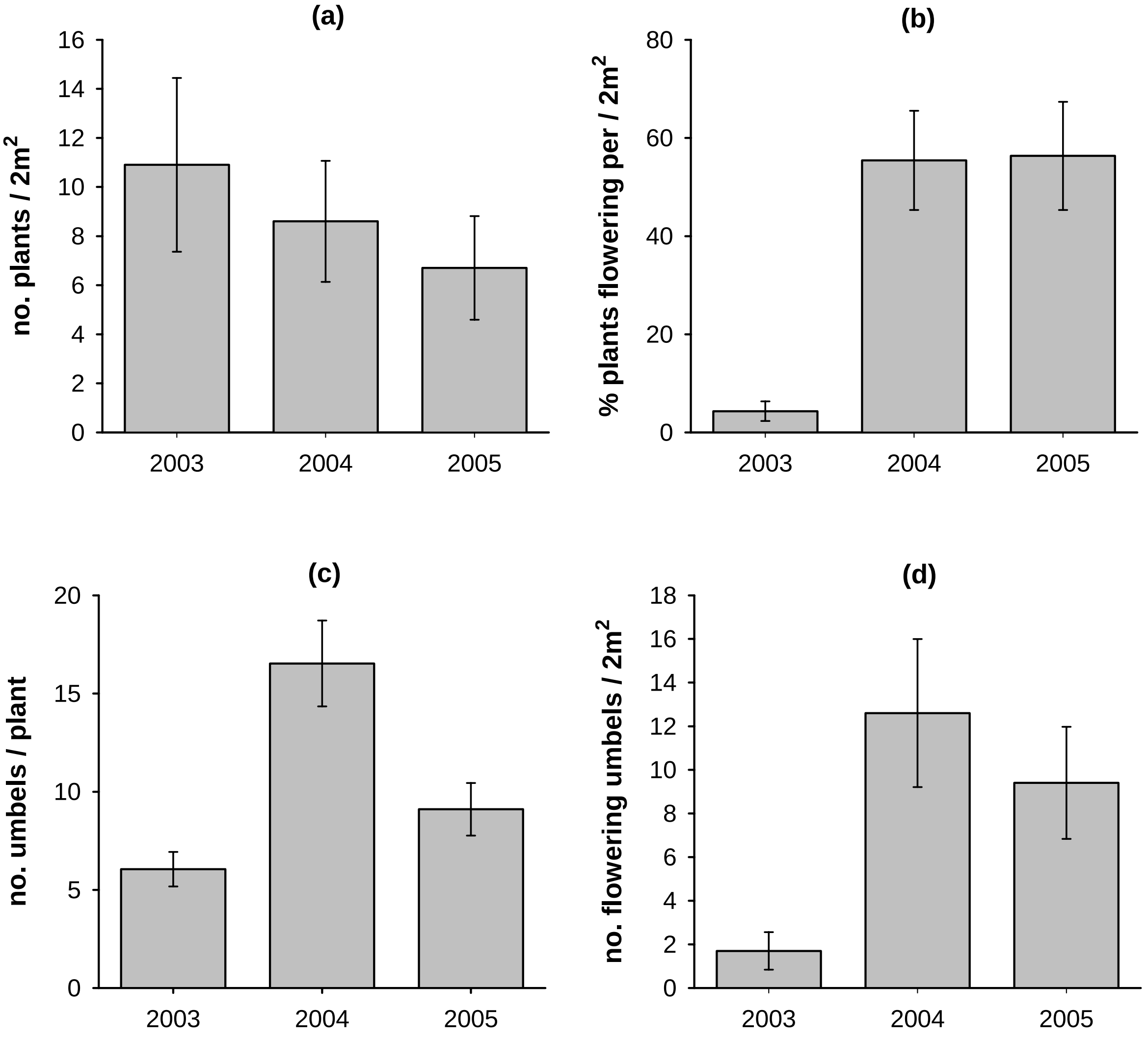

Fig. 4-4 Floral display of *T. incisa* in the Agnes Banks Woodland over three years: (**a**) plant density per 2 m^2 during peak flowering (not significant); (**b**) percentage of plants flowering per 2 m^2 during peak flowering ($F_{2,25} = 11.135$, $P = 0.0003$); (**c**) number of umbels per plant ($F_{2,56} = 10.873$, $P = 0.0001$); (**d**) flowering umbel density per 2 m^2 during peak flowering ($F_{2,27} = 5.001$, $P = 0.014$). Mean ± SE presented.

Reproductive output

Plant reproductive output, in terms of the mean number of umbels with seeds per plant, varied significantly among years ($F_{2,56} = 8.821$, $P = 0.0005$; Fig. 4-5a), with fewer umbels with seeds in 2003 compared to 2004 and 2005 (Tukey-Kramer post-hoc test, $P = 0.01$). Considering reproductive effort and output together, the percentage of umbels with seeds per plant varies significantly across years ($F_{2,56} = 7.884$, $P = 0.001$; Fig. 4-5b), with a general trend for an increase with time since fire (2003 < 2005, Tukey-Kramer post-hoc test, $P = 0.05$).

Percentage seed set per umbel (percentage of ovules developing into seeds) was consistent across years ($F_{2,42} = 0.647$, $P = 0.529$; Fig. 4-5c). There was no difference in the number of flowers per umbel across years ($F_{2,42} = 1.609$, $P = 0.212$; Fig. 4-5d) or the number of seeds per umbel ($F_{2,42} = 0.293$, $P = 0.747$).

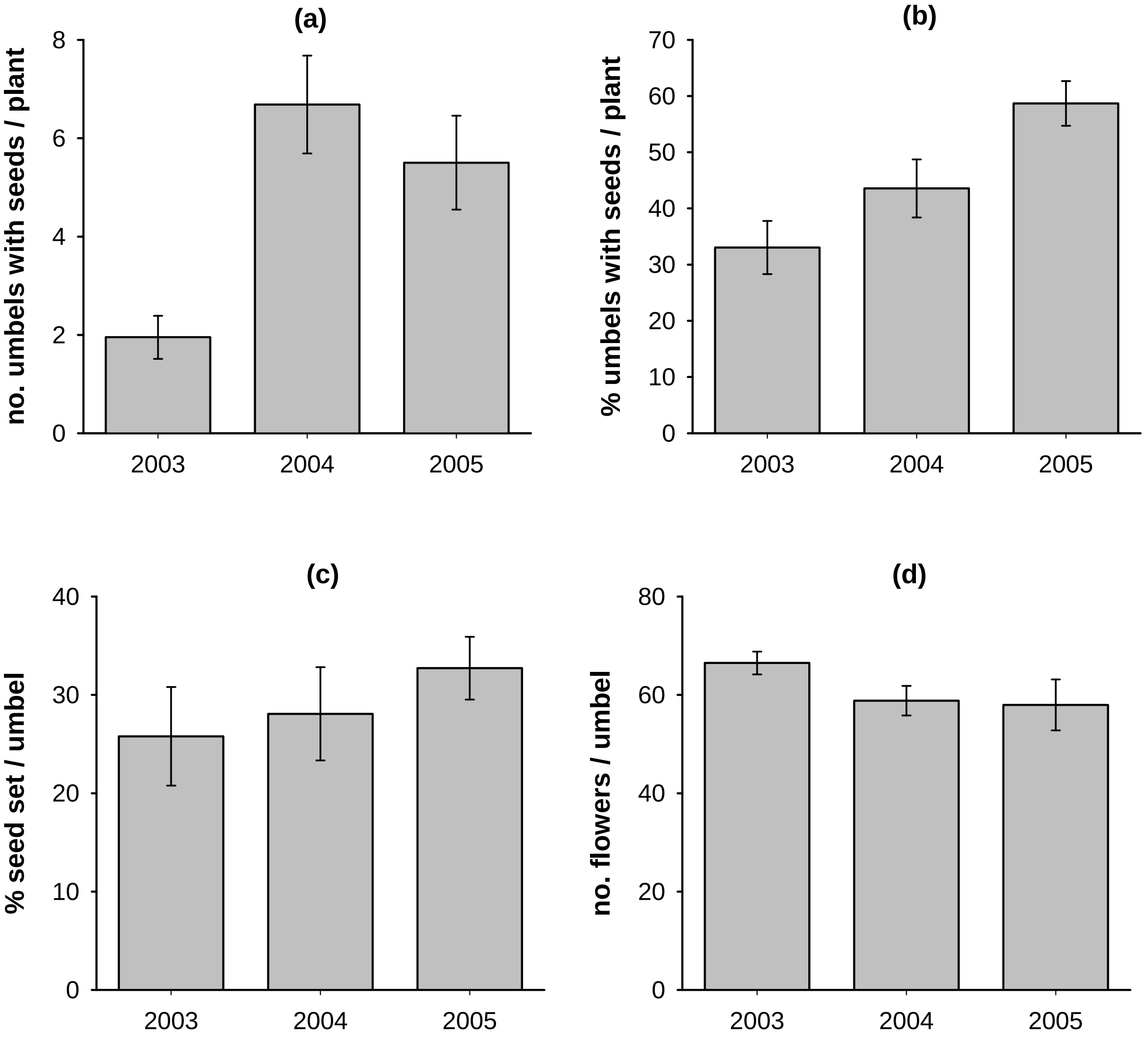

Fig. 4-5 Reproductive output of *T. incisa* in the Agnes Banks Woodland over three years: (**a**) number of umbels per plant producing seeds ($F_{2,\,56} = 8.821$, $P = 0.0005$); (**b**) percentage of umbels per plant producing seeds ($F_{2,\,56} = 7.884$, $P = 0.001$); (**c**) percentage seed set per umbel (not significant); (**d**) number of flowers per umbel (not significant). Mean ± SE presented.

DISCUSSION

Temporal variation in insect visitation and reproductive output

Despite significant differences in insect assemblage composition among all years, there were no overall differences in insect visitation rate or percentage seed set per umbel in *T. incisa* among years. This indicates that different assemblages of insects are providing pollination resulting in similar seed set per umbel within the Agnes Banks Woodland. This result is important given that *T. incisa* is not autogamous and requires pollinators for seed production (Davila and Wardle 2002). In contrast, other plant species have produced different levels of seed production resulting from visitation by assemblages composed of different insects (e.g. Herrera 2000; Ivey *et al.* 2003). Therefore, this result is unexpected given that over 94% of visits in 2003 were made by ants, which are unlikely to be pollinators because they forage at the base of flowers and do not contact the reproductive structures (Chapter 2). In 2004 and 2005, bee flies (Bombyliidae) were the most abundant morphospecies (Fig. 4-3a). Adult bee flies feed on nectar and pollen and are known to pollinate other plant species (reviewed by Larson *et al.* 2001).

Although different assemblages of insects achieve similar seed production in *T. incisa*, the mean percentage seed set per umbel was consistently less than 50%. Tests for pollen limitation were not undertaken during this study, however, Davila and Wardle (2002) found that in 2000, *T. incisa* plants in the Woodland were not pollen limited. Seed set in 2005 (32.7 ± 3.2%) was significantly less (df = 19, $t = 1.738$, $P = 0.049$) than seed set in 2000 (44.6 ± 7.5%). Similarly, populations of *T. incisa* at Myall Lakes and Tomago produced 20.4 ± 3.6% and 44.2 ± 5.3% seed set in 2003 respectively, and 27.6 ± 3.0% and 33.1 ± 4.7% in 2004 respectively (Chapter 3). Other Apiaceae are known to be resource but not pollen limited (Gaudeul and Till-Bottraud 2004). It is possible that resource limitation acts at the umbel level in *T. incisa*, such that not all flowers produce seeds and there is an upper limit to the proportion of ovules developing into seeds within an umbel.

Interestingly, the differences among years in reproductive output are due to differences in umbel production and proportion of umbels with seeds, and not seed set per umbel. Only 4.3% of plants flowered immediately after fire in 2003 (Fig. 4-4b). Therefore, most plants experienced their first flowering season after fire in 2004, which is when the number of umbels per plant (Fig. 4-4c) and the density of umbels (Fig. 4-4d) were greatest. Plants in 2005 produced significantly fewer umbels than plants in 2004 (Fig. 4-4c). This is similar to the post-fire pattern in pine forest at Mt Carmel, Israel, where widespread loss of bees and flowers immediately after fire was short-term (Potts *et al.* 2003). In the pine forest a peak in floral coverage was experienced two years after fire and steadily declined over the next 50 years. Flowering in plants often increases after fire due to increased nutrient availability, increase in sprouting of shoots and leaves, and removal of competing vegetation (Whelan 1995). Increased flowering was also observed in North-American Apiaceae exposed to fire compared to unburnt plots (Howe 1999). In addition, fire plays an important role in sustaining populations of some North-American Apiaceae by reducing competing canopy and ground litter (Menges and Kimmich 1996; Howe 1999). Despite the differences in flowering between 2004 and 2005, the actual number of seeding umbels per plant was similar (Fig. 4-5a) due to a higher proportion of umbels developing into seeds in 2005 (Fig. 4-5b).

Fire may also play a role in maintaining above-ground plant densities of *T. incisa* in the Woodland. There was a trend for plant density to decrease with time since fire (Fig. 4-4a). *Eryngium cuneifolium* (Apiaceae), a rare herb with similar habit to *T. incisa* that is restricted to open sand areas in the Florida scrub, suffers increased mortality and decreased fecundity with increased time since fire (Menges and Kimmich 1996). This is due to increased interspecific competition from dominant shrubs, which also reduces the areas of open patches available for *E. cuneifolium* to inhabit. A similar process may occur in the Woodland, where increased competition from larger shrubs results in decreased density of *T. incisa* with time since fire.

Comparison to flowering season after 1998 fire in the Agnes Banks Woodland

There have been at least six fires in the Agnes Banks Woodland between 1974 and 2006, with the last four fires occurring in November 1994, October 1998, December 2002 and January 2003 (New South Wales National Parks and Wildlife Service 1999; de Barse 2001; Y. C. Davila, personal observation). These fires vary in extent, resulting in areas of the Woodland with different burn histories. The ability to resprout and flower within a year of fire is an important strategy for *T. incisa*, given the frequency of fires in the Woodland. Due to fire in late December 2002, peak flowering was shifted to May in 2003, but returned to January in 2004. Similarly, following the fire in October 1998, peak flowering was shifted to March in 1999, and in 2000 peak flowering was in February (Davila and Wardle 2002). This indicates that changes in floral phenology appear to affect the first season after fire only, with the onset of flowering restored to summer (January-February) in following years.

The pattern of reproductive output differing among years due to umbel production also holds when including the 1999 season. Davila and Wardle (2002) found that the mean number of umbels per plant was 40.0 ± 3.8 in 1999, which is approximately three times greater than the flowering season of 2004 (Fig. 4-4c). In 1999, the percentage of umbels developing seeds was 44.9 ± 2.3% and percentage seed set was 35.8 ± 2.8%, which are both similar to the 2004 season (Figs. 4-4b and 4-4c).

In 1999 and 2000, the introduced European honey bee, *Apis mellifera*, was the most abundant floral visitor observed on *T. incisa* (Davila and Wardle 2002). However, the introduced honey bee was unexpectedly absent from the insect assemblage in all years of this study. This means the pollinator assemblage has changed from one dominated by a social super-generalist bee to an assemblage with a mix of bee flies and native bees, in less than four years. Seed set was not significantly different between 2000 and 2005 (discussed above under 'Temporal variation in insect visitation and reproductive output') and seed set in 1999 was within the range of 2005 (35.8 ± 2.8%; (Davila 1999), which indicates that these different assemblages also result in similar reproductive output at the umbel level. Differences in seed germination among years were not investigated in this study, although there is evidence for differences in seedling performance as a result of different pollinator assemblages in *T. incisa* (Chapter 2) and other species (e.g. Herrera 1988; Herrera 2000). It would be interesting to investigate the effects of variation in pollinator assemblage on demography and population persistence of *T. incisa* in the Woodland.

Role of fire in altering pollination in the Agnes Banks Woodland

Fire affected reproduction in *T. incisa* in several ways. Although plants resprouted after fire, only 4.3% of plants flowered in 2003, therefore the level of attraction for pollinators as well as the potential reproductive output was reduced. The fire also resulted in a shift in the onset of flowering to March and timing of peak flowering to May in 2003. During this time pollinators, such as native bees, were likely to be scarce due to inactivity in cooler temperatures (Dollin *et al.* 2000), fire-related mortality and a post-fire vegetation poor in nectar and/or pollen (Ne'eman *et al.* 2000). Native bees and flies were observed in low numbers in 1999 (Davila and Wardle 2002) and in 2003, which were both flowering seasons following fire in the Woodland. However, bees and flies were common visitors to

T. incisa at other populations in 2003 (Chapter 2) and returned to the assemblage in 2004. Similarly, the highest densities and diversity of bees in pine forest at Mt Carmel, Israel, were recorded soon after fire (Potts *et al.* 2003). These results indicate that pollinators can return quickly and in high densities to fire affected areas. Fire may also alter the flowering phenology other plants in the Woodland, and therefore the foraging behaviour of their pollinators.

The ability for plants and their pollinators to recover from fire is considerably variable, and dependent on many factors such as tolerance of plant and animal populations to fire, as well as the intensity and extent of the fire. *Trachymene incisa* can resprout after fire and flower within a year. Since only parts and not all of the Woodland were burnt, it is likely that insects responsible for pollination can return quickly to the burnt area from unburnt areas or adjacent woodlands. This helps explain the observed recovery of pollination of *T. incisa* within one year of fire. Fire management has been identified as an important consideration for conservation of the Woodland, particularly for the threatened and regionally rare species which are at their western range limit in the Woodland (New South Wales National Parks and Wildlife Service 1999). Both the recovery of pollinators and reproductive abilities of plants after fire must be considered as part of the fire management in the Woodland.

Due to the extent of the December 2002 and January 2003 fires in parts of the Agnes Banks Woodland located within the Agnes Banks Nature Reserve, comparisons between burnt and unburnt areas of the Woodland were unable to be made. Experimental burns to study effects of fire on interactions in the Woodland are unfeasible, given the endangered community status, restricted size, high flammability and close proximity to residential properties. Long term monitoring of interactions within the Woodland is needed, as well as continued assessment of the flora and fauna to ensure this unique community remains protected in the future (de Barse 2001). This study shows that pollination services to *T. incisa*, a characteristic species of the Agnes Banks Woodland, can be restored within one year of fire. Visitation by different assemblages of pollinators resulted in similar percentage seed set per umbel across all years and similar number of seeding umbels per plant between 2004 and 2005. This indicates that reproductive output is determined by umbel production, and less influenced by changes in insect visitor assemblage composition.

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BEE BOYS AND FLY GIRLS:
DO POLLINATORS PREFER MALE OR FEMALE UMBELS IN PROTANDROUS PARSNIP, *TRACHYMENE INCISA* (APIACEAE)?

A similar version of this chapter has been submitted to *Austral Ecology*:

Davila YC and Wardle GM (in review) Bee boys and fly girls: do pollinators prefer male or female umbels in protandrous parsnip, *Trachymene incisa* (Apiaceae)?

Photographs: left – native bee *Lasioglossum* sp. on male umbel; right – fly on female umbel

INTRODUCTION

Separate sexes and the division of reproductive activities along gender lines are prevalent in animals but less common in plants. Most flowering plants are hermaphroditic and only 6% of plant species are dioecious, exhibiting separate female plants that specialise in making seeds and male plants that provide pollen (Renner and Ricklefs 1995). An interesting alternative approach to achieving separation of reproductive functions is to have sequential gender functions within a hermaphroditic individual. Dichogamy is the temporal separation of pollen presentation (male) and stigma receptivity (female) within hermaphroditic flowers. There are two forms of dichogamy: protandry, where the male phase precedes the female phase, and the reverse, protogyny. Over 250 families of angiosperms exhibit dichogamy (Routley 2003), including both self-incompatible and self-compatible species (Bertin 1993; Routley *et al.* 2004). The advantages of dichogamy include reducing self-pollination (Lloyd and Webb 1986; Harder *et al.* 2000; Harder and Aizen 2004; Routley *et al.* 2004), reducing the interference between reproductive structures (Lloyd and Webb 1986; Routley *et al.* 2004), enhancing outcross siring success (Harder *et al.* 2000; Routley and Husband 2003), and reducing the incidence of pollen discounting (Harder *et al.* 2000).

Complete dichogamy occurs when there is no overlap in male and female function in a flower or larger unit (Lloyd and Webb 1986). Hemi-synchronous dichogamy occurs when there is synchronisation of gender among the flowers of one or more inflorescences or part-inflorescences, but not all flowers on the same individual or ramet (Lloyd and Webb 1986). A combination of complete dichogamy within a flower and hemi-synchronous dichogamy within a plant prevents within-flower and within-inflorescence self-pollination, but still allows self-pollination among flowers on different inflorescences or ramets (geitonogamy).

An immediate consequence of complete and hemi-synchronous dichogamy is the presentation of flowers with different floral rewards, since pollen is only available in male phase flowers. A visit from a pollinator is no guarantee of pollination, given that pollinators must move pollen from a male functioning flower to a female functioning flower. In addition, nectar volume, nectar production rate and/or nectar sugar content can vary between the male and female phases of dichogamous plants (Bell *et al.* 1984; Klinkhamer and de Jong 1990; Snow and Grove 1995; Aizen and Basilio 1998; Langenberger and Davis 2002). Pollinating insects have been shown to prefer flowers of one gender over the other when foraging on dichogamous species (Bell *et al.* 1984; Klinkhamer and de Jong 1990; Cresswell and Robertson 1994; McKone *et al.* 1995; Stone *et al.* 1999). In monoecious *Begonia tonduzii*, pollinators visited male flowers over 15 times more often than female flowers, and this resulted in reduced seed set due to pollinator limitation (Le Corff *et al.* 1998). Similarly, pollinator preferences in dichogamous plants could result in reduced transfer of pollen between male and female phase flowers, and the depletion of resources for more effective pollinators. Gender preferences may be increased as pollinators forage for the floral resource matching their specific needs at that time (Galen and Plowright 1985). For example, honey bees on nectar-collecting trips preferred and spent longer foraging on inflorescences with more female flowers of protandrous *Lavandula stoechas*, which contain two times more nectar than male flowers (Gonzalez *et al.* 1995). By contrast, honey bees on pollen-collecting trips preferred inflorescences with male and female flowers, and foraging time increased with a higher ratio of male flowers per inflorescence (Gonzalez *et al.* 1995). Pollinator preferences may also cause selection pressure on plants, because insects that are predominantly pollen or nectar foragers are expected to have different abilities to transfer pollen (Gonzalez *et al.* 1995).

Here I investigate whether floral insect visitors show preferences for male or female inflorescences of a generalist-pollinated plant exhibiting complete, hemi-synchronous dichogamy (i.e. synchronous dichogamy at the inflorescence level). *Trachymene incisa* subsp. *incisa* Rudge (Apiaceae, *T. incisa* hereafter) has completely protandrous flowers arranged in umbels. Although each umbel functions sequentially as a male and female, the extended longevity of the male phase (16 days compared to 4 days as female) results in a male-biased sex ratio across the population (Davila and Wardle 2002). Consequently, direct observations in the field on the frequency of visits by pollinators to each gender phase may be biased because more male umbels than female umbels are available. Therefore, in addition to direct observations I used experimental arrays consisting of equal numbers of male and female umbels to assess preferences. *Trachymene incisa* exhibits a generalist pollination system and requires insect pollinators for seed production (Davila and Wardle 2002). Therefore, pollinator behaviour and preferences can greatly influence pollination rates and seed set in *T. incisa*.

Due to the sequential opening of flowers within an umbel, only ~18 flowers offer pollen and nectar at any one time during the male phase (Y. C. Davila, personal observation). By contrast, during the female phase all flowers (~ 72 per umbel, Davila and Wardle 2002) are functional and offer only nectar. Given these differences in floral resources, I hypothesise that insect visitors will show a preference for male or female umbels in terms of (1) number of visits overall, (2) number of visits during a foraging trip and (3) mean foraging time per umbel visit. The first hypothesis will be tested using observations from natural patches and an experimental array. The second and third hypotheses will be tested using an experimental array only. For example, I expect native bees that collect pollen and nectar to visit more male umbels and forage for longer on male umbels because female umbels do not present pollen. By contrast, I expect butterflies that forage only for nectar to prefer female umbels because there are more nectar-presenting flowers open at any one time in female umbels compared to male umbels. Insects may prefer one gender during a trip whilst foraging for a specific resource, which directly affects pollen transfer capacity, whereas overall (over several foraging trips) the insects may visit both umbel phases to meet their dietary needs. Therefore, I made the distinction between the number of overall visits and the number of visits during a foraging trip to each umbel phase.

METHODS

Study species

Trachymene incisa subsp. *incisa* Rudge (Apiaceae) is a common perennial herb endemic to eastern Australia, with a large latitudinal range from Fraser Island, Queensland, south to Ulladulla and inland to the Nandewar Range near Narrabri, NSW (Powell 1992; Hart and Henwood 2006). It grows on infertile sandy soils in dry eucalypt woodland or scrub and in crevices of granite outcrops (Benson & McDougall 1993).

There are approximately 72 flowers per inflorescence, arranged in simple umbels (Davila 1999; Davila and Wardle 2002). Each flower has five white petals, five stamens and two equally sized carpels (bilocular) each of which are connected to an elongated style at female anthesis (Hart and Henwood 2006). The flowers open centripetally within the umbel (Davila and Wardle 2002). Within the completely protandrous flowers, the anthers dehisce sequentially while the styles remain curled and non-receptive, and after the anthers are spent the flower enters a quiescent phase. The female phase occurs when the styles have elongated and stigmas are receptive and begins after all flowers within the umbel have entered the quiescent phase (Davila & Wardle 2002). Nectar is present in open flowers that are functioning as either male or female, but not during the quiescent phase (Davila & Wardle 2002). Therefore, whole umbels function as either male (pollen presenting, ~16 days) or female (pollen receptive, ~4 days), which prevents self-pollination within the umbel (Davila 1999; Davila & Wardle 2002). Umbels develop sequentially on a floral stalk with the secondary umbels branching off the floral stalk of the terminal primary umbel, and the tertiary umbels branching off the floral stalk of the secondary umbels and so forth. Therefore, male and female phase umbels are usually present on the same plant at the same time (hemi-synchronous dichogamy, Lloyd & Webb 1986). Because gender is not synchronised across umbel orders, geitonogamous pollination is possible. *Trachymene incisa* is self compatible, although seed set resulting from self-pollination is less than seed set resulting from cross-pollination (Davila and Wardle 2002).

There are very few morphological changes in the umbel with the shift from male to female phase. Umbel height (mean = 5.1 mm) and diameter (mean = 19.7 mm) do not change significantly from the middle of the male phase (approximately 8 days after the first flowers open) through to the end of the female phase (Davila 1999). There is approximately 5% UV reflectance from petals during both male and female phases (Appendix 6). The height of dehiscing anthers and receptive stigmas from the base of flowers is similar (Davila 1999). Therefore, any differences in visitation rate by insects are more likely to be due to differences in floral rewards available, rather than minute morphological differences.

The umbels attract a large suite of insect visitors, including native bees, wasps, flies, butterflies and the introduced European honey bee *Apis mellifera* (Davila 1999;

Davila and Wardle 2002). The flowering period usually begins in late spring and continues through to late autumn, with peak flowering during summer (Powell 1992; Davila and Wardle 2002).

Study populations

Insect visitation was recorded at three populations of *T. incisa* occurring in New South Wales, Australia: Myall Lakes National Park, the Tomago Sandbeds and Agnes Banks Woodland. At Myall Lakes National Park (32°30′S, 152°21′E), 200 km north of Sydney, *T. incisa* grows in dry heath and dry heath forests on freely drained sands (Myerscough and Carolin 1986). The Tomago Sandbeds (32°47′S, 151°51′E) are located on the central coast of New South Wales, approximately 70 km south of Myall Lakes National Park. *Trachymene incisa* occurs within the dry open-forest, low woodland and in adjacent fire breaks of the sandbeds. Myall Lakes National Park and the Tomago Sandbeds share similar climate, geomorphology, soils and vegetation (Fox *et al.* 1996; Ross *et al.* 2002). The Agnes Banks Woodland (33°39′S, 150°41′E) is a unique low woodland community occurring on wind blown sand dunes, 55 km west of Sydney (Benson 1992). The Woodland is listed as an Endangered Ecological Community with *T. incisa* as an identifying species (New South Wales Scientific Committee 2000). Benson (1981) noted that 47% of the Agnes Banks flora is also found at Myall Lakes. Therefore, all three sites are floristically similar but geographically separated.

Insect visitation to natural patches of male and female phase umbels

Six observation patches consisting of 3-6 plants in an area of approximately 2 m^2 were located and tagged at all populations during the peak flowering seasons of 2003 and 2004. I labelled each flowering umbel in a patch with a sticker and unique number on its inflorescence stalk. Insects did not approach the labels and foraging did not appear to be affected by the labels (Y. C. Davila, personal observation). Flying insect visitors to male and female umbels were observed at each patch for 10 min, at three time periods per day in the morning (0930-1100 h), midday (1230-1400 h) and afternoon (1530-1700 h). Observations have been made at sunrise (0700-0800 h), sunset (1830-1930 h) and at night (2100-2200 h), however, insects were either absent or in very low numbers, indicating that pollination does not occur or is very limited during those times (Chapter 2; Appendix 4). Observations were made over three consecutive days during peak flowering for each population. During the observation periods, the morphospecies of flying insects (as a surrogate for species because identification was difficult in the field) and all umbels they visited were recorded.

Insect visitation to experimental arrays of male and female phase umbels

During the 2004 flowering season, experimental arrays of freshly cut umbels, consisting of five male phase and five female phase umbels, were placed in moist florist foam. The umbels were arranged in two rows of five umbels, in random order and spaced 4 cm apart. The density of umbels in the array was high but was not unlike high density patches, which are common at all populations (Y. C. Davila, personal observation). The array was placed approximately 60 cm above ground level, which is the average height of umbels above ground (Wardle 2003). The array was also located in close proximity to other flowering umbels to maximise visitation. One array was set up during peak flowering at each of the three populations during peak flowering in 2004.

Insect visitation to the array was recorded with a video-camera mounted on a tripod 1 m from the array. Visitation was recorded for one 90 min session during the middle of the day when insect visitation to umbels is greatest (Chapters 2 and 3). The advantages of using a video-camera to record visitation include: no insect observations are missed during the observation periods; the foraging time can be determined accurately; there was is need for marking the umbels with labels to identify gender phases; and the insects are not disturbed by the presence of observers.

The number of visits to each umbel and the foraging time per visit were recorded for each insect visitor. Foraging time was determined to the nearest 0.04 s (25 frames per second). Insect visitors were grouped into four categories that could be easily distinguished from video footage: native bee, fly, butterfly and wasp.

Data analyses

To compare the differences in number of visits to each umbel phase, in both the natural and experimental patches, data were analysed using goodness-of-fit tests for each insect type and population combination ($\alpha = 0.05$). For the natural patches, data were pooled across patches, time periods and days at each population to increase sample size (number of visits). The expected frequencies are based on the proportion of umbels that were male and female across all patches observed within each population in each year. The total number of umbels observed (pooled across six patches, three time periods and three days) at Myall Lakes, Tomago and Agnes Banks were 483, 696 and 459 in 2003, and 675, 719 and 669 in 2004 respectively. Analyses for native bees and flies are presented when the number of visits was large enough to show the contribution of each morphospecies to visitation. For the experimental arrays, the expected frequency of visitation is 50% to each gender.

I investigated whether insects visited each gender phase equally during a foraging trip by analysing visits by insects foraging on two or more umbels, using paired *t*-tests ($\alpha = 0.05$). Chi-squared analyses were used to investigate whether an umbel was chosen independently of the previous umbel phase in the foraging sequence ($\alpha = 0.05$). The null hypothesis is that an insect leaving an umbel had umbels of both genders to choose from and the next umbel in the foraging trip would be chosen independently of the previous umbel.

To compare foraging time per visit between male and female umbels, data were analysed using a three factor analysis of variance ($\alpha = 0.05$) (SuperANOVA v1.1, Abacus Concepts 1989-1990). The three populations, Myall Lakes, Tomago Sandbeds and Agnes Banks, were chosen because they vary minimally in latitude, occur in floristically similar habitats, are large enough to adequately record plant-pollinator interactions, and have been compared in previous studies (e.g. Benson 1981; Fox *et al.* 1996; Ross *et al.* 2002). Since these populations were chosen specifically for this study and comparisons are made between only these three populations, the population term was treated as a fixed factor (Bennington and Thayne 1994). Insect type and umbel phase were also treated as fixed factors. The unit of replication is an insect visit onto an umbel. Data were $\log_{10}$ transformed prior to analysis to improve normality and homogeneity of variances. Means ± standard errors (SE) are reported.

RESULTS

Insect visitation to natural patches of male and female phase umbels

Insect visitation did not deviate from expected frequencies at Tomago in 2003 and 2004, and at Agnes Banks in 2003 (Table 5-1). By contrast, insects visited significantly more male umbels than expected at Myall Lakes in both years and at Agnes Banks in 2004 (Table 5-1). At these populations, native bees (Halictidae and Colletidae) and flies (including Syrphidae and Bombyliidae) made the majority of visits. Native bees constituted 82.5%, 60.3% and 49.3% of visits at Myall Lakes in 2003 and 2004, and Agnes Banks in 2004 respectively, and visited male umbels significantly more than expected (Table 5-1). Similarly, visitation to male and female umbels by flies at Myall Lakes and Agnes Banks in 2004 deviated significantly from expected frequencies. Flies made 26.0% and 47.3% of the insect visits to Myall Lakes and Agnes Banks in 2004 respectively.

Table 5-1 Comparison of number of flying insect visits to male and female phase umbels in natural patches at three populations of *Trachymene incisa* over two years. Expected frequencies of visits are in parentheses and based on the proportion of umbels of each gender at each population at the time of observations.

Year	Population	Insect type	Male	Female	χ^2 (df = 1)	*P*
2003	Myall Lakes	all	60 (45)	3 (18)	18.199	0.001
		native bee	50 (37)	2 (15)	16.169	0.001
	Tomago	all	152 (146)	35 (41)	1.175	0.172
		native bee	124 (116)	25 (33)	2.367	0.124
		fly	23 (25)	9 (7)	0.700	0.403
	Agnes Banks	all	6 (7)	2 (1)	0.627	0.278
2004	Myall Lakes	all	343 (291)	57 (109)	34.323	0.001
		native bee	213 (175)	28 (66)	29.861	0.001
		fly	87 (76)	17 (28)	6.287	0.012
	Tomago	all	31 (30)	15 (16)	0.073	0.787
		native bee	12 (9)	2 (5)	2.532	0.112
		fly	18 (20)	13 (11)	0.758	0.384
	Agnes Banks	all	169 (145)	36 (60)	13.230	0.001
		native bee	84 (72)	17 (29)	7.368	0.007
		fly	81 (69)	16 (28)	7.470	0.006

Insect visitation to experimental arrays of male and female umbels

Native bees and flies were observed visiting the experimental arrays of *T. incisa* at all three populations. Several morphospecies of bees (families Halictidae and Colletidae) and flies (families Bombyliidae, Syrphidae, Muscidae and others) are known to visit *T. incisa* (Chapters 2 and 3), however, the video camera resolution was not sufficiently high enough to confidently identify insects beyond these broad categories. The butterfly *Zizina labradus labradus* and one unidentified wasp species were only observed at Myall Lakes.

Insect visitation and the abundance of insect types varied across the three populations (Table 5-2). There were no significant differences between male and female umbels in overall insect visitation for all insect types at all populations (Table 5-2). Insects that visited two or more umbels during a foraging trip also visited male and female umbels equally during the trip (Table 5-3). Bees visited 8.6 ± 1.9 (mean ± SE) umbels and flies visited 3.8 ± 0.3 umbels during a single foraging trip through the array, estimated from data pooled across all populations. Butterflies visited 3.9 ± 1.3 umbels and wasps visited 4.0 ± 0.6 umbels during a foraging trip through the array at Myall Lakes.

The gender of an umbel in a native bee foraging sequence was not independent of the gender of the previous umbel ($\chi^2 = 10.11$, $P = 0.002$). Bees leaving a male umbel were more likely to move to a female umbel than a male umbel (40 male to female transitions compared to 19 male to male transitions). Similarly, bees leaving a female umbel were more likely to move to a male umbel than a female umbel (40 female to male transitions compared to 24 female to female transitions). Therefore, native bees alternated umbel genders during a foraging trip. There was no significant association between successive umbels visited by flies ($\chi^2 = 2.11$, $P = 0.146$), butterflies ($\chi^2 = 0.02$, $P = 0.901$), and wasps ($\chi^2 = 1.18$, $P = 0.279$).

Table 5-2 Comparison of number of visits to male and female phase umbels in experimental arrays by different insect types at three populations of *Trachymene incisa*.

Population	Insect type	Individuals	Male	Female	χ^2 (df = 1)	*P*
Myall Lakes	Native bee	21	61	67	0.281	0.560
	Fly	10	17	15	0.125	0.724
	Butterfly	12	15	16	0.032	0.857
	Wasp	12	15	8	2.130	0.144
Tomago	Native bee	3	6	7	0.077	0.782
	Fly	27	30	27	0.158	0.691
Agnes Banks	Native bee	6	5	7	0.333	0.564
	Fly	10	12	12	0.000	1.000

Table 5-3 Comparison of the mean ± SE number of visits to male and female phase umbels during a foraging trip, by insects that visited two or more umbels whilst foraging in experimental arrays of *Trachymene incisa* (pooled across all populations).

Insect	Male	Female	*t*	df	*P*
Native bee	3.8 ± 0.8	4.1 ± 0.8	0.814	17	0.427
Fly	1.9 ± 0.3	1.9 ± 0.2	0.000	23	1.000
Butterfly	1.4 ± 0.2	2.0 ± 0.7	0.957	7	0.370
Wasp	2.8 ± 0.3	1.3 ± 0.5	3.000	3	0.057

The analysis of variance examining differences in foraging time per umbel visit revealed a significant interaction between population and insect type (Table 5-4). There was no significant difference in mean insect foraging time between male and female phase umbels and all interactions with umbel phase were not significant (Table 5-4). However, bees tended to forage for longer on male umbels than female umbels (Fig. 5-1). A separate analysis of these bee foraging time data for each population reveals that bees at Myall Lakes forage for significantly longer on male umbels (19.6 ± 2.8 s) than on female umbels (10.3 ± 2.3 s) ($t = 2.620$, df = 126, $P = 0.01$). No differences in bee foraging time between umbel phases were detected at Tomago ($t = 0.796$, df = 7, $P = 0.452$) or Agnes Banks ($t = 1.566$, df = 5, $P = 0.178$), however, this may be due to the low number of bee visits and hence the small sample size.

Table 5-4 Results of a three way analysis of variance examining differences in foraging time among populations, insects, umbel phases and all interactions. Data were $\log_{10}$ transformed prior to analysis.

Source	df	MS	*F*	*P*
Population	2	2.380	4.369	0.014
Insect type	3	3.701	6.793	0.001
Umbel phase	1	0.058	0.107	0.744
Population × Insect type	2	3.722	6.832	0.001
Population × Umbel phase	2	0.395	0.724	0.486
Insect type × Umbel phase	3	0.630	1.156	0.327
Population × Insect type × Umbel phase	2	0.489	0.897	0.409
Residual	304	0.545		

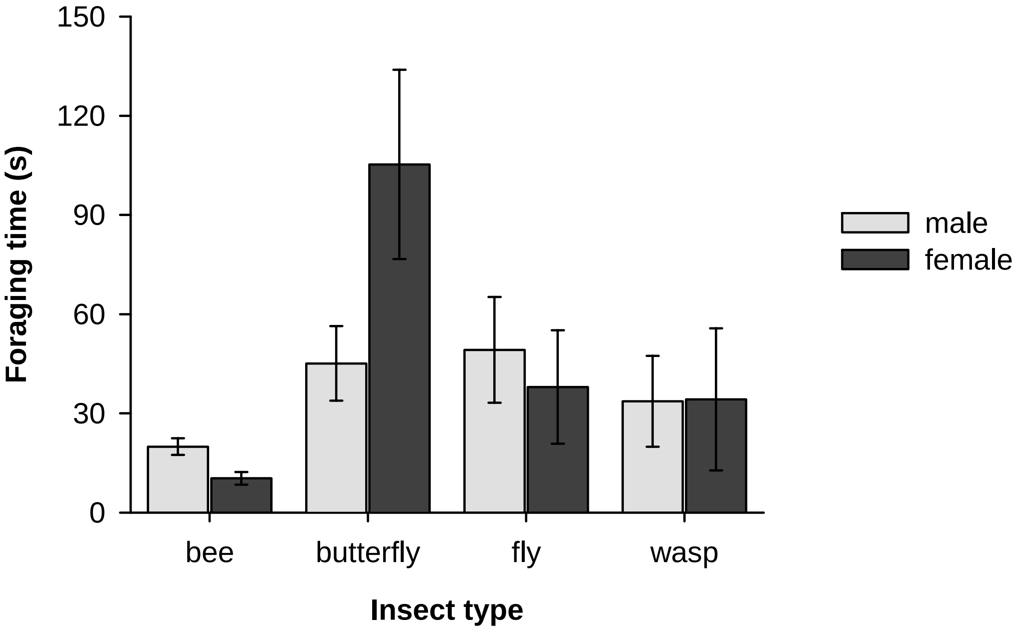

Fig. 5-1 Mean ± SE insect foraging time (s) per visit to male and female phase umbels of *T. incisa* in experimental arrays, pooled across populations. Sample sizes are (male, female): bee = 72, 81; butterfly = 15, 16; fly = 59, 54; wasp = 15, 8.

Foraging time per visit ranged between 0.1 and 114.4 s for bees (mean = 14.9 ± 1.6 s), and 0.1 and 905.2 s for flies (mean = 43.9 ± 11.7 s), pooled across genders and all populations. Butterfly foraging time ranged between 0.4 and 384.2 s per visit (mean = 76.2 ± 16.4), and wasp foraging ranged between 0.9 and 216.3 s per visit (mean = 33.9 ± 11.4) at Myall Lakes. During this time, insects were observed probing flowers for nectar and/or collecting pollen. The body of most insects also made contact with the reproductive structures of several flowers whilst foraging for pollen and/or nectar and walking around the umbel.

DISCUSSION

These results show an interesting combination of insect preferences and non-preferences for umbel phases in *T. incisa*, both supporting and opposing my hypothesis that insects will show a preference for male or female phase umbels. This variation in preferences by insects may be related to the local umbel gender ratio, where preferences occur when there is a gender bias but are not pronounced when the gender ratio is equal. Despite insects showing preferences for male phase umbels at some populations, 16.3% of all visits (pooled across populations and years) were to female umbels. Most insects visited both male and female phase umbels during a foraging trip, which is needed for successful pollination in completely protandrous *T. incisa*.

Insect visitors preferred male over female phase umbels at Myall Lakes and at Agnes Banks in 2004 (Table 5-1). At these populations native bees and flies made the majority of visits. Therefore, these results support my hypothesis that pollen-collecting insects would show a preference for male phase umbels. However, insects did not visit male phase umbels more often than expected at Tomago and at Agnes Banks in 2003. Native bees and flies showed no preference for male over female phase umbels at these populations. This variation in preferences in natural patches supports the need for the experimental test in which I manipulated umbel gender ratios.

Contrary to my expectation male and female phase umbels in experimental arrays were equally attractive to insect visitors. There was no difference between male and female umbels in terms of the number of visits received by each insect type at each population (Table 5-2). In addition, insects visited male and female umbels equally during a foraging trip (Table 5-3) and native bees alternated between male and female phase umbels while foraging through the experimental array. Overall insects spent similar amounts of time foraging on male and female phase umbels at each population (Table 5-4). Only native bees at Myall Lakes (when considered separately) showed a preference by foraging for significantly longer on male umbels compared to female umbels. However in accordance with my hypotheses, there was a non-significant trend for butterflies to spend more time on female phase umbels (Fig. 5-1).

When insects exhibited a preference in natural patches, the pattern was always that male phase umbels were visited more often than female phase umbels. Since male phase umbels offer both pollen and nectar, high visitation to male phase umbels by pollen-collecting and pollen-eating insects was expected. The majority of insect visitors were pollen-collecting native bees (Halictidae) and flies, which include pollen-eating hover flies (Syrphidae) and bee flies (Bombyliidae). Adult hover flies are known to exploit pollen and nectar from plants with large inflorescences and flat corollae such as the Apiaceae (Branquart and Hemptinne 2000). Adult bee flies are often highly specialised flower-feeders, and include both pollen and nectar in their diet (Proctor *et al.* 1996). In addition to visiting more male phase umbels in some natural patches, native bees had a tendency to forage for longer on male phase umbels compared to female phase umbels in the experimental array. These preferences for one gender can restrict pollen transfer between male and female inflorescences (e.g. Le Corff *et al.* 1998). This highlights the conflict between the advantages of complete dichogamy (increased outcrossing, decreased self-pollination, avoiding interference) and the need for insects to visit both umbel phases for successful pollination.

Pollinators have been shown to prefer either male or female flowers in dioecious (Kay 1982; Ashman 2000), monoecious (Le Corff *et al.* 1998) and dichogamous (Bell *et al.* 1984; Klinkhamer and de Jong 1990; Cresswell and Robertson 1994; McKone *et al.* 1995; Stone *et al.* 1999) species. However, there are plants in which pollinators show no preferences for male or female flowers or inflorescences (Aizen 2001; Bhardwaj and Eckert 2001; Galloway *et al.* 2002). When there are no preferences for umbel phases in *T. incisa*, native bees and flies are likely to be effective pollinators given their relatively high abundance (Chapters 2 and 3), this non-discriminatory foraging behaviour, that they contact floral reproductive structures and the ability to carry pollen on their body (Y. C. Davila, unpublished data). This behaviour is important because of the complete separation of male and female function within flowers and umbels, preventing autogamous pollination, and that *T. incisa* requires insect pollinators for seed production (Davila 1999; Davila and Wardle 2002).

The occurrence of preferences and non-preferences during insect foraging on *T. incisa* may be due to a differential response by insects when umbel gender ratios vary. Thomson *et al.* (1982) showed that bumblebees that previously foraged on male phase umbels preferred male phase umbels of *Aralia hispida* in experimental bouquets in 1979 but not in 1980. The authors suggested that the difference may be due to different population sex ratios, where in 1979 there was a male bias and in 1980 the genders were more equally represented. A similar pattern may occur with *T. incisa* where a large male bias in the umbel floral display results in insects visiting male phase umbels more often than by chance. By contrast, when the local umbel phase ratio is close to equal, insects do not show a preference, which was seen in the experimental arrays. Floral sex ratios can vary throughout the flowering season of dichogamous plants (Sargent and Roitberg 2000; Aizen 2001). A shift from a predominantly male to predominantly female floral display in protandrous species may not result in overall preferences, although it can increase the number of uninterrupted visits to female phase flowers causing a decrease in pollen deposition (Aizen 2001). Seasonal changes in umbel gender ratios have not been documented for *T. incisa*. However, as a protandrous species, it is predicted that the gender ratio will be highly male biased at the beginning of the flowering season and become less male biased throughout the flowering season. This study provided a snap-shot of preferences occurring during peak flowering at all populations in both years, where the floral display consisted of 65.5-85% male umbels. Further investigation into potential preferences throughout the flowering season is needed to determine the consistency of the preferences observed at Myall Lakes and Agnes Banks. In addition, manipulating the gender ratio in experimental arrays could be included to simultaneously investigate preferences when the local gender ratio varies.

Many plants incorporate protandry with vertical structure (Corbet *et al.* 1981; Bertin and Newman 1993; Harder *et al.* 2004). As pollinators forage upward on these inflorescences, they visit female flowers at the base and male flowers towards the top of each inflorescence. Therefore, the vertical floral architecture promotes pollinator visits to both male and female flowers. Interestingly, in this study and previous studies showing no gender preferences by pollinators (Aizen 2001; Bhardwaj and Eckert 2001), the male and female flowers or inflorescences were arranged randomly in three-dimensional space. In these plants, floral architecture has little influence on pollinator movements. Therefore, in plants where gender is not incorporated into architecture we may expect discrimination between male and female flowers to be more evident, because pollinators are not being guided to visit both genders. The absence of preferences in some dichogamous species indicates some form of cryptic specialisation, where the insects are specialised to visit both male and female flowers equally, showing floral constancy despite differences in floral rewards. Dichogamous species also need pollinators that will forage during both the male and female phases of flowers and inflorescences, a transition which may take days (e.g. Devlin and Stephenson 1985; Bhardwaj and Eckert 2001) or weeks (e.g. Snow and Grove 1995; Davila and Wardle 2002). This floral constancy should not be mistaken for pollination by deceit, which can occur in flowers where the male and female flowers are morphologically similar but the female flowers do not offer reward (e.g. Le Corff *et al.* 1998), because the female phase flowers of *T. incisa* produce nectar. Although nectar volume and concentration have not been quantified in *T. incisa* (the small flower size has prevented collection of nectar by conventional methods), other Apiaceae have been shown to produce more nectar in the female phase compared with the male phase (e.g. Koul *et al.* 1993; Langenberger and Davis 2002), and this has been interpreted as a way to compensate pollinators for the non-availability of pollen during the female phase (Koul *et al.* 1993). It would be interesting to determine whether there are any differences in nectar quantity and quality between male and female phase flowers of *T. incisa*.

In addition to the differing patterns of preference, the sample size (number of insects observed) for the experimental array was relatively low for some insects and populations. Therefore these results are not conclusive and increased sampling would be needed to investigate these trends further. The foraging time per visit was highly variable among individuals from each insect type, with the mean ranging between 14.9 s for bees and 76.2 s for butterflies. However, these data indicate that insects forage long enough to contact the reproductive structures of several flowers while they probe flowers and walk around the umbel.

The number of visits among populations and insect types was variable, and naturally low in some instances. While some visits may represent infrequent events for *T. incisa*, for example visits by butterflies and wasps which occurred only at Myall Lakes in 2004, they are still important in terms of removal of floral rewards and potential for pollen movement. Insect visitors to *T. incisa* differ significantly throughout the day, among populations and among years in terms of visitation rates and assemblage composition (Chapters 2 and 3). This study further illustrates the variability in pollinators of *T. incisa* in terms of visitation rates and foraging times, indicated by the significant population by insect type interaction (Table 5-4). Determining the effectiveness and importance of floral visitors in terms of pollination service involves many lines of evidence and is less understood for plants with diverse assemblages of pollinators. Completely dichogamous plants present an interesting case of conflict between the advantages of separating the genders in time and the need for pollinators to visit both genders for successful pollination. Here I have shown through observations and experimental arrays that there is spatial and temporal variation in preferences made by insect visitors to a protandrous generalist pollinated plant.

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CHAPTER 6

SYNCHRONOUS PROTANDRY IS EFFECTIVE AT AVOIDING SELF-POLLINATION AND INBREEDING DEPRESSION IN *TRACHYMENE INCISA* (APIACEAE)

Photograph: one month old *Trachymene incisa* seedling

INTRODUCTION

Most flowering plants species are hermaphroditic, with only 6% exhibiting separate male and female individuals (dioecy) (Renner and Ricklefs 1995). The expression of both genders within an individual presents the opportunity for self-fertilisation and inbreeding. Inbreeding depression is the reduction in fitness of offspring resulting from inbreeding relative to those resulting from outcrossing (Husband and Schemske 1996). Predominantly self-fertilising species exhibit significantly lower inbreeding depression than outcrossing species (Husband and Schemske 1996), due to excessive selfing purging populations of deleterious genotypes as they become exposed to selection (Charlesworth and Charlesworth 1987). The timing and magnitude of inbreeding depression is also variable, with most self-fertilising species expressing inbreeding depression late in the life cycle (growth or reproduction) and outcrossing species expressing inbreeding depression at either early (seed production) or late stages of the life cycle (Husband and Schemske 1996). Inbreeding depression is one of the major selective forces involved in the evolution and maintenance of plant mating systems (Charlesworth and Charlesworth 1987; Husband and Schemske 1996).

Many plants have evolved strategies that minimise self-fertilisation, such as self-incompatibility, herkogamy (spatial separation of anthers and stigmas) and heterostyly (flowers with more than one length of styles and anthers) (Charlesworth and Charlesworth 1987). Dichogamy, the temporal separation of pollen presentation (male) and stigma receptivity (female) within hermaphroditic flowers, is thought to have evolved to reduce interference between reproductive structures (Routley *et al.* 2004) as well as an anti-selfing mechanism (Harder *et al.* 2000). Dichogamy is effective at reducing self-pollination and inbreeding, including self-pollination between different flowers on the same plant (geitonogamy) in some species (Harder *et al.* 2000; Harder and Aizen 2004; Routley *et al.* 2004). However, geitonogamy remains an important source of self-pollination, particularly in species with large floral displays (de Jong *et al.* 1993).

Despite the many strategies to avoid self-pollination and uniparental inbreeding, biparental inbreeding (mating between relatives) can still lead to inbreeding depression. Biparental inbreeding is likely to occur in flowering plants with limited seed dispersal that are animal pollinated, particularly when animals restrict flight distances between plants (Delph 2004). Several studies have shown that the identity and relatedness of a plant's mates can affect offspring fitness (e.g. Waser and Price 1983; Dudash 1990; Byers 1998). It is important to document both the causes and consequences of selfing relative to outcrossing, in order to examine the relationship between inbreeding depression and mating systems (Dudash and Fenster 2001).

Trachymene incisa subsp. *incisa* (Apiaceae) is an interesting species in which to investigate the potential for and effects of uniparental and biparental inbreeding. *Trachymene incisa* is a generalist-pollinated herb (Chapter 2) and identifying species of three endangered ecological communities (New South Wales Scientific Committee 2000a; 2000b; 2000c). *Trachymene incisa* is a self-compatible perennial herb that experiences a reduction, although not statistically significant, in seed set, seed mass and germination as a result of self-pollination relative to cross-pollination (Davila and Wardle 2002), which indicates weak inbreeding depression. Flowers and umbels are protandrous (a form of dichogamy where the male function is followed by the female function), with the genders completely separated by a quiescent phase (Davila and Wardle 2002; Appendix 7). This synchronous protandry is effective at preventing self-pollination within a flower and umbel (Davila 1999). However, male and female phase umbels may be present on a plant at the same time, which provides the opportunity for self-pollination between flowers on different umbels (Davila and Wardle 2002). Due to the separation of genders at the umbel level, *T. incisa* requires insect pollinators to visit both male and female umbels for seed production (Davila and Wardle 2002). Primary seed dispersal occurs by gravity with most seed deposited on average 60 cm from the base of the maternal plant (Gill 2000). This indicates that nearest neighbours are likely to be closely related. Therefore, both uniparental and biparental inbreeding can occur in *T. incisa*, with the potential for inbreeding depression, despite synchronous protandry. *Trachymene incisa* is a model species for investigating the interaction between floral phenology, breeding system and inbreeding depression.

Here I used experimental analysis of inbreeding depression on seedling performance and observations of insect foraging within and between plants of *T. incisa* to investigate the following questions:

1. Does geitonogamous self-pollination and biparental inbreeding (mating between relatives) result in negative effects in terms of seedling emergence from soil, short-term survival and growth?

Self-pollination results in fewer and smaller seeds with lower germinability, compared to cross-pollination (Davila and Wardle 2002). I hypothesise that self-pollinated seeds will not perform as well as cross-pollinated seeds, in terms of seedling emergence, survival and growth. I expect biparentally inbred seeds (seeds produced where the pollen donor and maternal plants are siblings) will have intermediate performance between self- and cross-pollinated seeds. In addition, I expect naturally pollinated seeds will have intermediate performance because they have the potential to be outcrossed, inbred or selfed, and are likely to be a mixture of these.

2. What is the potential for geitonogamous pollination through insect visitation in *T. incisa*?

I used field observations of insect visitation and the number of plants with male and female umbels on a plant at the same time to investigate the potential for geitonogamy. I hypothesise that synchronous protandry significantly decreases the chance of geitonogamy and biparental inbreeding, compared to if flowers and umbels were not dichogamous.

MATERIALS AND METHODS

Study species

Trachymene incisa Rudge subsp. *incisa* (Apiaceae) is a common perennial herb endemic to eastern Australia, with a large latitudinal range from South-east Queensland to the south coast of New South Wales (Powell 1992). It grows on infertile sandy soils and in crevices of granite outcrops in dry eucalypt woodland or scrub (Benson and McDougall 1993).

There are approximately 72 small white flowers per inflorescence, arranged in simple umbels (Davila and Wardle 2002). The flowers are protandrous and open centripetally within the umbel. The male (pollen presenting) phase is followed by a quiescent phase until all flowers have dehisced pollen, then all flowers enter the female (pollen receptive) phase simultaneously (Davila and Wardle 2002). The female phase is characterised by elongated styles with wet, bulbous stigmatic tips (Appendix 7). Therefore, whole umbels function as either male (~16 d) then female (~4 d) which prevents self-pollination within the umbel (Davila 1999; Davila and Wardle 2002). Insect pollinators are required for seed production. Umbels are arranged in a hierarchy, where the secondary umbels branch off the stalk of the terminal primary umbel and the tertiary umbels branch off the stalks of the secondary umbels and so forth, usually up to four orders. There are on average four inflorescence stalks per plant, which grow to a height of approximately 60 cm (Davila and Wardle 2002). Male and female phase umbels from different orders and floral stalks are often present on a plant at the same time. *Trachymene incisa* is self compatible and self-fertilised seeds are viable (Davila and Wardle 2002). The umbels attract a large suite of native insect visitors, although the introduced European honey bee has been the most abundant visitor in recent years (Davila 1999; Davila and Wardle 2002).

The flowering period usually begins in late spring with a peak in flowering (highest density of flowering umbels) for approximately one month in summer (Powell 1992; Davila and Wardle 2002; Y. C. Davila, personal observation). Following this peak, many umbels set seed while a small proportion of plants continue to flower through to the end of autumn. Although *T. incisa* is common, it has been listed as an important component of at least three endangered ecological communities in New South Wales (New South Wales Scientific Committee 2000a; 2000b; 2000c).

Study populations

Seeds used to test for inbreeding depression were collected from a large population located in the Agnes Banks Woodland. Movement of insects within plants and patches was recorded at three populations: Myall Lakes National Park, Tomago Sandbeds and Agnes Banks Woodland.

The Agnes Banks Woodland is a unique low woodland community occurring on wind blown sand dunes, located in the Agnes Banks Nature Reserve, 55 km west of Sydney (Benson 1992). The Woodland is listed as an endangered ecological community with *T. incisa* as an identifying species (New South Wales Scientific Committee 2000a). The study site is located 250 m from the entry to the reserve (33°39′S, 150°41′E). Insect observations were made in May 2003, January 2004 and January 2005. Benson (1981) noted that 47% of the Agnes Banks flora is also found at Myall Lakes.

At Myall Lakes National Park, located on the east coast approximately 200 km north of Sydney, *T. incisa* grows in dry heath and dry heath forests on freely drained sands (Myerscough and Carolin 1986). The study area was located in dry heath along the side of an old mining road (32°30′S, 152°21′E). Insect observations were made in January 2003 and December 2003 (part of the 2004 season).

The Tomago Sandbeds are located on the central coast of New South Wales, approximately 70 km south of Myall Lakes National Park. *Trachymene incisa* occurs within the dry open-forest, low woodland and in adjacent fire breaks. The study site was located in a cleared area adjacent to shrubland and an access road (32°47′S, 151°51′E). Insect observations were made in February 2003 and January 2004. Myall Lakes National Park and the Tomago Sandbeds share similar climate, geomorphology, soils and vegetation (Fox *et al.* 1996; Ross *et al.* 2002). Therefore, all three sites are floristically similar but geographically separated.

Testing effects of inbreeding on seedling performance

Maternal plants were collected as naturally pollinated mature seeds from field plants in the Agnes Banks Woodland in April 2002. Field plants were randomly chosen over a distance of 100 m and approximate position in the field noted. Healthy seeds were sown into seedling trays containing moist river sand. Trays were placed in a growth chamber set to temperatures and day length resembling germination conditions in April, as recorded by the Bureau of Meteorology and Geoscience Australia (Appendix 2). Seedlings were transplanted into nursery pots filled with 6:1 sand:peat mix in December 2002, and returned to the growth cabinet. Plants were watered and fertilised regularly.

Between December 2004 and January 2005, flowering umbels from maternal plants were bagged and randomly assigned one of three pollination treatments: self-pollination, biparental inbreeding and cross-pollination. Self-pollinated seeds were produced by geitonogamous pollination between male and female umbels on the same maternal plant. In biparentally inbred seeds, the pollen donor and recipient maternal plant originated from seeds collected from the same field plant, and are at least half-siblings (sharing only a maternal parent) and possibly full siblings (sharing both paternal and maternal parents). Cross-pollinated seeds were pollinated using a pollen donor originally collected from a field plant located several metres away from the field plant where the recipient maternal parent was collected. Due to the complete separation of male and female function within the umbel, mechanical self-pollination does not occur (Davila and Wardle 2002) and bagging umbels in the growth cabinet ensures no pollen deposition prior to manual pollination. To guarantee adequate seed production for each treatment, pollen was applied by brushing the donor male umbel across the recipient female umbel, until all pollen was brushed off anthers and stigmas were saturated with pollen. Differences in seed set from different pollination treatments were not investigated because the amount of pollen deposited was not controlled and likely to be variable. In some cases pollen from a particular donor was in short supply, and seed set for some umbels may have been reduced by pollen limitation rather than inbreeding depression, further preventing a comparison of seed set among treatments. Pollinations were to some extent opportunistic depending on which plants were flowering and the availability of self, sibling or non-sibling pollen. More than one umbel was treated on most plants and the order of treatment application was randomised among plants. After pollination, umbels were bagged and collected in March 2005 after seed maturation. Not all pollinations resulted in adequate seed production, probably a result of pollen limitation or pollination of non-receptive umbels (receptive phase estimated by morphology). For a balanced design, eight umbels with at least fifteen seeds from a mixture of plants were chosen per treatment.

To compare seeds from hand-pollinations to seeds from natural pollination of the same age, mature seeds from eight plants were collected from the Agnes Banks Woodland in April 2005. Fifteen healthy seeds from eight umbels per pollination treatment (self, biparentally inbred, cross and natural) were weighed as groups. These seeds were sown into seedling trays containing moistened sieved sand collected from Agnes Banks. Seeds were randomly assigned wells and sown in pairs to save space (total of 480 seeds). Seedling trays were placed on a laboratory bench receiving ambient light and temperature, and were watered regularly. Emergence, survival and growth of true leaves were recorded every two days up to 70 days after sowing. A coefficient of inbreeding depression was calculated for each variable measured as $\delta = (W_O - W_S)/W_O$, where W_O and W_S are the mean trait values of offspring from cross- and self-pollinated flowers, respectively (Charlesworth and Charlesworth 1987).

Quantifying the potential for geitonogamy

Insect visitation to flowering umbels was recorded during peak flowering at Myall Lakes and Tomago between 2003 and 2004, and at Agnes Banks from 2003 to 2005. At each population, six observation patches consisting of 3-6 plants in an area of approximately 2 m^2 were located and tagged. A green or blue sticker to signify female or male phase umbels, respectively, was placed below each flowering umbel on the inflorescence stalk. Each umbel in a patch was given a unique number and its individual plant recorded. At the beginning of each day the flowering umbels were checked and labels changed if umbels had changed to a different phase (e.g. male to female, or female to post-receptive), and any newly flowering umbels were labelled and noted. Insect foraging did not appear to be affected by the presence of the labels (Y. C. Davila, personal observation).

Observations of insect visitors were made at each patch for 10 min, during the morning (0930-1100 h), midday (1230-1400 h) and afternoon (1530-1700 h) at all populations in all years. Observations were also made at sunrise (0700-0800 h) and sunset (1830-1930 h) in 2003 and at night (2100-2200 h) in 2004. Patches were observed in pairs by two observers and the order of paired patches and observers randomised at each time period. During the observation period, the morphospecies of insects (as a surrogate for species because identification was difficult in the field), which umbels they visited and the order of visitation within each patch were recorded. The observations were made over three consecutive sunny days during peak flowering for each population.

Data analyses

The null hypothesis that there are no differences among pollination treatments in *T. incisa* was analysed using analysis of variance (ANOVA, Abacus Concepts 1989-1990), with maternal plant as the unit of replication. Differences among pollination treatments were analysed in terms of percentage seed emergence, percentage of seeds alive as seedlings 70 days after sowing, percentage of seedlings (emergents) with true leaves by day 70, percentage of seeds alive as seedlings with true leaves 70 days after sowing and percentage of seedlings (emergents) surviving at least 30 days. Days to emergence was analysed as a nested ANOVA, with maternal family nested within pollination treatment and each seed as a replicate. Prior to analyses, a Cochran’s test (for balanced data) or Bartlett’s test (for unbalanced data) was performed to test for homogeneity of variances. Data with heterogeneous variances were transformed (details in Results) to improve homogeneity of variances. The performance of cross- and self-pollinated seeds was compared directly with *t*-tests. Prior to *t*-tests, an F test was performed to test for homogeneity of variances. Data with heterogeneous variances were analysed with *t*-tests assuming unequal variances (details in Results). The probability of Type I error (α) was set at 0.05. Means ± standard errors are reported.

RESULTS

Effects of self-pollination and biparental inbreeding on seedling performance

There were no significant differences among pollination treatments in terms of seed mass, percentage emergence, survival and growth (Table 6-1). However, there were some important patterns in seedling performance related to pollination treatment. On average, cross pollinated seeds performed better than self-pollinated seeds for all variables measured, particularly the percentage of seedlings with true leaves 70 days after sowing (Table 6-1). Biparentally inbred seeds were intermediate in performance in three out of six variables measured, and naturally pollinated seeds were intermediate in performance in four out of six variables measured.

Significant differences in days to emergence exist among families within pollination treatments (data square root transformed, $F_{28, 401} = 2.554$, $P = 0.001$). Overall, there were no significant differences in days to emergence among treatments ($F_{3, 28} = 2.549$, $P = 0.076$; Fig. 6-1). However, self-pollinated seeds took longer to emerge (20.6 ± 0.8 days) than biparentally inbred seeds (17.6 ± 0.4 days). The number of days to emergence for seeds produced from natural (18.8 ± 0.6 days) and cross-pollination (18.3 ± 0.4 days) were intermediate between self-pollinated and biparentally inbred seeds.

Table 6-1 Summary of means ± SE and ANOVA on seed mass, seedling emergence, survival and growth from four pollination treatments.

Variable	Self-pollination	Biparentally Inbred	Cross-pollination	Natural pollination	$F_{3,28}$	P
Mass of 15 seeds (mg)	19.1 ± 1.3	18.6 ± 0.7	20.8 ± 2.1	15.4 ± 0.8	2.693	0.065
% final emergence	87.5 ± 4.3	94.2 ± 2.3	91.7 ± 1.7	87.5 ± 2.9	1.236	0.315
% seedlings surviving 30d	87.5 ± 2.9	91.3 ± 3.4	92.0 ± 3.4	89.0 ± 3.3	0.391	0.760
% seeds alive as seedlings on day 70	72.5 ± 5.7	80.0 ± 4.4	79.2 ± 2.7	69.2 ± 5.6	1.219	0.321
% seeds alive as seedlings with true leaves on day 70	43.3 ± 6.7	59.2 ± 7.0	67.5 ± 4.3	55.8 ± 6.3	2.675	0.066
% seedlings with true leaves on day 70	49.7 ± 7.3	62.7 ± 7.0	74.2 ± 5.3	63.0 ± 5.7	2.467	0.082

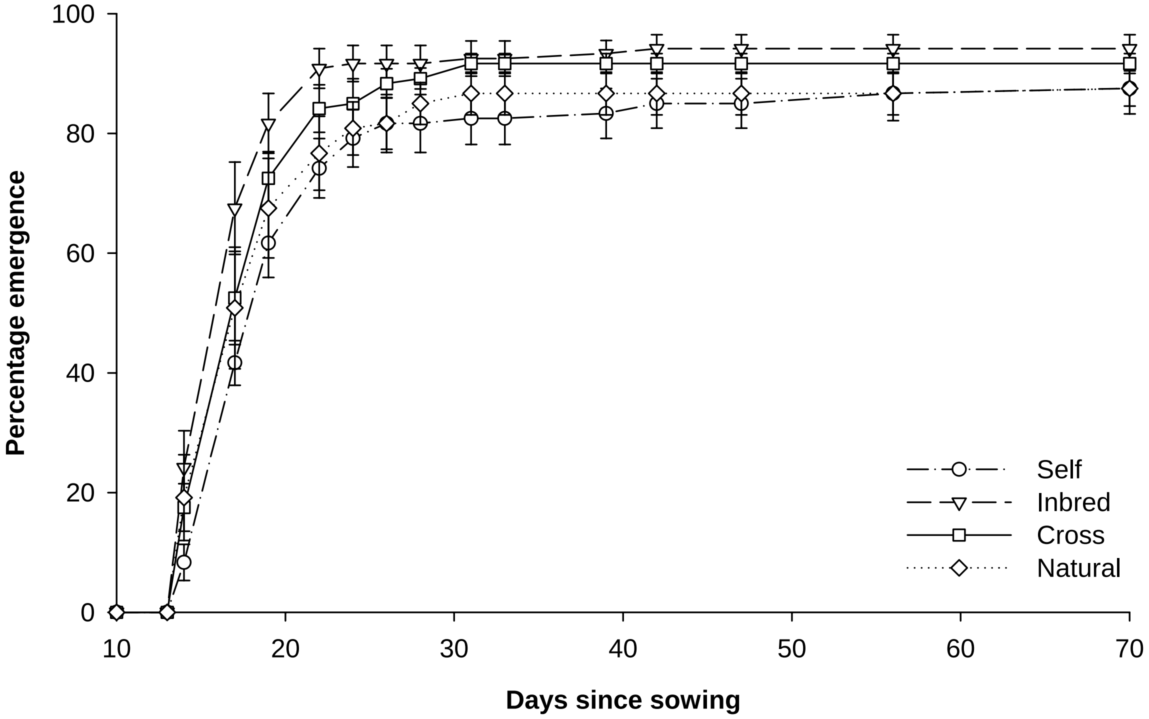

Fig. 6-1 Cumulative mean ± SE percentage emergence of *Trachymene incisa* seedlings from four pollination treatments: self-, biparental inbreeding, cross- and natural pollination.

Significant inbreeding depression was detected in self-pollinated progeny in terms of days to emergence, percentage of seeds alive as seedlings with true leaves 70 days after sowing and percentage of seedlings (out of all seedlings that emerged) with true leaves by day 70 compared to self-pollination (Table 6-2). Inbreeding depression was weak in early life cycle characters of seed mass, percentage emergence and short-term survival (seedling surviving 30 days).

Table 6-2 Summary of *t*-test results comparing self- and cross-pollinated seeds and coefficient of inbreeding depression (δ) for seed mass, seedling emergence, survival and growth from four pollination treatments. *P* values in bold are significant. † *t*-test performed assuming unequal variances. * Calculated assuming early emergence is advantageous, such that $\delta = (|W_O - W_S|)/W_O$.

Variable	df	*t*	*P*	δ
Mass of 15 seeds (mg)	14	0.707	0.491	0.085
% final emergence†	9	0.911	0.386	0.045
Days to emergence†*	142	1.429	**0.014**	0.121
% seedlings surviving 30d	14	1.612	0.129	0.066
% seeds alive as seedlings on day 70†	10	1.061	0.314	0.084
% seeds alive as seedlings with true leaves on day 70	14	3.054	**0.009**	0.358
% seedlings with true leaves on day 70	14	2.714	**0.017**	0.329

Opportunity for geitonogamy – presence of male and female umbels

Due to relatively low visitation rates (maximum mean observed = 0.6 ± 0.1 visits per umbel per 10 min, at Myall Lakes in 2004; Chapter 3), surveys of plants and observations of insect movements were pooled across populations and years. A total of 163 plants were used for insect foraging observations at Myall Lakes, Tomago and Agnes Banks between 2003 and 2005. Of these plants, 42.3% (69) had male and female umbels present at the same time over the survey period. There were 2.8 ± 0.2 male umbels and 1.8 ± 0.1 female umbels per plant when both male and female umbels were present (mean ± SE across all populations and years, n = 69 plants). The highest ratio of male to female umbels per plant observed was 8:1, and the highest ratio of female to male umbels per plant observed was 4:1.

Opportunity for geitonogamy – insect foraging

Ants contributed a large proportion of visits to flowering umbels at all populations (Chapters 2, 3 and 4) but are unlikely to be pollinators because they rarely contact the reproductive structures when they forage at the base of flowers for nectar (Chapter 2). Therefore, I restricted this survey to flying insects, including native bees, flies, butterflies and moths, which are likely to move pollen between umbels (for lists of morphospecies see Chapters 2, 3 and 4). Introduced honey bees were not observed visiting umbels during this study. A total of 700 flying insects were observed visiting flowering umbels. Of these, 32.6% (228) visited two or more umbels per patch during a foraging trip and 73 visitors (10.4% of all visitors) were observed visiting both male and female umbels within a patch during a foraging trip. However, only 46 visitors (6.6% of all visitors) were observed visiting male then female umbels (this order necessary for pollination) within a patch during a foraging trip.

Flying insects were observed moving between umbels on the same plant, with 169 individuals (24.1% of total) visiting two or more umbels on the same plant during a foraging trip. Only 30 visitors (4.3% of total) were observed visiting both male and female umbels from the same plant during a foraging trip and 21 visitors (3.0% of total) visited male then female umbels (order required for geitonogamous pollination) on the same plant during a foraging trip.

DISCUSSION

Trachymene incisa is self-compatible and suffers weak to intermediate levels of inbreeding depression (δ = 0.045-0.358) through early stages of the life cycle when seeds are self-pollinated and biparentally inbred. Cross-pollinated seeds performed consistently better than self-pollinated seeds in terms of percentage emergence and short-term survival and growth. However, it appears that self-pollination is avoided by umbels exhibiting synchronous protandry, which reduces the simultaneous occurrence of male and female function to only 42.3% of plants. In addition, synchronous protandry at the umbel level requires insects to visit at least two umbels on the same plant for geitonogamous pollination to occur, a male umbel to collect pollen followed by a female umbel to deposit pollen. Only 3.0% of insect visitors were observed visiting male then female umbels on the same plant during a foraging trip. Similarly, few insects were observed visiting male then female umbels between plants within a patch (6.6%), which suggests levels of biparental inbreeding between neighbouring plants are likely to be low.

The spot counts of insect visitation used in this study were adequate for recording within patch insect movements. However, since insects were not tagged and were not followed once they left the observation patch, this sampling design limits the ability to record insect movements between patches and movements where insects return to the observation patch. Therefore, these counts may under-estimate the within plant and within patch movements of some insects. Following insects with a video-camera was attempted but proved difficult with most insects because they were too small to focus on and flew quickly between umbels. Despite these limitations, my results still show that most consecutive insect movements are between plants that are not located adjacent to each other.

Insect visitation, geitonogamy and biparental inbreeding in *Trachymene incisa*

The reliance on insect pollinators that move relatively short distances increases the chances for geitonogamous pollination and biparental inbreeding. The majority of insects (75.9%)

visited only one umbel per plant during an observed foraging trip on *T. incisa*. In contrast, other species of Apiaceae have recorded high insect foraging within and between neighbouring plants. In two North American genera, *Thaspium* and *Zizia*, 35-40% of the 587 insect movements recorded were within plants and 25-71% of sequences included a female phase umbel (Lindsey and Bell 1985). Similarly, insects often moved among flowers on a plant or travelled less than 5 m when they moved between plants of *Eryngium cuneifolium* (Apiaceae) (Evans *et al.* 2003). In protandrous *Eryngium alpinum*, 70% of flights occurred within a single plant, and 72% of flights were less than 50 cm (Gaudeul and Till-Bottraud 2004). However, insects usually moved from male phase flowers to immature flowers or female- to male phase flowers, and consequentially genetic analyses of *E. alpinum* seeds showed that plants were primarily outcrossing (Gaudeul and Till-Bottraud 2003). This indicates that strong dichogamy can counteract the effects of intra-plant insect foraging by reducing the chances of geitonogamous pollination.

Synchronous dichogamy was effective at reducing the chance of geitonogamous pollination in *T. incisa*. Of all flying insects observed, 169 (24.1%) visited two or more umbels per plant during a foraging trip. If umbels were adichogamous, then each of these visits could lead to geitonogamous pollination. The potential for geitonogamy is very high in many species of Apiaceae, due to mass flowering (Evans *et al.* 2003; Gaudeul and Till-Bottraud 2003) and non-synchronisation of dichogamy among umbel orders (Schlessman and Graceffa 2002; Rovira *et al.* 2004) or separate flowering stalks (Lindsey 1982; Davila and Wardle 2002). However, due to the complete separation of genders at the umbel level in *T. incisa* and that only 42.3% of plants presented both male and female umbels at the same time, the chance of geitonogamous pollination is much lower than 24.1% of insect visits. Similarly, strong protandry reduced the occurrence of male and female flowers on the same plant to only 29% of the time in *Silene virginica* (Caryophyllaceae) and plays an important role in maintaining outcrossing in this species (Dudash and Fenster 2001). Harder and Aizen (2004) also demonstrated that synchronous protandry was effective at avoiding self-pollination within umbels and ramets, and hence inbreeding depression, in self-compatible *Alstroemeria aurea* (Alstroemeriaceae). The rate of self-pollination is likely to be low in *T. incisa* given that only 3.0% of insects visited male followed by female umbels on the same plant and that not all visits would result in successful pollination.

Biparental inbreeding is also likely to be low due to the small proportion of insects foraging among closely located plants. Most insect visitors (67.4%) to *T. incisa* only visited one umbel within a patch. This suggests that most pollen transfer is between plants that are not located adjacent to each other. Patterns of insect foraging and seed dispersal suggest short distance gene flow via gravity dispersed seed and longer distance gene flow via insect-mediated pollen dispersal. Genetic analyses of outcrossing rates and population genetic structure are needed to confirm this hypothesis.

Pollen carry-over, the number of subsequent flowers to which pollen of any one donor is carried (Galen and Rotenberry 1988), can affect the level of geitonogamy occurring within a plant (de Jong *et al.* 1993). Insects walk across the umbel of *T. incisa* during foraging and contact multiple stigmas when foraging on a female umbel. Therefore, if the insect is carrying pollen from a male umbel on the same plant, opportunity for carry-over onto multiple female flowers is possible. In this respect, pollen carry-over is a potential method for increasing geitonogamy in *T. incisa*, particularly if insects make return visits to the same plant. Investigation of pollen carry-over was beyond the scope of this study, although determining whether pollen is carried over multiple umbels, as well as multiple flowers, would be interesting in terms of multiple paternity within an umbel and outcrossing rates.

Inbreeding depression in *Trachymene incisa* and the Apiaceae

Environmental, developmental or genetic factors may contribute to the timing of inbreeding depression throughout the life cycle. Variation in environment may enhance differences in the coefficient of inbreeding depression among life stages (Husband and Schemske 1996). Alternatively, the expression of some deleterious alleles may be stage specific, and the magnitude of inbreeding depression will depend on the genes expressed at different life stages and the duration of their effects (Husband and Schemske 1996). *Trachymene incisa* suffers inbreeding depression which was most evident as a significant reduction in the percentage of self-pollinated seeds alive as seedlings with leaves after 70 days compared to cross-pollinated seeds (Table 6-1). This measure is analogous to cumulative inbreeding depression as it includes emergence, survival and growth of leaves. There was also a tendency for earlier emergence, higher seed mass, greater final percentage emergence and greater seedling survival to 30 days in cross-pollinated seeds compared to self-pollinated seeds. The differences between self- and cross-pollinated seedlings in mean performance also increased with further development. Husband and Schemske (1996) showed that in outcrossing angiosperms, maximum inbreeding depression is often (but not always) expressed late in the life cycle, during growth and reproduction. *Trachymene incisa* is a perennial herb and there is potential for greater expression of inbreeding depression in later stages of the life cycle, such as reproduction and life span.

As hypothesised, biparentally inbred seeds performed intermediate between self- and cross-pollinated seeds in terms of survival of emergents and growth of true leaves. This result indicates that increased inbreeding decreases seedling performance. Other studies have found similar results, with progeny as a result of pollination between plants located far apart performing better than progeny with parents located closer together (e.g. Price and Waser 1979; Dudash 1990; Waser 1993; Byers 1998).

Naturally (field) pollinated seeds have the potential to be outcrossed, inbred or selfed and performed intermediate between self- and cross-pollinated seeds for many characters. This implies some level of inbreeding within the Agnes Banks population and that pollen movement is limited. Alternatively, the parental neighbourhood may be larger than expected, given that insects travel between plants and patches regularly. The proportion of biparentally inbred seeds following natural pollination will partly depend on plant density, which is related to time since fire (Chapter 4). While plants resprout in high numbers, flower prolifically and produce copious seeds in the flowering season following fire (Chapter 4), the quality of seeds may be diminished due to increased biparental inbreeding. It would be interesting to investigate the degree of biparental inbreeding in response to fire frequency, since *T. incisa* grows in fire prone habitats. The importance of synchronous protandry in avoiding inbreeding depression also has consequences for the structuring of populations of *T. incisa*, particularly in populations located in endangered communities which are under immediate threat of extinction.

Several studies worldwide confirm widespread self-compatibility in the Apiaceae (Keighery 1982; Lindsey 1982; Davila and Wardle 2002; Gaudeul and Till-Bottraud 2003; Rovira *et al.* 2004). Few studies worldwide have investigated inbreeding depression in non-cultivated species of Apiaceae. The results have been mixed, with no difference in seed set (Evans *et al.* 2003), non-significant decrease in fruit/seed set (Lindsey 1982; Rovira *et al.* 2004), significant decrease in fruit/seed set (Lindsey 1982) and significant decrease in seed germination (Gaudeul and Till-Bottraud 2003) detected as a result of self-pollination compared to cross-pollination. A study of 12 Apiaceae genera from Western Australia, including *Trachymene*, revealed few differences in germination between self-, cross- and naturally pollinated seeds (Keighery 1982). One limitation is that all of these studies have been restricted to investigating inbreeding depression early in the life cycle, on fruit/seed set, seed mass and germination. If inbreeding depression is expressed in later stages of the life cycle, which has been suggested by Husband and Schemske (1996) and is indicated by my results, then longer term studies are needed to quantify this.

Studies of inbreeding depression in the glasshouse and similar benign habitats may underestimate the magnitude of inbreeding depression expressed during severe episodes of selection (Eckert and Barrett 1994; Husband and Schemske 1996). Therefore, seeds should ideally be germinated in the field. However, field conditions were extremely harsh during the course of this study with two previous attempts at germinating seeds in the field (Agnes Banks Woodland) unsuccessful due to drought conditions (Appendix 3) and fire destroying experimental plots. Preliminary results of germination trials in the field compared to the glasshouse and growth cabinet show that seed families with high percentage emergence in the glasshouse and growth cabinet do not necessarily do well in the field (Appendix 2). Therefore, future investigations of inbreeding depression in *T. incisa* should consider maternal effects and performance under field conditions.

Synchronous protandry and high inter-patch insect foraging suggests that *T. incisa* is a mostly outcrossing species. However, a change in insect-foraging to more within plant and patch movements can increase the amount of self-pollination and biparental inbreeding, and increase inbreeding depression. The introduced honey bee, *Apis mellifera*, was recorded as the most abundant visitor to *T. incisa* in recent years (Davila 1999; Davila and Wardle 2002; Y. C. Davila and G. M. Wardle, personal observation). However, during the course of this study, honey bees were absent from the pollinator assemblage at all populations (Chapters 2, 3 and 4). Honey bees have been observed visiting both male and female umbels on the same plant of *T. incisa* and travelling short distances between umbels

(Davila 1999). This indicates that variation in the pollinator assemblage by the addition or return of honey bees can potentially change the amount of inbreeding and inbreeding depression expressed in populations of *T. incisa*. England *et al.* (2001) found that when birds were excluded from *Grevillea macleayana* (Proteaceae), allowing only honey bees access to flowers, outcrossing rates were significantly lower. It would be interesting to investigate whether synchronous protandry in *T. incisa* is effective at avoiding inbreeding in populations and years when the introduced honey bee is a dominant member of the pollinator assemblage.

Temporal variation in rates of inbreeding is likely to occur within the Agnes Banks population, due to years dominated by honey bees and years dominated by native bees or flies. As a result, the current adult population of *T. incisa* at Agnes Banks is a combination of many cohorts produced by different pollinator assemblages. It would be interesting to compare the current levels of inbreeding in seeds with this adult population. Since floral visitors vary spatially and temporally in *T. incisa* (Chapter 2, 3 and 4), it would also be interesting to determine how genetic diversity is distributed within and among populations of *T. incisa* across its large geographic and habitat range.

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CHAPTER 7

UNDERSTANDING THE POLLINATION ECOLOGY OF GENERALISED PLANT-POLLINATOR SYSTEMS

Photograph: *Trachymene incisa* at Bald Rock National Park

SPATIAL AND TEMPORAL VARIATION IN POLLINATORS AND EFFECTS ON PLANT REPRODUCTIVE SUCCESS

One of the central issues regarding the evolution and ecology of mutualistic interactions is the degree of specialisation or generalisation that occurs among interacting species (Thompson 2002). Biotic pollination is a mostly mutualistic interaction between plants and animals, and most flowering plants depend on animal pollinators for pollination and hence seed production (Buchmann and Nabhan 1996). In order to understand floral adaptation, pollinator foraging, gene flow, community interaction webs and the evolution and maintenance of pollination systems, we need a comprehensive understanding of the range of specialisation and generalisation of plants and pollinating animals, and the temporal and spatial variation in these relationships (Waser *et al.* 1996; Herrera 2005; Waser 2006). Significant progress towards this understanding can be achieved by examining generalised pollination systems and determining:

1. How variable are pollinators? To what degree do insect visitation rates and assemblage composition vary spatially among populations and temporally among flowering seasons?
2. How does variation in pollinators affect plant reproductive success?

I chose to do this using a model system, *Trachymene incisa* subsp. *incisa* (Apiaceae), which is a widespread herbaceous species with simple flowers that attract a high diversity of insect visitors. The Apiaceae are considered to be highly generalist in terms of pollination, due to their simple and uniform floral display and easily accessible floral rewards. Three populations of *T. incisa* located between 70 km and 210 km apart were studied over 2-3 years.

How variable are pollinators in a generalised pollination system?

Considerable variation among populations in pollinator assemblages has previously been documented (Lindsey 1984; Herrera 1988; Eckert 2002). My study supports these findings, with different populations of *T. incisa*, growing in similar habitats that are geographically separated, experiencing significant variation in pollinator visitation rates and assemblage composition (Chapter 2). The introduced European honey bee was unexpectedly absent from the pollinator assemblages during this study, providing the unique opportunity to study the native Australian pollinators.

The few studies investigating spatial and temporal variation simultaneously over geographic and yearly/seasonal scales indicate that there is a trend for more spatial than temporal variation in pollinators of generalist-pollinated plants (Gómez and Zamora 1999; Herrera *et al.* 2002; Cane *et al.* 2005; Herrera 2005). In contrast, my study showed both spatial and temporal variation in insect visitation rates and assemblage composition (Chapter 3). A significant population by year interaction was driven by an increase in insect visitation at Myall Lakes and a decrease in insect visitation at Tomago over consecutive years (2003-2004). The most notable change in assemblage composition was due to a shift from predominantly ant visits in 2003 to mostly native bee and fly visits in 2004 at Agnes Banks. Ants were the most abundant visitor at all three populations in 2003 and at Tomago in 2004. However, removing ants from the analyses to restrict the assemblage to flying insects and the most likely pollinators, resulted in no difference in assemblage composition between the Myall Lakes and Tomago populations located 70 km apart. At these populations, the flying insect assemblage was dominated by native bees

Hylaeus (Prosopisteron) woyensis and *Lasioglossum* sp. The overall visitation rate of flying insects in 2004 was greater than in 2003 (Chapter 3). These results show more temporal than spatial variation in the flying insect visitor assemblage of *T. incisa*.

Significant variation in pollinators among years occurs in several plant species (e.g. Price *et al.* 2005), however, some plants show consistency in core floral visitors over long time scales (e.g. Cane *et al.* 2005). Although there were no significant differences in visitation rate to *T. incisa* at the Agnes Banks Woodland between 2003 and 2005, the assemblage composition varied over the three years, with each year different to the other years (Chapter 4). In 2003, the assemblage was dominated by ants; in 2004, visits were made by bee flies and the native bees; and in 2005, the assemblage was dominated by bee flies. Temporal variation in the assemblages of populations among years maintains generalisation at the population/local level, and spatial variation in assemblages among populations maintains generalisation at the species level.

Foraging behaviour provides another source of variation when considering the pollinator assemblage of generalist-pollinated plants. This is important for *T. incisa* which exhibits umbels that function as either male or female at any one time and offer different floral rewards. Insects showed both preferences and non-preferences for umbel phases in natural patches where the gender ratio was male biased. In contrast, insects showed no bias in visitation during a foraging trip or in time spent foraging on male and female umbels in experimental arrays where the gender ratio was equal. (Chapter 5).

Several important features of generalised pollination systems are highlighted as a result of my study. The large differences among populations and between years indicate that populations are not equally serviced by pollinators and are not equally generalist. This supports Herrera’s (2005) findings that generalisation in pollination systems is not an invariant, species-level property. Insect visitation rates also varied significantly throughout the day, highlighting that sampling of pollinators at one time will result in an inaccurate estimate and usually underestimate the degree of generalisation. Fire plays an important role in altering pollination, by shifting the flowering season and reducing the abundance of flying insects. However, pollination services to *T. incisa* can be restored within one year of fire. Fire may also play a role in maintaining spatial and temporal variation in this fire-prone system.

How does variation in pollinators affect plant reproductive success in a generalised pollination system?

In many pollination studies there has been emphasis on determining the most effective pollinator and how individual pollinators differ in their effectiveness in pollen deposition (e.g. Stebbins 1970; Wilson and Thomson 1991; Mayfield *et al.* 2001). However, few studies have looked at the effects of variation in pollinator assemblages on plant reproductive success and mating (Galen 1996; Herrera 2000; England *et al.* 2001; Eckert 2002). High insect visitation rates do not guarantee high plant reproductive success (Chapter 2). There was no positive correlation between insect visitation rate and reproductive success, with the Agnes Banks population receiving the highest visitation rate but producing the lowest reproductive output, and the Myall Lakes population receiving the lowest visitation rate and producing the highest seedling emergence in 2003. These results suggest that the quality of visit, rather than the rate of insect visitation, is important in determining reproductive output in *T. incisa*.

Interestingly, populations with different assemblage composition can produce similar percentage seed set per umbel (Chapters 3 and 4). However, similar percentage seed set does not mean similar offspring performance. This is demonstrated by comparing the Agnes Banks and Myall Lakes populations in 2003, which were visited by assemblages with different taxonomic composition and produced similar percentage seed set, but had significantly different percentage seedling emergence (Chapter 2). Percentage seed set per umbel was unexpectedly consistent among populations and years (Chapters 3 and 4). Therefore, differences among years in reproductive output (total seed production) are due to differences in umbel production (reproductive effort) and proportion of umbels with seeds, and not seed set per umbel (Chapters 3 and 4).

Trachymene incisa is self-compatible and suffers weak to intermediate levels of inbreeding depression through early stages of the life cycle when seeds are self-pollinated and biparentally inbred (Chapter 6). Floral phenology, in the form of synchronous protandry, plays an important role in avoiding self-pollination within umbels (Davila and Wardle 2002) and reducing the chance of geitonogamous pollination between umbels on the same plant (Chapter 6). Assemblages containing pollinators that increase the rate of inbreeding may be less effective compared to assemblages containing pollinators that reduce the risk of inbreeding. Pollinators can increase the rate of inbreeding in *T. incisa* by foraging on both male and female phase umbels on the same plant or closely related plants, which is more likely to occur by insects foraging for nectar which is available during both umbel phases. Therefore, variation in pollinators can affect seedling performance and has the potential to alter rates of inbreeding in *T. incisa*.

Potential pollinators of *Trachymene incisa*

In order to determine whether a floral visitor is an effective pollinator requires evidence that the visitor can pollinate by picking up pollen and depositing pollen onto stigmas; evidence that a visitor pollinates by showing exclusion of all visitors causes lack of seed set and visitor access results in seed set; and evidence that a particular visitor contributes to pollination (Pellmyr 2002). I was not able to determine whether the most abundant visitors are effective pollinators of *T. incisa* (discussed in Chapter 2). However, many of the floral visitors to *T. incisa*, such as native bees and flies, are potential pollinators based on visitation rates, foraging behaviour on both male and female umbels and ability to carry pollen.

Native bees from the families Colletidae (which ingest pollen) and Halictidae (which carry pollen externally, Fig. 1-3b, c) are likely to be effective pollinators of *T. incisa*. Many species of native bee have been recorded visiting umbels at several populations of *T. incisa* (Chapters 2 and 3; Appendix 5; Y. C. Davila, unpublished data). These bees were observed foraging on both male and female phase umbels, and contact the reproductive structures of both gender phases during visits. The Hymenoptera are not always well represented in the insect assemblages of many Apiaceae, although they often carry significant amounts of pollen and therefore are considered important pollinators (Bell and Lindsey 1977; Grace and Nelson 1981; Lindsey 1984; Zych 2002).

The introduced European honey bee (Fig. 1-3a) was not observed visiting *T. incisa* umbels during the course of this study. However, in 1999 honey bees that were caught foraging on *T. incisa* were found to have a very high proportion of *T. incisa* pollen in their corbiculae (Davila 1999), indicating they actively forage on *T. incisa*. In addition, *T. incisa* pollen was found on other parts of the honey bee body that would normally contact the stigmas, indicating they are likely to transfer pollen. Introduced honey bees were observed visiting both male and female umbels during foraging trips. This evidence suggests that honey bees can be major pollinators of *T. incisa* when present in large numbers.

Pollination studies of Apiaceae in Australia (Pickering 2001) and worldwide (Bell and Lindsey 1977; Grace and Nelson 1981; Lindsey 1984; Lamborn and Ollerton 2000; Larson *et al.* 2001; Zych 2002) confirm a high proportion of insect visitors to be flies.

Hover flies are also known to exploit pollen and nectar of several Apiaceae species (Branquart and Hemptinne 2000). Hover flies (Fig. 1-3g), bee flies (Fig. 1-3e) and muscid flies (Fig. 1-3f) could be effective pollinators of *T. incisa* because they were common visitors to both male and female phase umbels, and were observed at all populations studied (Chapters 2 and 3; Appendix 5).

Beetles have been reported to pollinate over 88% of flowering plants worldwide (Buchmann and Nabhan 1996). Beetles (Fig. 1-3h) were infrequent visitors to *T. incisa*, but may be effective pollinators because they can carry *T. incisa* pollen externally (Y. C. Davila, unpublished data) and contact the reproductive structures of flowers (Y. C. Davila, personal observation). Butterflies were also infrequent visitors to *T. incisa*, and were observed foraging on male and female phase umbels. Butterflies may be responsible for longer distance matings.

While many insects are potentially effective pollinators, a large proportion of floral visits were attributed to ants (Fig. 1-3d), which are unlikely pollinators (discussed in Chapters 2 and 3). Ants rarely contact the anthers and stigmas of flowers as they forage for nectar in the base of the flower, and do not appear to carry pollen externally. Ants have been shown to have a negative effect on pollination in several other species (e.g. Beattie *et al*. 1984; Galen and Butchart 2003; Ashman and King 2005).

Although the Apiaceae are generalists in terms of pollination, there appears to be an upper limit on the size of the floral visitors to *T. incisa*, which can restrict the number of potential pollinators. Introduced honey bees appear to be the biggest floral visitors to comfortably forage on *T. incisa*, although they do cause the floral stem to bend and the umbel to bounce when they land on an umbel (Y. C. Davila, personal observation). Larger insects would certainly make the stem buckle under their weight. The flowers of *T. incisa* are small and shallow, and are likely to be more suited to short-tongue bees and flies, rather than long-tongue bees and butterflies. Finally, individual flowers offer relatively small amounts of nectar, and larger insects with higher nutritional needs may not find *T. incisa* satisfactory as a nectar source.

Limitations to this study

Insect visitation rates and reproductive output were reported for three populations in this study, which span approximately 210 km. While this is a relatively large geographic scale, it is only one sixth of the entire range of *T. incisa*. Two other populations were surveyed with the same level of detail in 2004, however, the insect visitation rates were extremely low and no transformations could improve the skewed data (Appendix 5). As a result these data were excluded from the analyses. These populations were located at Bald Rock National Park, located on the border between the states of NSW and Queensland, and in crown land near Ulladulla, on the south coast of NSW. This latter population is located on the southern-most edge of the range. In addition, a one day sampling effort was undertaken at a small population located on the Sunshine Coast, in south-east Queensland, although the insect visitation rates were again very low. These populations extend the coverage of my sampling to approximately two thirds of the range of *T. incisa* and although they do not provide data for statistical analyses, they do provide qualitative data on insect morphospecies and confirm the absence of introduced honey bees from the floral visitor assemblage across this range (Appendix 5). Ultimately, surveying populations throughout the entire range was not feasible due to time and logistic constraints. Future studies could incorporate populations in Queensland and the north coast of NSW in order to characterise the diversity of pollinators of *T. incisa* and the extent of generalisation throughout the range. An altered sampling design which incorporates longer observational periods to record insect visitation is needed at these populations.

Similarly, this study was limited to 2-3 years and ideally more years are needed to document the extent of temporal variation in pollinators. However, the three year study in the Agnes Banks Woodland coupled with a previous one year study (Davila 1999) does indicate substantial temporal variation in floral visitors and consistent seed production levels.

With the recent advances in molecular ecology and population genetics, the effects of variation in pollinators on pollen flow and outcrossing rates can be investigated in detail. Microsatellite markers were in development but due to limited funding and technical difficulties, were not completed in time to be used in this study. Therefore, I was unable to determine the levels of outcrossing resulting from variation in pollinator assemblages. The implications of this line of study are discussed further in “Other future directions”.

Despite these limitations, my study highlights the importance of variation in pollinators for determining the degree of generalisation, the scales at which variation in floral visitors occurs and the effects on plant reproductive success. Pollination is a dynamic interaction, where in-depth examination of pollination systems is required in order to understand the range of strategies utilised by plants and their pollinators. These results lead me to discuss two concepts: a way to categorise the degree of generalisation of a plant at different spatial scales, and an assemblage approach to investigating the consequences of variation in pollinators on plant reproductive success.

DEFINING GENERALISATION IN POLLINATION SYSTEMS

Generalisation and specialisation in ecology usually refer to niche breadth and are defined by the number of different resource items used (Renner 2006). What does it mean to be specialised or generalised in terms of pollination? Firstly, specialisation and generalisation is a continuum rather than a dichotomy, although there is no universal terminology to describe the continuum of pollination interactions (Waser *et al.* 1996). There is also no consistent relationship between the degree of specialisation of a flower and the degree of specialisation of its interaction partners (Renner 2006). This is reflected in the different terminology and organisation used to describe plant and pollinator (particularly bee) specialists and generalists. The definition can be from the plant or pollinator perspective or describing the actual interaction (Appendix 1). For example, specialist plants may be pollinated by a single pollinator, but this pollinator may be generalist on several plants, which means the interaction is also generalised (Minckley and Roulston 2006). The simplest way to define generalisation is the number of interaction partners of a given species, that is, the fewer the interacting partners, the more specialised a species (Vázquez and Aizen 2006). However, the number of interaction partners seems inadequate given that plant species can be functionally specialised, where they are pollinated by one type of pollinator, and/or phenotypically specialised, showing specific adaptations to a particular pollinator, but at the same time be ecological generalists by being pollinated by more than one species of that type of pollinator (Ollerton *et al.* 2006). The scale or taxonomic level at which generalisation is defined is often inconsistent, and the degree of generalisation can change between these levels. For example, Herrera (2005) showed that extensive generalisation was not an invariant, species level trait for *Lavandula latifolia* (Labiaceae), with populations and individual plants within populations varying in degree of pollinator generalisation.

In an attempt to categorise the degree of generalisation of a plant species, I have developed a model which includes the degree of ecological and functional specialisation of a plant species on pollinators and the variation encountered across different plant scales. We start with a two-dimensional plot. The first axis is the degree of ecological generalisation, and is simply the number of pollinator species (Fig. 7-1). The second axis is the degree of functional generalisation, and records the number of pollinator types present. A pollinator type can be based on function, morphology, behaviour or higher level taxonomy. The need to specify both the number of species and the number of pollinator types was inspired by the term “eclectic oligolege” developed by Cane and Sipes (2006) to describe a category of bee specialist. An eclectic oligolege is a bee that obtains pollen from few fixed plant genera that belong to different clades, and is different from an oligolege that obtains pollen from several plant genera in the same clade. Consider two plants that are pollinated by the same number of pollinator species, plant A is pollinated by five bee species and plant B is pollinated by three bee species and two bee-flies species. These plants would be equally generalised if only the number of species was considered. Including the number of pollinator types distinguishes the plants, with plant B more generalised than plant A. Functional diversity of pollinators and plants also plays an important role in plant community persistence (Fontaine *et al.* 2006), and is therefore an important categorical trait.

On this two-dimensional plot we can determine where a plant species sits with regards to ecological and functional specialisation (Fig. 7-1). Extreme specialists are pollinated by one pollinator species (and one pollinator type) and are located in the bottom left corner. Extreme generalists are pollinated by several pollinator species and types and are located in the top right corner. Functional specialists are pollinated by few to many pollinator species that are the same pollinator type, and are located along the bottom axis. Intermediate generalists are pollinated by few to many pollinator species of few to many pollinator types, and will be located within the middle of the plot (Fig. 7-1).

This two-dimensional plot illustrates where on the continuum between extreme specialisation and extreme generalisation a plant species sits with respect to both the number of pollinator species and the number of pollinator types. However, below the species level, variation in pollinator species and types exists at the regional, population and individual plant level. Therefore, the position of the species points on this two-dimensional plot is the accumulation of variation at these varying geographical extents and shows the maximum number of pollinator species and types for a given plant species.

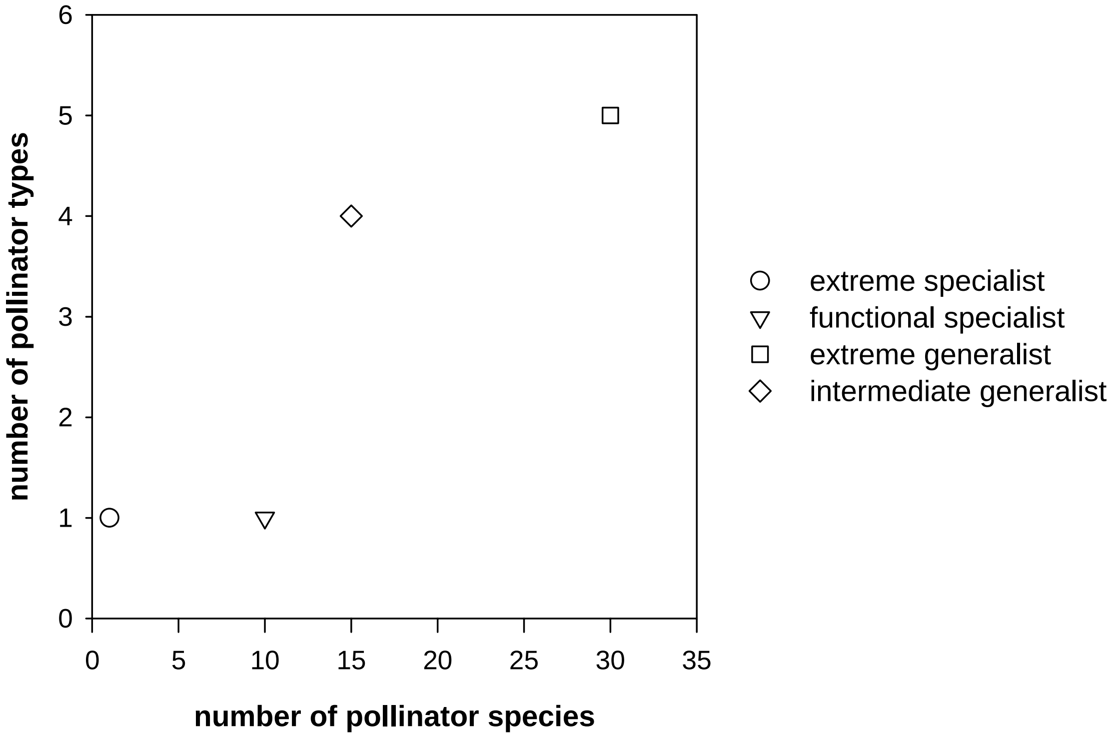

Fig. 7-1 Two-dimensional plot of the continuum between specialisation and generalisation of plant species on their pollinators, based on the number of pollinator species and pollinator types.

It is well documented how variable pollinators can be among individual plants in a population, among populations and among regions within the range of a plant species. If we include the variation in pollinator species and types among individual plants, populations and regions, we have an expanded area rather than a single point on this plot for most species (Fig. 7-2).

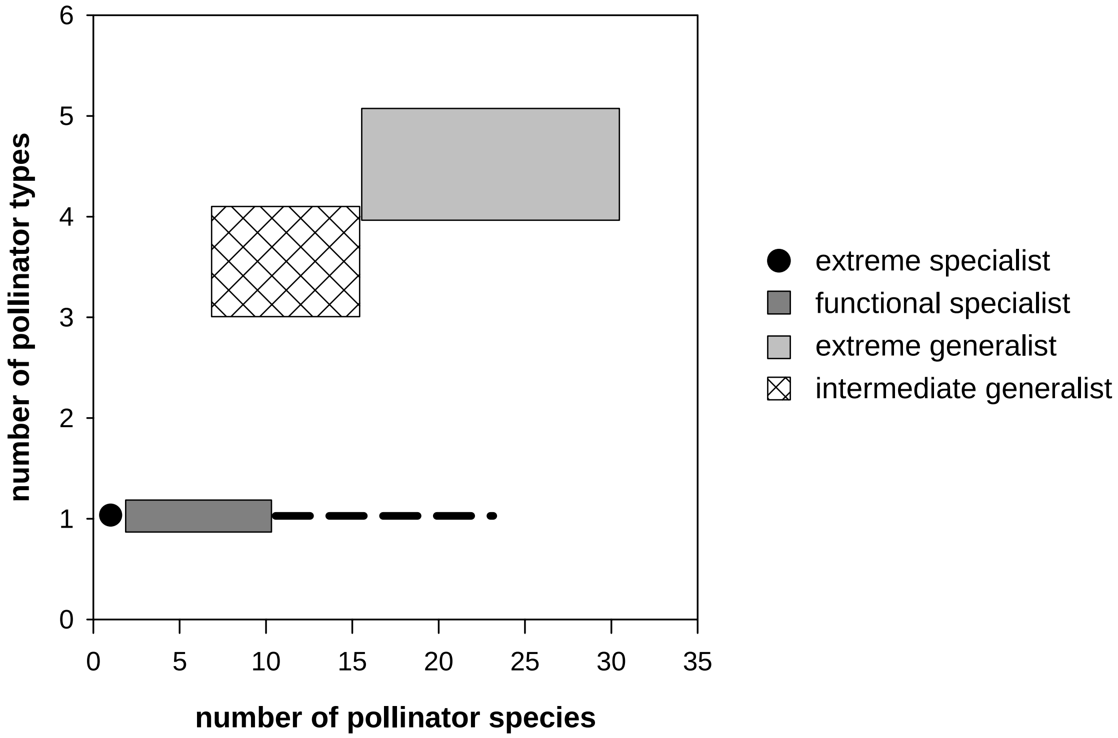

Fig. 7-2 Two-dimensional plot of the continuum between specialisation and generalisation of plant species on their pollinators, based on the number of pollinator species and pollinator types, including variation at levels below species (individual, population and region).

Extreme specialists remain a single point on the plot, with one pollinator species and type. Extreme generalists cover an area incorporating a range of pollinator species and types, still located in the top right corner. Functional specialists occupy a narrow strip along the plane of one pollinator type and can vary in length depending on the number of species. Some functional specialists may also be ecological generalists, because they are pollinated by several species of the same type. Intermediate generalists occupy an area within the middle of the plot (Fig. 7-2). This new plot gives a visual representation of how generalised a plant species is in terms of its pollinators by the position of the box on the plot, and how variable these pollinators are by the size of the area of the box.

To visualise at which levels variation in pollinators occurs, the plot can be expanded into a third dimension, geographical extent (Fig. 7-3). The four plant categories included in my plot are the individual, population, regional and species levels. These categories are useful for determining whether the degree of generalisation is consistent across different spatial scales or if, for example, populations are less generalised compared to the regional or species level. Extreme specialists are characterised by having the same pollinator from individual to species level (Fig. 7-3). Extreme generalists are pollinated by several species from many pollinator types from individual to species level (Fig. 7-3). In the two dimensional plot (Fig. 7-2), extreme generalists were represented by a plant pollinated by 15-30 pollinator species and 3-4 pollinator types and the scales of this variation were not specified. In the three-dimensional plot (Fig. 7-3), individual plants are pollinated by 15-18 species and 3-4 types each; populations are pollinated by 20-21 species; at the regional level there are 25-27 pollinator species; and at the species level there are 30 pollinator species and four pollinator types. In this extreme generalist example, individuals and populations are less generalised than the regional and species level, and shows that sampling one population would not characterise the full degree of generalisation of this species.

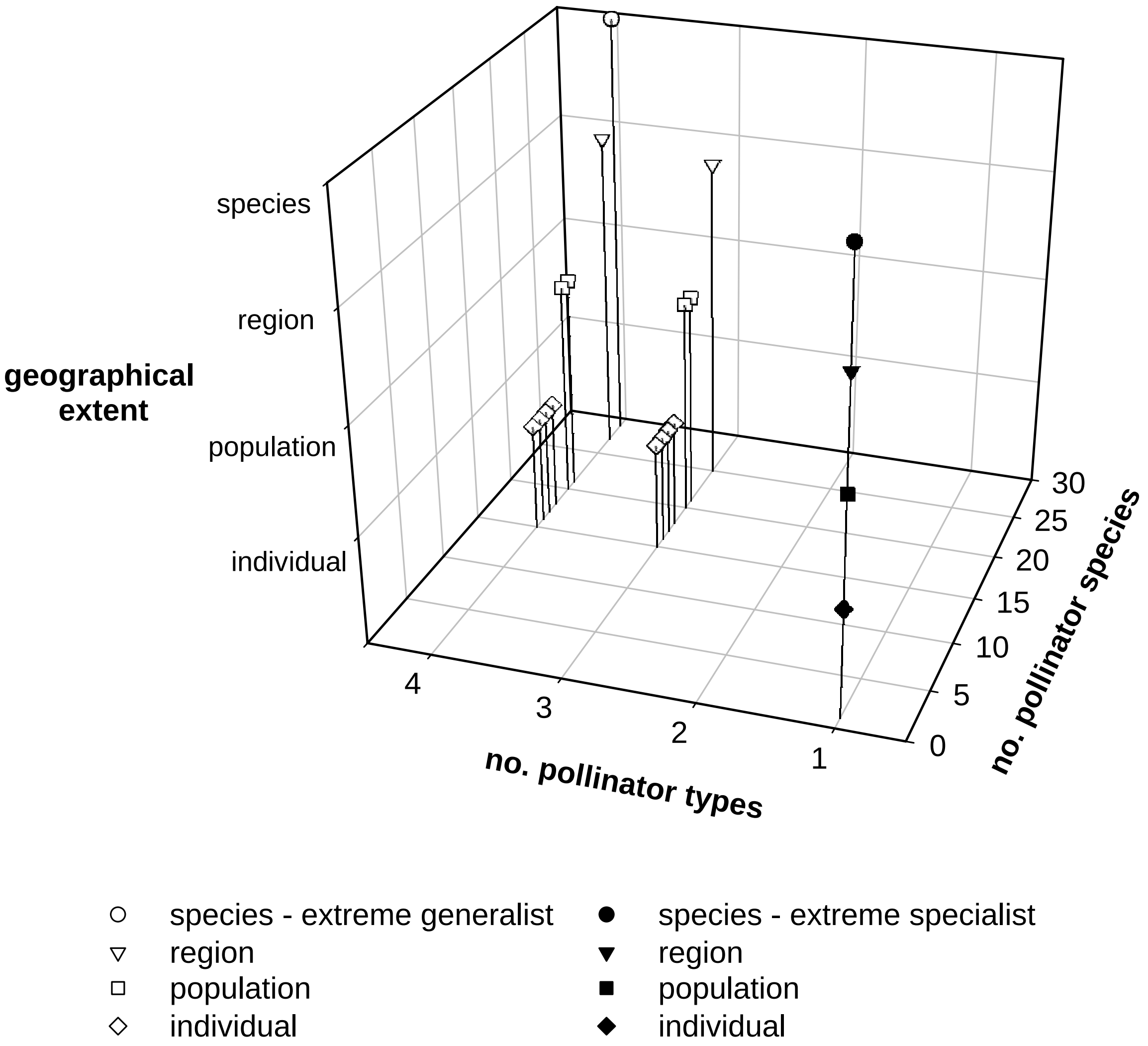

Fig. 7-3 Three-dimensional model summarising the degree of ecological and functional generalisation of a plant on its pollinators across different geographical scales. Extreme specialists (solid points) utilise one species that represents one pollinator type, and the same pollinator occurs at all levels of plant organisation. Extreme generalists utilise several pollinator species and pollinator types at all levels of plant organisation.

Ecological generalists (plants pollinated by several species) can be functional specialists (Fig. 7-4a). In this case, the number of pollinator types is constantly one, but the number of pollinator species can increase with an increase in geographical extent, by accumulating pollinator species at larger spatial scales. Alternatively, some plants may be functional specialists and relatively specialised ecologically if the number of pollinator species is low and consistent across all geographical scales.

Intermediate generalists are probably a fair description of most plant species. In the example in Fig. 7-4b, individual plants can be specialists or generalists, with 1-4 pollinator species from 1-2 pollinator types. In the case of the specialist that is pollinated by one species, this is an example of realised specialisation (Vázquez and Aizen 2006), which is specialisation under a particular ecological context. Moving up in spatial scale, plants are pollinated by 6-7 species from 2-3 types at the population level; there are 10-11 pollinators from 3-4 types per region; and 13 pollinator species and four types at the species level. Therefore, with this new three-dimensional model we can determine the degree of generalisation of a plant species at multiple spatial levels as well as whether generalisation is due to the number of pollinator species and/or the number of pollinator types (also possible in the two-dimensional model).

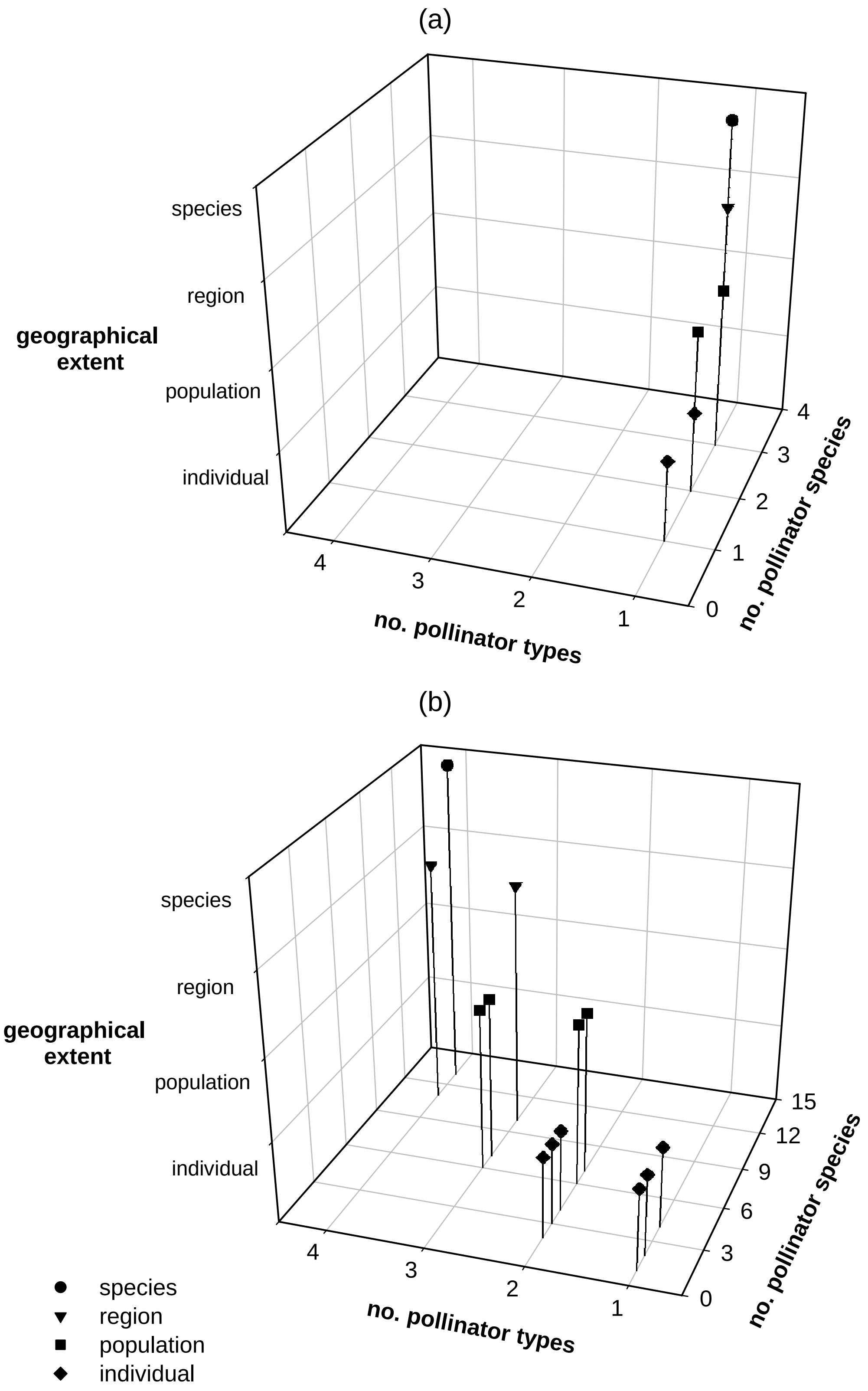

Fig. 7-4 Degree of generalisation of a plant on its pollinators. (**a**) Functional specialists / ecological generalists are pollinated by few to many pollinator species that are the same functional type; (**b**) Intermediate generalists are pollinated by more than one pollinator species and type, generalisation increases from individual through to species level.

Knowing the scales at which pollinators vary has important ecological and evolutionary consequences. Individuals are the unit that is involved in interactions and individual interactions can vary spatially and temporally. Local adaptation to pollinators occurs at the population and sometimes regional level, although these are also dynamic and may change over time. Variation in pollinators at the population and regional level is the basis for specialisation and coevolution in pollination systems. This model can illustrate how populations differ spatially in terms of specialisation on a few pollinators or generalisation on several pollinators. By plotting the differences among populations in degree of specialisation, we can start to hypothesise about the direction populations will deviate from each other. Alternatively, we can use this tool to investigate whether populations that appear to be specialised are actually specialising on few pollinator species and types or if this specialisation is enforced by the environment or a depauperate floral visitor fauna. We can then make predictions about the stability of pollination interactions in that population.

This model is a categorical tool and has some limitations. It does not have the detail of a community web, which lists the plant and pollinator species and the realised links between them (e.g. Memmott 1999). My model could utilise the information for a particular species from several community webs in order to show the extent of variation in generalisation among populations, and whether populations differ slightly or extensively to give rise to the degree of generalisation at the species level. As a result, this model relies on information on the pollinator assemblage from several populations to be useful, which is a basic requirement for describing the pollination ecology of a plant species.

Other community web studies focus on the number of interaction partners and whether these are a random selection of the available partners (e.g. Olesen 2000; Medan *et al.* 2006; Petanidou and Potts 2006). There is no inclusion of the randomness of pollinators in my model. However, the number of potential pollinators is assumed to increase with an increase in populations. If the number of pollinator species or types stays consistent across several populations and moving up to regional and species levels, then it can be assumed that the assemblage is not random and is a specific set of pollinators.

Specialisation in pollination systems is often implied with the use of the pollination syndrome concept (Waser *et al.* 1996). In addition, there is the potential for “special” to be confused with “specialised”, for example, mammal and bird pollinated species compared to insect pollinated species. Compare a plant pollinated by three bird species versus a plant pollinated by three bee species – are they equally specialised? Under the basic definition of number of pollinator species and according to my model which also considers number of pollinator types, the answer is yes. This highlights another aspect of defining specialisation and generalisation: the ecological (current state) and evolutionary (process) perspective (Appendix 1). Ecologically, the plant species in the previous example are equally specialised. Evolutionary, these plant species may not be equally specialised, due to different degrees of phenotypic specialisation or adaptive generalisation, where different pollinators act in a similar way to cause evolution of floral adaptation (Gómez and Zamora 2006). My model describes the ecological or current state of plant species and their pollinators. However, it also presents the pattern of generalisation across a range of populations, which can provide important information on the evolution and maintenance of the system.

Ultimately, a meaningful definition of specialisation would require measuring the reproductive consequences of interactions between a given plant species and its flower visitors (Vázquez and Aizen 2006). Therefore, it is critical to consider the whole visitor assemblage when determining the consequences of variation in pollinators on plant reproductive success.

ASSEMBLAGE APPROACH TO POLLINATION STUDIES

Most plants receive visits from many different animals to their flowers. The floral visitors can vary in their ability to collect and deposit pollen. It is important to study all floral visitors, including the less abundant visitors, because they can contribute a fair proportion towards plant reproduction (e.g. Lamborn and Ollerton 2000). Rare floral visitors may also be responsible for altered patterns of pollen flow which increases genetic variation in offspring and the population. For example, butterflies are rare visitors to *T. incisa*, occurring only at Myall Lakes (Chapter 3). Butterflies spend longer periods of time foraging on umbels than the more abundant native bees (Chapter 5), and are likely to collect and deposit some pollen grains whilst foraging for nectar on both male and female umbels. Butterflies also travel larger distances between umbels and overall during foraging trips (Y. C. Davila, personal observation) and can disperse pollen over large distances. This suggests that butterflies will be responsible for long distance pollen flow and contribute to higher levels of cross-pollination between unrelated plants. Cross-pollinated seeds perform better than inbred seeds in *T. incisa* (Chapter 6). Therefore, butterfly pollination can result in higher seed quality. In addition, a shift towards more butterfly pollination is likely to alter population structure through larger pollen dispersal distances.

Although pollination is a mostly mutualistic interaction, underpinning it is a series of competitive interactions between floral visitors. Foraging behaviour in the presence of competitors will be different relative to that in the absence of competitors (Aigner 2006). For example, Australian solitary bees are more effective pollinators of *Melastoma affine* (Melastomataceae) than introduced honey bees, however, honey bees disturb foraging of solitary bees and also remove pollen from stigmas previously deposited by solitary bees (Gross and Mackay 1998). Therefore, the addition of honey bees to the assemblage has altered the way the pollinator assemblage functions, because in the presence of honey bees, native bees are less effective pollinators.

Pollination has a degree of conflict between the plant that offers rewards and the pollinator that provides the service of transporting pollen. However, it is susceptible to cheating because some floral visitors remove ("rob") nectar through a hole bitten or pierced in the corolla and avoid transporting pollen (Maloof and Inouye 2000). Similarly, many ants are nectar thieves, entering flowers legitimately but feeding at the base of flowers and not contacting the reproductive structures (Galen and Butchart 2003). Pollinators may avoid flowers depleted of nectar, visit fewer flowers per inflorescence and fly longer distances between flowers when nectar levels are low (reviewed by Maloof and Inouye 2000). This may translate to lower visitation rates and/or higher outcrossing rates in the presence of nectar robbers. Therefore, nectar robbers and thieves can influence plant fitness by changing the behaviour of legitimate pollinators.

These examples clearly illustrate that pollinator assemblages are not simply the sum of their parts, because floral visitors interact with each other and can alter foraging and effectiveness of other visitors in the assemblage. Therefore, the focus of studies addressing the effects of variation in pollinators on plant reproductive success should be on the effectiveness of whole assemblages. It is the way the assemblage functions as a whole that will determine plant reproductive output. In my study, I took the whole assemblage approach by recording all floral visitors and the resulting plant reproductive output across multiple populations. If I had restricted my survey to the most abundant visitor, which was ants, then the contributions of flies and native bees would be ignored. Similarly, if I restricted my survey to flies and native bees as the most likely pollinators based on flower shape and rewards, I would miss the small but important contributions from butterflies and beetles, and the role ants play in the pollination ecology of *T. incisa*.

Variation in assemblage function will also shape the evolution and maintenance of specialised or generalised plant-pollinator interactions. The first step towards this approach is community interaction webs, which records all interactions between plants and animals within a community. In reality, most mutualistic webs have a core of strong interactions and a large network of weak relationships (Gómez and Zamora 1999). However, constructed interaction webs are based on the presence of pollen on the floral visitor and ignore other floral visitors that do not carry pollen. The contribution of other floral visitors, such as nectar robbers and thieves, is not considered and the potential for interaction between floral visitors is not recorded.

To test the effectiveness of visitor assemblages requires populations of plants with different assemblage composition. Due to the selective pressure that pollinators can exert on plants, populations with different pollinators may also have morphology reflecting adaptation to those pollinators (Galen 1996). In this case, populations differ in both pollinator assemblage and floral specialisation, and this confounding makes teasing apart the effects of a different pollinator assemblage impossible. An alternative method is to use plants that show high floral uniformity, such as the Apiaceae. In such plants, the phenotype remains constant and the environment (pollinator assemblage) changes over the range of the plant, which enables one to test the effect of variation in assemblage on plant reproduction in a more controlled way. This is analogous to reciprocal transplanting and having similar genotypes exposed to different environments. For example, the genus *Trachymene* shows high floral uniformity; flowers each have five petals, five anthers, two styles and two ovules, and flowers are grouped into simple umbels. Flower size and colour, and the number of flowers per umbel vary among species, as does species range and habitat (Hart and Henwood 2006). Using *Trachymene*, its features and recently constructed phylogeny (Lu-Irving 2005), we can apply a comparative approach and take advantage of the increased sample size (multiple species) to further understand the dynamics of generalised pollination systems: are widespread species more generalist than restricted species? Could this partly explain the reason why restricted species are restricted? We may expect widespread species to be more generalised because they are exposed to more climates and habitats, and potentially more floral visitors. An experimental test of the effects of functional diversity of plants and pollinators on functioning and persistence of plant communities, showed that plant reproductive success (seeds per fruit, fruits per plant and recruitment) increased with increasing pollinator functional diversity (Fontaine *et al.* 2006). However, widespread species can be habitat specific, such as *T. incisa* on sandy soils, which may also apply to the visitor fauna. It would be interesting to compare the pollination ecology of *T. incisa* subsp. *incisa* with the restricted *T. incisa* subsp. *corrugata* as a first step to understanding how two closely related plants can vary dramatically in range.

OTHER FUTURE DIRECTIONS

The pollination ecology of *T. incisa* has proven to be an interesting system in which to investigate the degree and effects of variation in pollinators. However, many questions have arisen concerning the pollination of *T. incisa*, and these are also contemporary issues relevant to other pollination systems. For example, what is the role of introduced honey bees in the pollination of *T. incisa*? In previous years, introduced honey bees have been the most abundant visitor to *T. incisa* (Davila and Wardle 2002). Introduced pollinators, such as the honey bee and bumble bee *Bombus terrestris*, have the potential to alter foraging of native insects, affect seed production and gene flow (Paton 1997; Hingston and McQuillan

1998; Paini 2004), particularly in Australia because the flora and fauna have evolved without these large social insects. These factors have led “Competition from feral honey bees” and the “Introduction of the large earth bumble bee *Bombus terrestris*” to be listed as key threatening processes under the Threatened Species Conservation Act in New South Wales, Australia (New South Wales Scientific Committee 2002; New South Wales Scientific Committee 2004). Honey bees have been observed to restrict their foraging to individual plants of other species (e.g. Paton 1997) and forage on umbels of *T. incisa* from the same or closely located/related plants (Y. C. Davila, personal observation). Therefore, introduced honey bees have the potential to alter the mating system and seed quality in *T. incisa*, by increasing the chance for inbreeding through their restricted foraging patterns.

The success of introduced honey bees and bumble bees can be attributed to their generalisation on plants and ability to occupy many habitats. The displacement of native pollinators by these introduced super-generalists is more likely to occur in plants with generalised floral systems. After displacement these plants become functionally specialised on honey bees or bumble bees for pollination (Waser *et al.* 1996). Although there have been no studies documenting the complete displacement of native pollinators by introduced pollinators (Gross 2001), simplification of systems through introduced pollinators may render them vulnerable to further change (Waser *et al.* 1996). Given this potential, are plant communities robust to such changes in pollinators? Pollination networks appear to be robust to losses of pollinators due to the redundancy in pollinators per plant and nestedness of pollination networks (e.g. Memmott *et al.* 2004). Plants are thought to be well-buffered against the loss of their dominant, highly efficient pollinator (Aizen 2001), and have been documented to persist after the extinction of their pollinators (Cox 1983; Bond 1994; Schmidt-Adam *et al.* 2000). While pollinators are considered to be more vulnerable to human-induced extinction than plants (Memmott *et al.* 2004), pollination networks can collapse with the loss of a small fraction of plant species (Jordano *et al.* 2006). This suggests that plants and pollination networks can cope with the loss of some pollinators, but pollinators and pollination networks cannot persist with the loss of some plants. This highlights another angle of asymmetry in pollination networks and warrants further research, especially since plant-pollinator systems are being disrupted worldwide (Kearns *et al.* 1998).

Pollination ecology combines the studies of pollination biology and breeding systems to determine the mechanisms through which plants donate and receive pollen. Pollinators are responsible for a significant proportion of gene flow within and between populations (seed dispersal being the other source). Therefore, variation in pollinators can lead to variation in gene flow. It remains unclear how variation in the pollinator assemblage across the range of a plant affects plant mating (Eckert 2002). Having determined that pollinator assemblages and reproductive output vary spatially and temporally in *T. incisa*, determining the outcrossing rates of populations of *T. incisa* with different pollinator assemblages is the next step in investigating the effects of variation in pollinators on plant reproduction. The recent advances in developing microsatellite markers provide an excellent opportunity to study direct measures of gene flow and determining the source of pollen. This will enable the assessment of changes caused by the activity of different pollinators and the consequences of such changes for the overall fitness of populations. Specifically, it would be interesting to determine the level of outcrossing within an umbel, compared to between umbels within a plant. There is a high potential for outcrossing, with approximately 70 flowers per umbel that are receptive to pollen over at least four days. In addition, it would be interesting to determine whether outcrossing rates increase with an increase in the number of pollinator species or pollinator types in the assemblage. If so, then being generalist may confer a fitness advantage by increasing the amount of outcrossing and reducing the amount of inbreeding in *T. incisa*.

Finally, the field of pollination ecology should continue to include detailed studies of single species, such as this study, to determine the patterns and strategies that lead to interactions between plant and pollinator species, and the geographic differences in these interactions. It is these high resolution studies that will confirm the links in community interaction webs and other larger scale analyses, reveal patterns used to decipher the evolution of pollination systems, and provide information needed to conserve these interactions that are central to ecosystem function.

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DEFINITIONS OF SPECIALISATION AND GENERALISATION IN POLLINATION ECOLOGY

Table A1-1 Multiple definitions for specialisation and generalisation in pollination ecology used by authors in Waser and Ollerton (2006).

Perspective	Definition	Source
Plant	Highly specialised plants are successfully pollinated by one or a few animal species	(Gómez and Zamora 2006)
Plant (flower)	Specialised flowers are those that attract few pollinator species Simple flower describes an open floral morphology with readily accessible rewards, and complex flower describes those that package their rewards in a way that excludes access for many potential visitors	(Minckley and Roulston 2006)
Plant (island plants)	Island plants are specialised because few potential pollinator species are found on islands relative to mainland areas Island plants are also generalised (non-selective) because they will use whatever animals are available as pollinators	(Petanidou and Potts 2006)
Plant	Generalisation is the possession of a phenotype that is not singularly optimised for any one pollinator type	(Aigner 2006)

Table A1-1 continued

Perspective	Definition	Source
Plant	*Ecological specialisation*: pollinated by one species *Functional specialisation*: pollinated by one type of pollinator (but can be ecologically generalised by being pollinated by more than one species of that type of pollinator) *Phenotypic specialisation*: showing specific adaptations to a particular pollinator	(Ollerton *et al.* 2006)
Plant	*Adaptive generalisation* is where different pollinators act in a concordant fashion to cause evolution of floral adaptation *Non-adaptive generalisation* is where pollinators are not real selective agents and ecological forces constrain development of adaptive specialisation	(Gómez and Zamora 2006)
Plant	Generalisation is the tendency for plants to use a large proportion of the available flower-visiting fauna as pollinators, as defined by species or functional groups Specialisation occurs when plants use a relatively small proportion of the available flower-visiting fauna as pollinators, as defined as species or functional groups *Ecological specialisation* is the state of being specialised *Evolutionary specialisation* is the process of evolving toward greater specialisation in response to selection generated by pollinators	(Armbruster 2006)
Pollinator (bees)	Specialised and generalised are used based on the number and relatedness of each species' floral hosts, without regard to adaptive traits or to the presence of covisiting species on the same hosts	(Minckley and Roulston 2006)
Pollinator (bees)	*Narrow oligolege* – obtains pollen from single plant genus *Oligolege* – obtains pollen from several genera in the same clade (families) *Eclectic oligolege* – obtains pollen from few fixed genera that belong to different clades *Mesolectic* – obtains pollen from more than four genera in 2-3 different clades	(Cane and Sipes 2006)

Table A1-1 continued

Perspective	Definition	Source
Plant and Pollinator	The inverse of the number of interaction partners of a given species i.e. the fewer the interaction partners, the more specialised a species *Fundamental specialisation*: potential interactions that would lead to positive fitness gains for a given species under any ecological circumstance *Realised specialisation*: actual specialisation attained under a particular ecological context	(Vázquez and Aizen 2006)
Plant and Pollinator	Generalisation and specialisation in ecology refer to niche breadth, the number of different resource items used e.g. number of pollinator species used by a plant or number of plant species used by a pollinator	(Renner 2006)
Plant and Pollinator	Generalists are any plant or animal that meets its needs by using a high proportion of the partners available in the community Specialists are species that satisfy their needs using a low proportion of the potential mutualists	(Medan *et al.* 2006)
Interaction	*Reciprocal specialisation* is where a plant is pollinated by one pollinator and that pollinator only visits that one plant *Specialised interaction* is where flowers are pollinated by a narrow range of pollinators that harvest resources from very few plant species *Generalised interaction* is where specialists interact with generalists and vice versa	(Minckley and Roulston 2006)

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APPENDIX 2

EFFECT OF ENVIRONMENT ON SEEDLING PERFORMANCE: GERMINATION OF SEEDS IN THE FIELD, GLASSHOUSE AND GROWTH CABINET

BACKGROUND AND AIM

Many germination studies are conducted in glasshouses and controlled growth cabinets because they are easy to access and maintain. However, the glasshouse and growth cabinet environments may be relatively benign compared to the natural field environments experienced by developing seedlings, and results may not be consistent between natural and artificial environments. Therefore, it is important to know how much seedling development in artificial conditions deviates from that experienced in natural conditions.

The aim of this pilot study was to determine whether differences in rate of seedling emergence and final emergence percentage vary among three environments: field, glasshouse and growth cabinet. Seeds sown in the field were exposed to natural light and temperature, and only received additional watering at time of sowing. Seeds sown in the glasshouse received ambient light but maximum temperature was regulated by a cooling system which restricted maximum temperature to approximately 30°C. Seeds also received watering as needed. Seeds sown in the growth cabinet had light and temperature regulated to resemble field conditions during seedling emergence, and received watering as needed.

The original aim of this study was to follow plants with known relatedness in the field, glasshouse and growth cabinet, through to flowering and use them for hand-pollinations to test effects of inbreeding on offspring performance. The field sown seeds, sown in April 2002, suffered harsh drought conditions and all seedlings that germinated were recorded as dead by September 2002. The plots were destroyed by fire in January 2003. The growth cabinet suffered several malfunctions, including variations in temperature and temporary shutdowns, which required moving seedlings to another growth cabinet while the original one was fixed. Due to these interruptions, data analyses were restricted to the data collected during the first 100 days after sowing.

MATERIALS AND METHODS

Mature *T. incisa* seeds were collected from plants in the Agnes Banks Nature Reserve in April 2002. Seeds were collected from two umbels per plant, and from 13 plants randomly chosen over a distance of 100 m. Ten seeds per umbel were sown randomly into seedling trays containing moist river sand (total 260 seeds). Replicate trays were placed in the field, glasshouse and growth cabinet. Trays in the field were placed flush with the ground and watered after sowing in seeds on 25 April 2002. Trays in the glasshouse were sown in May 2002, and placed on a bench receiving, that was partly shaded throughout the day and were watered regularly. The growth chamber was set to temperatures and day length resembling germination conditions in March-April (when most seedlings are observed to germinate), as recorded by the Bureau of Meteorology and Geoscience Australia (Table A2-1, Fig. A2-

1). Emergence of seedlings was monitored weekly in the field and three times per week in the glasshouse and growth cabinet, up to 110 days after sowing.

Table A2-1 Climate averages for Richmond (RAAF) from the Bureau of Meteorology and sunrise/sunset times for Agnes Banks from Geoscience Australia.

	March	April
Mean daily maximum temperature	27°C	23.9°C
Mean daily minimum temperature	15.5°C	11.8°C
Mean 9 am air temperature	20.1°C	16.6°C
Mean 3 pm air temperature	25.9°C	23.1°C
Sunrise (15th of each month)	5:56 am	6:19 am
Sunset (15th of each month)	6:16 pm	5:36 pm

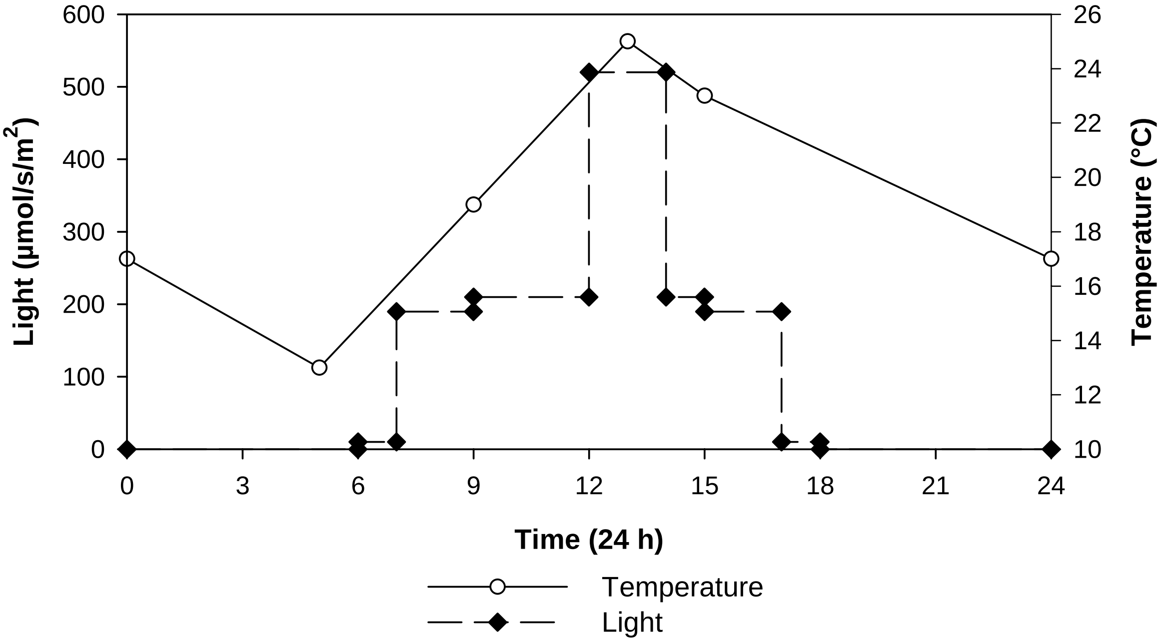


Fig. A2-1 Temperature and light conditions of growth cabinet.

RESULTS AND DISCUSSION

Seedling emergence varied significantly among environments (ANOVA, $F_{2, 36} = 7.718$, $P = 0.002$; Fig. A2-2), with fewer field sown seeds (4.5 ± 0.7) emerging compared to the glasshouse (10.2 ± 1.7) and growth cabinet (12.2 ± 1.6) after 110 days. Seeds sown in the field were also slower to start emerging through the soil (ANOVA on square-root transformed days to emerge, $F_{2, 347} = 406$, $P < 0.0001$). The mean number of days to emerge was 28.3 ± 0.7 days in the growth cabinet, 32.8 ± 0.7 days in the glasshouse and 71.6 ± 2.1 days in the field.

Not all families performed well, with some families recording low emergence in all environments. Families also had variable emergence in different environments, which indicates a G × E interaction (Fig. A2-3). This study highlights that seeds sown in different environments can perform significantly differently in terms of final germination/emergence and days to emergence. Therefore, consideration of maternal effects and environment should be taken when studying seedling performance.

ACKNOWLEDGEMENTS

I thank C. Newman for help with the growth cabinet and glasshouse, and H. Giragossyan and A. Divljan for watering my plants and seedlings while I was away. Field work was carried out under permit from the NSW National Parks and Wildlife Service, part of Department of Environment and Conservation (NSW). Support was provided by the University of Sydney, Botanic Gardens Trust and an Australian Postgraduate Award.

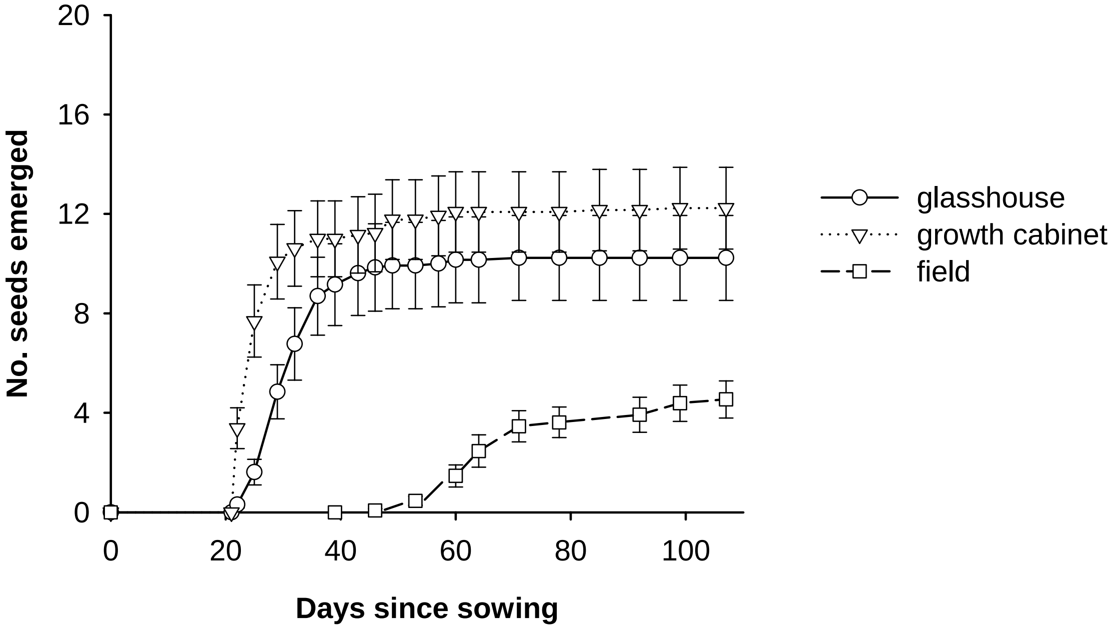


Fig. A2-2 Cumulative mean ± SE *Trachymene incisa* seedling emergence across three environments (n = 13 families, 20 seeds per family sown).

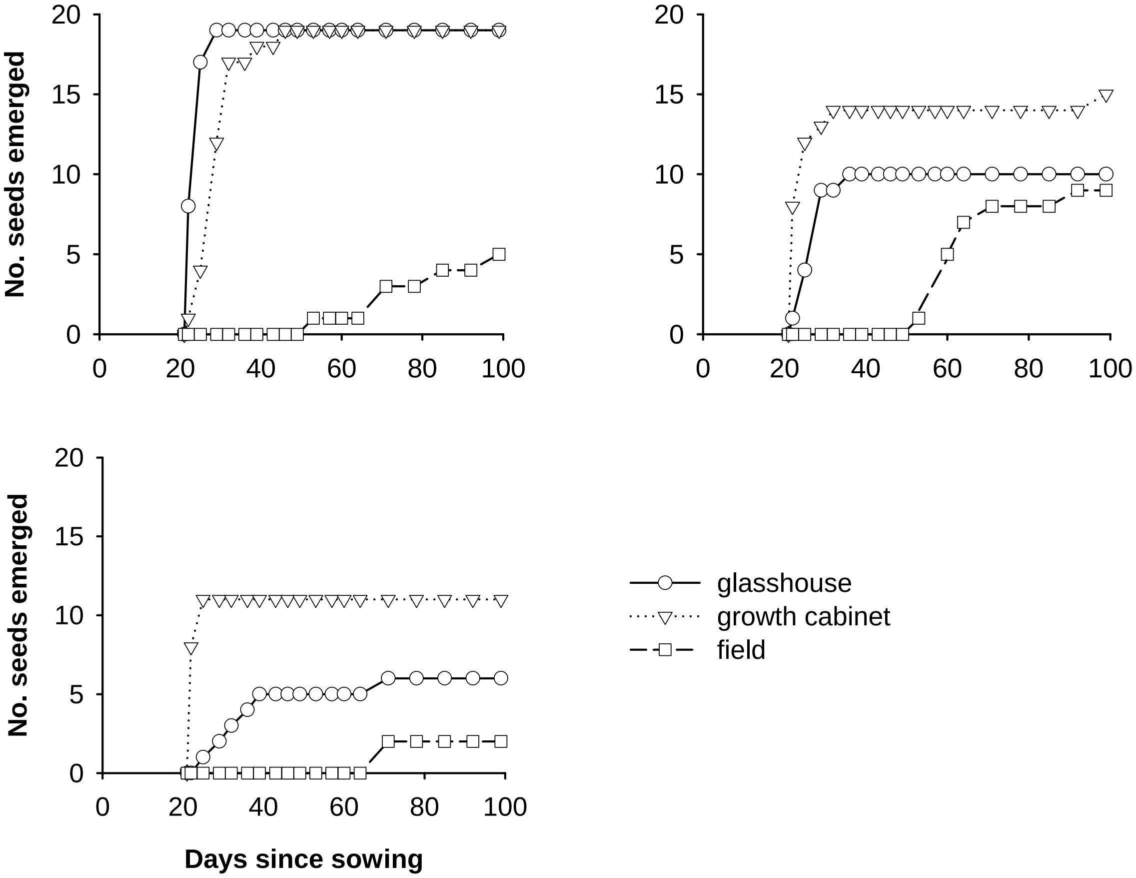


Fig. A2-3 Seed emergence for three different seed families of *Trachymene incisa* in three environments.

APPENDIX 3

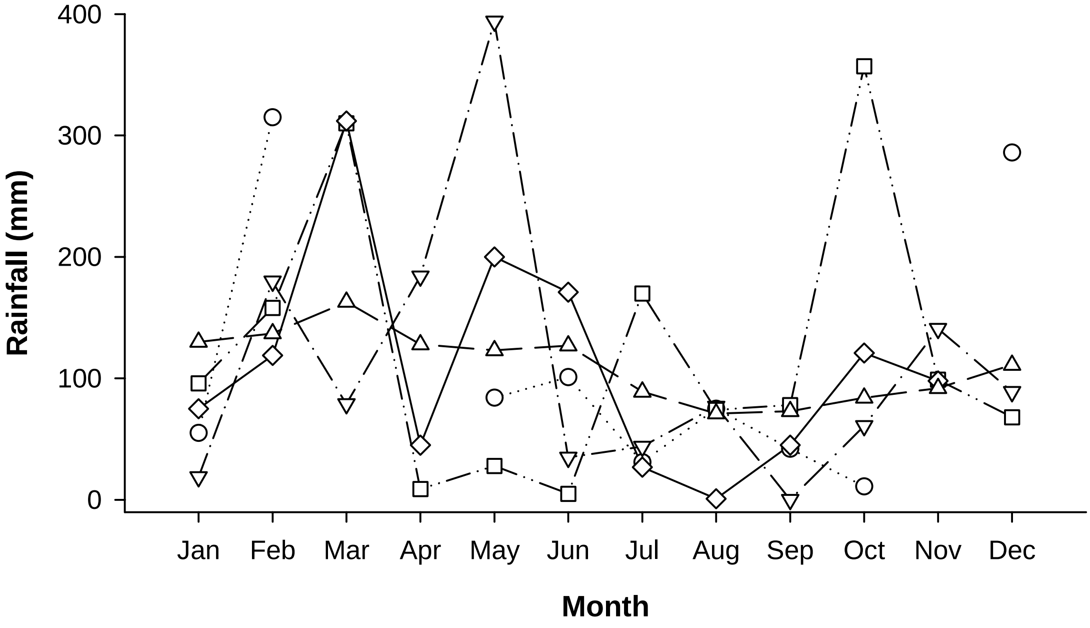
MONTHLY RAINFALL AT THREE STUDY POPULATIONS OF *TRACHYMENE INCISA* BETWEEN 2002 AND 2005

STUDY POPULATIONS AND RAINFALL PATTERNS

The three study populations of *Trachymene incisa* used in this study were located at Myall Lakes National Park, Tomago Sandbeds and Agnes Banks Nature Reserve. Weather stations were not located at these sites, however, the approximate rainfall received can be determined from nearby weather stations of the Bureau of Meteorology. The closest station to Myall Lakes was at Bulahdelah. The closest station to Tomago was located at the Williamtown RAAF. The closest station to Agnes Banks was at Richmond. The monthly rainfall between 2002 and 2005, and the long term averages are presented in Fig. A3-1. Rainfall data is missing for some months because it was not presented in the Monthly weather review (Bureau of Meteorology, 2002-2005).

Rainfall was not consistent throughout the year or among years. One notable pattern is the low rainfall in 2002 and the beginning of 2003 at all sites compared to the long term average. Monthly rainfall was often below the long term average at Agnes Banks (Richmond) throughout the course of this study.

(a) Myall Lakes (Bulahdelah weather station)



(b) Tomago (Williamtown RAAF weather station)

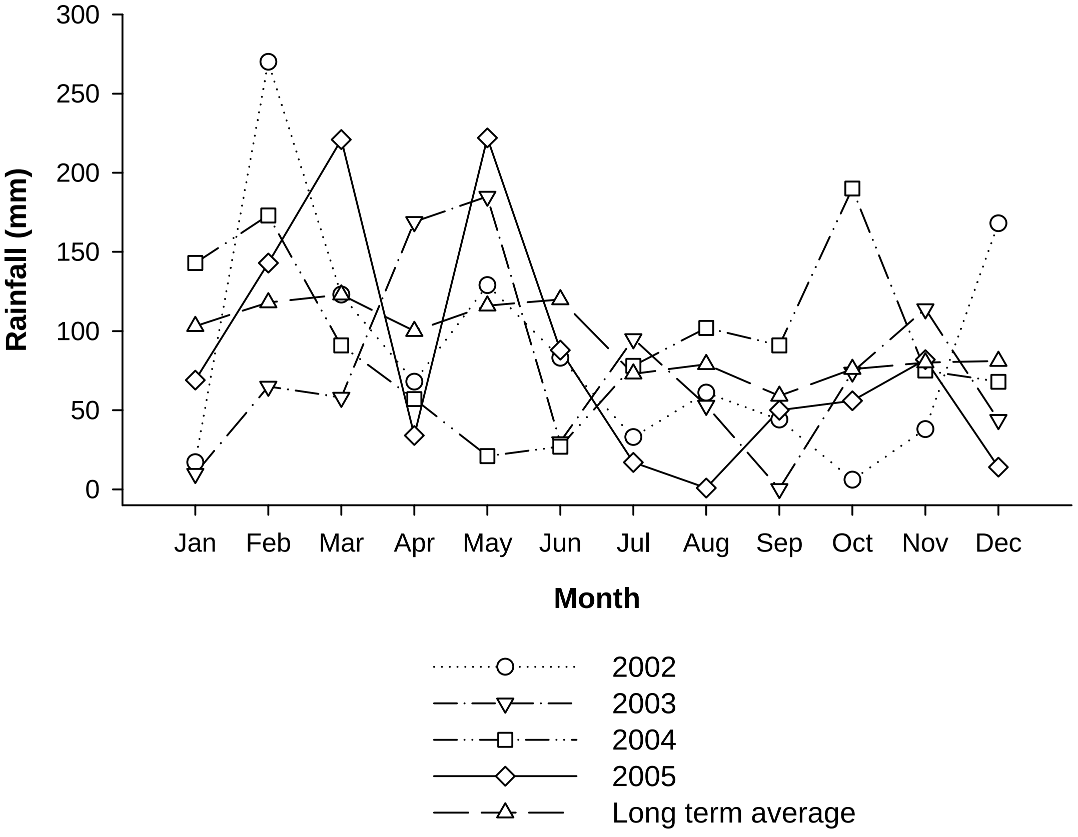
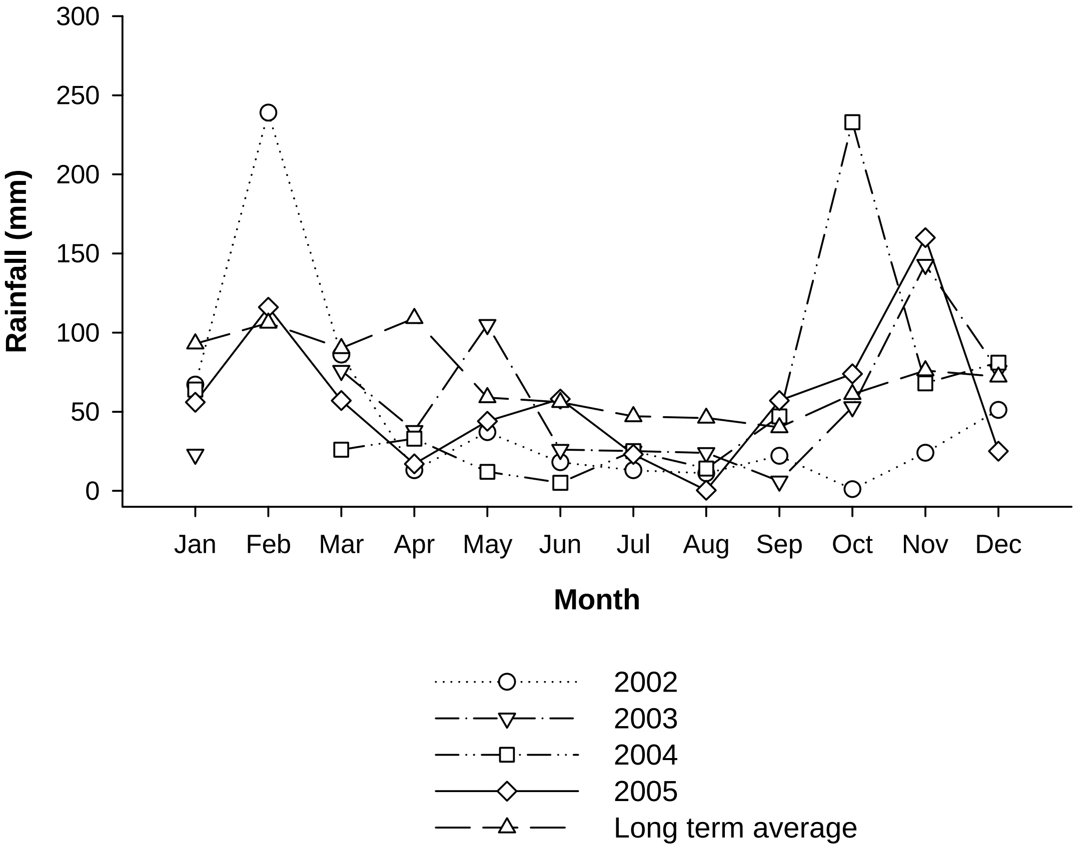


Fig. A3-1 Monthly rainfall between 2002 and 2005 at three study populations of *Trachymene incisa*: (**a**) Myall Lakes (Bulahdelah weather station); (**b**) Tomago (Williamtown weather station).



(**c**) Agnes Banks (Richmond weather station)

Fig. A3-1 continued. Monthly rainfall between 2002 and 2005 at three study populations of *Trachymene incisa*: (c) Agnes Banks (Richmond weather station).

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ABSENCE OF NOCTURNAL POLLINATION IN *TRACHYMENE INCISA*

BACKGROUND AND AIMS

Flowers that are open more than 12 hours per day are exposed to potential visitation by a variety of nocturnal insects (Young 2002). It is possible that nocturnal insects play an important role in pollination of *Trachymene incisa*, because the white flowers in the umbels remain open for up to three weeks and during the night (Davila 1999). There have been no published studies on the nocturnal pollination of Apiaceae to date.

The aim of this study was to determine whether nocturnal pollination occurs in *T. incisa*, by evaluating whether insects visit umbels during the night.

MATERIALS AND METHODS

Insect visitation to umbels was observed between 2100 h and 2200 h at five populations of *T. incisa* in 2004. The northern-most population was located at Bald Rock National Park, NSW (28°51′S, 152°03′E), on the sandy soils and in eucalypt woodland near the base of

Bald Rock. The southern-most population was located in crown land adjacent to Wairo Beach, near Tabourie Lake, NSW (35°25′S, 150°26′E). This small low density population is located in eucalypt woodland that is heavily invaded by *Pinus radiata*, and is located at the edge of *T. incisa*'s range. The Myall Lakes, Tomago and Agnes Banks populations are described in detail in Chapter 3. Insects were observed visiting tagged umbels in patches at each population over three days. Details of observation patch-setup and methods are in Chapter 3.

In addition to observations, I set up a video camera to record visitation to a patch of flowering umbels at Myall Lakes in 2004 continuously for 1.5 hours one night. The advantage of the video recorder is that insects are not disturbed by the presence and movements of people.

RESULTS AND DISCUSSION

Insect visitors to umbels were recorded at all five populations (Table A4-1). However, the visitation rate was extremely low, with the maximum recorded at Tomago, where eight visitors were recorded over three nights (3 hours). There were no insect visits recorded using the video camera. This indicates that nocturnal pollination is rare or does not occur simply because the visitation rate of insects is very low.

Most of the nocturnal visitors were ants and spiders (Table A4-1). Ants are unlikely to be pollinators because they forage for nectar at the base of flowers and rarely contact the reproductive structures (Davila and Wardle 2002). Spiders are also unlikely pollinators because they are predators on other floral visitors and are not moving between umbels. Beetles and moths were commonly caught in light traps setup at these populations during the same sampling period (Y. C. Davila, unpublished data), which indicates that these insects were present but not visiting *T. incisa* umbels during the night.

Table A4-1 Number of insect visits to *Trachymene incisa* recorded between 2100 h and 2200 h at five populations. Visits are pooled across six patches and three nights per population (3 h of observations).

	ant	spider	fly	other
Myall Lakes	2	1	1	2
Tomago	5	1	1	1
Agnes Banks	1	1	2	0
Bald Rock	1	0	0	2
Wairo Beach	1	3	0	2

Interestingly, I noticed that the umbels do not emit a scent at night but smell strongly during the day. Scent plays an important role in attracting pollinators to flowers (Proctor *et al.* 1996), and the absence of scent at night indicates the flowers are not attracting insects using scent during this time. I was unable to determine whether nectar was secreted during the night, however, the presence of ant visitors indicates there may be some nectar present in the flowers at night.

The very low visitation rates at night indicate that nocturnal pollination is rare or does not occur in *T. incisa*.

ACKNOWLEDGEMENTS

I thank A. Divljan, C. Phu, M. Williams and R. Ilsley for assistance in the field. Field work was carried out under permit from the NSW National Parks and Wildlife Service, part of Department of Environment and Conservation (NSW) (Myall Lakes, Agnes Banks and Bald Rock), the Hunter Water Corporation Ltd. (Tomago Sandbeds) and Department of Lands NSW (Wairo Beach). Support was provided by the University of Sydney, Botanic Gardens Trust, an Australian Postgraduate Award and grants from the Ecological Society of Australia, Linnean Society of New South Wales, Australian Federation of University Women (SA Inc.) and Royal Zoological Society of New South Wales.

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FLORAL VISITORS OF *TRACHYMENE INCISA*:

SUNSHINE COAST, BALD ROCK NP AND WAIRO BEACH

BACKGROUND

Trachymene incisa is a perennial herb with a large distribution along the east coast of Australia, from Fraser Island, Queensland south to Ulladulla and inland to the Nandewar Range near Narrabri, NSW (Hart and Henwood 2006). As part of my study investigating variation in floral visitors of *T. incisa*, I surveyed six populations covering approximately two thirds of the natural range of *T. incisa*. Data for three populations, Myall Lakes, Tomago and Agnes Banks, are reported in the main section of this thesis. The remaining three populations, Sunshine Coast (opposite Mooloolah River National Park), Bald Rock National Park and Wairo Beach are reported here. These populations had extremely low visitation rates and no transformations could improve this extremely skewed data. Therefore, these data were not included in the main analyses but the morphospecies of floral visitors observed are reported here.

MATERIALS AND METHODS

Insect visitation to umbels was observed at three other populations of *T. incisa* in 2004: Sunshine Coast in crown land opposite Mooloolah River National Park, Queensland (26°43′S, 153°05′E), Bald Rock National Park, near Tenterfield, NSW (28°51′S, 152°03′E), and in crown land adjacent to Wairo Beach, near Tabourie Lake/Ulladulla, NSW (35°25′S, 150°26′E). The Wairo Beach population represented the southern-most population of *T. incisa*. Insects were observed visiting tagged umbels in patches at each population over multiple days. Details of observation patch-setup and methods are in Chapter 3. At Bald Rock, the observations were extended to five days instead of three days, due to heavy fog in the morning and intermittent rain throughout the day. At the Sunshine Coast population, only one day of sampling was performed due to time limitations.

RESULTS AND DISCUSSION

A total of 16 insect visits were observed during one day of observations at the Sunshine Coast population (3 hours). At Bald Rock, 118 insect visits were observed over five days of observation (over 12 hours). At Wairo Beach, 112 insect visits were observed over three days of observation (9 hours). A large diversity of floral visitors were observed visiting *T. incisa* umbels at these three populations (Table A5-1). The day-flying moths observed at Wairo Beach were not observed at any other population of *T. incisa* surveyed between 2003 and 2005.

Table A5-1 Floral visitors to three populations of *Trachymene incisa* at three times during the day.

Population	**Morning**	**Midday**	**Afternoon**
Sunshine Coast	Native bee	Fly	Native bee Fly
Bald Rock	Ant	Ant Native bee - black Native bee – green Native bee – red abdomen Bee fly Hover fly Other fly Butterfly	Ant Native bee - black Native bee – green Native bee – red abdomen Bee fly Hover fly Other fly Beetle
Wairo Beach	Ant Bee fly Other fly Beetle	Ant Native bee Bee fly Hover fly Other fly Butterfly Moth	Ant Bee fly Other fly Butterfly

These observations confirm the generalised pollination system of *T. incisa*. These data also confirm that native bees, bee flies and ants are common visitors throughout most of the range of *T. incisa*.

ACKNOWLEDGEMENTS

I thank G. Wardle. M. Wardle, R. Ilsley and M. Williams for assistance in the field. Field work was conducted under permit from the NSW National Parks and Wildlife Service, part of Department of Environment and Conservation (NSW) (Bald Rock National Park), Department of Lands NSW (Wairo Beach), and Queensland Parks and Wildlife Service (Sunshine Coast). Support was provided by the University of Sydney, Botanic Gardens Trust, an Australian Postgraduate Award and grants from the Ecological Society of Australia, Linnean Society of New South Wales, Australian Federation of University Women (SA Inc.) and Royal Zoological Society of New South Wales.

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UV REFLECTANCE OF THE PETALS OF MALE AND FEMALE UMBELS

BACKGROUND AND AIMS

Ultraviolet (UV) light is reflected by the flowers of approximately 25% of angiosperms (Chittka *et al*. 1994, cited by Johnson and Andersson 2002). Most insects are sensitive to ultra-violet radiation and their visible spectrum covers the wavelengths 300 nm to 650 nm (Proctor *et al.* 1996). UV reflectance, in the form of floral guides and marks, is believed to play an important role in attracting and guiding certain pollinators to flowers.

The aim of this pilot study was to determine whether flowers of *Trachymene incisa* reflect in the UV range, and if reflectance differs between male and female umbels.

MATERIALS AND METHODS

Spectrometric measurements were taken over a wavelength range of 300-700nm (general insect visible spectrum) using a portable spectrometer (Ocean optics S2000) and illumination was provided by a high power xenon strobe light source (Ocean Optics PX2).

Reflectance was expressed relative to a SpectralonTM 99% reflection standard. Measurements were taken from the open petals of one male and one female umbel from plants grown in a growth cabinet (Appendix 2) from field (Agnes Banks Woodland) collected seeds.

RESULTS AND DISCUSSION

Male and female umbels reflected approximately 5% in the UV range, below 400nm (Fig. A6-1). This indicates that *T. incisa* is not using UV reflectance to guide insects or signal differences between the gender phases. The shapes of the reflectance curves are similar although the magnitude of reflectance differed between the male and female umbels between 400 nm and 700 nm. Since only one sample per gender phase was measured, it is not feasible to generate conclusions about differences among gender phases. However, these differences do generate questions regarding differences among the gender phases in terms of overall reflectance that may signal floral resource differences, gender or age. Further study is warranted and opens up an interesting line of study into the differences between the umbel gender phases, which can relate to gender function and fitness.

ACKNOWLEDGEMENTS

I thank I. Nelson who kindly provided instrumentation, information and discussion for this pilot study. Seeds were collected under permit from the NSW National Parks and Wildlife Service, part of Department of Environment and Conservation (NSW). Support was provided by the University of Sydney, Botanic Gardens Trust and an Australian Postgraduate Award.

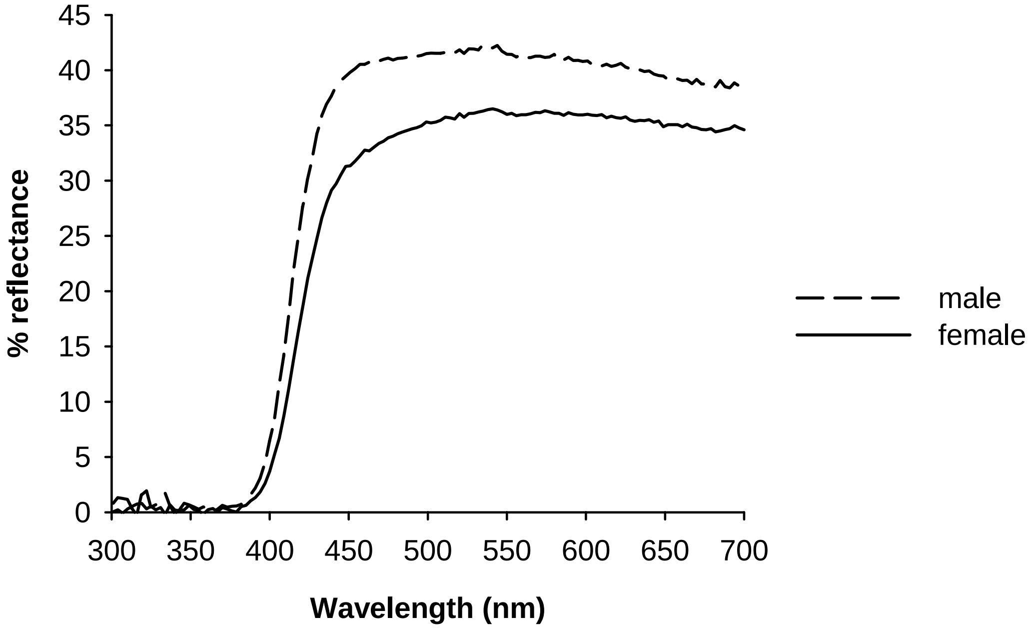

Fig. A6-1 Spectral reflectance of petals from a male and female umbel of *Trachymene incisa*, between wavelengths of 300nm and 700 nm.

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APPENDIX 7

STIGMA RECEPTIVITY IN *TRACHYMENE INCISA*: DETERMINED BY ESTERASE TEST, MORPHOLOGY AND POLLINATION

BACKGROUND AND AIMS

Previous observations of the onset of stigma receptivity in *Trachymene incisa* have been based on morphology (Davila 1999; Davila and Wardle 2002). Specifically, the onset of receptivity has been characterised as the elongation of the style and a change in the stigma from a dry tip to a wet bulbous tip. It is important to determine the onset and length of stigma receptivity for hand pollination experiments. The length of stigma receptivity also has implications for outcrossing and pollen competition. The aim of this study was to determine the onset and length of stigma receptivity in *T. incisa*, using a chemical esterase test and hand pollination and to confirm that the morphological changes observed in the style and stigma correlate with stigma receptivity.

MATERIALS AND METHODS

Several flowering umbels from plants grown in the glasshouse and growth cabinet (Appendix 2) were used in this study. Umbels were divided into circular regions (outer, intermediate and inner) to determine whether stigma receptivity is varies with positions within the umbel. Umbels were also divided into 10 “pizza” sections, and tests applied to one section each day. Tests for stigmatic receptivity (esterase test and hand pollination) and observations of morphology were carried out daily on flowering umbels from the day the umbels entered the quiescent phase (the first day after the last anthers fell off the umbel). Tests and observations continued until the umbels dried out and lost all their petals, which was usually by day 10.

Morphology

On each day the following floral morphological characters recorded: elongation of the styles, expansion of the stigma (“bulbous stigma”, Fig. A7-1a), secretion on stigma (“wet stigma”), presence of nectar, presence of floral smell, and wilting or loss of petals.

Stigmatic esterase activity

The onset of stigma receptivity was investigated using esterase as an indicator of receptivity (Kearns and Inouye 1993, p69). This test uses α-naphthyl acetate as the substrate, with fast blue B salt in a coupling reaction. A drop of solution was applied directly to one the stigmatic tips on a flower. Approximately five flowers per umbel were tested each day. Receptive stigmatic surfaces stain red (Fig. A7-1b).

Hand pollination

The remaining stigma on each esterase tested flower was hand pollinated with pollen from another plant. Pollen was applied by brushing an anther with pollen against the stigmatic tip. The ovaries were observed for swelling and initiation of seed production.

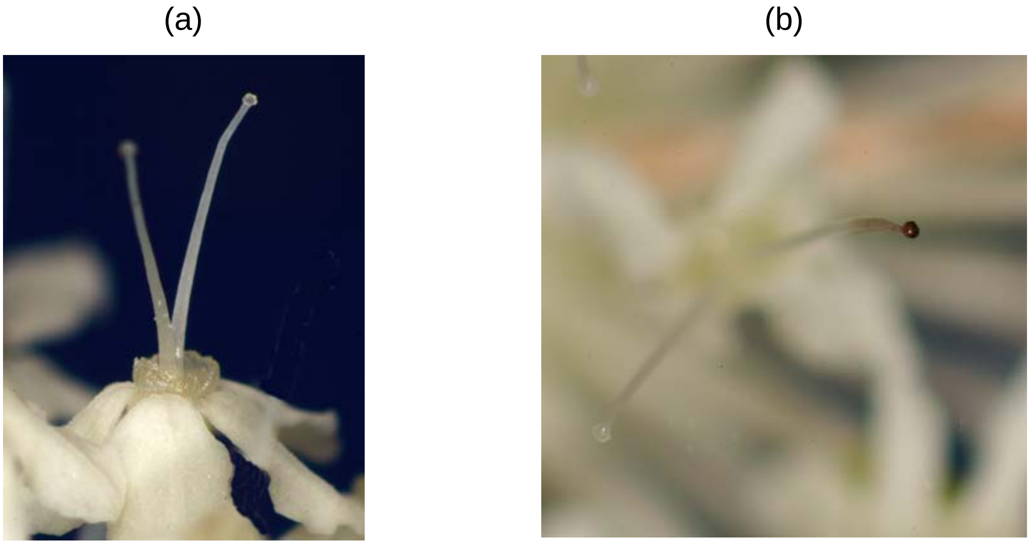

Fig. A7-1 (**a**) Female flower with elongated styles and pollen adhering to bulbous, wet stigmatic tips; (**b**) Stigmas viewed from above, with stigma on the right stained for receptivity using the esterase test and stigma on the left not stained.

RESULTS AND DISCUSSION

The morphological changes and receptivity as determined by esterase test and hand pollination are summarised in Table A7-1. The results were consistent across several umbels tested, therefore a general summary is provided.

Table A7-1 Phenology of the female phase and stigma receptivity. Results of tests for receptivity using esterase reaction, morphology and hand pollinations over 10 days, starting from the first day after last anthers have dehisced.

Day	Phase	Morphology	Nectar and smell	Petals	Stigmas stain with esterase test?	Ovaries swell after hand pollination?
0	quiescent	Styles elongated, stigmas not bulbous, stigmas dry	No nectar; no smell	Present	no	no – pollen did not adhere to stigma
1	female	Stigmas bulbous and wet	Nectar present; flowers smell	Present	yes	yes
2	female	Stigmas bulbous and wet	Nectar present; flowers smell	Present	yes	yes
3	female	Stigmas bulbous and wet	Nectar present; flowers smell	Present	yes	yes
4	female	Stigmas bulbous and wet	Nectar present; flowers smell	Present	yes	yes
5	female	Stigmas bulbous and wet	Nectar present; flowers smell	Present	yes	yes
6	female	Stigmas bulbous and wet;	Nectar present; flowers smell	Earliest pollinated flowers start to lose petals	yes	yes

Table A7-1 continued

Day	Phase	Morphology	Nectar and smell	Petals	Stigmas stain with esterase test?	Ovaries swell after hand pollination?
7	female	Stigmas bulbous and wet	Nectar present; flowers smell	Pollinated flowers losing petals; non-pollinated flowers keep petals	yes	yes
8	Post-receptive	Stigmas bulbous but dry	Nectar present in non-pollinated flowers only; faint smell	Pollinated flowers losing petals; non-pollinated flowers keep petals	yes	no
9	Post-receptive	Stigmas bulbous but dry	Nectar present in non-pollinated flowers only; faint smell only	Pollinated flowers losing petals; non-pollinated flowers keep petals	yes	no
10	Post-receptive	Stigmas bulbous but dry	Nectar dried out; flowers do not smell	Shrivelled or no petals	yes	no

The quiescent phase lasted one day, during which the styles had elongated but the stigmas were not receptive. The female phase (stigmas receptive) began two days after the male phase (pollen presentation) ended. Flowers entered the female phase simultaneously throughout the umbel, with no differences in morphology, staining ability or pollination among flowers in different positions within the umbel.

Based on morphology and hand pollination, the female phase lasts up to seven days. Pollinated flowers begin losing their petals five days after pollination, while non-pollinated flowers can retain their petals up to eight days after the female phase begins. In the field, the female phase appears to last four to five days based on morphology, and this is probably due to pollination early in the female phase and subsequent loss of petals after five days. The esterase test continued to stain stigmas well after receptivity ceased, and is not a good indicator of receptivity length.

Conclusion: morphological changes are a good indicator of stigma receptivity. The male and female umbel phases are completely separated by a quiescent phase, during which the stigmas are not receptive and pollen does not adhere to the stigma. This may be a way to guarantee avoidance of self-pollination within the flower, because the stigma and anther sit at approximately the same height (Davila 1999).

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