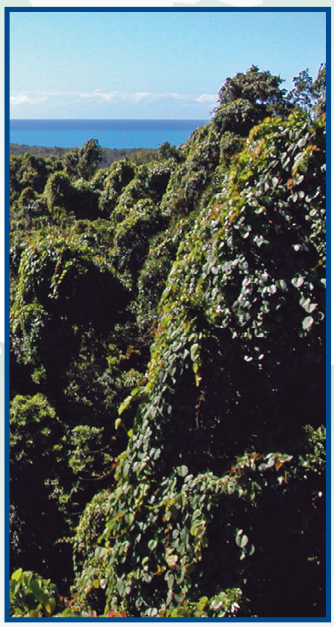
BEST PRACTICE MANUAL



Reproductive Biology and Pollination in Rainforest Trees: Techniques for a Community-level Approach

S. L. Boulter, R. L. Kitching,
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Rainforest CRC

Cooperative Research Centre for Tropical Rainforest Ecology and Management

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May 2006

Cover Images ©
(*Top*) Flower wasp on flowers of *Syzygium gustavioides* (Photo: Sarah Boulter).
(*Centre*) View of the Coral Sea from the gondola of the Australian Canopy Crane, located in tropical forest abutting the Daintree National Park at Cape Tribulation, far north Queensland (Photo: Sarah Boulter). See also Figure 3 on page 6.
(*Bottom*) Flowers of *Syzygium sayeri* (Photo: Sarah Boulter).

Layout by Shannon Hogan

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1. INTRODUCTION

1.1 BACKGROUND

The process of pollination is fundamental to the long-term sustainability of a plant. It is through pollination that seed set occurs and on which depends the genetic future of the individual. Ultimately, these interactions are expressed in the phenology and flower morphology we can identify for each plant. Plants have more reproductive options than do most animals. They can reproduce vegetatively: essentially perpetuating almost the exact genome of the parent tissues (apart, presumably, from a few mitotic copying errors). They can reproduce sexually from gametes, which they themselves produce ('selfing'), resulting in a remixed genome which will, on average, but not individually, be identical with that of the parent. Lastly, they can do as most animals do: reproduce sexually by outcrossing with a different individual of a greater or lesser level of relatedness to the focal plant. The science of pollination biology has focussed substantially on this process of outcrossing and the agencies that facilitate the gene transfer involved.

It has been clear, at least from the time of Sprengel's pioneering work in 1793, that animals are involved in a majority of angiosperms as the agents by which male gametes (pollen) are transferred to female receptive surfaces (stigma). The physiological, behavioural, ecological and evolutionary aspects of this process form the substance of pollination biology. When we add to this subject issues dealing with the growth, survivorship and mortality of the flowers themselves, we circumscribe so-called 'Pollination Biology'.

Like most aspects of field biology, pollination biology has its historical roots in the temperate zone. In his authoritative history of the subject, Baker (1983) defines two phases of the development of the subject:

- The 'Old Testament' reflects the compilations of anecdotal observations on whole floras culminating in the massive three-volume work of Knuth (1898-1905).
- The 'New Testament', as defined by Baker, attempted to draw from the preceding encyclopaedic knowledge base sets of generalisations that allowed predictions on pollination mechanisms even for those species for which detailed observations were not available.

These 'pollination syndromes', as defined by Faegri and van der Pijl (1979), identify sets of characters associated with particular known classes of pollinators. This 'syndrome approach' to generalisations about pollination is still very much alive, although it can 'straight-jacket' thinking on the topic if applied in too polemical a fashion. Throughout this period, however, pollination biology was undoubtedly approached in an autecological fashion with individual, species-based accounts accumulated so that generalisations emerged in a 'bottom-up' fashion (where they emerged at all).

It was during this phase of development, also, that studies of tropical pollination systems began to emerge as access and interest in tropical forests, in particular, emerged. Refocussing on the tropics drew attention to novel systems, rare or absent in the temperate zone (such as pollination by bats and other mammals) while underlining further just how great was our ignorance of pollination in all but a few highly selected species.

Baker, writing in 1983, introduces the idea of ecosystem-level approaches to pollination and reviews important early work on the topic by Moldenke (1975, 1976) in California, Moldenke and Lincoln (1979) in Colorado and by Hocking (1953, 1968) in the Canadian Arctic. These syntheses represented the first steps in community-level anthecology and represent a

quantum leap forward in understanding both the ecosystem level emergent properties of floral biology and the amount of data needed to produce useful generalisations. This 'top-down' approach permits a wide range of 'new' interrelated questions to be addressed, including:

- Are there patterns of flowering phenology characteristic of particular ecosystem types, or of particular spatial components within ecosystems, or levels of disturbance?
- Does the taxonomic composition of a plant assemblage affect its flowering phenology?
- Are particular sets of pollinators more or less dominant in particular ecosystem types, or the same ecosystem type in different biogeographical regions?
- Do the pollination mechanisms of plants species within an assemblage compliment each other?
- Are the flowering and pollination patterns of flowers within an assemblage co-evolved, or assembled accidentally?
- To what degree are the pollination mechanisms of a focal species of plant affected by the comparable mechanisms of surrounding plants?
- How are pollination patterns and mechanisms affected by habitat fragmentation and other anthropogenic changes in ecosystem quality?

Over the past seven years we have been engaged in a research programme on the pollination landscape of the Wet Tropics bioregion of tropical Queensland. Our approach has been a ‘top-down’ one. The rainforests of Australia's Wet Tropics form a well-defined and floristically well-known set of ecosystems that are well served by keys (see Hyland *et al.* 2003), herbaria and taxonomic specialists. A handful of autecological studies of pollination in the region exist (see Boulter *et al.* in review for a summary), as do attempts at broad generalisation stemming from single species studies and pollination syndrome-style estimates (Irvine and Armstrong 1990). An approach beginning with the phenology and phenomenology of the whole woody flora, however, has not hereto been attempted. Our 'top-down' approach does include selected autecological studies of single species of woody plant (Figure 1).

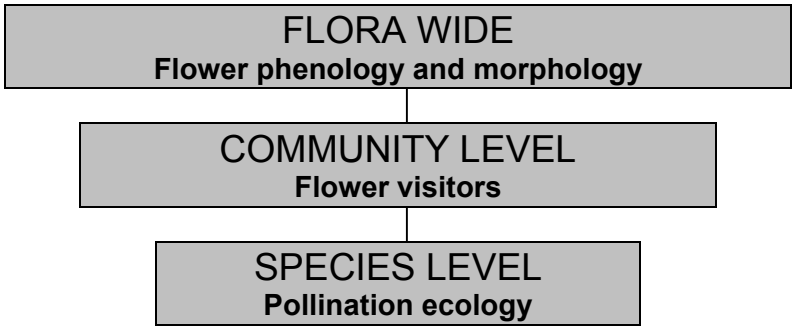

Figure 1: Schematic of the ‘top-down’ approach of the Rainforest CRC pollination project.

In 1998, the Cooperative Research Centre for Tropical Rainforest Ecology and Management (Rainforest CRC) successfully attracted funding to establish the Australian Canopy Crane Research Facility. Subsequently, a fixed tower crane was erected in the lowland rainforest of the Daintree in North Queensland. This structure provides complete three-dimensional access to a hectare of primary (although cyclone-impacted) lowland rainforest. This has become the local focus of our pollination research and the place where we have performed experimental manipulations of selected target species.

This manual summarises the techniques we have used since 1998. We have approached the manual by first describing techniques for studying the flora and flowers, and include accounts for studying animal associates of flowers before moving on to techniques designed specifically to examine the animal-plant interactions. A brief account of the physiological and genetic techniques we have used to support our field studies follows. We conclude by discussing the technical aspects of assessing the conservation implications of our studies.

This manual is intended as a resource for other researchers and students of rainforest plant reproductive ecology, land managers wishing to understand the reproductive ecology of forested areas under their care, and landholders wishing to better understand native crop pollinators.

1.2 THE WET TROPICS

Although rainforests cover only about 0.3% of Australia, they contain about 60% of all Australian plant families and about a third of Australia's mammal and bird species.

The Wet Tropics bioregion of far north Queensland stretches from the Black Mountains in the north (south of Cooktown), south to the Paluma Ranges region (north of Townsville) (Figure 2). Its rainforests make up a mere 0.18% of Australia, but contain about 30% of Australia's marsupial and frog species and 62% of its butterfly species. The flora of the Wet Tropics bioregion is unique for a number of reasons. First, rainforest is dominant throughout the entire bioregion (approximately 1.8 million hectares, Sattler and Williams 1999). Second, the area has a high floristic diversity and a high species to area ratio (Myers *et al*. 2000), with approximately three thousand species of plants recorded in the bioregion. Third, the area has a high level of endemism, with more than seven hundred (23%) species endemic to the region (Sattler and Williams 1999). In addition, there is a high generic diversity, a high (66%) incidence of monotypic genera (Gross 2005), and a high diversity of woody, phylogenetically basal, angiosperms (Worboys and Jackes 2005). The forests are also notable for their distinctive Gondwanan taxa (Webb and Tracey 1994), particularly in the uplands. Unlike many equatorial areas of rainforest, the Wet Tropics region can be categorised as 'seasonally dry' (van Schaik *et al*. 1993), with at least five months of the year receiving less than sixty millimetres of rainfall on average (Gross 2005).

Figure 2: Map of the Wet Tropics bioregion showing the location of the Australian Canopy Crane research facility at Cape Tribulation.

1.3 CANOPY ACCESS

A significant proportion of flowering and fruiting occurs in the canopy of a rainforest. Traditionally, access to these flowers has been restricted to the use of single rope techniques, although the obvious limitation of this method is the frequent positioning of flowers or inflorescences at the extremes of branches not readily accessible from a climbing position close to the tree trunk. As a result, a preference for the study of understorey species or cauliforous species is seen in the literature (see Table 1, Boulter *et al*. in review). As an alternative, pollination ecologists have climbed neighbouring trees (Worboys and Jackes 2005) and hauled traps into the canopy to access flowers (House 1989). The development of permanent canopy access structures such as canopy walkway systems (van Dulmen 2001) and canopy cranes (Mitchell *et al.* 2002; Boulter *et al.* 2005) have allowed improved access to canopy studies in a number of locations worldwide and this has been the case for this research programme.

The fieldwork for this project was conducted at the Australian Canopy Crane Research Facility. The crane is located at forty metres elevation in lowland tropical rainforest at Cape Tribulation, 140 kilometres north of Cairns in Queensland, Australia (16° 17' S, 145° 29' E). The crane is a Liebherr 91 EC, freestanding construction tower crane, and is 47 metres tall with a radius of 55 metres, enabling access to just over one hectare of rainforest. A gondola is suspended from the jib of the crane, which allows three-dimensional access to the canopy (Figure 3) and, in particular, ready access to flowers on the outside of the canopy. The crane plot supports approximately 680 trees of *dbh* >10 cm from 34 families and some 86 species. Tree identifications, tree *dbh* and tree heights have been surveyed twice now (first in 2000 and again in 2005) and the resultant database is held at the Australian Canopy Crane Research Facility.

For sites not accessible by the canopy crane (e.g. fragmentation studies), we have hauled traps into the canopy. Many alternatives exist for getting an initial line into the canopy (see Mitchell *et al.* 2002 for some other techniques). We used two simple alternatives. For flowers high in the canopy we used a compound bow and a modified arrow. The arrow has a blunt weighted head (instead of a point) and a trace line along its length to which a fishing swivel is attached. This swivel can slide up and down the length of the arrow. The bow has a reel attached to it to enable fishing line to be attached to the arrow. The advantage we found with using the bow and arrow was the accuracy we could achieve at all heights. With practice, grabbing the fishing line once the arrow is over the branch will halt the arrow and help drop it down on the other side of the branch for easy hauling. The second method was simply to attach a line to a rock and use a slingshot. This technique was useful for low branches where the bow and arrow provided excessive force. Once an initial length of fishing line was over a suitable branch we could attach a length of nylon sash cord and pull it over the branch using the fishing line. This nylon line was then used to pull up the traps. It is important to have a guide rope/line attached to the traps in order to steer traps past other branches and retrieve traps at the end of sampling.

Figure 3: The tower of the Australian Canopy Crane research facillty, and (inset) the crane gondola. Photos courtesy of Craig Lockhart and Sarah Boulter.

1.4 TECHNIQUES FOR POLLINATION STUDIES

Pollination ecology describes the relationship between a plant and its pollinator and as such must consider the contribution and restraints each brings to the relationship. This relationship inevitably involves a degree of conflict. For example, Feinsinger (1983) proposed that the optimal pollen vector, from the plant's perspective, would move rapidly between plants and remain faithful regardless of any other species flowering within the community. A "harried, underfed, yet constant pollinator" would be ideal. On the other hand, foraging theory suggests that the pollinator will seek to remain well fed and minimise effort for reward. It is the dynamic conflict between these two optimal states (one from the view of the plant, one of the pollinator) that is expected to drive plant-pollinator evolution.

The pollination tradition has been built on the assumption that plants offer floral rewards and evolve morphological adaptations to attract and/or accommodate pollinators. By attracting the most effective pollinator to a specialised structure, the animal gains rewards and the plant facilitates successful reproduction. The floral trait therefore, is assumed to be the result of selection pressures from effective pollinators. The division of disparate plant lineages into relatively few floral types or syndromes (as alluded to above) and the predictable association with higher taxa of animals has been taken as *a priori* evidence of the adaptive nature of plant-pollinator relationships (Herrera 1996). But this is essentially a circular argument. Observation may be useful to generate hypotheses about the value of a floral trait, but it cannot be used to test these hypotheses (Waser 1983). More specific evidence to confirm the impact of selection pressures is often difficult to obtain (Waser 1983). It does however emphasise the integral relationship between the characteristics of the plant and its associated pollen vector. The study of pollination ecology must therefore consider features of both the plant and its flower visitors in order to understand the impact of one on the other and acknowledgement of this has underlain the approach we have taken to our autoecological pollination studies. A general schematic of this is presented in Figure 4 and some of the questions associated with each aspect noted.

There are many techniques that can be employed to determine the pollination system of a species (see Kearns and Inouye 1993 for an extensive treatment of this subject) but time, resources and desired outcome will limit those techniques used. We present in this manual those techniques that we have used successfully in the course of our rainforest canopy pollination project.

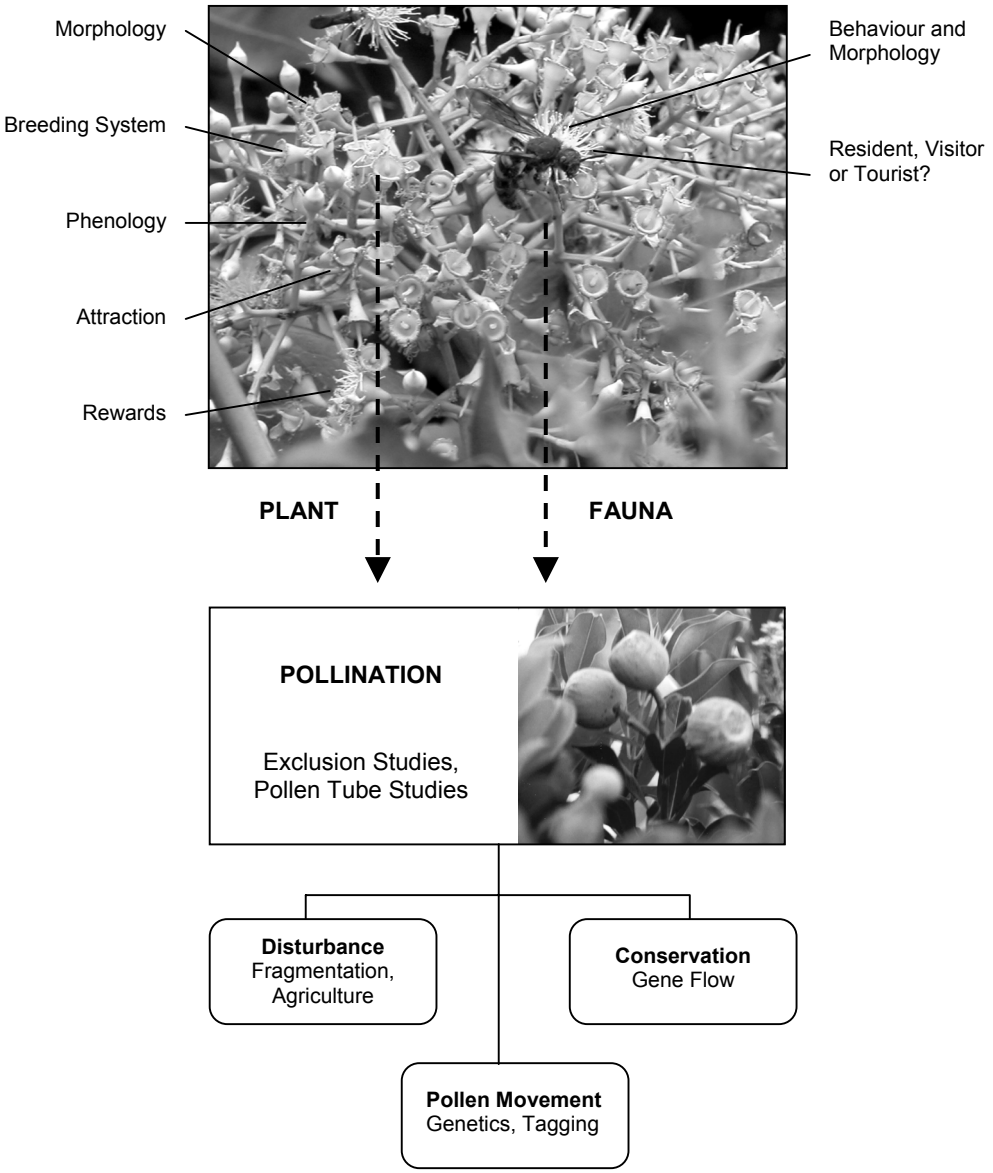

Figure 4: Conceptual diagram of studies in pollination ecology.

2. THE FLOWERS

2.1 GENERAL INTRODUCTION

Flower morphology, physiology and flowering phenology all play a role in the reproductive fitness of individual plant species. Morphology – the shape, colour, flower architecture and offering of rewards – determines a flower's attractiveness to visiting fauna and the efficiency of pollen transfer to those visitors (Faegri and van der Pijl 1979; Muchhala 2003). The level of self-compatibility determines the effectiveness of different potential pollen vectors (Williams and Adam 1994; Murawski 1995; Kenta *et al.* 2002), and the simple timing of flowering will determine the available array of potential visitors (Auspurger 1981; Rathcke and Lacey 1985; Bishop and Schemske 1998).

In this section we consider the function of flower morphology, the timing of flowering, sexual systems, how some these features might act as attractants or rewards to flower visitors and how they might operate in favour of some flower visitors and the plant itself.

2.2 PHENOLOGY

2.2.1 Introduction

Plant phenology is concerned with the timing of recurring events such as leaf flushing, flowering and fruiting. Understanding the timing of these events is important for understanding the ecology and evolution of species and communities (Newstrom *et al.* 1994). For example, the timing, intensity and duration of flowering among plants dictate the success of a plant's reproductive cycle and in turn the success of those animals relying on the plant resources resulting from this process (e.g. pollinators and frugivores).

In temperate regions, climatic conditions show marked seasonal variation and phenological events show a distinct seasonal rhythm (e.g. flowering in spring). In this case the correlation between the timing of fruiting and flowering and climatic conditions is clear. In contrast, tropical systems have conditions favourable to flowering (i.e. temperature and rainfall) available year round and a diversity of flowering patterns is observed (Newstrom *et al.* 1994; Bawa *et al.* 2003). Understanding these patterns requires large data sets spanning many years to understand the full breadth of the rhythm of flowering

Flowering patterns in tropical floras can vary in a number of ways. First, they can vary in intensity, timing and duration. Newstrom *et al.* (1994) outline a classification system of flowering phenology that incorporates these flowering patterns, namely:

1. **Frequency** (the number of on/off cycles per year):
   - *continual* (flowering with sporadic brief intervals).
   - *sub-annual* (flowering in more than one cycle a year).
   - *annual* (only one major cycle per year).
   - *supra-annual* (one cycle over more than one year).

2. **Duration** (length of time in each cycle or phase):
   - *brief flowering (less than one month).*
   - *intermediate flowering (one to five months).*
   - *extended flowering (longer than five months).*

3. **Amplitude** (intensity or quantity of flowering).

In addition, Newstrom *et al.* (1994) suggest the variables 'regularity', 'date' and 'synchrony' can be used to describe flowering patterns. Measuring any or all of these variables will assist in understanding the interaction of a plant's flowering and the activities of its pollinators.

The timing of flowering dictates the array of available visitors. For example, flowering may coincide with the seasonal movement of migrating vertebrates or periods of heightened insect activity (Rathcke and Lacey 1985; van Shaik *et al.* 1993). Indeed, a number of studies have gone so far as to suggest that the flowering patterns of plants are, in general, an adaptive response to the availability of suitable pollinators (Waser 1983). Whether flowering phenology is under strong pollinator or predator selection or is responding to optimal abiotic factors (i.e. climate), or whether flower phenology might be best explained by phylogeny (i.e. evolutionary history) is a matter of considerable debate (Ollerton and Lack 1992; Wright and Calderon 1995; Boulter *et al.* 2006).

Pollinators also may respond to the intensity of flowering. The occurrence of mass flowering in some floral groups (e.g. Dipterocarpaceae) or aggregate flowering is thought to increase the overall attraction of pollinators (Ashton *et al.* 1988; Gross *et al.* 2000; Ghazoul 2006). This in turn might increase the probability of a pollen vector visiting the flowers of any particular plant (Rathcke and Lacey 1985).

Flowering patterns can be observed at different time scales. Some phenological observations are made of *daily phenomena*, such as time of anthesis, time of nectar production and so on. Others are interested in *yearly occurrences*, such as timing and duration and intensity of flowering (records may be kept for several years). Finally, *long-term* behaviour may be of interest (e.g. mass-flowering episodes) (records are kept for more than ten years). By the same token, analysis can be performed at a variety of levels, i.e. from flower, to whole plant, to population, to community.

2.2.2 Techniques for Studying Flower Phenology

See Appendix 1 for a suggested list of equipment for observing flower phenology.

In our project we looked at three levels of flowering information; (a) flowering information for the entire Wet Tropics flora (Boulter *et al.* 2006); (b) flowering trends in individual trees at our study sites over one to two years; and (c) tracking the opening of individual flowers. Our interest lay largely in looking at the timing and intensity of flowering patterns. These techniques could be employed over multiple years or seasons to give a greater understanding of long-term trends.

Entire Flora

With little known about the patterns of flowering in the Wet Tropics, we set about identifying flowering patterns for individual plant species and looking at flowering patterns for the entire Wet Tropics flora (Boulter *et al.* 2006). In the absence of available long-term monitoring records, we relied on herbarium records to identify the timing of flowering. Using a list of trees, shrubs and, later, vines (Boulter *et al.* in review) derived from Hyland *et al.* (2003), we sifted through all herbarium specimens collected in the Wet Tropics. Where flowers or buds were present, the month, altitude and latitude of collection were recorded. These data have been incorporated into a large database of some 30,000 flowering records. From this, we can characterise the flowering phenology of individual species, see some indication of flowering intensity at any time of the month, identify different flowering trends at different altitudes or latitudes and identify flora wide trends. All of the above is dependent on the availability of sufficient records to show trends accurately.

Individual Trees

During each visit, we record the number of buds, male flowers, female flowers and fruits. For branches with greater than two hundred buds / flowers / fruits, an estimate of the total number can be made by counting the flowers / buds / fruits on a number of branches or sub-branches and multiplying the average count per unit (branches or sub-branches) by the total number of units.

Individual Flowers

By observing the phenology of individual flowers, we can determine several characteristics of a plant's reproductive ecology. These include timing of bud opening, anthesis, pollen dehiscence, and flower abscission. These observations simply require marking or tagging individual flowers, visiting them at regular intervals and recording the physical state of the flower.

In our experiments we used retail swing tags each marked with individual numbers and looped around individual flowers (Figure 7). We recommend using pencil to number the tags, as this will survive wet weather. Retail swing tags in various sizes are readily available from newsagents and stationery suppliers. Other researchers have used coloured thread to code individual flowers (Kearns and Inouye 1993). We found the swing tags allowed a very large number of flowers to be tagged and were simple to attach to flowers. A few fell off, but generally only in extremely wet weather.

Individual flowers are tagged at the bud stage. The date, time and state of the bud are recorded at this time and on subsequent visits (see Table 1 for an example of a data recording sheet). Return visits are made every two to three hours, depending on the ease of access to the field site. We recommend visiting in the early morning, at midday and in the late afternoon as a useful minimum, particularly if night visits are impractical.

Table 1: Example of a phenology record sheet for tracking the opening and senesce of individual flowers.

Flower Number	27/03/06 06:00	08:00	10:00	12:00
24	bud	bud	bud splitting	bud splitting
25	Open; 5 stamen	10 stamen; Style protudes 2 mm	20 stamen; Style protudes 4 mm	25 stamen; Style protudes 5 mm

The kind of information recorded at each visit includes splitting of the bud; protrusion of the style or stigmas; signs of anthesis; the presence of nectar; deterioration of the flower (e.g. loss of anthers, browning); and senescence and abscission of the flower.

This phenological observation was combined with nectar measurements and testing for stigmatic receptivity (see Section 2.5).

Flowering Patterns in the Wet Tropics

Using herbarium data, we can see patterns of flowering for some 1,575 species of tree, shrub and vine from the Wet Tropics bioregion. A simple analysis of the number of species recorded flowering in any given month shows that an annual rhythm in flowering exists for the Wet Tropics flora. An increase in flowering activity coincides with the beginning of the wet season (October to November) (Figure 5). This pattern is equally represented in the vines, trees and shrubs. We have also used more complicated methods of calculating the peak flowering month for every species and these results show a similar trend (see Boulter *et al.* 2006 and Boulter *et al.* in review for further discussion of these results).

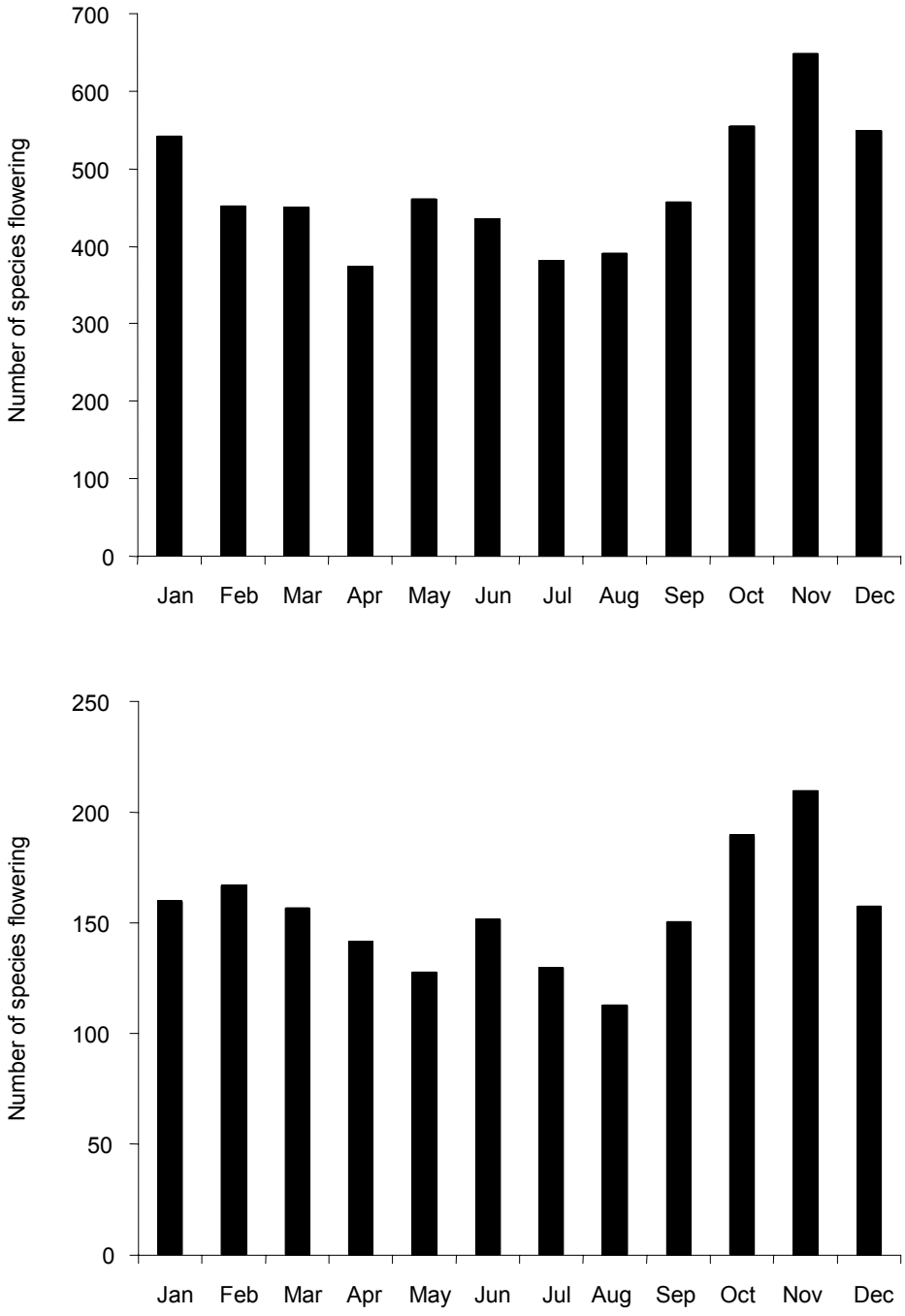

Figure 5: Total number of (*top*) tree and shrub species (reproduced from Boulter *et al.* 2006), and (*bottom*) vine species recorded flowering in any given month in the Wet Tropics bioregion.

Case Study – Flowering Patterns of *Syzygium gustavioides*

Our interest in the phenology of individual trees was to determine the population level flowering patterns of trees found within the canopy crane plot. We followed the flowering of three plant species in detail. All reproductive trees of the species of interest were visited fortnightly, and an estimate of the number of inflorescences on each tree as well as the proportion of those in flower or bud was recorded.

In the case of *Normanbya normanbyi*, all reproductive trees within the study plot were visited at least fortnightly from the start of flowering in February 2003 through to the end of flowering in October.

The canopy giant *Syzygium gustavioides* seemed to flower year-round at the canopy crane site. We visited the five individuals known to flower on site every fortnight over the course of two years. For each tree, we recorded the approximate number of inflorescences and the proportion of those in bud, flower or fruit. Over the period of two years we built up a profile of flowering, which showed two increases in flowering intensity, first in March and again in November to December (Figure 6).

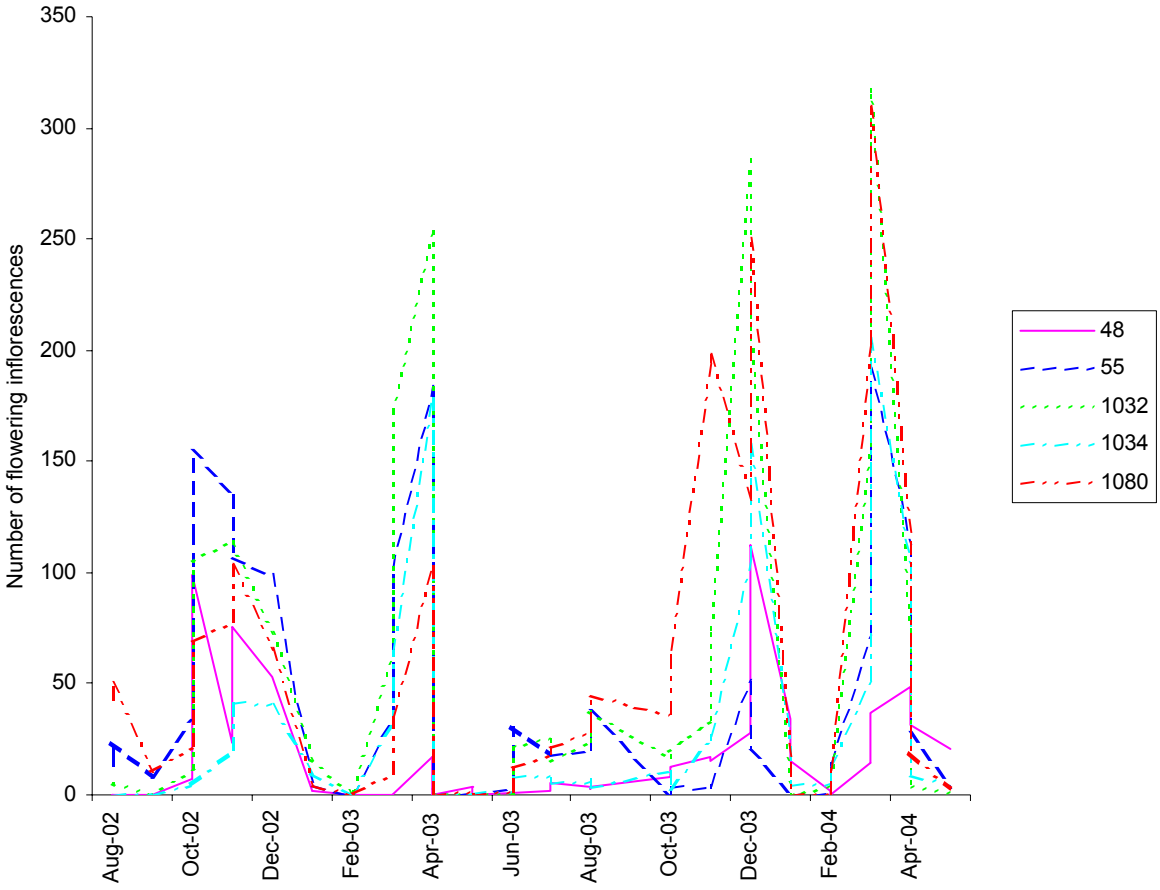

Figure 6: Approximate number of inflorescences in flower per *Syzygium gustavioides* tree within the one-hectare canopy crane plot. Each coloured line represents an individual tree.

Figure 7: Individual flowers of *Syzygium sayeri* marked with retail swing tags for phenological observations.

Case Study – Flowering Patterns of *Normanbya normanbyi*

The male and female flowers of the Wet Tropics endemic monoecious palm, *Normanbya normanbyi*, were closely observed every two hours over a 48-hour period. Using the phenological techniques described above, we identified that the male flowers opened just before dawn and abscised at dusk of the same day (see Figure 8 below). Knowing this meant we could exclude night-active moths as potential pollinators of this species.

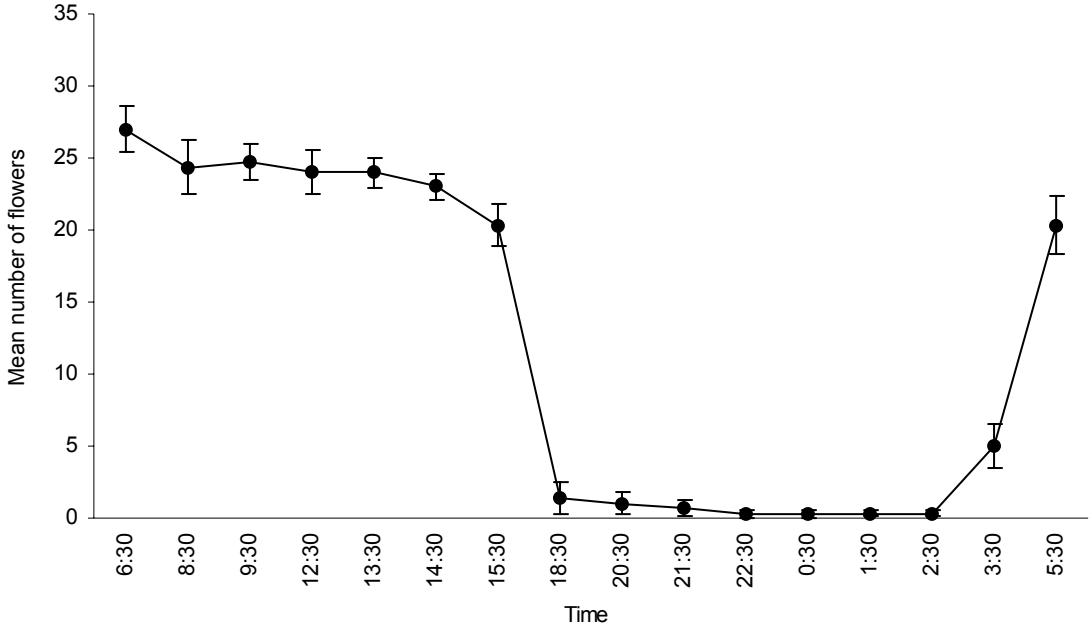

Figure 8: Mean number of open male flowers on four individual *Normanbya normanbyi* branchlets observed over a 24-hour period.

2.3 FLOWER MORPHOLOGY AND ATTRACTION

2.3.1 Introduction

Flower Structure

There is a diverse array of flower morphologies in the angiosperms. Some of these morphologies represent slight variations on a basic floral structure, while others offer examples of extreme modification, fusion or loss of various floral parts (e.g. *Ficus, Pseuduvaria*). Nonetheless, the flower has become a distinctive feature of this phylum and an important diagnostic feature with respect to identification and classification. More importantly, the floral features play a pivotal role in sexual reproduction. Ultimately, the form or morphology of the flower influences the removal and deposition of pollen and hence the success of sexual reproduction in the plant.

The basic structure of the angiosperm flower consists of four whorls of modified leaves, calyx, corolla, androecium and gynoecium (Figure 9). These whorls are attached to the **receptacle** – the swollen tip of the peduncle (a modified stalk). The first two whorls are infertile and have various functions with respect to pollination. The first whorl consists of the sepals (together, the **calyx**) that generally enclose the flower bud and offer some form of protection during this early stage of flower development. The calyx can also form part of the floral display after the flower has opened. The petals (together, the **corolla**) make up the second whorl and are often coloured and function as a form of advertisement or visual attractant to various animal visitors. Different size and structure / arrangement of the petals can influence the size class of visitors able to access any nectar offered. This is particularly the case if the nectar is located in nectaries or glands at the base of long spurs or in other locations where access is limited to animals with mouthparts of a particular size (e.g. short-billed birds or insects with a long proboscis) or shape. Other modifications include petals that offer landing platforms or guides that direct the visitor to nectar or pollen offered by the flower. The **perianth** is the collective term for these first two infertile whorls in the basic flower. In some groups of plants these first two whorls are fused or show little differentiation in structure, as evident in the Myrtaceae.

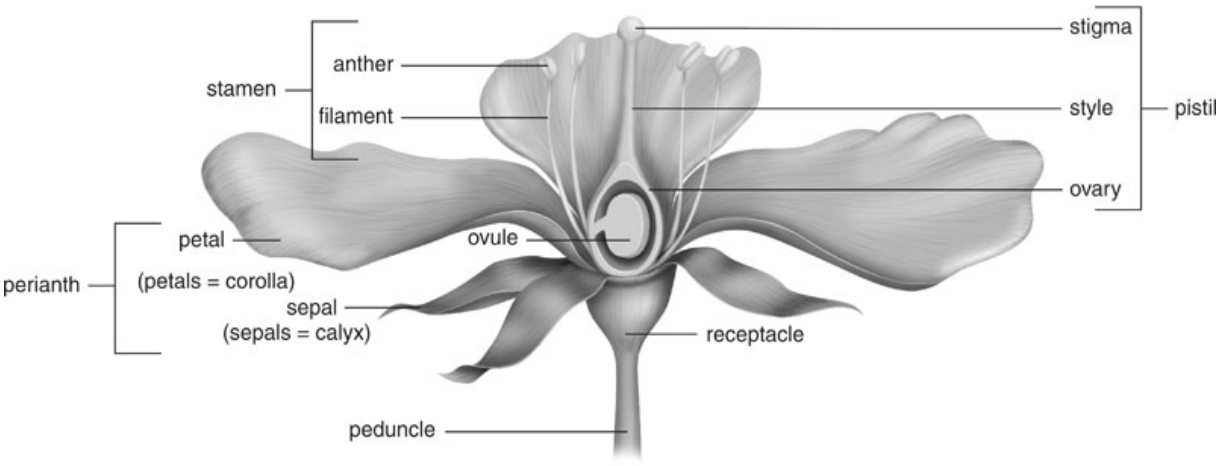

Figure 9: Schematic of the basic structure of the angiosperm flower presented here with the major function parts labelled. (Source: Stern *et al.* 2006)

The second two whorls represent the fertile parts of the flower, the male and female parts. The **anthers** produce the pollen grains and are attached to the flower via a stalk or filament. The way the anther is attached to the filament and the height of the filament determine the orientation of the anther with respect to the other floral parts. Plants that rely on a biotic vector for pollen movement require the visitor to come into contact with the anthers at the time of pollen release. Generally, pollen production is such that many visitors potentially can act as pollen dispersers. The Orchidaceae and some members of the Apocynaceae have pollen packaged into a single structure – the pollinium. Orchids have only one joined pair of such pollinia and successful pollination is dependent on a single visitor transferring the pollinia to another flower. It is not surprising that in this family of plants very specific relationships between plant and pollinator have evolved. In contrast, the Apocynaceae produce up to five pollinia per flower, although this still represents a strategy of relying on relatively few visitors for successful pollen transfer. Plants using wind as a vector generally produce large quantities of pollen and have anthers that are exposed to the air.

The final whorl of the flower contains the female structures, the **stigma**, **style** and **ovary** (together, the **pistil**). Compatible pollen grains germinate on the stigmatic surface and grow through the tissue in the style towards the ovary. Successful fertilisation causes the ovary to expand in preparation for fruit development. Considerable variation exists in the size, shape, position and orientation of the carpel. The function of the carpel is to enable pollen to be deposited onto the stigmatic surface and this can only occur if the pistil comes into contact with a visitor carrying pollen.

The basic floral structure contains both male (anthers) and female (pistil) parts and is functionally hermaphroditic. Differences in the maturation times of these parts will separate these functions temporally and, by so doing, promote outcrossing. Some flowers are unisexual and are only ever functionally male or female. The arrangement of these unisexual flowers within and among plants influences the mating system in these groups of plants. Ultimately the same process is required – transfer of pollen from male structures to female structures among flowers of the same species.

Flowers can be solitary or grouped into inflorescences on individual plants. Considerable variation exists in flowering patterns with respect to the number of flowers produced, the spatial arrangement of flowers and the timing of maturation of the reproductive structures. The physical size of the flower or inflorescence will have a bearing on the size and weight of the animal that is able to visit and not dislodge or damage the flower. The large sturdy inflorescences of some taxa allow small mammals and birds to visit as well as the smaller invertebrate visitors (e.g. Proteaceae, Proctor *et al.* 1996; *S. sayeri,* Boulter *et al.* 2005). Other flowers with more delicate structures can only cope with smaller, lighter invertebrate visitors (e.g. *S. gustavioides,* Boulter 2003).

Many other unique morphological characteristics or specialised structures in flowers have been identified that enable successful pollination by particular vectors (e.g. Sazima *et al.* 1993; Sakai *et al.* 2000). These specialised modifications do not necessarily exclude other successful pollinators, but certainly floral structural attributes or filtering mechanisms (Table 2; Stiles 1981) can allow access by certain visitors or exclude others.

Flower Morphology as Advertisement

The type of floral display influences the types of visitors likely to be attracted to the flowers. The structure of the flower in turn influences how the visitor comes into contact with the reproductive structures. Attraction of flower visitors is usually achieved through a combination of advertisement (e.g. colour and scent) and rewards (e.g. pollen and nectar). Visual features, such as colour, scent and shape are assumed to act as an attractant to flower visitors (Faegri and van der Pijl 1979). Extensive experimental work has sought to test the strength of these associations (Weis 1991). The role of colour has almost certainly been overemphasised (Johnson and Steiner 2000), with colour seen differently through the insect eye to the human eye. Colour is more likely to be used as a cue for identifying rewards such as nectar (Waser 1983). The role of flower symmetry (Muller 1995; Giurfa *et al.* 1996) and olfactory cues (Dobson 1987) in guiding pollinators is more widely supported.

Any or all of these characteristics can determine the attraction and success of a flower as a pollinator, and so careful study of the flower's morphology is an important component of pollination studies. In our work we have looked at flower morphology at two levels. First, we sought to characterise the flower morphology of the entire Wet Tropics flora (see Boulter *et al*. in review). Second, and integral to understanding the pollination system of individual systems, we examined the morphological characteristics of individual flowers (e.g. Boulter 2003). We provide the techniques we used in those two studies in Section 2.3.2.

Table 2: Examples of floral traits that may attract or filter pollinators.

Function	Floral Trait	Example	Reference/s
Advertisement	Shape	Symmetry preference in bumblebees.	Muller 1995
	Colour	Colour change following fertilisation.	Weis 1991
	Odour	Odour imitating female wasp to attract male wasp to 'copulate' with flower.	Sands and House 1990
	Motion	Filiform appendages.	Faegri and van der Pijl 1979
	Sound	Acoustic guide in bat-pollinated flowers.	von Helversen and von Helversen 1999
Filtering Mechanisms	Nectar Guides	Concentric markings around nectar source.	Faegri and van der Pijl 1979
	Landing platforms	Lower lip of gullet flowers used for alighting.	Faegri and van der Pijl 1979
	Traps	Exit barred by reflexed inner petals following anthesis.	Faegri and van der Pijl 1979
	Flower shape	Long narrow corolla used by long billed birds.	Sazima *et al.* 1996

2.3.2 Techniques for Studying Flower Morphology

See Appendix 1 for a list of equipment required.

Flora-wide Morphology

In order to make some generalisations about the morphological characteristics of the flowers of the Wet Tropics rainforest, we wanted to be able to summarise some key floral features of the trees, shrubs and vines. To do this, we undertook a data mining exercise. A database of key morphological features (e.g. habit, inflorescence form and position, flower size and colour, flower symmetry, sexual system) was constructed for the species list of trees, shrubs and vines of the Wet Tropics, as recorded in Hyland *et al.* (2003). The data was drawn from existing floras (Cronin 2000; Hyland *et al.* 2003; Cooper and Cooper 2004). Using this extensive database, we were able to summarise these key morphological characteristics for the majority of the Wet Tropics tree, shrub and vine flora. In addition, we looked for relationships between key habit and morphological features (Boulter *et al*. in review). Some of the results are detailed on the following page.

Morphology of Individual Flowers

In order to better understand development and maturation, flowers were collected at different times of the day and at different stages of development, and were examined in the laboratory under the microscope to understand growth and development of the flowers. We used flowers that were collected for nectar extractions (see Section 2.5). Measurements were made using either a digital vernier or graticule of a dissection microscope. We were interested in the general dimensions of the flowers, the position, and accessibility by different organisms to reproductive organs. Using this information, we were able to determine at what stage the anthers dehisced (i.e. released pollen).

Case Study – Morphology Measurements of *Syzygium gustavioides* and *S. sayeri*

Measurements were made of flowers of *Syzygium gustavioides* and *S. sayeri*. The difference in size and development of the two taxa can be seen from these measurements (Table 3). In addition, we could determine the rate of growth of morphological features. For example, measurements of *S. gustavioides* show that although the stigma appeared to lengthen over the course of the first day of opening, based on our observations in the field, measurements of dissected flowers showed no significant increase.

We can also use the analysis to describe the general features of the flower. For example, we see that when fully open, the stigma of *S. gustavioides* protrudes beyond the staminal filaments; the anthers appear to have dehisced regardless of flower age; and again, pollen is released from longitudinal slits in the anthers (Figure 11).

Table 3: Average morphological measurements of *Syzygium sayeri* and *S. gustavioides* flowers.

Flower Character	*Syzygium sayeri*		*Syzygium gustavoides*	
	Mean (SE) mm	Sample Size	Mean (SE) mm	Sample Size
Diameter of open flower	4047 (0.04)	43	4.22 (0.09)	16
Depth of receptacle	5.48 (0.09)	43	3.55 (0.11)	18
Length of Stigma (> 24 hrs since anthesis)	16.19 (0.91)	29	7.73 (0.11)	18
Diameter of Stigma	6.44 (0.12)	41	1.09 (0.02)	18

Flower Morphology in the Wet Tropics

For the 1,459 species of tree, shrub and vine surveyed, nearly 75% of flowers have a diameter less than 10 mm, and the overwhelming majority are a dull colour such as white or green. When we look at these characteristics together we see that smaller flowers are more likely to be white or green than larger flowers. We also note that vines often have more colourful flowers than shrub and tree species.

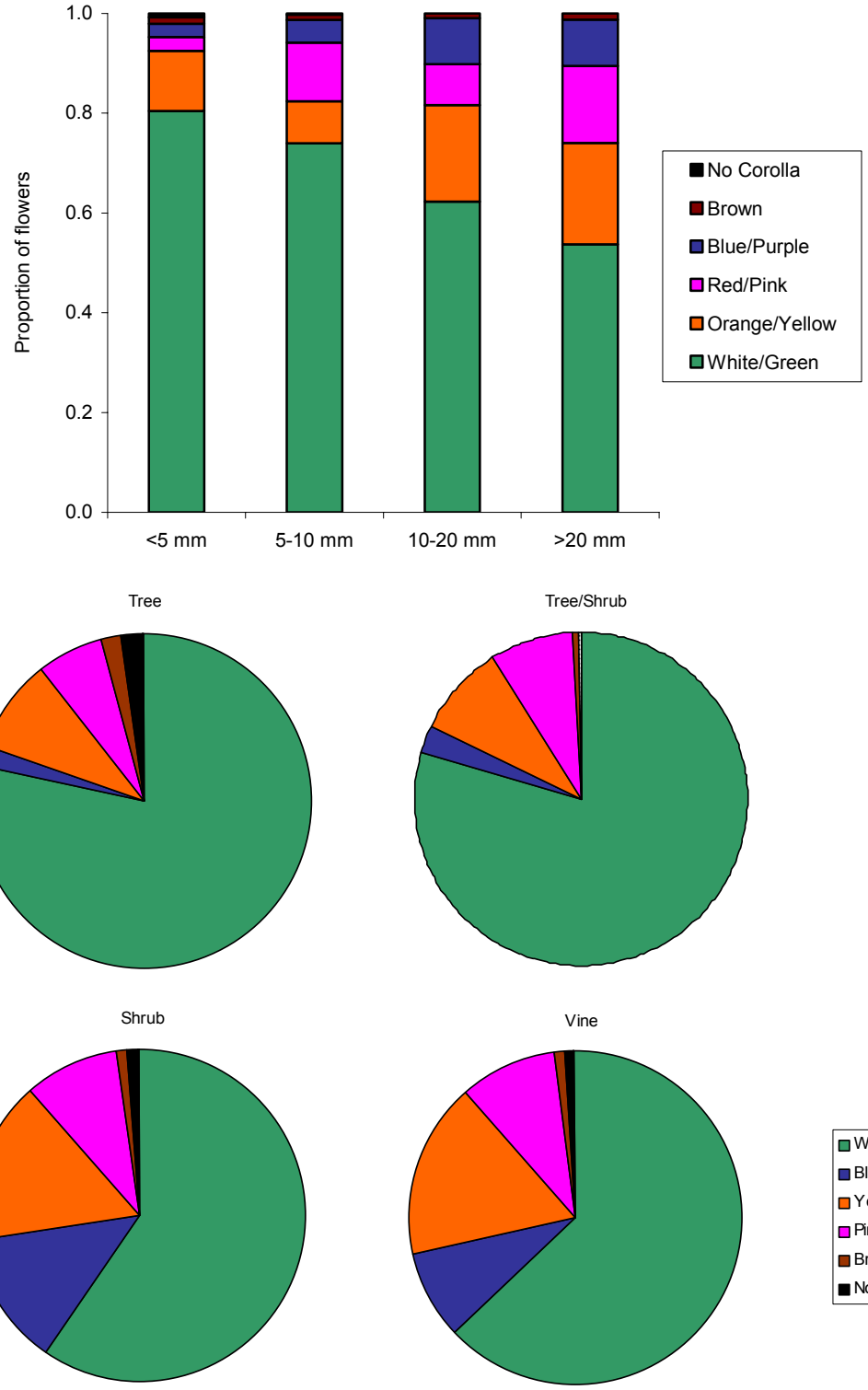

Figure 10: The proportion of Wet Tropics flowers (trees, shrubs and vines only) in (*top*) four size categories and colour groupings; and (*bottom*) in each colour category for each of four habit types. Source: Boulter *et al.* in review.

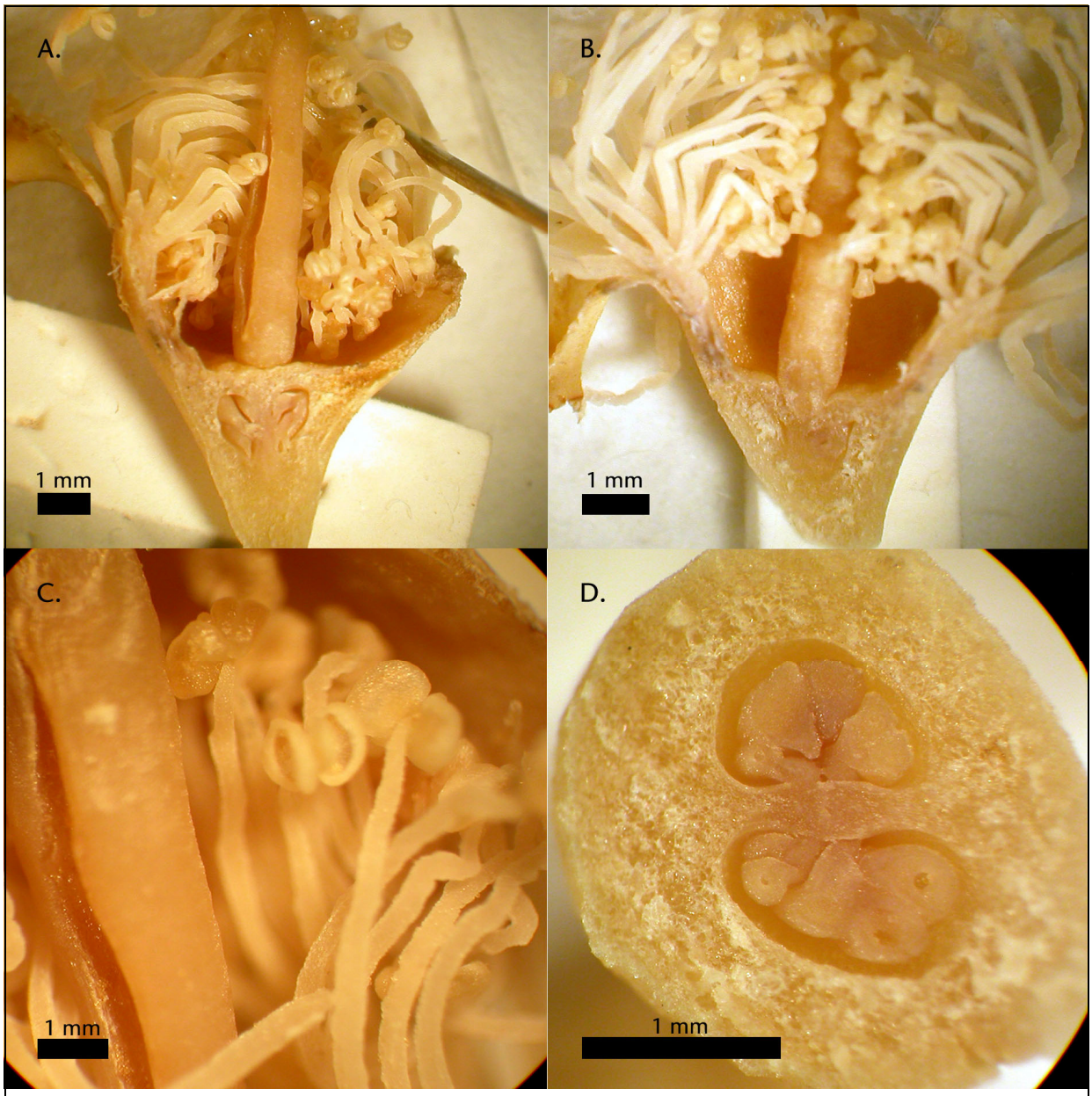

Figure 11: *Syzygium gustavioides* (a) flower at early opening stage; and (b) fully open in longitudinal section; (c) anthers (front and back); and (d) locule and ovaries in cross-section.

2.4 BREEDING SYSTEMS

2.4.1 Introduction

We refer to "breeding systems" as the self-compatibility (or otherwise) of a particular species. A plant's capacity to be pollinated by its own genetic material determines the need for a pollen vector and the optimal pollen vector. For example, obligate outcrossing species (those that physically cannot be self-pollinated, e.g. dioecious and self-incompatible hermaphroditic species) require pollen to be transferred between individual trees. Avoidance of self-pollination may occur in several ways including physical separation of the reproductive organs (e.g. dioecey or monoecey) or chemical avoidance (see Table 4 for a full description of these mechanisms). This has considerable implications for the success of potential pollinators, particularly if individual plant species are spatially distant. Self-incompatibility is thought to be widespread in tropical trees (Bawa 1982; Sands and House 1990; Johnson and Steiner 2000), although low or variable levels of self-compatibility may occur in otherwise outcrossing species (Crome and Irvine 1986; Gross 1993). This may result in some successful pollination where pollinators are absent or cross-pollination is unpredictable for some other reason (Williams and Adam 1994). On the other hand, self-compatibility can result in reduced offspring vigour or inbreeding depression (Shapcott 1998).

The sexual system of a plant can be described at three levels – the flower, the individual plant or a group of plants. Regulation of the outcrossing rate of a species may occur by the spatial arrangement of the male and female organs, the temporal or spatial isolation of the male or female organs within a flower, the biochemical rejection of self-incompatible pollen and variation in style and stamen length (Dafni 1992; Table 4 this volume)

Some Key Terms

Self-compatible..... Capable of self-fertilisation.
Self-incompatible ..... Incapable of self-fertilisation.
Dioecious ..... Having staminate (male) and pistillate (female) flowers on separate plants.
Monoecious ..... A plant with both staminate (male) and pistillate (female) flowers.
Agamospermy ..... The production of seeds without sexual reproduction.
Geitonogamy ..... Interflower pollination on the same plant.
Inbreeding depression..... Poor performance and low fertility in inbred individuals.

2.4.2 Techniques for Understanding Breeding Systems

See Appendix 1 for a list of equipment required.

Testing for Stigma Receptivity

The stigma of a flower must be receptive to pollen in order for the pollen to germinate. For some flowers, this phase may not start until some time after the opening of the flower and may cease before the flower senesces. Testing for this phase can be demonstrated by chemical reactions. We used a simple test using hydrogen peroxide.

In our experiments, we use the same flowers as those monitored for their individual phenology (Section 2.2.2) to test for stigma receptivity. By using these flowers we have a record of the stage of development of age of the flowers, which can then be correlated to the receptivity of the stigmatic surface. To test the receptivity of the stigma, apply a drop of 3% hydrogen peroxide to the tip of the stigma using a pipette. The presence of bubbling is then observed to indicate peroxidase activity and therefore the receptivity of the stigma (Kearns and Inouye 1993). For small stigmas it is useful to use a hand lens to see the presence of bubbles at the tip of the stigma.

Testing for Self-compatibility

Testing for the self-compatibility of a species can be done using combinations of artificial pollination, bagging and emasculation to mimic a set of pollination scenarios (e.g. cross-pollination versus self-pollination). For example, to test the levels of self-incompatibility in two of our target trees found at the canopy crane plot – *Syzygium sayeri* and *S. gustavioides* – we used a modified version of the regime described in Dafni (1992). Treatments are a combination of bagging, emasculation and pollination of individual inflorescences as described in Table 5.

We used the following methods to perform these manipulations as follows:

Bagging: Flowers are bagged in several of the treatments to prevent animal visits and possible pollination. Bagging is a common technique used by pollination ecologists, and different materials and methods can be used. We use a mesh sock created from a fine nylon fabric (hole diameter < 0.5 mm) drawn over a plastic “cage”, which prevents the fabric bag from coming into contact with the flowers. Once drawn over the plastic cage, the fabric sock is secured around the stem and above the cage by tying two lengths of string above and below. The cage is constructed of five strips of plastic acetate stapled to two circles. The bottom circle of the cage has a hole at its centre and a slit from one point on the edge to that hole. This allows the cage to be slipped around the stem of the inflorescence at this point and stapled closed to form a balloon around the inflorescence (Figure 12). A make-up pad (a circle of cotton wool) is wrapped around the stem to prevent the cage from damaging the stem. The inflorescence can then be quite simply accessed by untying the top string and drawing the mesh sock off the cage.

Emasculation: This manipulation involves the removal of the anthers from the flowers, which needs to be done before the flower is open (can often be done by opening a splitting bud to access the anthers). As some of our focal species had over one hundred stamens, we used a small pair of sharp scissors to cut off the anthers. By emasculating the flowers we control the source of pollen. A control for the effect of emasculation is included, and this test for any negative impact of the manipulation on the fertility of the flower.

Artificial Pollination: To test the various levels of compatibility, different forms of artificial pollination must be performed. We used another flower and brushed the anthers of the donor flower against the stigmatic surface of the flower to be pollinated. For the artificial self-

pollination treatments, the anthers of the subject flower were pushed onto the individual's stigma to transfer pollen. Other methods that could be used include using a fine paintbrush to transfer pollen (Kearns and Inouye 1993). Care must be taken with the latter method to avoid pollen contamination on the brush between treatments.

In the field, *S. gustavioides* proved to be extremely fragile, and all attempts to emasculate these flowers resulted in the loss of the flower immediately upon being touched. A modified treatment was adopted that provides a partial indication of self-compatibility. This schema is described in Table 6.

To conduct breeding experiments, we recommend performing a set of the treatments as listed in Tables 5 or 6 on each of at least three trees. For each treatment, a single inflorescence of buds, or largely of buds, is selected. Any open flowers should be removed and all unopened buds counted. Flowers are visited once or twice a day for approximately one week and pollinations and emasculations are carried out according to the treatment prescription (Table 5 or 6). Repeated visitation ensures that pollen is transferred when the stigma is receptive. At the end of the period, any unopened flowers should be removed and subtracted from the original bud count. Flowers can then be revisited several weeks later and scored for the appearance of a swollen receptacle or immature fruit to indicate successful pollination. We used swollen receptacle as an indicator of successful fertilisation, rather than successful seed-set to avoid possible effects of predation and abortion prior to seed-set. In our case, our interest lay in successful pollination (Crome and Irvine 1986).

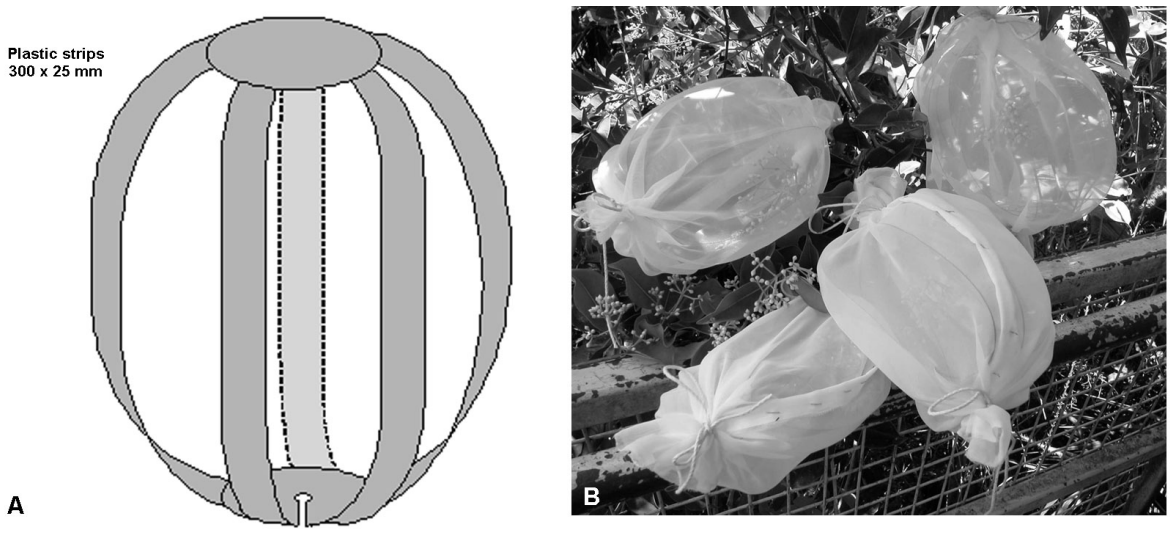

Figure 12: (a) Design of "balloon" cage, constructed from plastic acetate and placed around inflorescence stem; and (b) the cage is then covered in a fine mesh sock and tied with string to exclude visitors and allow daily access.

Table 4: The regulation of outcrossing (Modified from Dafni 1992).

(a) Spatial arrangement of male and female organs.

1. Individual plants:
   i. *Hermaphroditic*: each plant bears only bisexual flowers;
   ii. *Monoecious*: each plant bears male and female organs (flowers bisexual, or unisexual flowers);
   iii. *Andromonoecious*: individual plants bear bisexual and male flowers (male flowers dominant);
   iv. *Gynomonoecious*: individual plants bear bisexual and female flowers (female flowers dominant);
   v. *Polygamomonoecious*: individual plants bear bisexual flowers, male and female flowers.
2. Group of plants:
   i. *Dioecious*: each plant bears male or female flowers only;
   ii. *Androdioecious*: each plant bears either bisexual or male flowers;
   iii. *Gynodioecious*: each plant bears either bisexual or female flowers;
   iv. *Polygamodioecious* (trioecious): each plant bears either bisexual, female or male flowers.

(b) Temporal or spatial isolation of male and female organs either within hermaphroditic flowers or on co-occurring unisexual flowers on a single individual plant (monoecious).

1. *Protandry*: pollen released before stigmas receptive;
2. *Protogyny*: stigmas receptive before pollen released;
3. *Herkogamy*: male and female organs mature simultaneously but spatially isolated.

(c) Biochemical recognition / rejection self-incompatibility alleles

1. *Self-incompatibility*: plants are polymorphic in respect to the presence of self-incompatibility alleles. Pollinations involving pollen and stigma sharing the same self-incompatibility alleles, including self-pollinations, do not result in fruit set.
2. *Self-compatibility*: all pollinations, including self-pollinations, result in fruit set.

Table 5: Treatments designed to test levels of self-compatibility (Modified from Dafni 1992).

Trap	Treatment	Emasculated	Bagged	Pollinated
A	Control	No	No	No
B	Spontaneous selfing	No	Yes	No
C	Induced selfing	Yes	Yes	With self
D	Geitonogamy	Yes	Yes	Same tree
E	Cross-artificial	No	Yes	Different tree
F	Cross natural	Yes	No	No
G	Emasculation control	Yes	No	No
H	Emasculation control 2	Yes	Yes	Different tree
I	Agamospermy	Yes	Yes	No

Table 6: Modified treatments used to test levels of self-compatibility in *Syzygium gustavioides*.

Trap	Treatment	Bagged	Pollinated
A, G	Control	No	No
B, I	Spontaneous selfing	Yes	No
C	Induced selfing	Yes	With self
D	Geitonogamy	Yes	Same tree
E, H	Cross-artificial	Yes	Different tree
F	Cross-artificial	No	Different tree

The Breeding System of *Syzygium sayeri*

We show here the results from the breeding system experiment on *S. sayeri* (Figure 13).

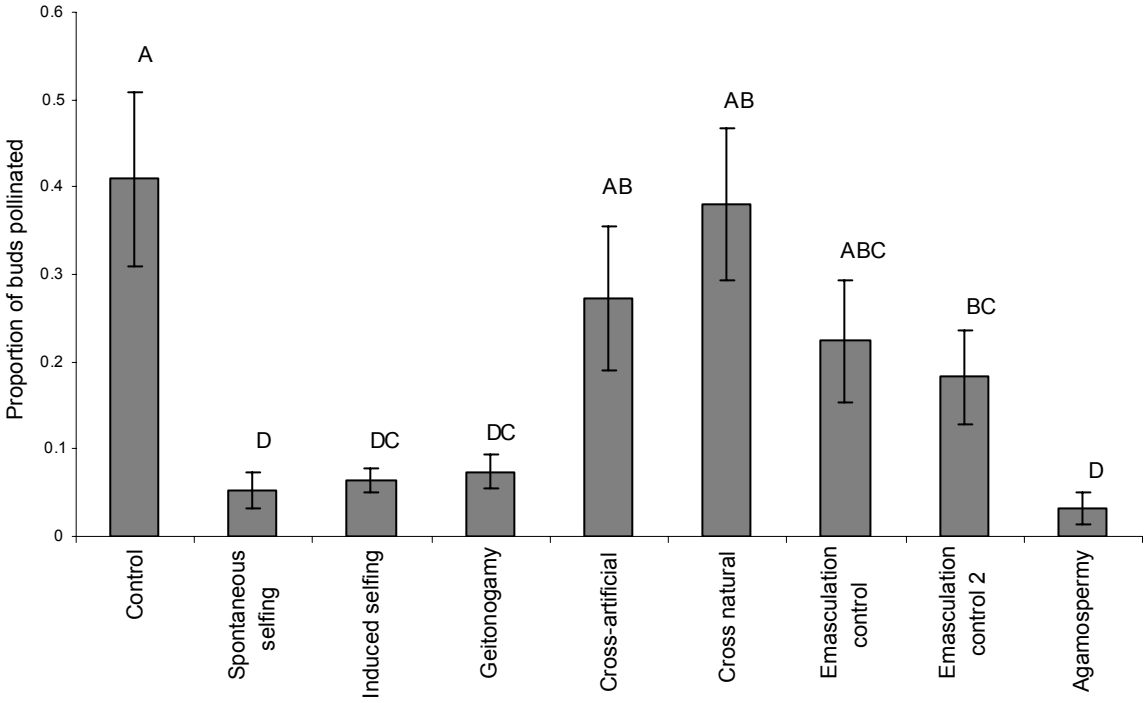

Figure 13: Proportion of *Syzygium sayeri* flowers demonstrating successful fertilisation using nine treatments to determine breeding system. Different letters indicate significant differences ($P < 0.05$).

Approximately 40% of the untreated *S. sayeri* control flowers were successfully pollinated by natural vectors (i.e. where we simply counted the starting number of buds in an inflorescence and the proportion which demonstrated successful fertilisation). Pollination was significantly lower than the untreated control for inflorescences that were, (a) artificially selfed; (b) artificially pollinated with donors from the same tree (geitonogamy); or (c) left to self-pollinate spontaneously (Extended T-test, $P < 0.05$). Pollination success in cross-pollinated flowers – both artificial and natural – was not significantly different from the open, non-manipulated control levels. Emasculation of the flowers did not have a statistically significant impact on levels of pollination although the average level of pollination in the emasculated controls was lower. In this case, then, we conclude that *S. sayeri* has a low level of self-compatibility (less than 10%) and must rely on pollen vectors for most of its reproduction.

2.5 REWARDS

2.5.1 Introduction

Nectar and pollen are the rewards most commonly sought by flower visitors, although other rewards include larval brood sites, food bodies, oils, resin and gum (Table 7). Nectar is the primary reward for many flower visitors and as such has been well studied (Kevan 2003). It is known that both the quantity and composition of nectar vary enormously, not only among species, but also across time and with the age of the flower (Pacini *et al.* 2003). Quantities of nectar range from an almost undetectable fraction of a microlitre, to thousands of microlitres (Opler 1983), and may be produced for short or long periods, from as little as a few minutes to many days (Pacini *et al.* 2003). The production of both different quantities and specific compositions of nectar have been associated with the attraction of different guilds of visitors (e.g. large quantities of nectar are associated with large vertebrate visitors such as birds and bats; Faegri and van der Pijl 1979, Wyatt 1983). Nectar is mainly a sugar solution, although other elements are found, sometimes in trace quantities. These include amino acids, proteins, enzymes, lipids, transfructosidases, transglucosidases and phenolics (Kearns and Inouye 1993). The components of the nectar solution will give the nectar its specific taste and odour that may be important in attracting specific pollinator groups.

Pollen is a major attractant for many pollinators and an important dietary element for many flower visitors. The pollen is a very reduced male gametophyte. The pollen develops in the anthers and is shed from openings in the anther. The pollen grain is made up of a sculptured exine, the intine or cell wall and internal cellular material. The pollen morphology (size, external exine sculpturing, aperture and polarity) in angiosperms can be used to identify the species of origin and can provide clues to the mode of pollination. For example, drawing on pollen samples from 130 species of trees, shrubs, vines and herbs, Williams and Adam (1999) used exine sculpture to predict those species that might be facultatively wind pollinated.

Table 7: Categories of floral rewards offered by flowers to animal visitors.

Floral Trait	Example	Reference/s
Pollen	Collection by bees	Faegri and van der Pijl 1979
Nectar	Nectar feeding	Baker and Baker 1983
Oil / Resin / Gums	Oil collecting bees	Faegri and van der Pijl 1979
Food bodies and Tissues / Brood sites	Development of beetle larvae in abscised flowers	Listabarth 1996
Basking places / Temperature	Visitor changes in response to radiance levels	McCall and Primack 1992
Sexual attraction	Mate rendezvous	Faegri and van der Pijl 1979

2.5.2 Methods

See Appendix 1 for a list of equipment required.

Nectar

There are a number of aspects of nectar production that can be investigated, and this subject fills entire textbooks. We wished to know the following information about nectar production in our target trees:

- The timing of nectar production;
- The quantity of nectar produced;
- The sugar quantity in the nectar; and
- Any changes of these factors over time.

In order to test these qualities, nectar can be extracted, measured, and its sugar content determined. In our nectar experiments, individual unopened flowers were tagged, as they were for the phenology study using retail tags (see Section 2.2.2). The inflorescences should be bagged to prevent flower visitors from altering nectar quantities. We used the "balloon cages" described above, as they allowed ready access to the flowers. These inflorescences were visited regularly and floral development recorded as described in the individual flower phenology protocol. We removed selected individual flowers at different times of the day and at various times since opening, and collected nectar measurements. All nectar was drawn from the flowers using a 10 µl micro syringe (SGE Graduated, blunt needle) introduced into the corolla. Capillary tubes can also be used in this way, but we found the syringe allowed better control. The quantity of nectar collected can then be recorded. To test the sugar concentration of the nectar, several drops of the collected sample can be placed on the prism surface of a hand-held 0-50% BRIX refractometer (Atago N50E). The measurement is taken by viewing the scale through the eyepiece of the refractometer held to the light. The prism needs to be carefully cleaned using water and a soft cloth between measurements to avoid contamination. If nectar quantities are very low, a known quantity can be added to the nectar to increase volume (but dilute sugar), enough to take a set of measurements and calculate sugar concentration.

Average nectar quantities and sugar concentration can be plotted for individual trees or across several individuals at different times of the day and at different stages of development.

Timing and Quantity of Production and Sugar Concentration in Nectar of *Syzygium sayeri*

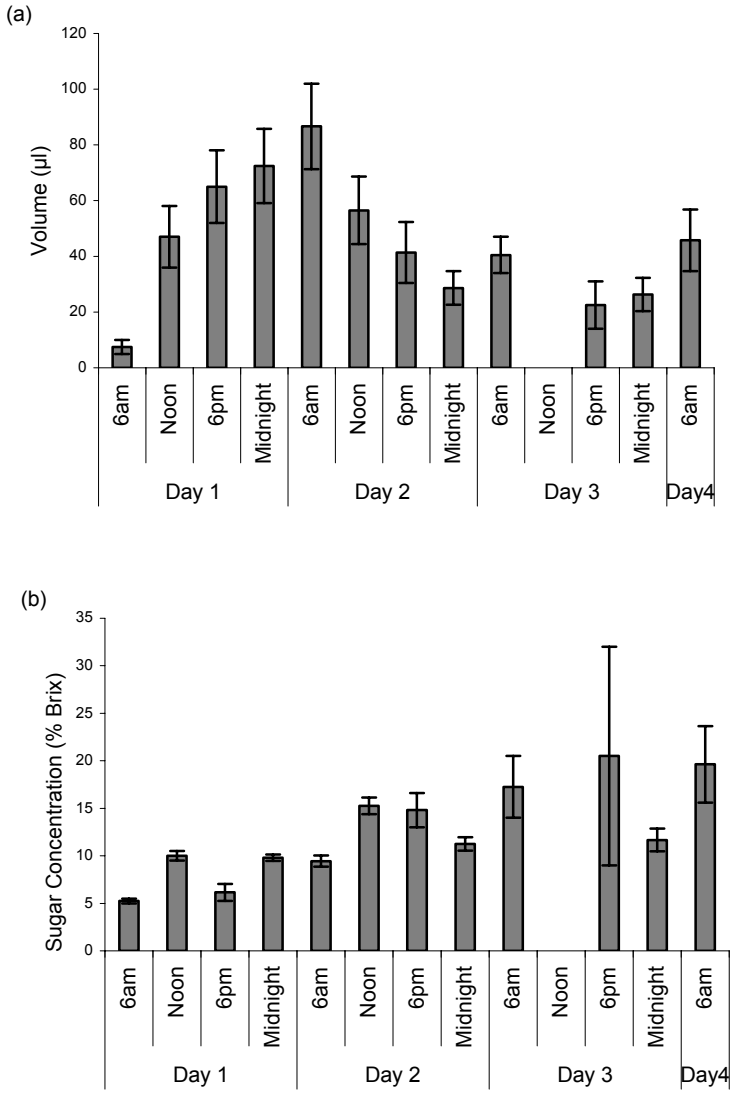

Figure 14: (a) Mean volume of nectar collected from *Syzygium sayeri* flowers at different times after opening; and (b) sugar concentration of nectar collected from *S. sayeri* flowers at different times after opening.

Nectar was collected and measured early to mid morning ('6am'); in the middle of the day ('noon'); late afternoon to early evening ('6pm') and late evening to midnight ('midnight'). The quantity of naturally available nectar varied considerably within similarly aged flowers. Flowers sampled on the first day of opening could have as much nectar as 163 µl, or as little as 4 µl. Tracking average nectar quantities from the time of opening and across each succeeding 24-hour period gives the impression that nectar volume increases across the first 24 hours after flower opening, reaching a peak early on the second day of opening, then declining (Figure 14). A two-way ANOVA indicated that the individual effects of day and time of day were not significant, but that the interaction of these two effects was ($P < 0.01$).

Although the sugar concentration of nectar from *S. sayeri* appears to increase with age, considerable variation among samples meant no significant difference was detected across any samples.

Pollen Morphology

The morphology of pollen varies considerably among plant genera and species. The morphology of a pollen grain can be used to identify the source plant of pollen carried by insect visitors and so determine the variety of plant species visited. Pollen from different angiosperm species can be very distinctive, and identification can be based on size and sculpturing.

Preparing Pollen for Viewing

To describe its morphology, pollen needs to be viewed under a microscope. Pollen samples need to be free from contamination. To do this, an inflorescence of buds close to opening should be collected and taken to the laboratory. Here, the stem of the inflorescence should be placed in a jar of water and set upon a large filter paper to catch the pollen as the flowers open. A large plastic bag to prevent contamination from foreign airborne pollen should loosely cover the inflorescence. In this state, flowers can be left to open. Two to three days following opening, use a small lump of basic fuchsin jelly (Kearns and Inouye 1993) dabbed onto the filter paper to collect the released pollen. Place the lump of jelly on a microscope slide and warm the slide on a slide warmer until the jelly has melted. Cover the melted jelly with a cover slip and cool at room temperature until firm. The pollen grains can then be examined using a compound light microscope to enable measurement and description of the pollen grains.

Basic Fuchsin Jelly Recipe (modified from Kearns and Inouye 1993)

Ingredients:

Distilled water	175 ml
Glycerine	150 ml
Gelatine	50 g
Crystalline basic fuchsin stain	as desired

To make:

Add gelatine to distilled water in a beaker and warm until dissolved. Add glycerine and stir gently while warming. Add basic fuchsin crystals to make a claret colour. Filter through glass wool into sterile containers. Refrigeration is recommended to avoid mould.

Pollen Morphology of *Syzygium gustavioides* and *S. sayeri*

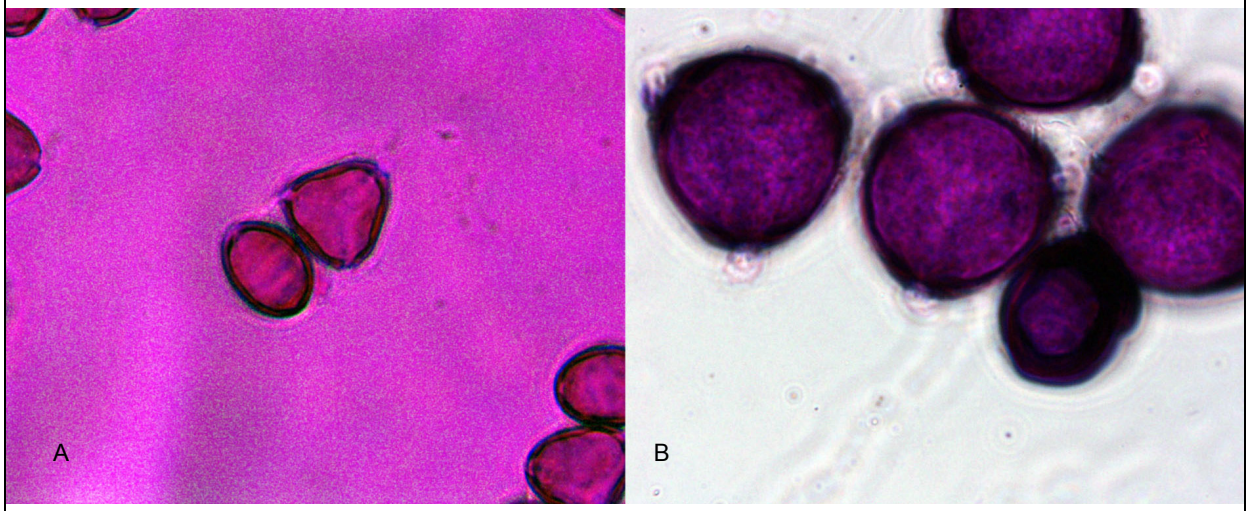

Figure 15: Pollen grains of (a) *Syzygium sayeri*; and (b) *S. gustavioides* (100x magnification).

Figure 15 shows images of pollen grains collected from (a) *Syzygium sayeri*, and (b) *S. gustavioides.* Pollen grains from both species were triangular in polar view and oblate-elliptic in lateral view and tricolporate (Shivanna and Rangaswamy 1992). Pollen of *S. sayeri* was however smaller, with an average polar diameter of 14.1 ± 0.18 µ and equatorial diameter of 14.1 ± 0.26 µ. Pollen of *S. gustavioides* had an average polar diameter of 27.7 ± 0.49 µ and equatorial diameter of 27.6 ± 0.54 µ. The surface exine of *S. sayeri* appeared smooth, while that of *S.* gustavioides was faintly patterned.

2.6 DISCUSSION

Understanding the morphology, breeding system and flowering morphology, as well as the natural variations in those features, provides essential clues in understanding the limits and boundaries of the plant's pollination system. Examination of these factors must be critical and we suggest here some questions that might arise in these studies:

- Are some structures fused, e.g. are anthers free or fused to the inside of the petals?
- What is the relationship of the male and female structures? Are they all of similar height so that a visitor is likely to come into contact with both whilst foraging on the flower?
- Is nectar available to all size class of visitors or is it only accessible to individuals with particular mouthparts?
- Are flowers available only at particular times (e.g. daytime only)?
- Will pollen have to come from another individual plant? How far away might that be?

There are many more questions that are essential when trying to understand the role of the plant in a pollination system.

3. THE ANIMALS

3.1 GENERAL INTRODUCTION

At flower opening, new resources become available to the local fauna. Animals may visit these flowers for numerous reasons – to feed upon pollen or nectar (Faegri and van der Pijl 1979, Baker and Baker 1983), to shelter or brood larvae (Listabarth 1996, Sakai *et al*. 2000, Williams *et al*. 2001) via pseudosexual or sexual attraction (Faegri and van der Pijl 1979), to prey upon other faunal visitors (Dukas 2001, Suttle 2003) or any combination of these. These visits or inhabitations may influence the success of the host plant species in a number of ways. Foremost is the successful transfer of con-specific pollen. However, flower visitors may have no impact on the flower, that is, they may be 'tourists'. In the alternative, they may reduce the plant's success by preying upon other potential pollinators (Dukas 2001, Suttle 2003), or consuming the flowers parts or products itself (e.g. nectar robbers) (Maloof and Inouye 2000, Lara and Ornelas 2001).

Ecologists have used data derived almost exclusively from direct observations to record, identify and quantify flower visitors (e.g. Kato 1996, Momose *et al.* 1998, but see House 1993). Many of these studies have focussed exclusively on particular taxa of pollinators such as bees, birds or bats. Frequently, the most conspicuous or numerous species is identified as the pollinator, yet a wider faunal array often visits the flowers of the subject plant (see Boulter *et al*. in review). With the emerging perception that generalised pollination systems are more widespread than previously thought, greater emphasis is now placed on the importance of profiling the entire visitor fauna to a flowering plant (Bronstein 1995, Ollerton 1996, Waser *et al*. 1996) – even flowers that appear to be specialised are often visited by a diverse array of animals (Johnson and Steiner 2000). Yet few studies have considered the entire plant-visitor system of a plant species (Memmott 1999, Hingston and McQuillan 2000). Small insects are often missed or excluded due to the difficulties of identification when using direct observation methods, and the number of species in a plant-insect visitor system is often underestimated as a consequence (Dicks *et al.* 2002, Howlett *et al.* 2005).

We used a comprehensive approach to determine the total array of animal visitors to the flowers using a combination of techniques. We have classified the flower fauna into two categories, (a) the "in-fauna", or those insects expected to be living or brooding in the flower, were sampled, and (b) the more active flower visitor fauna were observed and collected using trapping techniques.

3.2 IN-FAUNA

Many small insects often inhabit the open flowers of a plant. These insects are usually residents in the flower or use the flower as shelter or a brood site. They are often not seen moving between flowers, but may play some role in the pollination of self-compatible flowers. This role has been observed for flower residing thrips. Resident fauna, or "in-fauna", may offer no benefit to the plant's reproductive success, or perhaps may even have a negative impact on its reproductive success (e.g. predating on the flowers).

3.2.1 Washing Technique to Sample In-fauna

See Appendix 1 for a list of equipment required.

To determine the in-fauna associated with flowers, we use a branch clipping and washing technique (Southwood 1978, Basset *et al.* 1997). Selected individual inflorescences are enclosed in plastic bags and the stem clipped at the closure of the bag. The contents of the

bag are immediately sprayed with a commercially available pyrethrum insecticide (Slayafe®) for ten seconds. In the laboratory, the samples can then be transferred to a bath of ethanol to be washed and brushed using soft artist quality paintbrushes to remove all arthropods.

We conducted sampling on a pair of inflorescences, one with open flowers and the other with unopened buds, in order to make pair-wise comparisons of the fauna on buds versus flowers. This allows us to determine if the number and composition of insects associated with the flower changes with the presentation of new resources (i.e. the opening of the flower). We recommend sampling a pair of inflorescences on at least five individuals of a species.

3.3 VISITOR FAUNA

3.3.1 Introduction

Flower visitors in tropical floras vary from the minute and cryptic to the large and conspicuous. Animals that alight at a flower are invariably either seeking food resources (i.e. of nectar, pollen or the flowers themselves), using the flowers as a concourse to enhance the likelihood of encounter with prey or mates, or they may be merely casual visitors. Identifying flower visitors has been traditionally made through painstaking observations of flowers across a range of times. These observations can be enhanced by the addition of trapping of insects at flowers. The use of one technique to the exclusion of the other is likely to overlook a component of the flower-visiting fauna (Howlett *et al.* 2005). Some of the visitors, and even elements of the in-fauna, may be involved in gametic transfer from androecium to gynoecium within the flowers themselves – these are the pollinators.

In order to understand what fauna visits the flowers of a plant species as part of pollination studies, we have used trapping and observation techniques. We have also used extensive trapping to identify the insect visitors to twelve tree, shrub, palm and vine species at the canopy crane site and have used a subset of that data to address a number of inter-related hypotheses (see Kitching *et al.* in review). In particular, we have been interested to know (a) if the set of arthropods visiting the flowers of any particular species of canopy plant is a unique subset of all the canopy arthropods available, and (b) within selected species, whether or not the assemblage of visitors differs with the time of year. We do this through comparison of visitor assemblages associated with co-flowering species and by resampling flower visitors of a given species at different times of the year.

Case Study – "In-fauna" of Two Canopy Species

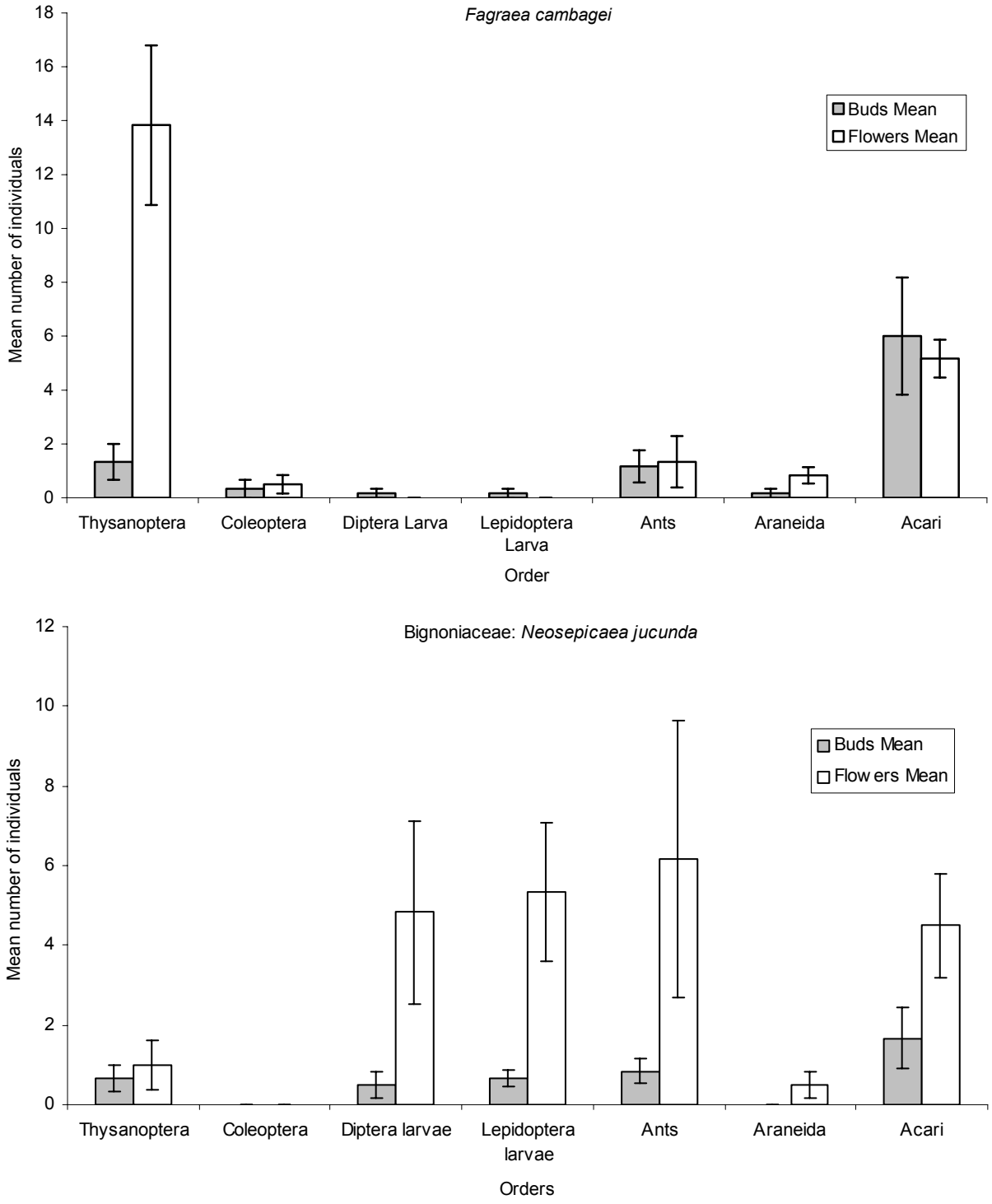

Figure 16: In-fauna sampled from (*top*) *Fragraea cambagei*; and (*bottom*) *Neosepicaea jucunda* using ethanol washing.

Throughout our project, we have sampled the in-fauna of some twelve plant species. We present here the results of two of these species (Figure 16), *Fragraea cambagei* and *Neosepicaea jucunda.* These sample results demonstrate firstly (left column) the response of some taxa to the opening of the flower (e.g. Thysanoptera on *Fragraea cambagei*), and secondly (right column), that the group of taxa that respond to flower opening vary among plant species (e.g. lepidoptera larvae increase in open *Neosepicaea jucunda* but not on *Fragraea cambagei)*.

3.3.2 Techniques for Determining Flower Visitors

See Appendix 1 for a list of equipment required.

Trapping Methods

Trapping techniques can be used to capture insects close to or visiting a flower. We have employed two types of traps to capture small and medium-sized insects visiting flowers (Howlett *et al*. 2005). The first trap type, hereafter referred to as 'PAS' traps (plastic acetate strips), consist of 80x30 mm strips of transparent plastic acetate (0.2 mm thick) with a hole at one end through which tie-wire is used to attach the trap to an inflorescence or flower (Figure 17). These traps are coated on both sides with the commercially available Tangletrap®, a sticky paste commonly used to trap insects and which can be dissolved using mineral spirits. The clear advantage of these traps is the ability to position them very close to the flower. They do tend, however, to catch only smaller insects (Howlett *et al.* 2005), so we use a second larger trap in conjunction with this method.

The second type of trap is a small interception trap. This trap is an all-in-one construction, with a small interception screen (140x130 mm) constructed from 0.2 mm transparent acetate plastic, mounted over a plastic collection tray (takeaway food container, 150x100x54 mm) (Figure 18). Wire stays are attached from the corners of the takeaway container to a top wire. A short length of string can then be attached to the top wire, and the container suspended from this. The size and container construction mean that these traps can be suspended near or on an inflorescence, or in our case, in the canopy. The catching screen is coated in petroleum jelly and the collection tray filled to a depth of 20 mm with water and a little detergent. Pilot studies demonstrated that the addition of petroleum jelly to the interception screen improved container capture rates. Several small holes are punched into the ends of the catch container, close to the rim. This prevents water from over flowing out of the top of the trap and washing out insects during rain.

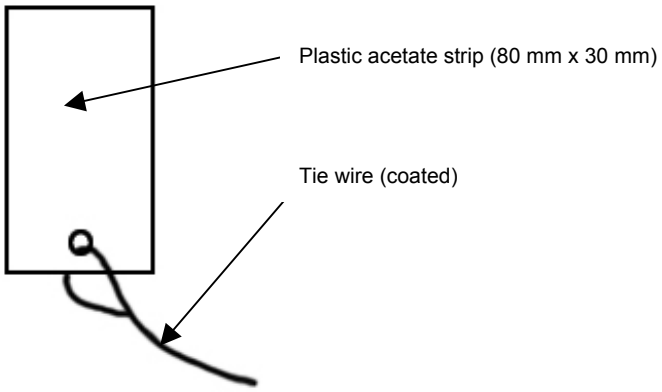

Figure 17: The PAS trap design is simply a strip of plastic acetate and a length of coated tie wire to attach to the inflorescence.

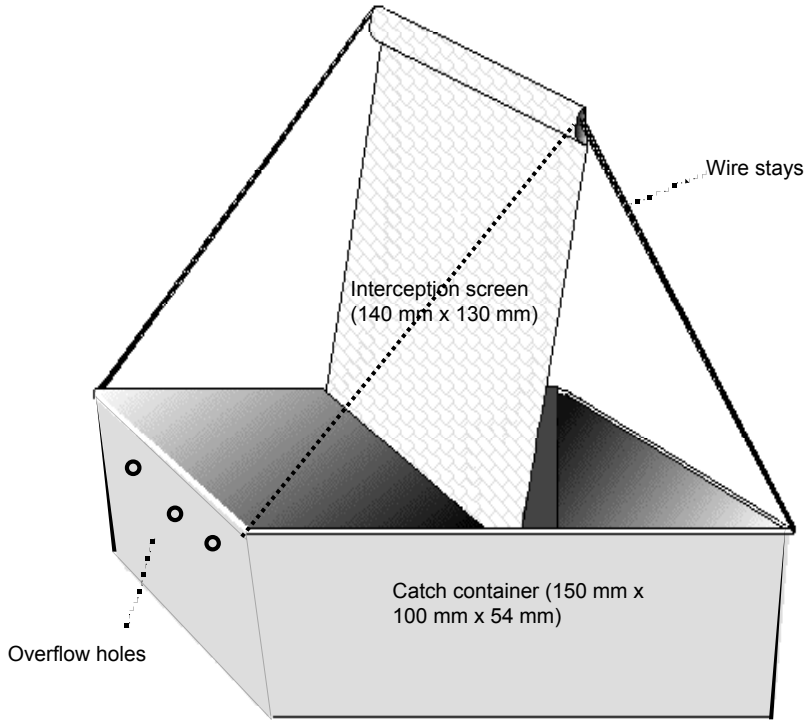

Figure 18: Design of the interception trap.

Preparing Interception Traps

Traps should be constructed in advance of usage, as they need to be set simultaneously to avoid any weather or other biases in samples. PAS traps need to be primed with Tangletrap®, which is available as either a paste or spray (we use the paste, as it is the least expensive option). To apply, we simply add some paste to a flat surface (e.g. a plastic lid) and then, holding the trap by its wire (but close to the trap to avoid tearing the plastic), pat the trap into the paste. The idea is to get a thin and even coating. If a trap has an excessive coating, it can be patted onto another trap. Both sides of the trap need to be coated. Gloves should be worn throughout the application process, as Tangletrap® is an insecticide and is extremely sticky. To transport the traps, we simply wrap a bundle in plastic wrap (used for covering food), leaving the wires protruding so they can be picked up when needed.

Interception traps should be fully constructed and the interception screen thinly smeared with petroleum jelly before use in the field. The traps can be carried in bundles held by the strings at the top.

Installing Interception Traps

We place one interception trap among an open flowering inflorescence and one among unopened buds of a similar sized inflorescence that is at least two metres from the next nearest inflorescence. This allows us a non-flower test. Four individual PAS-traps are also paired with each interception trap. Interception traps were usually tied to the stem of the inflorescence or a nearby branch, so that the trap sits just beneath or behind the selected inflorescence. The wire of the PAS traps can then be wound on to the inflorescence or the wire stays of the interception trap, and the PAS trap bent such that the catching surface is among or close to the flowers of the inflorescence. Label traps by writing on the catch container of the interception trap in permanent marker pen. Use a code for the individual plant and indicate whether the traps were placed on buds or flowers (e.g. 1F would be tree 1,

flowers). Once the traps are in place, add water with a few drops of detergent to the catch container of the interception trap. Record the location of the trap and mark its position using brightly coloured flagging tape. Traps are left in place for 72 hours.

We used this combination of traps to catch flower visitors in the canopies of trees at fragmented sites (i.e. not accessible by the canopy crane). By simply attaching the PAS traps to the wire stays of the interception traps, we could haul the complete trapping unit into the canopy (see Section 1.3 for a description of how to place traps in the canopy).

Trap Collection

Once collected, traps need to be processed ready for sorting. Insects should be stored in at least 70% ethanol to ensure their preservation. For interception traps, this means syphoning off the water and detergent mix. We pour our samples into a fine gauze fabric lining a funnel to remove the water and detergent mix. Insects collect on the gauze and can then be washed off the gauze into a collection vial using a spray bottle of 70% ethanol. We invert the gauze over the vial and wash through from the back. Wash the catch container and screen into the same vial to ensure all insects are collected. Labels with collection date, location and trap code should be put into this vial (see Figure 19).

PAS traps can be returned to the laboratory in small zip lock plastic bags in a bunch of four, but be sure to put a sample label in the bag that reveals the collection date, location and trap code (e.g. Figure 19). To remove insects from PAS traps, the Tangletrap® needs to be dissolved in mineral spirits. We place about 300 ml of mineral spirits into a takeaway container, and soak the PAS traps in the container for a few minutes. Ensure that the sample label stays with the soaking traps. Gently agitate the traps and brush off any stubborn insects with a soft artist's brush until the traps are clean. Filter the insects out of the mineral spirits in the same way as insects are removed from the water and detergent mix from the interception traps (see above). When pouring the sample through the fine gauze fabric in a funnel, place the funnel over a bottle to collect the now clean mineral spirits for reuse. Again, insects can simply be washed off the gauze into a collection vial using a spray bottle of 70% ethanol. Wash the takeaway container in which the trap was cleaned into the same vial to ensure all insects are collected. Transfer the sample label into the vial.

16° 07.30S 145° 26.30E
Cape Tribulation

N. normanbyi

Trap: Nn 5B PAS
13-15 April 2003
Kitching/Boulter

Figure 19: Example of a collection label for vials of specimens collected from visitor traps.

Samples are then sorted to Order, and each Order examined in greater detail if appropriate. When analysing data we pool the data for the PAS traps and single interception trap.

Case Study – Visitor Fauna of *Syzygium gustavioides* and *S. sayeri*

Table 8: Mean number of individuals by taxonomic grouping collected in PAS and interception traps at the flowers and buds of *Syzygium sayeri* and *S. gustavioides* during July 2002. The difference between the number of individuals at flowers and buds is tested using paired T-tests.

	S. sayeri			***S. gustavoides***		
	Mean No. Individuals (SE)		***P***	**Mean No. Individuals (SE)**		***P***
	Flowers	Buds		Flowers	Buds	
Collembola	0	0.17 (0.17)	n.s.	0.17 (0.17)	0.09 (0.09)	n.s.
Blattodea	0	0	-	0.50 (0.29)	0.27 (0.13)	n.s.
Orthoptera	0	0	-	0.33 (0.19)	0.09 (0.09)	n.s.
Dermaptera	0	0	-	0.08 (0.08)	0	n.s.
Psocoptera	1.17 (0.40)	1.33 (0.67)	n.s.	0.58 (0.23)	0.54 (0.27)	n.s.
Homoptera	6.83 (1.72)	1.83 (0.75)	*	6.50 (1.75)	5.64 (0.67)	n.s.
Heteroptera	0	1.0 (0.52)	n.s.	0.33 (0.33)	0.09 (0.09)	n.s.
Thysanoptera	13.0 (3.14)	3.17 (0.79)	**	8.08 (4.07)	1.27 (0.61)	n.s.
Neuroptera	0.83 (0.54)	0.83 (0.40)	n.s.	0	0	-
Coleoptera	41.33 (21.62)	1.33 (0.95)	n.s.	104.33 (31.12)	12.64 (0.01)	*
Diptera	65.83 (22.01)	49.17 (23.52)	**	37.08 (7.53)	16.55 (3.72)	*
Lepidoptera	7.5 (1.89)	14.33 (9.89)	n.s.	2.0 (0.73)	2.36 (0.95)	n.s.
Trichoptera	0	0.33 (0.33)	n.s.	0	0.09 (0.09)	n.s.
Ants	0.50 (0.34)	0.50 (0.34)	n.s.	3.0 (1.45)	0.91 (0.42)	n.s.
Other Hymenoptera	24.67 (8.2)	9.0 (3.30)	*	12.42 (3.44)	5.18 (2.23)	n.s.
Araneida	4.0 (1.44)	0.67 (0.33)	*	5.58 (2.01)	2.64 (0.58)	n.s.
Acari	36.0 (17.20)	1.0 (0.45)	*	0.33 (0.26)	0.09 (0.09)	n.s.
Total Individuals	203.83 (46.73)	83.67 (33.89)	**	181.67 (38.38)	48.55 (8.06)	**

* 0.01<P<0.05; ** 0.001<P<0.01; n.s. = no significant difference.

Trapping visitors to the flowers of *S. gustavioides* and *S. sayeri* demonstrated not only the increase in some taxa on the opening of the flower resource, but also the response of different insect taxa to each of the two plant species (Table 8). This was seen across a number of different plant species surveyed concurrently. We also saw changes in the taxa visiting the same species at different times of the year (Kitching *et al*. in review).

Observation

The patient observation of all animals that visit a flower of interest across all times of the day is the standard technique of most pollination biologists. Little equipment, but much time, is needed. Visiting fauna can be expected to change at different times of the day and observations should cover those times. In our study, flower visitors were observed from the gondola of the canopy crane. We used observation periods of twenty minutes, and tried to have at least two sets of observations (twenty minutes each) for every two-hour segment of the day starting from midnight (e.g. 00:00 hrs to 02:00 hrs). This could be reduced to early morning, midday, late afternoon and late night.

During observation periods, we recorded the identity of animals visiting the flower and any associated activity or behaviour that might suggest the capacity of the animal to pollinate the flower. Specifically, we recorded if the animal touched the stigma, sipped nectar or collected pollen. In addition, notes were made of the number of flowers in an inflorescence visited and the total number of inflorescences an individual visited. Figure 20 provides an example of a data sheet of the kind used during observation periods. Where possible, individual visitors were collected using a hand net, killed using a killing jar, individually labelled and stored in a vial of 70% ethanol to permit later identification. Killing jars can be made by adding a few drops of ethyl acetate to cotton wool in the bottom of a glass jar. The lid of the jar needs to be metal as ethyl acetate dissolves many types of plastic. Rather than trying to transfer the insect from the net to the killing jar (particularly if it is likely to sting the handler!), simply put the part of the net with the insect in it into the jar and screw the lid onto the net and jar. If using this method, it is best to have a couple of nets and killing jars or you will miss the next visitor while waiting for the previous one to die. Once the insect is dead, transfer it to a small vial of ethanol and include a label for the specimen. You should include the date, time and host plant ID from which it was collected, as well as an individual visitor number that relates to the observation sheet (Figure 20).

Tree No. / Position:	4065	Start Time:	11:00
Date:	12/03/06	End Time:	11:20
Weather:	cloudy	Observer:	Sarah

Visitor No.	Taxa	No. of Flowers Visited	No. of Inflorescences Visited	Time	Collected	Other Notes
1	native bee	1	1	11:09	yes	crawled over anthers, into corolla, sip nectar?
2	small fly	2	1	11:15	no	landed briefly on each flower

Figure 20: Example of a partially completed observation data sheet.

Case Study – Fauna Observed Visiting *Syzygium sayeri*

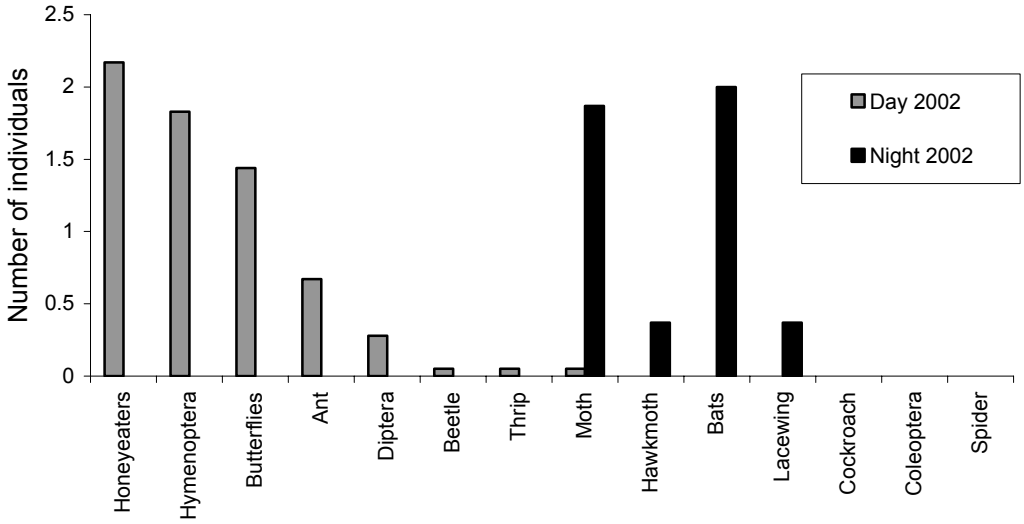

Figure 21: Average number of visitors by taxonomic group observed visiting the flowers of *Syzygium sayeri* at the Australian Canopy Crane.

Figure 22: Bridled honeyeater (*left*) observed feeding from the flowers of *Syzygium sayeri*; and beetles (*right*) feeding at the flowers of *S. gustavioides*.

Observations were made of visitors to four tree species within the access area of the Australian Canopy Crane throughout our project. We provide here examples of the observations made of *S. sayeri* flowers. This species had an apparent day fauna and night fauna, with few groups of taxa found both day and night (Figure 21). Honeyeaters were conspicuous daytime visitors and included Macleay's Honeyeater (*Xanthotis macleayana*), Graceful Honeyeater (*Meliphaga gracilis*), Dusky Honeyeater (*Myzomela obscura*), Yellowspot Honeyeater (*Meliphaga notata*) and Bridled Honeyeater (*Lichenostomus frenatus*). The dominance of different bird species changed between years of observation (Boulter *et al.* 2005). Visiting birds were observed to perch on adjacent branches or the stem of the inflorescence itself to feed on nectar (Figure 22). Probing of flowers was multiple and rapid within an inflorescence. Nighttime observations provided an opportunity to witness blossom bats visiting flowers. The bat visitors also fed from multiple flowers, but in their case were more aggressive foragers, pushing their faces deep into the flower's receptacle to feed on the nectar.

Video Surveillance

Because of the long time that must be spent in the field to make observations, the difficulty in interpreting and recording all visitor behaviour and the logistical problems of making observations across a 24-hour period, many pollination ecologists have taken advantage of surveillance technology to record visitor identity and visitor behaviour. Automated surveillance systems can be based around digital still cameras, video camcorders or cameras.

Video surveillance systems are used widely in laboratory studies and are currently becoming popular in field environments. In general, a video surveillance system consists of a video camera with or without infrared illumination; a video recorder, either digital (DVR) or cassette (VCR); a video multiplexer for multiple cameras; a viewing monitor; and lastly, a power supply. In field conditions, sealed lead acid batteries in waterproof housing can be used and for longer-term monitoring, solar panels can be fitted to provide extra power to the batteries.

Video recording can be continuous in either real time or time lapse, set by an internal clock or operated by external sensors. The most popular external sensors are infrared beams, which are used to monitor movement and trigger recording. Infrared beams can be active with a narrow accurate beam, or passive, sensing movement in a larger area. Other external sensors such as pressure mats, seismic sensors and manual remote controls can be used.

Time-lapse video recorders, especially DVRs, are becoming more popular as numerous hours of footage from individual or multiple cameras can be downloaded to a hard drive or videotape and viewed on a monitor over a smaller time frame. In addition, with the advance in digital technology, a radio link between the video camera and the video recorder can be fitted in place of cable links. The down side to this advance in the field is the limited range of the wireless signal and the interference of surrounding objects.

One of the advantages of video surveillance is that all observations are non intrusive, so disturbance to visitors is reduced and behaviour is not affected by the presence of an observer. Continuous monitoring means that diel behavioural patterns can be identified and the ability to identify species-specific detailed behaviours is enhanced. The disadvantages of video surveillance systems are that they can be quite large and heavy, so transport into the field at long distances can be an issue. Video cameras also only focus on a specific area, so once out of the view of the camera, the visitor's behaviour cannot be observed. Finally, video surveillance systems can be expensive, especially where multiple cameras are required for replication.

The video surveillance systems need to be encased in weatherproof housing. The extreme weather conditions often experienced in the Wet Tropics means that humidity and moisture could be a problem. Native wildlife such as rodents can damage the cable links, although stainless steel casing or coating cables with white diesel can alleviate this problem. Feral pigs can also damage camera systems that are located close to the ground.

We have begun a trial of video surveillance techniques and have deployed an infrared camera and digital video recorder for this purpose. This provides over 24 hours' recording and the flexibility to save sections of footage. The system does require very large heavy batteries and so must be used in sites with reasonably good access.

3.4 DISCUSSION

Identifying the entire suite of flower visitors requires a combination of techniques. We found that many of the very small flower visitors caught by insect traps were not observed by eye during periods of flower observation. Similarly, larger insects were infrequently trapped (e.g. butterflies, hawkmoths), but often observed, and vertebrate visitors were, of course, only identified through patient observation of the flowers. Identifying visitors to a flower does not of course help to determine the function of the visitor. The role of visitors can include pollination, but equally nectar robbing, flower feeding and predation on other visitors. Careful observations of visitor behaviour will provide clues to the role of a visitor and trapping techniques will expose other visitors not identified during observations.

4. POLLINATION

4.1 GENERAL INTRODUCTION

In many plant-pollinator systems, flowers are visited by a variety of pollen vectors. As discussed earlier, not all visitors will be pollinators. Those that are will have different levels of effectiveness (Stebbins 1970, Herrera 1987, 1989). The successful transfer of pollen, in most cases, relies on contact between the reproductive organs of a flower and its visitor in such a way that pollen is picked up on the visitor's body and deposited on the stigmatic surface of the host plant (Faegri and van der Pijl 1979). For obligate outcrossing species, pollinator movement between plants is also required. As a consequence the morphology of the flower and the behaviour of the visitor together determine the success of this process (Muchhala 2003).

Numerous approaches to identification of pollinators and assessment of their efficiency have been employed – none can be said to be the perfect test, and most add to the mounting evidence collected through observations of flower morphology and phenology as well as identifying candidate visitors. Observation of visitor behaviour and abundance has been the traditional approach, and remains the most frequently used technique to identify a plant's pollinators (Hopper 1980, Sazima *et al*. 1996, Sakai *et al*. 1999, Yumoto 2000). In addition the relationship between floral structure, floral rewards and typical faunal associations (i.e. pollination syndromes) has been used to predict pollinators (Hansman 2001, Highan and McQuillan 2000, Hingston and McQuillan 2000); to discount some visitors as effective pollinators (Muchhala 2003); or even to replace field observations (Ibarra-Manriquez and Oyama 1992). Understanding flower structure, identifying the visitor array and observing visitor behaviour are all essential precursors to making testable predictions and designing useful pollination experiments. The difficulty with the approaches listed above is the failure to demonstrate actual pollen flow (Muchhala 2003).

Alternative techniques seek to track the movement of pollen as a measure of pollination success. Examples of these include the capture of visitors and quantifying of con-specific pollen loads (House 1989, Kato *et al*. 1995, Williams and Adam 1995, Bernal and Ervik 1996, Nagamitsu and Inoue 1997, Birkinshaw and Colquhoun 1998, Sakai and Inoue 1999); the use of fluorescent powders as a proxy for pollen and hence pollen movement (Kearns and Inouye 1993, Kwak and Vervoot 2000); and the deposition of pollen on pollen traps within a flower (Muchhala 2003). In this way, positive identification of pollen vectors and quantification of pollen moved can be made. Again, successful pollination is not confirmed. These techniques are also highly labour intensive, requiring extreme field and laboratory hygiene protocols to avoid pollen contamination (House 1989) and dependent on identification of donor plant species using pollen morphologies. For con-specific species, the latter can be very difficult (Pike 1956).

Manipulative exclusion experiments are designed to identify pollinators by partitioning visitor groups (e.g. day versus night visitors) and use signs of fertilisation (e.g. seed set) to identify and quantify successful pollination (Crome and Irvine 1986, Bernal and Ervik 1996). The design and execution of these experiments is determined by the question asked, and relies on some understanding of visitors and flower morphology. These techniques rely on pollinator arrays that can be partitioned in a testable way. The vagaries of post-fertilisation failure can reduce the success of these types of experiments (Crome and Irvine 1986).

The capacity of individual visitors to transfer viable pollen can also be assessed by examining pollen tube growth within a visited style (Nagamitsu and Inoue 1997). By opening bagged flowers to a single visitor only, examination of the style using fluorescence microscopy can demonstrate the success of that individual visitor (Wilfred Morawetz *pers.*

com. June 2002). While this technique demonstrates successful pollen transfer and germination, it should not be mistaken for reproductive success, as post-fertilisation reproductive isolating mechanisms (Kenta *et al.* 2002) and predation are not accounted for.

4.2 POLLEN ON VISITORS

We know that visitors must move pollen between the male and female reproductive organs of a flower or flowers in order to be a pollinator. A visitor can be examined to see if indeed it is carrying pollen and is therefore a likely pollinator. This does require being able to identify the pollen of the species of interest. If a library of pollen exists, it may be possible to identify the array of plants visited by an animal. Pollen libraries can be established using the technique for collecting pollen described in Section 2.5.

4.2.1 Techniques for Examining Pollen Loads

See Appendix 1 for a list of equipment required.

Often, insects visiting flowers can have pollen on their body. To determine which species the pollen is from, and the quantity of pollen, pollen needs to be removed from the body and examined under a microscope. For insect visitors to *Normanbya normanbyi*, we used basic fuchsin jelly (see page 29 for recipe). Small lumps of the jelly can be speared onto a pin or other fine pointy instrument and dabbed over the insect's body. Transfer the jelly to a microscope slide. Place the slide on a slide warmer until the jelly is melted and cover with a cover slip. Leave to cool and then examine under a microscope, using the pollen library to help identify the pollen source. Refrigerate slides if they are to be kept for more than a few months.

4.3 EXCLUSION STUDIES

Exclusion studies offer a quantifiable method of assessing the success of a class or classes of visitors in pollinating a particular plant species (e.g. Crome and Irving 1986, Boulter *et al*. 2005). These techniques usually rely on being able to partition visitors into different classes. For example, we used exclusion studies to test the differing effectiveness of day and night visitors as well as visitors of different size classes.

Exclusion Trap Design

Our exclusion cages consisted of a collar constructed from a semicircle (radius 150 mm) of 0.2 mm thick plastic acetate. Two holes of diameter 70 mm were cut into the semicircle and circle mesh fabric was glued over these windows. Traps were assembled *in-situ*. The plastic cage was wrapped around the stem beneath each inflorescence to form a cone with the seam secured firstly with waterproof craft glue and then stapled. The bottom was sealed around the stem using waterproof tape. A cotton "make-up" pad was wrapped around the stem at the point of contact with the cage before placement to avoid damaging the stem. A cylinder of mesh fabric was attached to the top of the trap by gluing the long edge of a rectangle of fabric around the circumference of the trap and gluing and stapling the two shorter edges together. The bag was sealed by tying closed the cylinder with a length of string. The same type of cage was used for both *Syzygium gustavioides* and *S. sayeri.* In the latter case, an additional curved wire stay was attached across the top of the trap to prevent the fabric touching the larger inflorescences. For *Normanbya normanbyi*, a cylindrical trap was constructed using acetate and plastic lids.

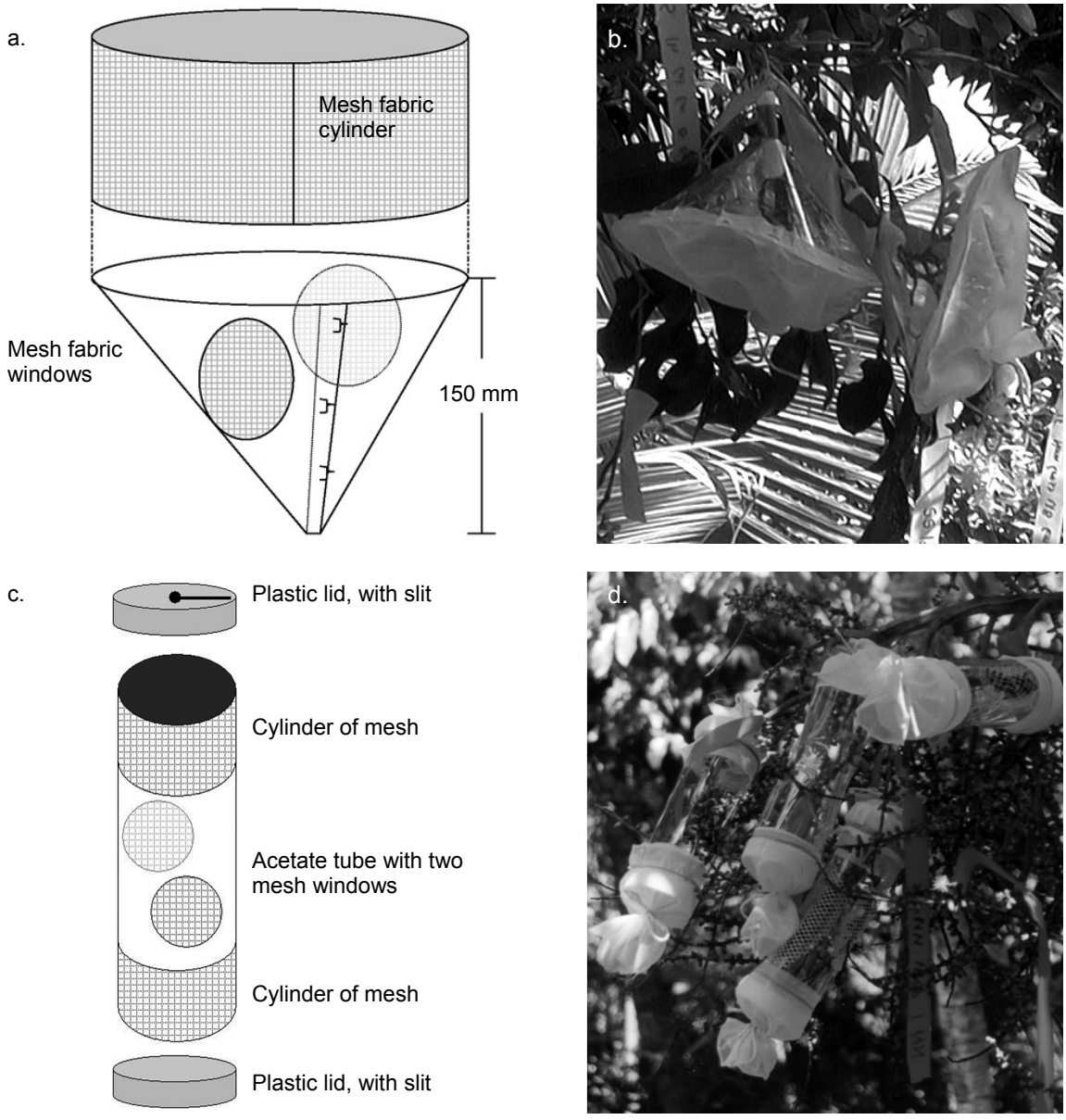

Figure 23: (a) Design of "bagging" cages, constructed from plastic acetate sheets with fine mesh windows; (b) assembled cages seen here on *Syzygium sayeri* during the breeding system experiment; and (c) the modified design used on inflorescences of (d) *Normanbya normanbyi*.

4.3.1 Size Class Exclusion Experiment

See Appendix 1 for a list of equipment required.

We have used exclusion cages with different levels of access to determine the capacity of different sized visitor groups to successfully pollinate a species. In this study, we designed cages that allow visual and physical access by visitors. Details of construction design are outlined on page 45 (Exclusion Trap Design). We used four treatments (i.e. four different mesh sizes) and two controls for our experiments. What you choose to use will depend on what animals you wish to differentiate between and the availability of suitable mesh fabric. For example, testing for the impact of a large introduced flower visitor (e.g. bumblebee) might be done with one mesh size that excludes only larger visitors including the bumblebee. For each treatment, the exclusion cage fabric mesh windows and the mesh at the top or ends of the cone / cylinder were replaced with one of four different sized mesh fabrics. Mesh hole diameters were 0.5 mm, 1.5-2.5 mm, 4.5 mm and 8-10 mm. A plastic cage with no mesh acted as the control for cage effect, and a non-manipulated inflorescence provided the control for natural out-crossing rates.

Traps are generally constructed around the inflorescence. Prior to trap construction, the number of buds on each inflorescence must be counted and recorded. Mark each trap with brightly coloured flagging tape on which the tree number and treatment type is recorded. Be sure to record the location of each tree. The cages are then left in place until all flowers on the inflorescence have opened and senesced. Cages can be removed at this stage. Retie the flagging tape to the stem of the inflorescence so it can be rechecked for successful pollination. In order to determine the success of pollinators reaching the flowers, signs of fertilisation need to be assessed. We chose the presence of a swollen receptacle in individual buds for *Syzygium gustavioides*, which had very large but low numbers of mature what, to avoid the effects of predation. In the case of other species, the development of fruit was rapid, and immature fruits could be used. We were able to return to manipulated inflorescences approximately eight to nine weeks after flowering to score for successful fertilisation (although regular observations should be made). The timing of scoring fertilisation should be based on the life history of the target plant.

In total, we used three to six sets of the six treatments on each of five to six trees for three of our target species. The resulting successful pollinations (i.e. swollen buds or fruits) were taken as a proportion of the original number of buds. Because the results of the experiment are a proportion, they must be arcsin transformed (arcsin $\sqrt{}$ proportion) for statistical analysis. We compared the various treatments and controls using ANOVA based on the transformed proportions (Sokal and Rohlf 1995).

4.3.2 Day / Night Exclusion

See Appendix 1 for a list of equipment required.

The exclusion experiment idea can also be used to test the different (if any) success rate of day versus night visitors. For this experiment, we use four treatments consisting of two controls – one with no cage (Control 1); the second with a cage that remained on for the entire experiment (Control 2) – and two treatments – first, diurnal pollination, for which the cage was removed at 06:00 hrs and reconstructed at 18:00 hrs, and second, nocturnal pollination, for which the cage was removed at 18:00 hrs and reconstructed at 06:00 hrs. For this experiment we simply used the “balloon cages” (described in Section 2.4.2), as they were very easy to remove and reapply.

Setting up the Experiment

For each set of treatments, select four similar sized inflorescences and count the starting buds. Choose buds that are close to opening. For Control 1, simply mark the inflorescence with brightly coloured flagging tape with the tree number and treatment number written on it in permanent marker pen. For Control 2, construct a trap around the inflorescence. This will stay on until the end of the experiment. Build traps on the inflorescences of the other two treatments. Label the three treatments with the tree number and the treatment (Control 2, day-pollinated or night-pollinated), again by writing these codes on brightly coloured flagging tape and tying around the stem of the inflorescence. This initial setting up can be done at any time of the day. Return the following day at dawn and remove cages from day-pollinated treatments. To remove the trap, undo the strings at top and bottom, remove the fabric sock, and remove the staples from the plastic cage. Leave only the flagging tape to denote the inflorescence and treatment. Return the same day at dusk, reconstruct the cages on the day-pollinated inflorescences and remove the cages from the night-pollinated treatments. Continue returning to the trees at dawn and dusk every day until most flowers have opened (this may take up to fourteen days). Do not miss any visitation or the experiment will be invalidated. At the end of the experimental period, remove any unopened buds and deduct from the original bud count, and reconstruct all cages to prevent any further pollinator access. The cages can be removed once all flowers have senesced. Leave the flagging tape on the inflorescence.

Results of Exclusion Experiments on *Syzygium sayeri*

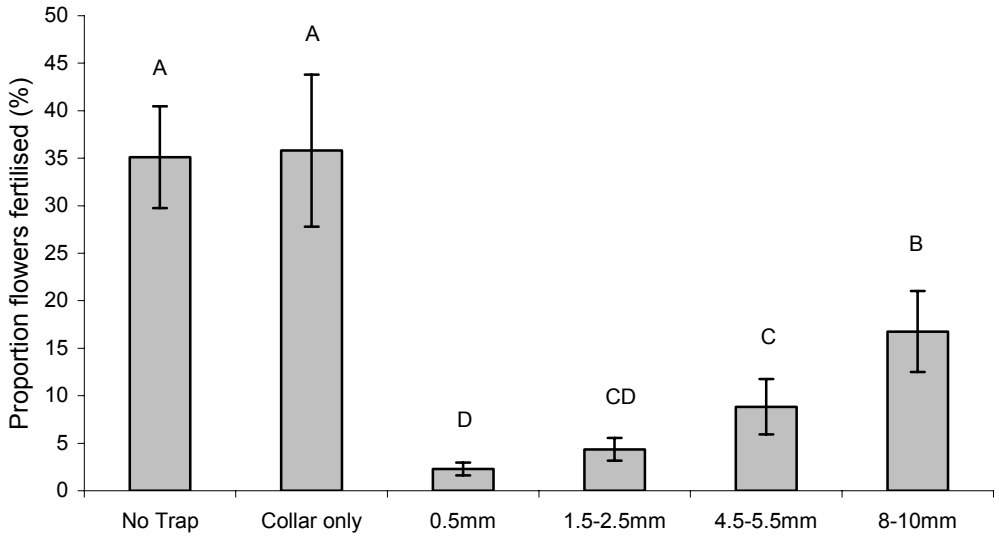

Figure 24: Mean proportion (percent) of *Syzygium sayeri* flowers fertilised under different mesh size exclusion cages. Different letters represent significant differences (P < 0.05).

The size exclusion experiment on *S.sayeri* demonstrated that large visitors have an important role in its pollination. The results clearly show that the largest mesh size did not allow access to all visitors that contribute to the successful pollination of this species. When smaller insect visitors alone have exclusive access to these flowers, pollination still occurs, but at a level lower than naturally found among these trees. The largest mesh size used would be expected to completely exclude bats from feeding on flowers, although some honeyeaters were observed to feed on nectar by poking their beaks through the mesh (K. Goodall *pers comm.* July 2002). The results of the day and night exclusion experiment in this case offer no statistically significant results, however the general trend does suggest that both night and day time visitors contribute to the pollination success of *S. sayeri*, with night time possibly slightly greater. Interestingly, the number of birds visiting flowers per observation period was greater than recorded for any night visitor (Boulter *et al*. 2005).

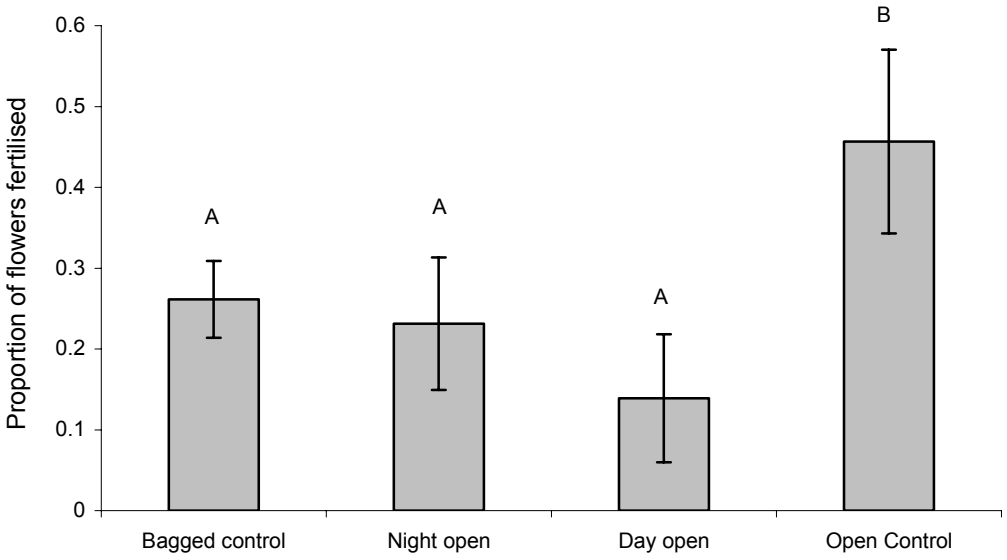

Figure 25: Mean proportion of *Syzygium sayeri* flowers successfully pollinated by day visitors, night visitors or for controls. Different letters represent significant differences (P < 0.05).

4.4 POLLEN TUBE STUDIES

Obtaining definitive evidence that a visitor has pollinated a flower during its visit is extremely difficult, and often observations of behaviour and quantification of the success of a group of visitors is circumstantial at best. One fairly labour-intensive technique that provides greater evidence of pollination success looks at the growth of pollen tubes in the stigma following the visit of a single individual. The basic idea is to exclude all but one visitor to a flower and then test this flower for the successful transfer and germination of pollen in that flower. Once compatible pollen is deposited on the stigmatic surface, it can germinate, sending a tube down into the stigma to the ovaries. The tube can be examined by using one of a number of alternative staining techniques and microscopy.

Kearns and Inouye (1993) provide a number of alternative staining techniques useful for examining pollen tubes. The most commonly used of these methods is epifluorescence, but requires access to a fluorescence microscope. Alternative staining methods are available for use with a stereomicroscope, although can be more difficult to detect pollen tube growth. We used two methods and these are described here. The non-fluorescence staining technique proved to be sufficient under field conditions (e.g. during the pilot study), but where samples could be returned to the laboratory for examination, the latter method was preferred.

4.4.1 Pilot Study: How long does it take for pollen tubes to grow?

The rate of pollen tube growth varies among species. Some will take a matter of a few hours, others over 24 hours. Before commencing the pollen tube experiment, the length of time a pollen tube will take to grow in the species of interest needs to be established. This is done by examining artificially cross-pollinated flowers at different times following pollination to determine pollen tube growth. The following protocol can be used:

1. Bag an inflorescence of unopened buds (bagging prevents flower visitation by potential pollinators in order to avoid pre-existing tubes giving a false positive).
2. Visit flowers regularly and, when several are open, cross-pollinate using pollen from another tree (do this early in the morning to allow for sufficient return visits).
3. Re-bag the inflorescence.
4. Collect a pollinated flower every two hours for the rest of the day. Collect a final flower 24 hours after the initial pollination.
5. Place flowers immediately into a fixative to prevent further pollen tube growth.
6. Stain the stigmas (see the two alternative methods described in Section 4.4.2, Techniques for Viewing Pollen Tube Growth).
7. Examine the stigmas for pollen tube growth.

Using this protocol, you will be able to establish the time that elapses between the deposit of pollen and pollen tube growth.

4.4.2 Techniques for Viewing Pollen Tube Growth

See Appendix 1 for a list of equipment required.

Method 1: Aniline blue epifluorescence

This is a four-step process of fixing, softening, staining and viewing the styles to see pollen tube growth.

Fixing: Place flowers in a solution of 1:3 acetate:ethanol for 24 hours. Once fixed, the flowers can be transferred to 70% ethanol for storage until ready to process.

Softening and clearing: Remove the style from the flower. Softening can be done with sodium hydroxide (NaOH). The time required to soften will depend on the species. For *S. gustavioides*, we placed the styles in 8N NaOH for 24 hours, as these were fairly thick styles. We suggest monitoring the styles for signs of deterioration, particularly if they are fine.

Staining: Decolorise aniline blue by dissolving 0.1% mass / volume aniline blue in 0.1mol/L K_2HPO_4. Rinse the styles in tap water to remove the sodium hydroxide. Stain the styles in decolorised aniline blue for 24 hours. Mount in a drop of glycerol and cover with a cover slip, squashing gently. Store in a lightproof box until ready for examination.

Viewing: Finally, styles were viewed with a fluorescence microscope and UV filter (excitation wave length: 450-490 nm). Pollen-tube walls and callose plugs fluoresce a bright yellow-green (Figure 26).

Modified from Martin 1958; Kearns and Inouye 1993

Method 2: Basic fuchsin / fast green

This method does not require epifluorescence, and tubes can be viewed under white light.

Fixing: Place flowers in a solution of 1:3 acetate:ethanol for 24 hours. Once fixed, the flowers can be transferred to 70% ethanol for storage until ready to process.

Staining: Stain the styles in 1% basic fuschin:1 % fast green (4:1) for at least 24 hours.

De-stain and soften: De-stain and soften tissues in lactic acid for twelve hours. Mount on a microscope slide and squash under a cover slip. Pollen tubes will stain maroon against a white background under white light.

Source: Kearns and Inouye 1993.

Setting up the Experiment

Bag a number of inflorescences of unopened flowers using a “balloon cage” and fine mesh sock (see Section 2.4.2 for a description of these cages). Visit the inflorescences daily and search for open flowers. If open flowers are found, remove the bag and cage. Observe flowers until the first visitor arrives (we recommend using a data sheet for observations similar to that shown in Figure 20). Note which flower is visited and the behaviour of the visitor. Once the visitor has departed, tag the visited flower / flowers using numbered retail swing tags and record the flower number next to behaviour observations and the time of the visit. Re-bag the inflorescence to prevent any further visitation. Collect the flower at the allotted time required for the pollen tube to grow (established in the pilot study), and treat using the chosen method of staining (Method 1 or Method 2, above). Flowers can be immediately fixed in 1:3 acetate:ethanol for both methods, but be sure to transfer to 70 %

ethanol after 24 hours if not able to complete the staining protocol immediately. Control flowers should also be sampled. Two types of controls can be made. First, simply collect unvisited flowers, and second, artificially cross-pollinate flowers. In the former, no pollen tubes are expected to grow and the latter should show pollen tube growth.

Examining Pollen Tubes

Following staining of the styles, they should be mounted on microscope slides in preparation for examination. Label each slide using a small sticker, recording the visitor number, date, time of visit and visitor taxa. Note, slides for epifluorescence should be kept in a lightproof box until they are ready to be examined, as the fluorescence has a limited excitation time and will fade after a period of exposure to UV light. The visitor is scored as “successful” if pollen tubes can be seen growing down the style.

Results of Pollen Tube Study on *Syzygium gustavioides*

Table 9: Proportion of styles from *Syzygium gustavioides* flowers, demonstrating the presence of pollen tubes following a single insect visit.

Visitor	Pollen Tubes Present (Percent)
Artificial Cross	100 (n = 5)
No Visitors	0 (n = 5)
Apis mellifera (Feral Honeybee)	90 (n = 20)
Small Hymenoptera	86.36 (n = 44)
Native Bees	100 (n = 8)
Nitidulidae sp.	100 (n = 3)
Thysanoptera	100 (n = 1)
Small Diptera	66.67 (n = 3)
Large Hymenoptera	100 (n = 2)
Curculionidae sp.	50 (n = 2)

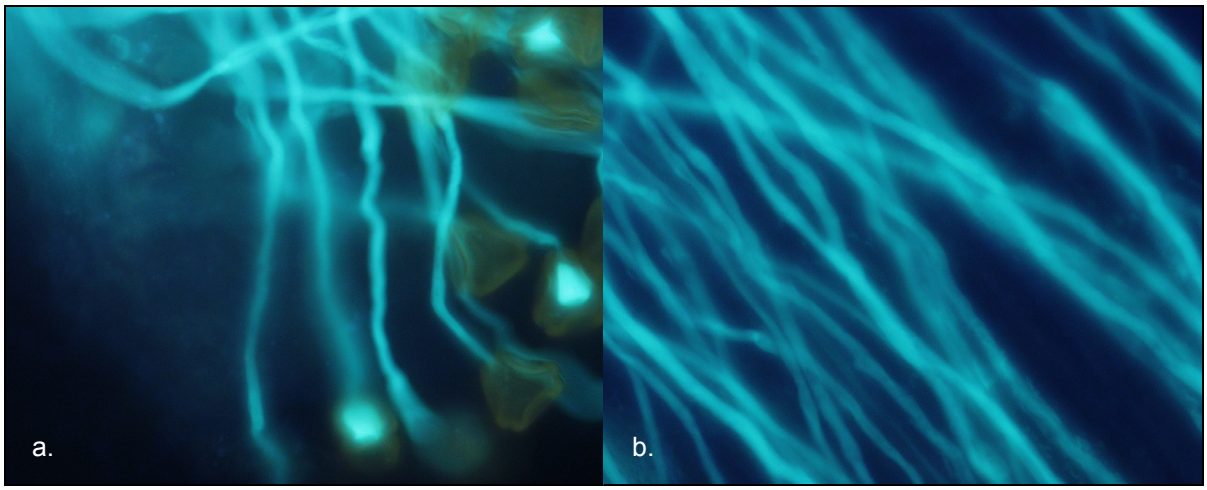

Figure 26: (a) Pollen tube germination; and (b) multiple pollen tube growth in *Syzygium gustavioides*. Style examined using fluorescence microscopy (100x magnification).

We bagged over one hundred *S. gustavioides* inflorescences and observed visitors to individual flowers from within those cages over the period from November 2002 to February 2003. In addition, five flowers were collected and examined that had not been exposed to visitations, and a further five were artificially cross-pollinated and collected as above, for scoring of pollen tube growth. We see from the results that all visiting taxa were capable of pollinating this species. The controls ('artificially cross-pollinated' and 'no visitors') suggest that this technique is a good indication of the pollination success of the treatment.

5. THREATS TO POLLINATION SYSTEMS

The collapse of pollinator mutualisms has been identified as one potential consequence of anthropogenic land use change (Kearns and Inouye 1997; Allen-Wardell *et al.* 1998; Kearns *et al.* 1998; Wilcock and Neiland 2002). Declines in pollinators have been reported from most continents (Kearns *et al.* 1998; Kevan and Phillips 2001). Land clearance, fragmentation, agricultural practices, herbicides, pesticides and the introduction of exotic plant and pollinator species (Table 10) have all been implicated in a serious decline in pollinators that has been referred to as a "pollination crisis" (Buchman and Nabhan 1996).

Loss of or interruption to pollinator services may have several outcomes. The most obvious result is a loss or reduction in seed set, however, impacts may also extend to reduced offspring vigour as a result of self-pollination, decreasing heterozygosity, and in the increased expression of deleterious traits, resulting from inbreeding (Kearns and Inouye 1997). Ultimately, loss of seeds, fruits or plants will affect animals that rely on these resources.

Table 10: Summary of threats to pollination systems.

Threat	Effect	Impacts
Fragmentation	• Reduced population size • Isolation • Hostile matrix • Alteration of visitor behaviour	• Increased genetic drift, in-breeding depression, increased threat of extinction, reduced pollen dispersal, reduced fitness (Rathcke and Jules 1993; Kearns *et al.* 1998). • Increased reproductive success (Cunningham 2000). • Temporary reduction in pollinator activity (Becker *et al*. 1991). • Genetic erosion of small populations (Cane and Tepedino 2001, Ghazoul *et al*. 1998, Oostermeijer *et al.* 1998). • No reduction of reproductive success, substantial between- and within-site variability (Costin *et al.* 2001). • Effect of isolation tied to pollinator mobility (Law 2001). • High genetic differentiation among geographically close patches (Dutech *et al.* 2002). • Pollen clogging by generalist pollinators (Kunin 1997, Groom 2001).
Agricultural Practices	• Land clearing • Pesticide spraying • Herbicide spraying • Extensive monocultures • Grazing • Resource depletion	• Pesticides reduce pollinator numbers (Batra 1981). • Poisoning of pollinators resulting in death, behavioural changes and reduced mobility (Johansen 1977). • Contamination of pollen and honey (Kearns *et al.* 1998). • Herbicides reduce availability of nectar plants, remove nesting sites, destroy larval food sources for pollinators (Kevan 1975, Kearns *et al.* 1998, Richards 2001). • Grazing changes nesting sites, decreasing water availability, and replacement of native grass species with introduced pasture grasses (Kearns and Inouye 1997).
Invasive Species	• Displacement of pollinators by feral competitors • Displacement of native plants	• Feral honeybees compete for pollen normally available to native pollinators, altering pollen dispersal patterns through foraging that differs from native pollinators, and depleting nectar supplies to nectar feeding pollinators (England *et al*. 2001). • Introduced bees implicated in successful spread of exotic plant species where native animal species are not suitable pollinators (Stout *et al.* 2002).

Like most tropical landscapes, the Wet Tropics have been subjected to processes of fragmentation over the last one hundred years or so. Plant species 'marooned' in these fragments may or may not be part of viable populations – and it may take much longer than one hundred years before this becomes evident. Pollination and the subsequent reproductive performance of plants in fragments becomes a crucial issue. Understanding the changes that will occur to pollination processes and outcomes in fragments is an essential first step in managing these changes and attempting to ensure the long-term future of our forests. That having been said, there is almost no data available on this topic. The study by Law and Lean (1999) on *Sygygium cormiflorum* did demonstrate that visits by vertebrates to the flowers were skewed in favour of bats over birds in fragmented situations. Far more work has been carried out in other countries (see references in Table 10), and what is clear from this work is that the little work that has been done shows that the impacts are complex.

We do have in progress studies on the visitor assemblages of isolated and garden individuals of *Syzygium sayeri* and *Normanbya normanbyi*, as well as investigations into the movement of pollen in continuous forest, but no results are yet available. There is no question that much more work of this nature is required and, indeed, is crucial to future management decisions. The key questions for the pollination system of any disturbed system include:

- Has the flowering pattern been altered?
- What level of successful pollination occurs?
- What is visiting the flowers?
- How far is pollen being moved?
- How does any of this differ from continuous forest?

Many of the methods already described can be used to investigate these questions. We briefly describe some additional methods we are currently using in the rest of this section.

5.1 POLLEN MOVEMENT

Understanding how far pollinators can move pollen can give some understanding of gene flow and the potential for pollinators to move pollen between isolated trees (especially in fragmented systems).

Tagging or Marking

There are a number of ways to tag or mark pollen to determine the movement of pollen between individual trees. We have used fluorescent powder to determine the movement of pollen amongst individual *S. gustavioides* trees at the Australian Canopy Crane site. In this case, we marked flowers on each tree using a different coloured powder for each tree. We then returned to the trees at night and used a UV torch to detect the fluorescent powder on other flowers. Using this, we saw that while most movement was within a tree, there was certainly some movement between trees. This method could be used on understorey trees elsewhere, however without crane access this method is not suitable for use in canopy trees.

Genetic Techniques

Genetic markers can be a useful tool for determining the flow of pollen between individual plants, and the rates of outcrossing. These techniques have been used extensively throughout the neotropics, based on allozyme and microsatellite data (Ward *et al.* 2005). The difficulty with this technique lays in the cost, and in some cases the failure, to obtain sufficient variability to successfully determine gene and pollen flow (Squirrell *et al.* 2003).

We are currently using micro-satellite markers in the hope of determining the distance that pollen is transferred within a continuous forest by pollen vectors. Our expectation is that by determining the paternity of fruit from focal tree, where the pollen donor tree can be identified we will know the distance pollen has been transferred.

To date, we have mapped and sampled all *S. sayeri* and *S. gustavioides* trees within a 250-metre radius of the canopy crane. Mapping was done using a Garmin Gecko GPS, which proved reasonably reliable within the rainforest. To find trees, we set up a series of 500-metre transects running north-south at every ten metres. We searched along the transects, looking approximately five metres on either side of the transect for the target trees. When trees of either species were located, they were tagged using tree tags and an individual number. To profile the DNA of each potential father tree, we collected a small core of cambium (Colpaert *et al.* 2005). A 10 mm leather punch was hammered into the tree trunk to extract the cambium. The core was stored in a labelled plastic vial with silica gel crystals until analysed.

Fruit has been collected from the maternal trees within the access area of the canopy crane, and then frozen and stored in a freezer at -80°C prior to analysis. A library of micro-satellites is developed for a plant species, in our case, we have a library of 6 loci for *S. sayeri*, but where less successful with *S. gustavioides*. The cambium will be analysed and compared to the DNA of the fruit to determine paternity. Identifying the father will give us a distance travelled by the pollinator. For those with no identified father, we can speculate that pollen has travelled further than 250 metres.

5.2 CONCLUSIONS – CONSERVATION AND MANAGEMENT

It is apparent from the literature that at present we do not know the net effect of anthropogenic disturbance on plant-pollinator interactions. It seems likely that activities such as habitat fragmentation, agriculture and changes to habitats caused by introduced species will be detrimental to some native species; potentially beneficial to others; and will sometimes have subtle and counter-intuitive effects (Cane and Tepedino 2001). The difficulties in understanding plant-pollinator interactions are matched by the difficulties of managing and conserving for these interactions. Conservation and management is likely to be frustrated by a lack of basic information on the reproductive ecology of individuals; detection of declines in populations and the interactions of individual populations; and the different scales at which processes operate. There is a clear lack of empirical evidence. The number of review papers on the subject of pollinator declines and the impacts of disturbance on the pollinator almost outweigh the number of field studies on which those reviews are based. The few studies that have been done provide conflicting and inconsistent results.

The recognition that most pollinators are far from obligate changes how they should be conserved (Kearns *et al*. 1998). The notion that the loss of one species will cause the linked extinction of another (Rathcke and Jules 1993) can no longer be maintained. Kearns *et al*. (1998) argue that if the fundamental nature of plant-pollinator interactions is that of a complex web, varying both in time and space, then the job of conservation is made more subtle and complex. But in turn, this may mean that these systems are more robust to change than previously thought. Consider the fundamental evolutionary nature of pollination. Plants and their pollinators are mutualists, both benefiting from the relationship. However, each has separate goals. The plant desires reproductive success via pollination; the pollinator is rewarded with a source of food, shelter or a mate. The selfish rather than cooperative nature of pollination is evidenced by nectar robbers and flower cheaters (Maloof and Inouye 2000). The result of conflicting interests is divergent natural selection, with plants responding with different floral phenotypes, and animals, their phenotype. The resulting morphologies are not optimal for the other, and in this way the relationship is more opportunistic and flexible (Kearns *et al*. 1998). This can create conflicts in conservation

management between the needs of endangered animal species and that of endangered plant species (Simon *et al*. 2001). While generalist pollinators may respond better to disturbance than specialists, the isolation and reduced densities of individual plant species created in disturbed areas may result in increased pollination failure in these systems (Wilcock and Neiland 2002). Both generalist and specialist pollination syndromes are likely to be impacted by disturbance, but the impacts will be different and will require different conservation and management strategies.

The demographic and genetic consequences of habitat fragmentation and land use change are likely to be species-dependent (Cane 2001). Determining the impacts on individual species will require the uncoupling of factors such as habitat management practices, population size, isolation, genetic effects, pollination and sexual systems. It would be unwise to assume that pollination systems are robust enough to adapt to current rates of anthropogenic change and disturbance. With implications for essential ecological processes, potential for flow-on effects to other species and serious economic implications, the conservation and management of pollination systems requires serious attention. Pollinators may not only be important for the long-term success of our forests. For example, on the Atherton Tablelands, native pollinators found in rainforest fragments played an important role in crop pollination (Blanche and Cunningham 2005). Conservation should not be placed on hold for lack of consistent evidence, but better understanding of the variation should be sought. Further information on pollination systems, carefully designed studies of potentially impacted systems and protocols for measuring pollinator declines are urgently needed.

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APPENDIX 1 – EQUIPMENT LISTS

Suggested suppliers of equipment are provided in Appendix 2.

Section 2.2.2 – Observing and Recording Flower Phenology

- Small swing tags
- Lead pencils
- Data sheet
- Clipboard

Section 2.3.2 – Morphology of Individual Flowers

- Dissecting microscope with graticule
- Dissection kit
- Verniers

Section 2.4.2 – Testing Stigma Receptivity

- 3% hydrogen peroxide
- Pipette
- Watch glass
- Hand lens

Section 2.4.2 – Testing Self-compatibility

- Plastic acetate sheets
- Fine mesh fabric
- Stapler and staples
- String
- Cotton pads
- Flagging tape
- Permanent marker pens
- Pencils
- Note book
- Scissors
- Fine paint brush

Section 2.5.2 – Nectar Measurements

- Micro-syringe or capillary tubes
- Handheld refractometer
- Soft tissues
- Water

Section 2.5.2 – Pollen Viewing and Morphology

- Beaker or small vase
- Large filter papers
- Plastic bag big enough to cover an inflorescence
- Fuchsin jelly (see recipe on page 29)
- Microscope slides
- Cover slips
- Slide warmer (we use an old pie warmer)

Section 3.2.1 – Washing Technique to Sample In-fauna

- A4 plastic zip lock bags
- Slayafe® or equivalent spray insecticide
- 70% ethanol
- Fine artist's brushes
- Takeaway containers

Section 3.3.2 – PAS (plastic acetate strip) Traps

- Plastic acetate sheets
- Plastic coated wire
- Tangletrap®
- Mineral spirits
- Takeaway containers
- Soft artist's brushes
- Funnel
- Fine gauze
- Collection bottle for cleaned mineral spirits
- Ethanol
- Vials
- Labels

Section 3.3.2 – Interception Traps

- Plastic acetate sheets
- Wire
- Petroleum jelly
- Takeaway containers
- String
- Permanent marker pens
- Funnel
- Fine gauze
- Ethanol
- Vials
- Labels

Section 3.3.3 – Observations

- Data sheets
- Pencils
- Ethyl acetate
- Cotton wool
- Glass jars
- Large butterfly net
- Small vials
- 70% ethanol
- Binoculars
- Stopwatch / timer

Section 4.2.1 – Examining Pollen Loads

- Fuchsin jelly (see recipe on page 29)
- Pin
- Slide
- Cover slip
- Slide warmer

Section 4.3.1 – Size Class Exclusion Experiment

- Plastic acetate sheets
- Various sized mesh fabric
- Stapler
- Waterproof glue
- Waterproof tape
- Cotton pads
- Flagging tape
- Scissors
- Permanent marker pens
- Pencils
- Notebook

Section 4.3.2 – Day / Night Exclusion Experiment

- Plastic acetate sheets
- Fine mesh fabric
- Stapler
- Waterproof glue
- Waterproof tape
- Cotton pads
- Flagging tape
- Scissors
- Permanent marker pens
- Pencils
- Notebook

Section 4.4.1 – Pollen Tube Fieldwork

- Plastic acetate sheets
- Fine mesh fabric
- Stapler and staples
- String
- Cotton pads
- Swing tags
- Flagging tape
- Permanent marker pens
- Pencils
- Notebook / data sheet
- Small vials
- Fixative (1:3 acetate:ethanol)

APPENDIX 2 – RESOURCES

REFERENCE BOOKS

Pollination – General

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EQUIPMENT AND SUPPLIERS

Fluorescent Dye Powders .....Brada Fine Colour Group (www.brada.com.au)

Hand lens ..... Australian Entomological Supplies (www.entosupplies.com.au)

Micro-syringe.....Alltech (www.alltechaust.com.au)

Refractometer.....John Morris Scientific (www.johnmorris.com.au)

Stains (e.g. basic fuchsin) .....ProSciTech (www.proscitech.com.au)

Swing tags..... newsagents, stationers

Tangle trap ..... Australian Entomological Supplies (www.entosupplies.com.au)

UV light..... Dick Smith Electronics (www.dse.com.au),
Australian Entomological Supplies (www.entosupplies.com.au)