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).

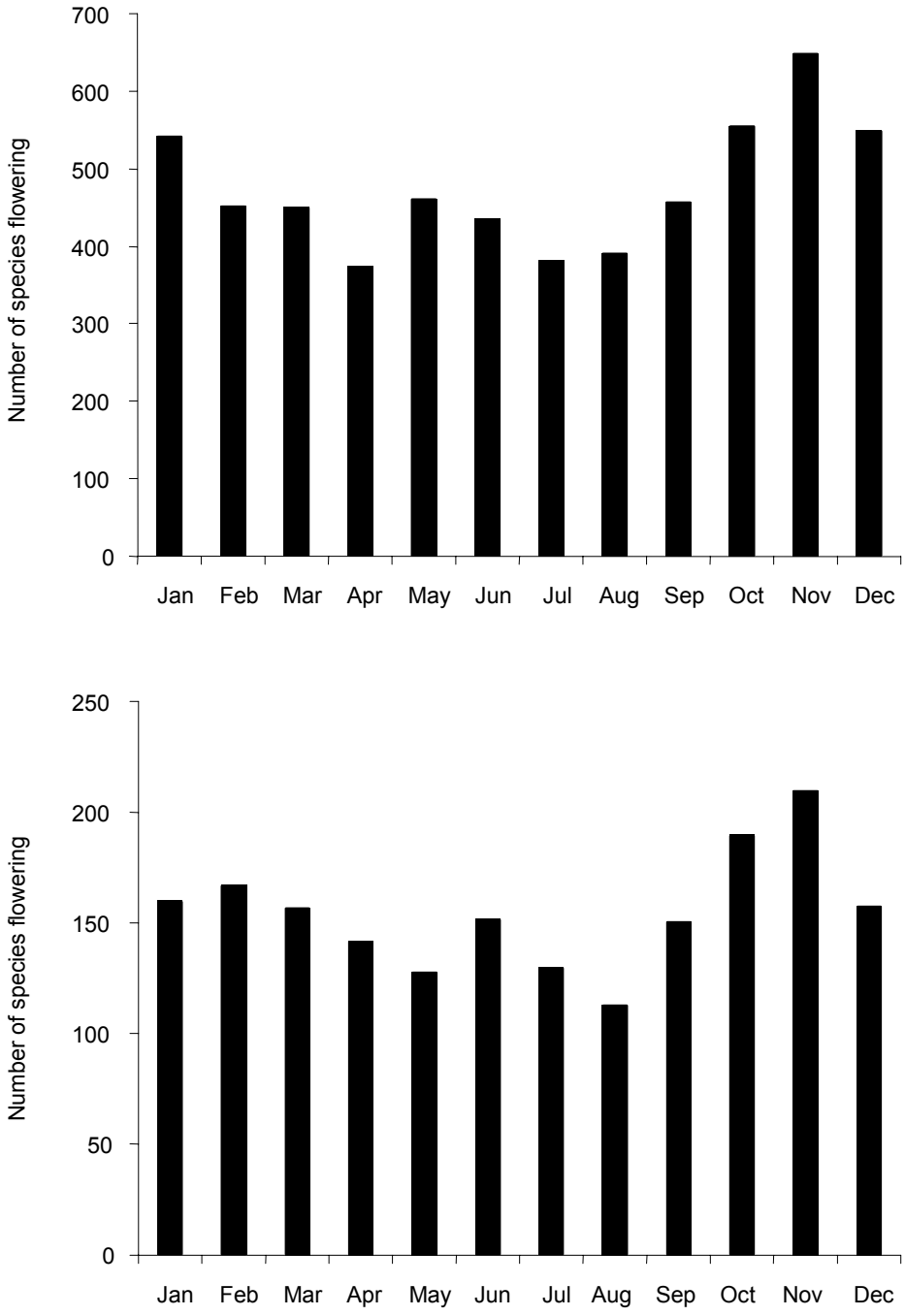

Figure 5: Total number of (*top*) tree and shrub species (reproduced from Boulter *et al.* 2006), and (*bottom*) vine species recorded flowering in any given month in the Wet Tropics bioregion.

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).

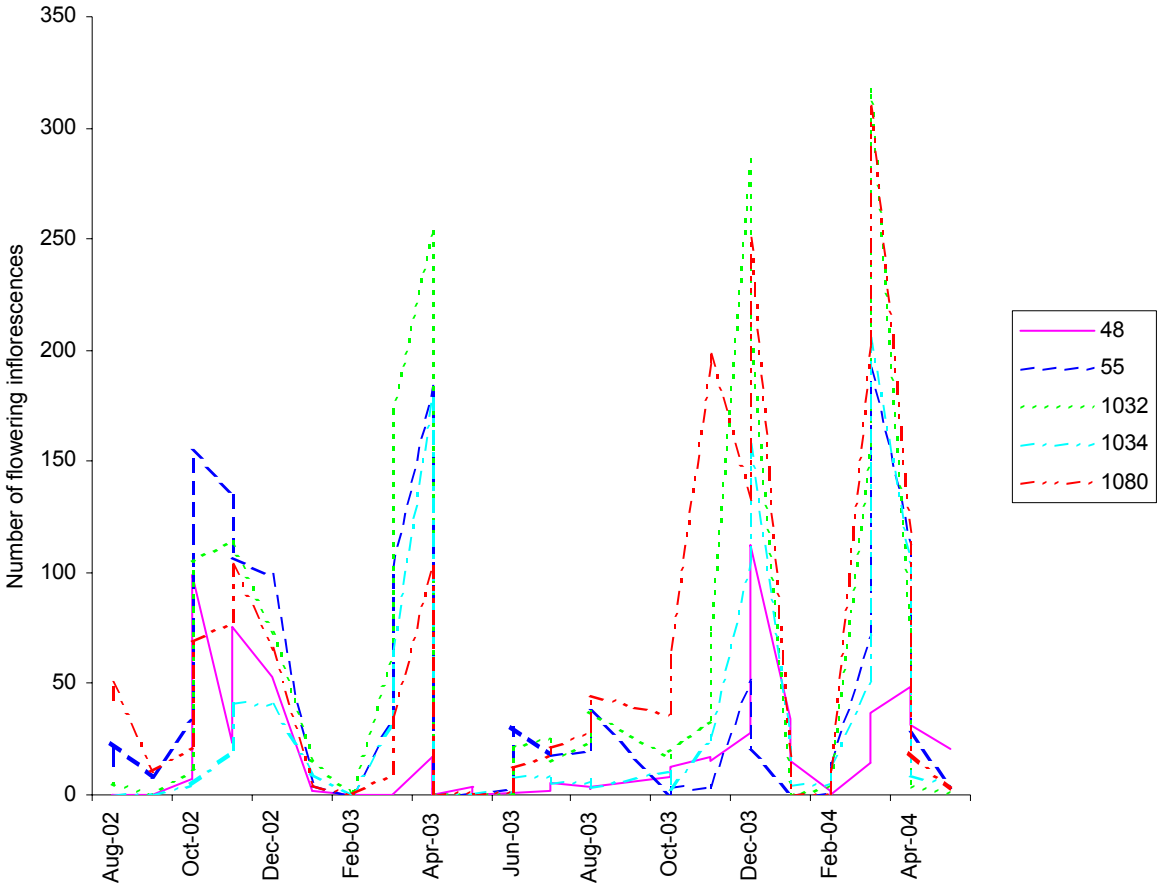

Figure 6: Approximate number of inflorescences in flower per *Syzygium gustavioides* tree within the one-hectare canopy crane plot. Each coloured line represents an individual tree.

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.

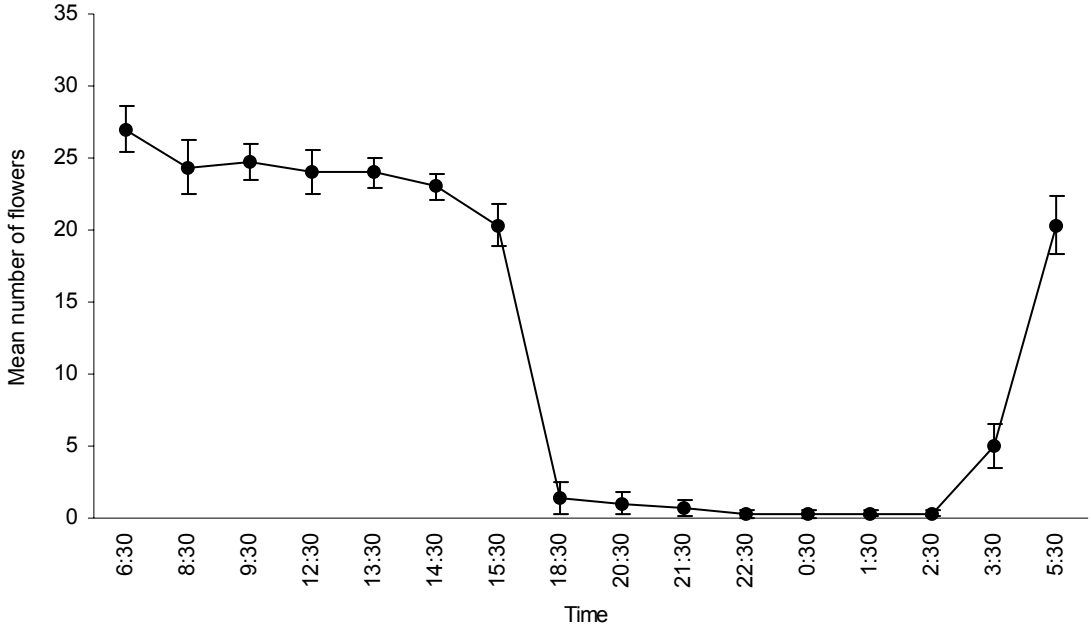

Figure 8: Mean number of open male flowers on four individual *Normanbya normanbyi* branchlets observed over a 24-hour period.

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.

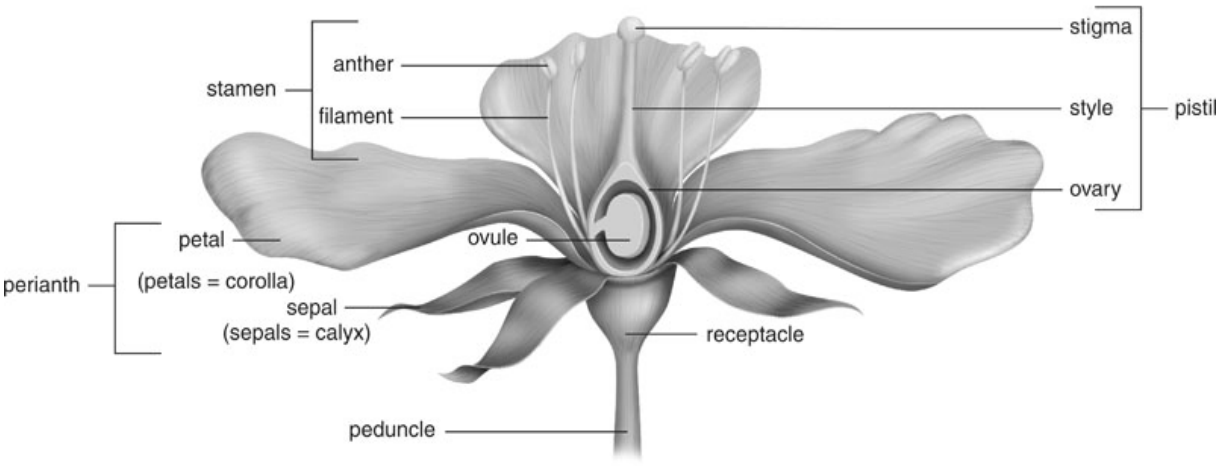

Figure 9: Schematic of the basic structure of the angiosperm flower presented here with the major function parts labelled. (Source: Stern *et al.* 2006)

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.

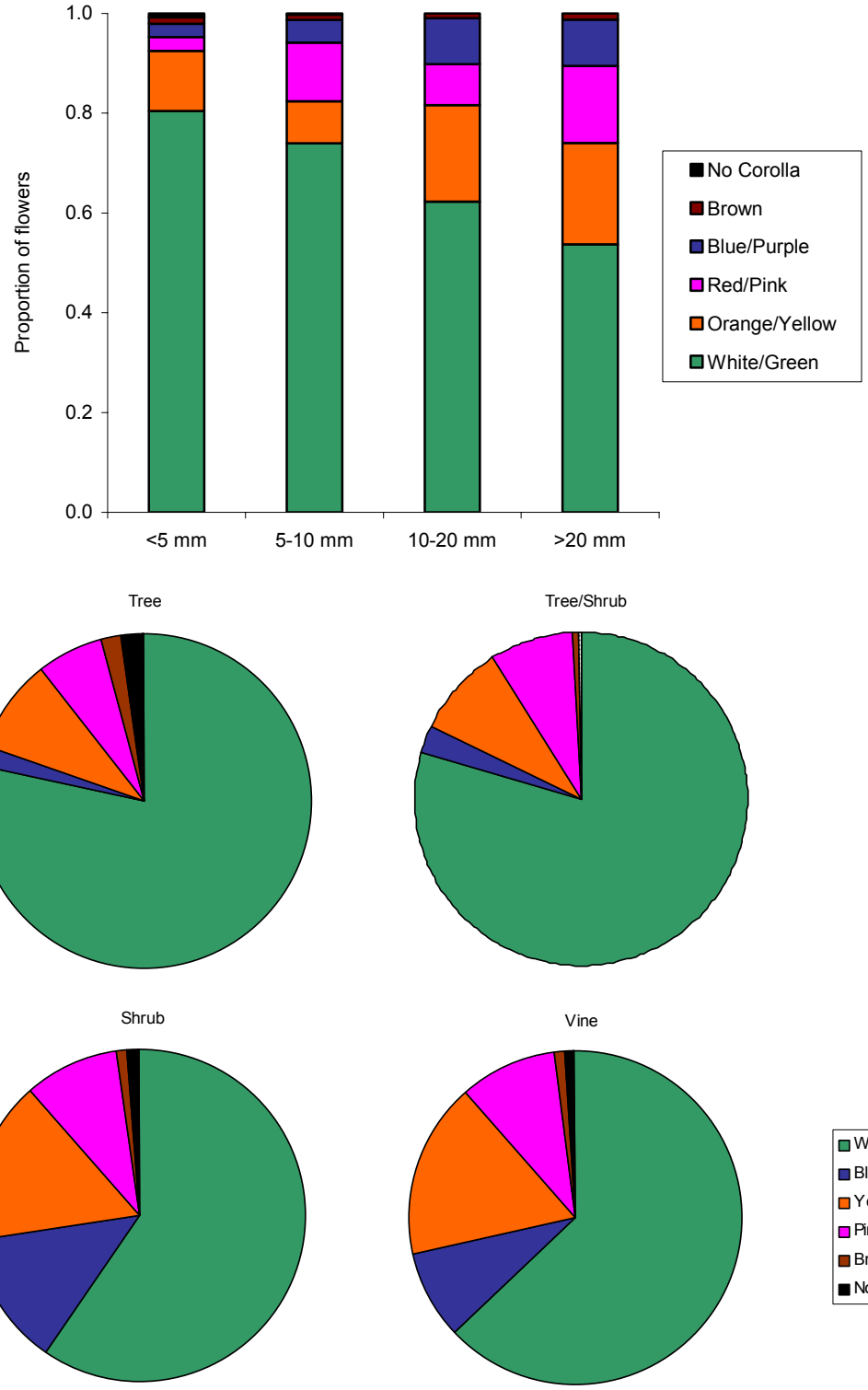

Figure 10: The proportion of Wet Tropics flowers (trees, shrubs and vines only) in (*top*) four size categories and colour groupings; and (*bottom*) in each colour category for each of four habit types. Source: Boulter *et al.* in review.

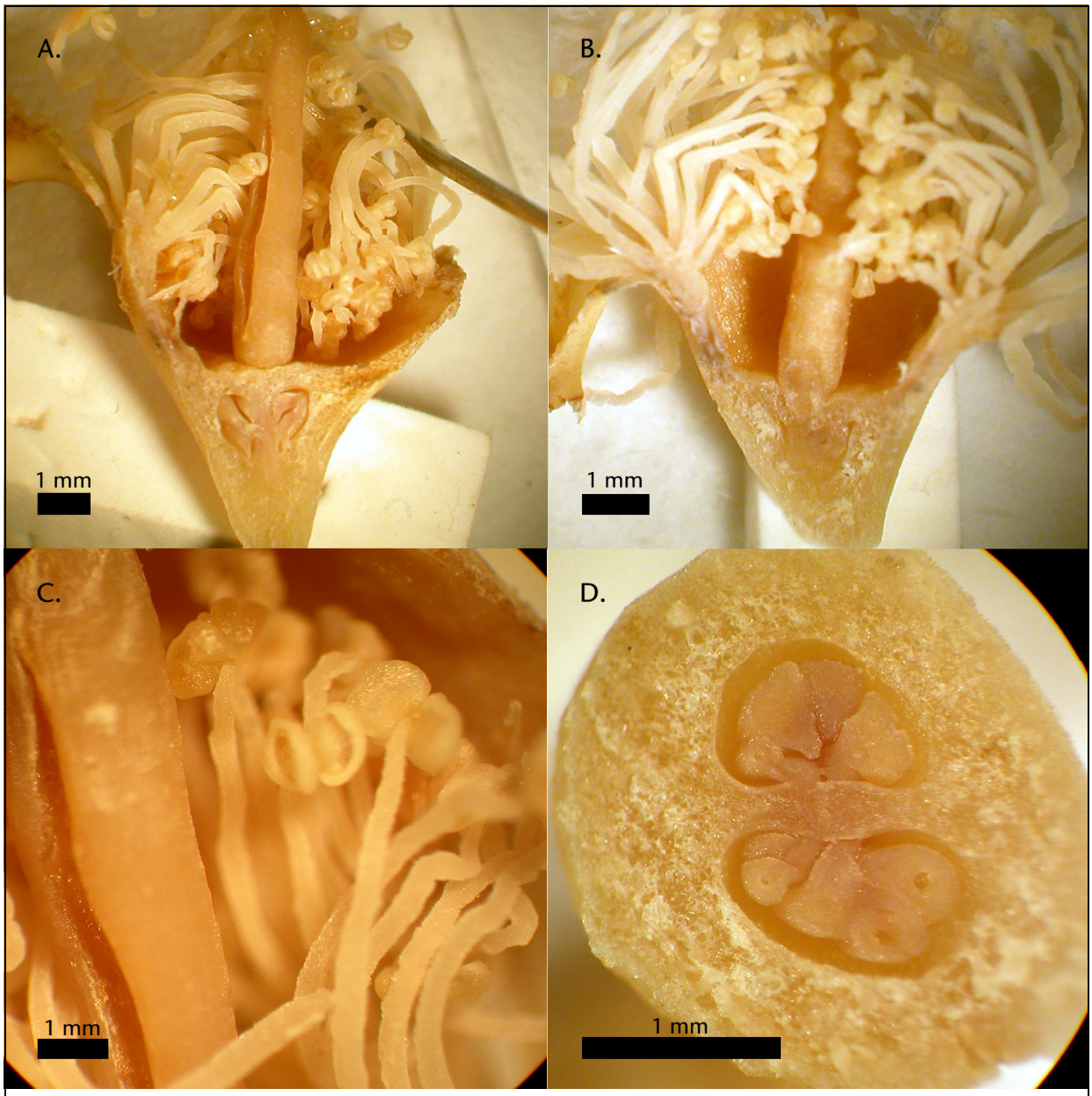

Figure 11: *Syzygium gustavioides* (a) flower at early opening stage; and (b) fully open in longitudinal section; (c) anthers (front and back); and (d) locule and ovaries in cross-section.

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).

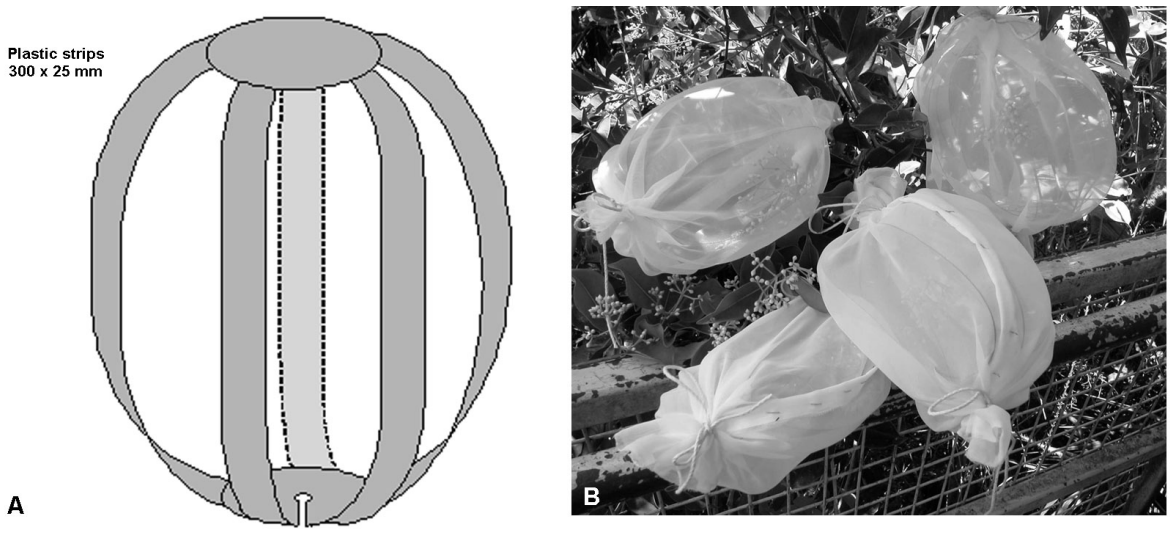

Figure 12: (a) Design of "balloon" cage, constructed from plastic acetate and placed around inflorescence stem; and (b) the cage is then covered in a fine mesh sock and tied with string to exclude visitors and allow daily access.

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).

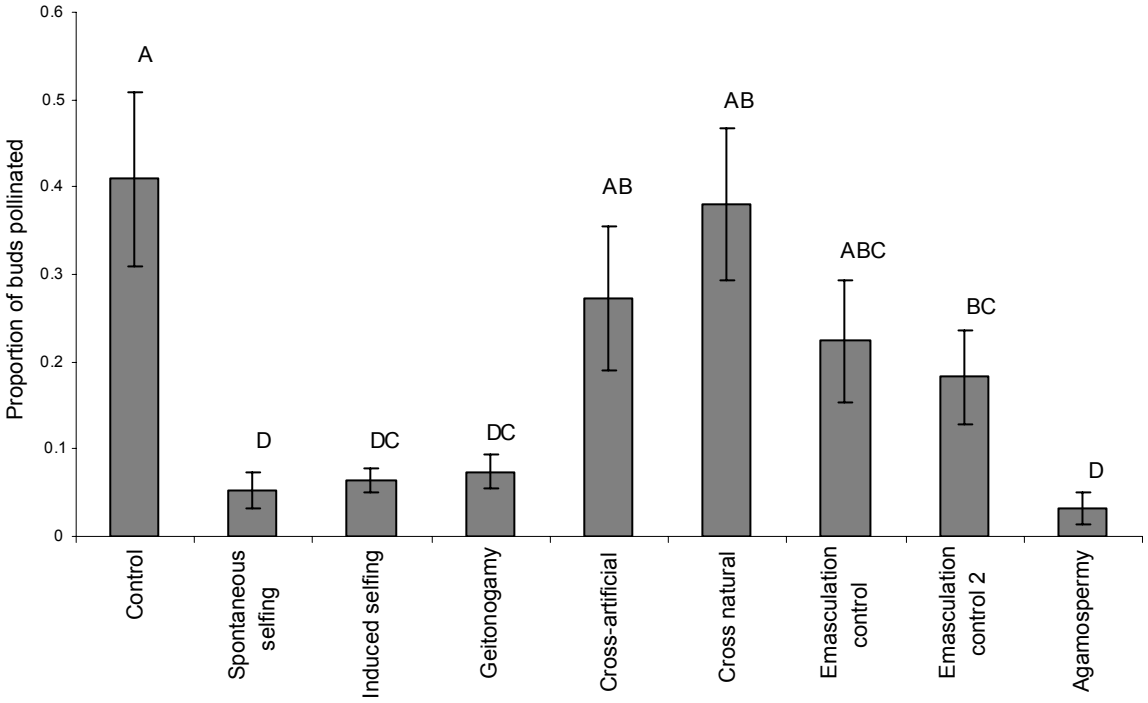

Figure 13: Proportion of *Syzygium sayeri* flowers demonstrating successful fertilisation using nine treatments to determine breeding system. Different letters indicate significant differences ($P < 0.05$).

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*

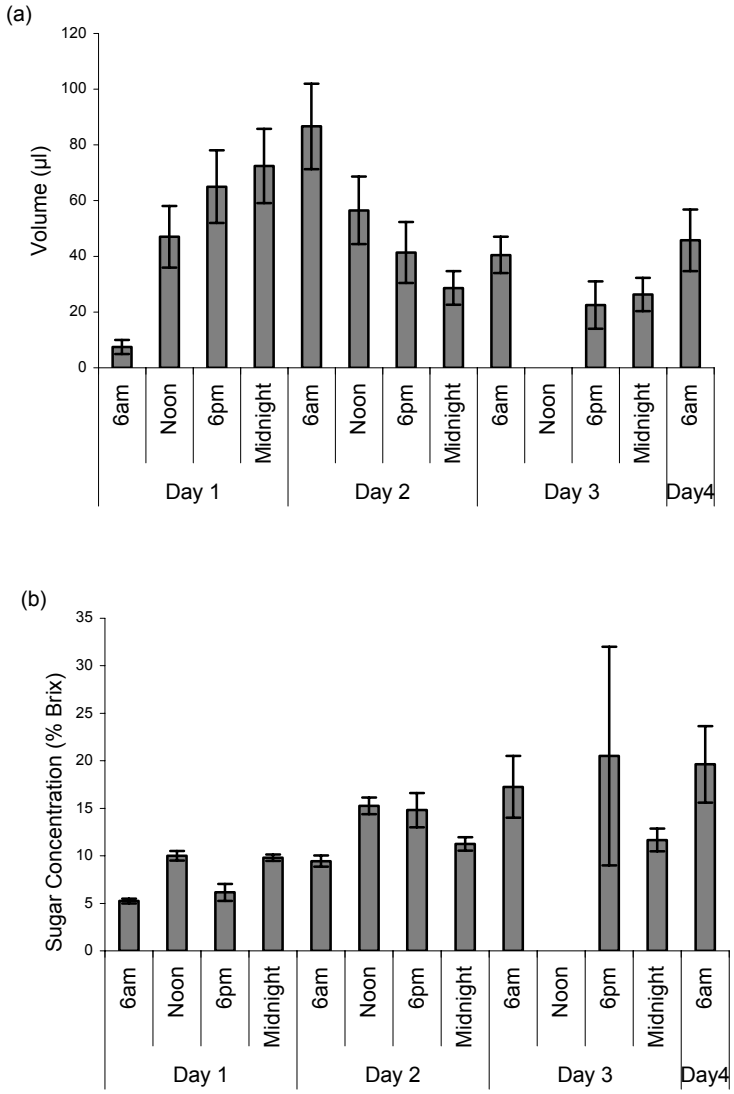

Figure 14: (a) Mean volume of nectar collected from *Syzygium sayeri* flowers at different times after opening; and (b) sugar concentration of nectar collected from *S. sayeri* flowers at different times after opening.

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*

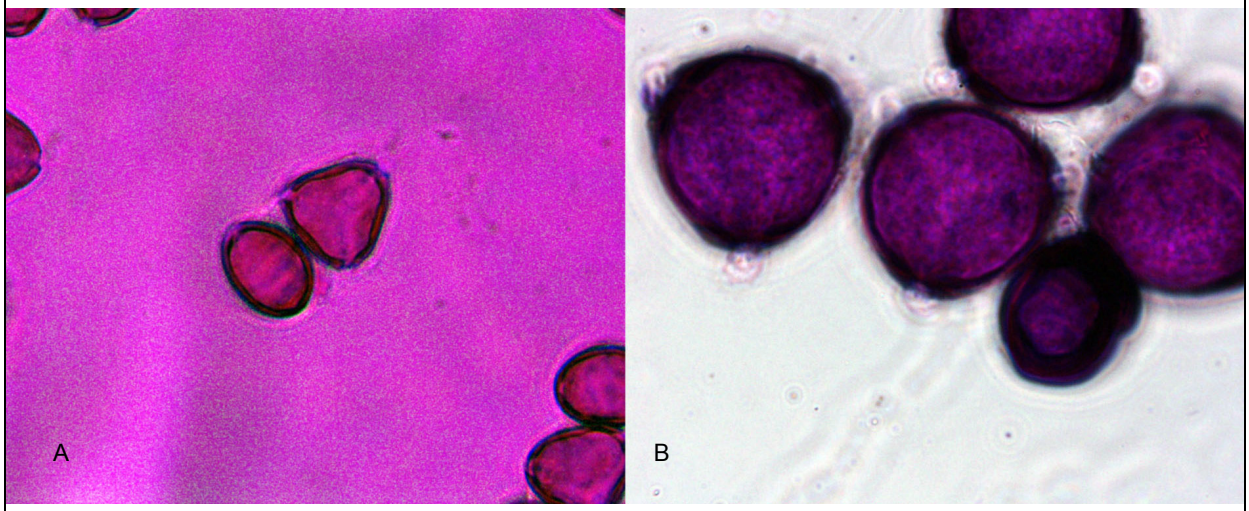

Figure 15: Pollen grains of (a) *Syzygium sayeri*; and (b) *S. gustavioides* (100x magnification).

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.