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

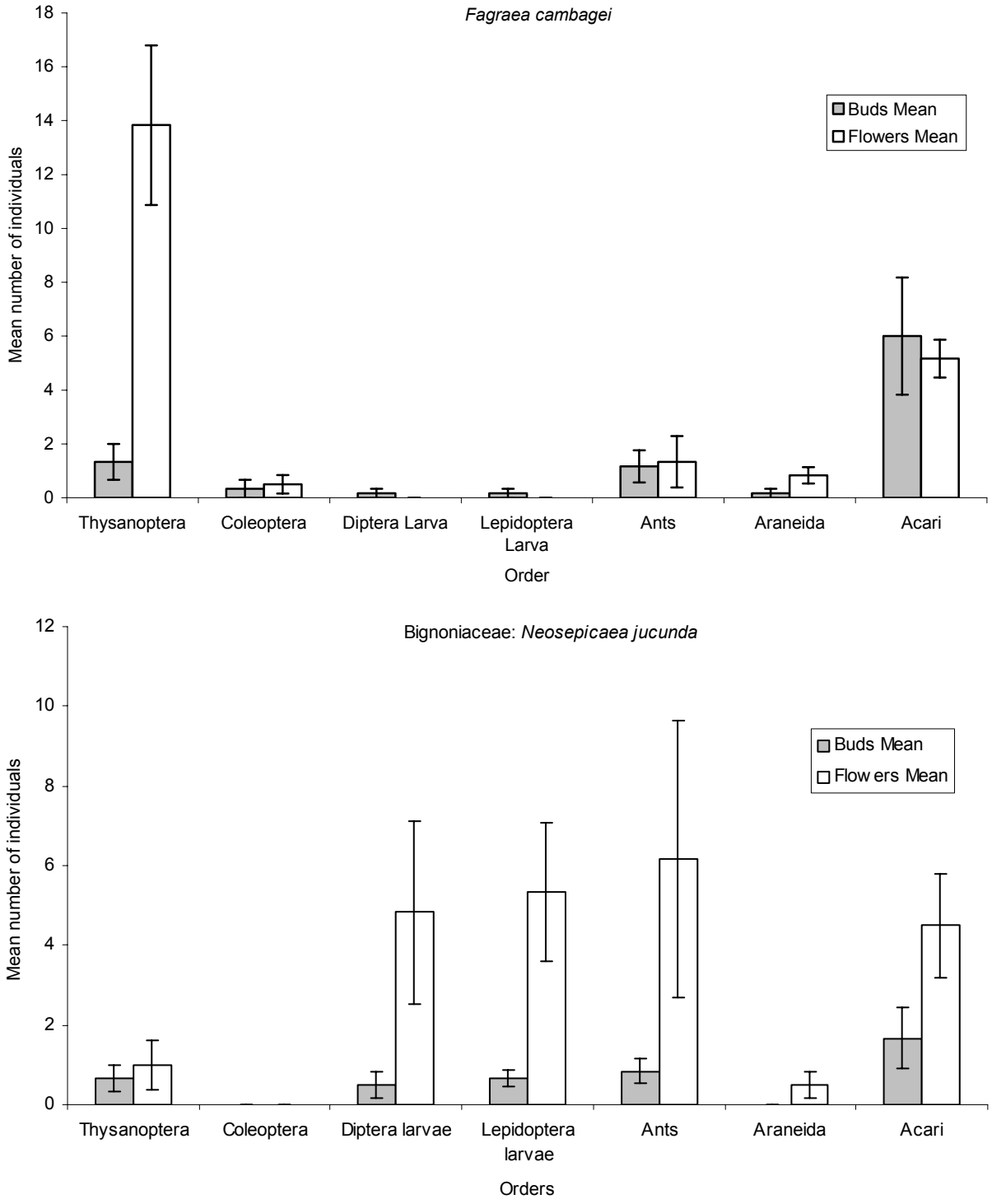

Figure 16: In-fauna sampled from (*top*) *Fragraea cambagei*; and (*bottom*) *Neosepicaea jucunda* using ethanol washing.

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.

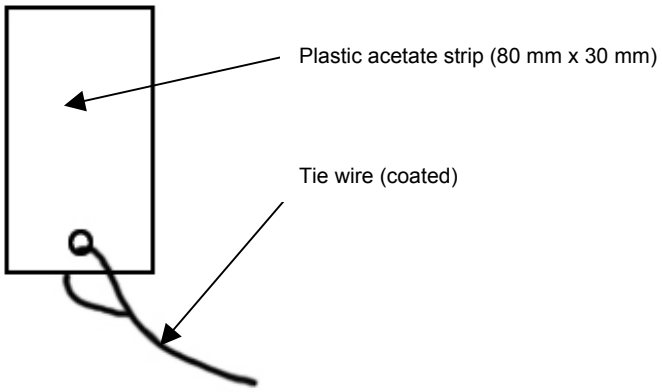

Figure 17: The PAS trap design is simply a strip of plastic acetate and a length of coated tie wire to attach to the inflorescence.

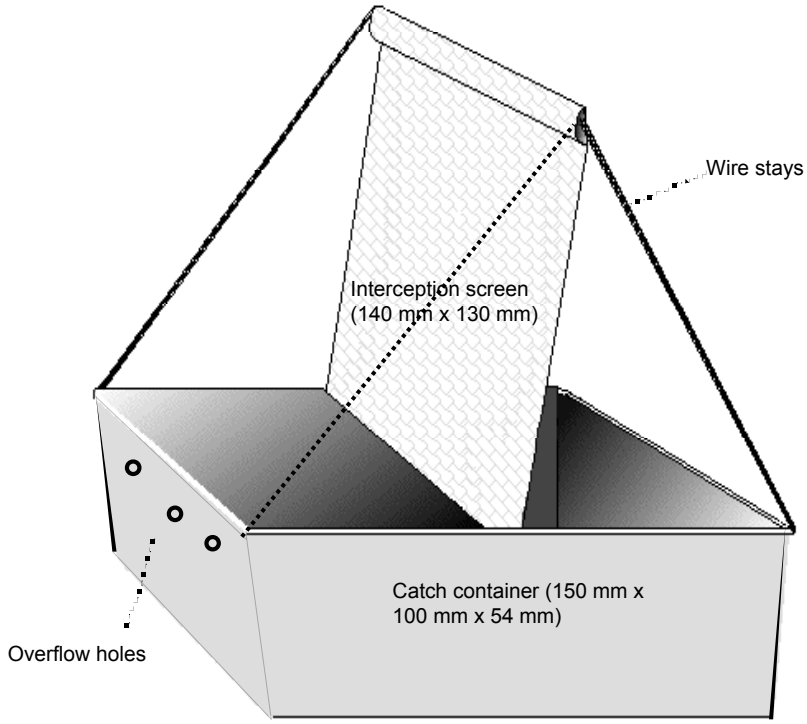

Figure 18: Design of the interception trap.

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*

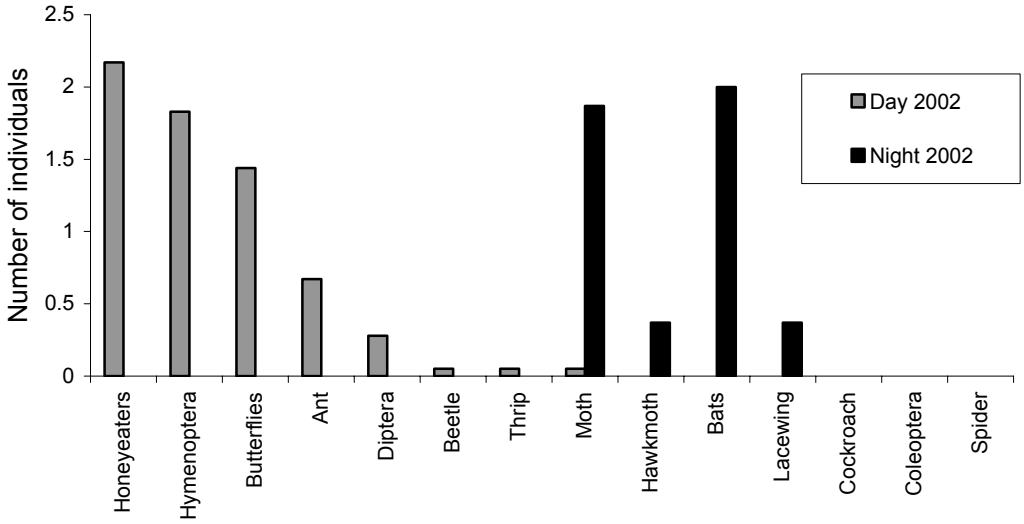

Figure 21: Average number of visitors by taxonomic group observed visiting the flowers of *Syzygium sayeri* at the Australian Canopy Crane.

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.