

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).

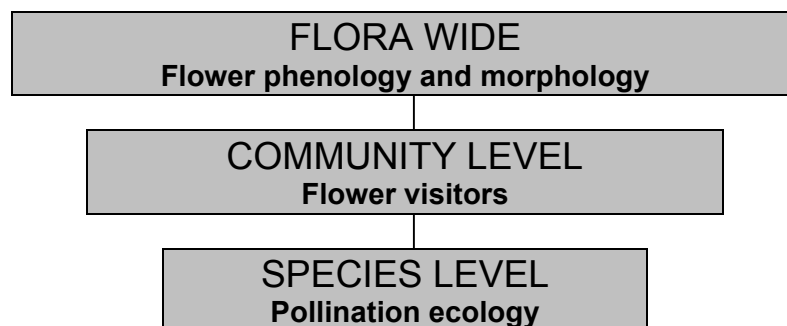


Figure 1: Schematic of the 'top-down' approach of the Rainforest CRC pollination project.

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 cauliflorous 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 facility, 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.

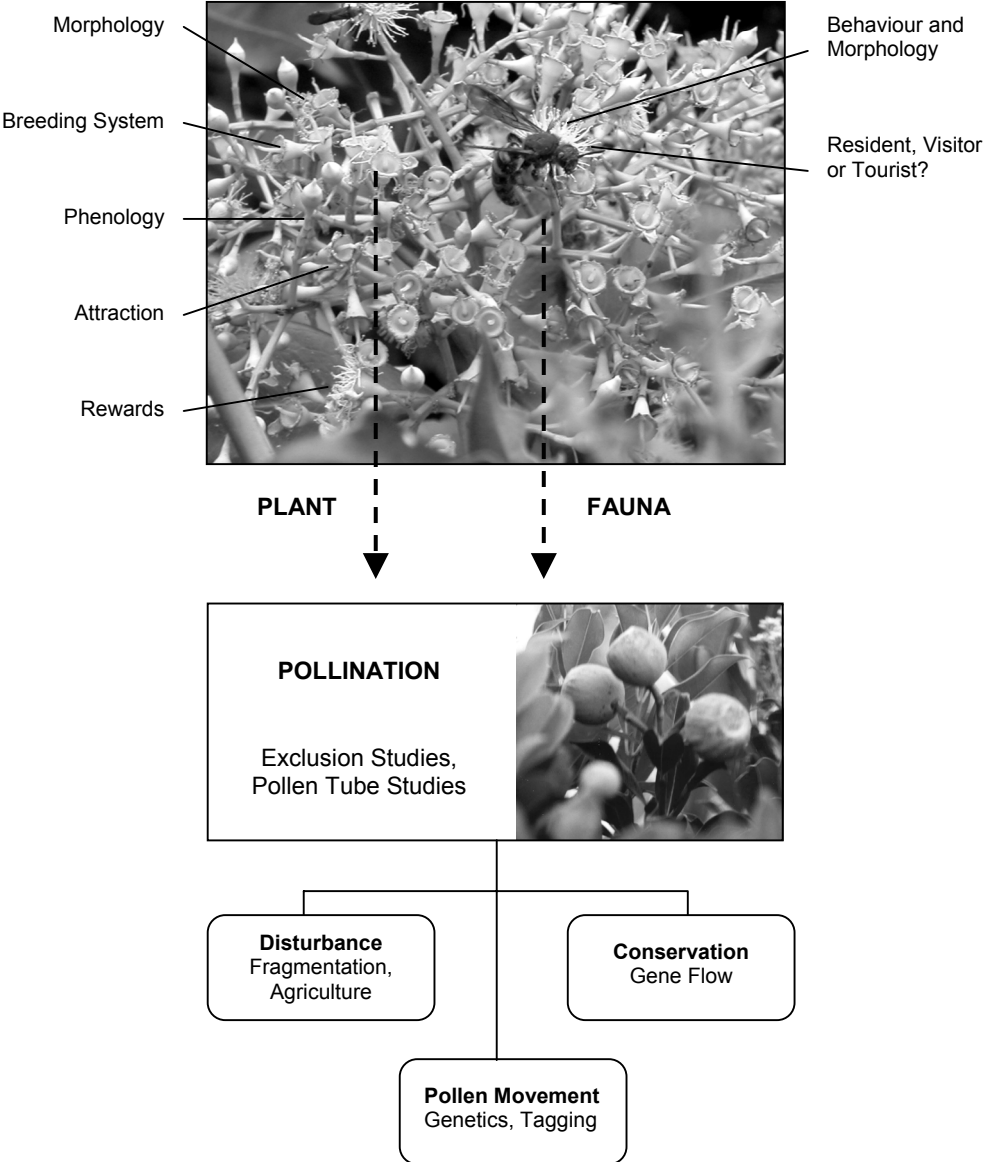


Figure 4: Conceptual diagram of studies in pollination ecology.