

## CHAPTER 7.

### SYNTHESIS AND CONCLUSIONS

#### 7.1 INTRODUCTION

Habitat fragmentation has been associated with many adverse effects on resident biota including disruptions to plant-pollinator interactions, which serve to maintain reproductive processes for sexually reproducing plants (see Kearns & Inouye 1997). Much research, utilising a variety of parameters at a variety of scales, has been undertaken to elucidate associated patterns and processes. This study focused on small-scale local density effects (sparse versus dense) (Chapter 2) on components of floral visitation (Chapter 4), plant fecundity (Chapter 5) and fitness (Chapter 6). Study sites occurred in the fragmented New England Tablelands, Australia (Chapter 1). Plant breeding systems were incorporated when selecting the plant study species, allowing for a comparative approach to be employed (Chapter 3) and reflected three different points along the breeding system spectrum (Chapter 1). The comparative approach extended to the use of multiple sites (three per species), which were examined over two years. The three study species (*Thesium australe* (Santalaceae), *Wahlenbergia luteola* (Campanulaceae) and *Dillwynia sieberi* (Fabaceae) were used as vehicles to examine the following broad questions:

1. Is visitation rate and visitor behaviour influenced by local density? If so then...
2. Does this regulate plant fecundity and fitness? And...
3. To what degree does breeding system influence fecundity and fitness responses?

#### 7.2 STUDY DESIGN

Several authors have expressed the need for fragmentation studies to incorporate multiple species and breeding systems and to take habitat and temporal variation into consideration (see Hobbs & Yates 2003; Aguilar *et al.* 2006); researchers who study fragmentation effects in natural systems often find among-population and among-season variability. It was therefore important to incorporate multiple sites and years into the present study. Thus, for each study species, data were collected from three discrete populations, across two years. Among site

(and between density, see below) comparison of several habitat attributes (e.g. species richness, grass and leaf litter cover) was overviewed to detect obvious among-site (or between density) variation, which may have indicated substantial differences in habitat quality or resource availability. No major inconsistencies could be identified for the variables measured (Chapter 2). However, more robust measures of habitat variability would have been desirable, but these were not feasible within the study.

Density effects can be investigated utilising a variety of scales. In this study, the focus was on small-scale local density, which has been shown to influence plant-pollinator relationships both in artificial arrays (e.g. Kunin 1997a; Knight 2003) and under natural field conditions (e.g. Kunin 1992; Roll *et al.* 1997). Measures of both relative density (i.e. sparse versus dense) and absolute density (i.e. individuals per unit area) were incorporated into the study design. Sparse and dense plots were established for each species at each site and were the main focus for data collection and analysis. A focal plant (FP) was designated at the centre of each plot and measurements of near neighbour distances (NND) to the FP were undertaken. The practical utility of these experimental units varied with each species, for instance the flowering phenologies of *T. australe* and *W. luteola* individuals did not lend themselves to data collection from FPs as individuals flower intermittently. For the mass flowering *D. sieberi* however, data collection could be reliably taken at both the FP and plot levels, and differences were occasionally observed between these data sets. For example, the incidence of native bees in the presence of honeybees at the FP and plot levels (Chapter 4, section 4.5.15).

### 7.3 BREEDING SYSTEMS

It is expected that a plant's breeding system will influence its response to fragmentation-induced influences on plant-pollinator interactions. Thus, baseline information regarding this factor for all study species was necessary. In Chapter 3, the breeding systems of *T. australe* and *D. sieberi*, which other researchers had previously examined were confirmed, and *W. luteola*'s breeding system was determined using field studies. In addition, breeding systems for all species were estimated using pollen to ovule ratios. Thus the breeding systems for the three species were as follow: *Thesium australe* (Santalaceae) is strongly self-compatible and autogamous, *Wahlenbergia luteola* (Campanulaceae) is self-compatible,

protandrous and a facultative outcrosser and *Dillwynia sieberi* (Fabaceae) is strongly self-incompatible and obligate outcrossing.

Since *T. australe* is strongly self-compatible and autogamous fruit set is frequent, interruptions to pollination were not likely to pose a barrier to reproduction for this species. Whilst *W. luteola* was found to be self-compatible, autogamous seed set can occur thus providing limited reproductive assurance under pollinator limitation. However, autogamy produced few seeds in two populations and was not detected at all in the third (section 3.5.3). The results illustrate the requirement for facilitated outcross pollination to maintain high levels of seed set in *W. luteola*. *Dillwynia sieberi* is completely reliant on pollinators for seed set and is expected to be the most vulnerable in situations where disruptions to plant-pollinator interactions arise.

#### 7.4 THE RESPONSE OF FLORAL VISITORS TO DENSITY

In Chapter 4, floral visitor composition and visitation rates to sparse and dense patches were ascertained for all plant species. Furthermore, it was possible to address within patch foraging behaviour for *D. sieberi*.

After observing >1200 *T. australe* flowers over >13 hours, only two thrips larvae (Thysanoptera) resident in flower buds and a single ant were observed, indicating that *T. australe* lacked a visitor fauna altogether. However, thrips may actually be more important to this species than indicated, if thrips populations vary greatly over seasons. Baker (1998) found thrips to be problematic on *T. australe* plants in her study, yet they were seldom encountered in the years this study was undertaken. It's possible that over time, the breeding system of *T. australe* has been moulded by an unpredictable pollinator fauna (section 4.6).

The visitor fauna to *W. luteola* comprised a suite of native bees, wasps and hoverflies (Syrphidae) (section 4.5.3). Although oligolecty has been reported for visitors to Australian *Wahlenbergia* (Michener 1965), the floral visitors encountered in this study tended to indicate generality, since some overlap in native visitor fauna was observed between *W. luteola* and *D. sieberi*. The major visitor to *D. sieberi* flowers however, was the introduced honeybee *Apis mellifera* (section 4.5.8).

For *W. luteola*, density was a consistent and significant positive influence to visitation rate (Figure 4.6). There was some indication that this was also the case for *D. sieberi*, but results

among sites and between years were less consistent (Figures 4.13 & 4.14). These data, particularly for *W. luteola*, support the work of Kunin (e.g. 1992; 1997a) and add to the growing body of research that attests to the importance of small scale density effects on visitation. Density effects were also apparent when within patch foraging behaviour of visitors to *D. sieberi* was assessed (section 4.5.16) where both native bees and *A. mellifera* visited more flowers on sparse individuals than on dense and thus potentially promoted geitonogamous pollinations. Although *D. sieberi* is self-incompatible and cannot produce selfed-seed, other negative effects may result from an increase in intraspecific pollen movement. Since several authors have reported a relationship between visitation rate and fecundity and that this varies with plant breeding system, the influence of density on both visitation rate and within patch foraging behaviour reported in Chapter 4 was expected to impact on plant fecundity. To assess this, reproductive output was measured for the three study species (Chapter 5).

## 7.5 REPRODUCTIVE OUTPUT, DENSITY AND VISITATION

The lack of visitors to *T. australe* negated visitation rate or behaviour as factors that may influence fecundity within the study populations. However, given *T. australe*'s strong self-compatibility and autogamy, a complete absence of pollinators is likely to promote the production of inbred progeny. Fruit production was considerable for this species, indicating that *T. australe* enjoyed substantial reproductive assurance. However when seed production was assessed, high levels of fruit abortion and partially filled fruit were encountered, which are traits characteristic of inbreeding depression (section 5.5.5.1). There were no indications that *T. australe*'s fruit or seed output was influenced by local density.

Whilst visitation was clearly influenced by density for *W. luteola*, the results for fruit and seed production were more ambiguous (section 5.5.1.2). Almost all flowers produced a fruit, whether containing seed or not, thus FR: FL ratios were not good indicators of reproductive success for this species. Seed to fruit ratios (S: FR) were almost always higher in dense plots, but this difference was never significant. Furthermore, regression analysis did not reveal any relationship between visitation rate and seed production. Yet, when S: FR ratios in sparse and dense plots was compared against S: FR ratios calculated from fruits that had been supplemented by hand with outcross pollen (Table 5.2) there was again, a trend for plants

from sparse plots to produce less seed than their dense counterparts, due to a reduction in pollen quantity. Overall, the data failed to reveal solid evidence that the density effects on *W. luteola* visitation had flow-on consequences to its reproduction. However, these populations may be saturated with pollinators and it is conceivable (especially given the trends observed), that under a certain threshold, density-dependent visitation could influence fecundity.

Although visitation to *D. sieberi* was significantly related to density at one site (for both absolute and relative measures), there was an overall difference in fruit production in both years. Fruit to flower ratios were higher in dense than in sparse plots (section 5.5.1.3). Thus, FR: FL ratios were positively related to density and moreover, FR: FL ratios were also related to visitation rate (at least in 2004). Again, these findings bolster those of other investigators. However, there was no relationship between density and S: FR ratios. Therefore, had this study used only FR: FL ratios as indicators, very different conclusions would have been drawn about the effects of density-dependent visitation on the reproduction of *D. sieberi*. Furthermore, and although *overall* S: FR ratios were not related to density, closer inspection of seed production revealed a tendency for the incidence of 1- and 2-seeded fruits to differ between sparse and dense plots providing further evidence for the utility of this ratio (Figure 5.17). A factor that may be more important in moulding realised fecundity than visitation for the self-incompatible *D. sieberi* is the incidence of seed/fruit predation by Coleopteran larvae (section 5.5.1.3). The incidence of fruit predation for this species did not appear to be density dependent however, since predation was observed to reduce seed output by up to 50%, its role in moderating realised fecundity cannot be underestimated.

## 7.6 FITNESS COMPONENTS

In Chapter 6, female fitness components were assessed which included: seed viability and germinability, and seedling survivorship and growth. Furthermore, stigmatic pollen loads and the fitness of self versus outcross pollen were investigated.

Seed from *T. australe* failed to germinate, thus offspring fitness traits could not be investigated. There was little indication that density influenced fitness traits measured at the seed level, however there were indications that seed produced autogamously was less viable on average than open seed. This was surprising considering stigmatic pollen loads and pollen

tube growth in this species did not indicate that outcross pollen movement was occurring among individuals, nor that outcross pollen behaved more vigorously than self-pollen.

Fitness traits measured for *W. luteola* seeds and seedlings showed that seed arising from both densities performed similarly. However, one site (UNE) outperformed the remaining sites in all traits measured, exemplifying the fact that responses can unexpectedly vary among populations. Unfortunately, pollen dynamics (pollen loads, germination and growth) proved difficult to quantify in this species. However, it is apparent that self- and outcross-pollen germinate and grow to similar degrees in this species. This is not surprising given its self-compatibility.

For *D. sieberi*, heavier seed weight appeared to impart an advantage to seedlings; seed mass was positively related to cotyledon size and seedling growth rates. Of note for this species was the fact that investigation of stigmatic pollen loads indicated density dependent pollen-tube growth, which implicates pollinators in provisioning flowers in dense plots with better quality pollen compared with flowers in sparse plots.

## 7.7 CONCLUSIONS AND IMPLICATIONS FOR FUTURE RESEARCH

The results presented in this dissertation support the premise that small-scale density influences visitation and furthermore, that these influences are apparent in natural systems. The potential exists therefore, for density-dependent visitation to influence plant reproduction, fitness and persistence. Although, there is little doubt that visitation is influenced by density in these systems, the extent to which this factor shaped reproductive output and offspring fitness was difficult to ascertain. However, to conclude unequivocally that density-dependent visitation is never a driver of reproductive success for these species would be shortsighted; other factors may be clouding what was anticipated to be a relatively clear relationship. One problem may be that the populations under study were saturated with pollinators and visitation was not limiting at the scales measured; even for *W. luteola* where significant reductions in visitation rates with sparsity were revealed, fruit and seed output was maintained. There may yet be a threshold under which such apparently resilient species may begin to display density-induced limits to reproduction. Furthermore, genetic substructuring in populations may be influential. For example if biparental inbreeding increases with density, this may serve to mask density-dependent effects on fecundity and fitness.

Whilst this study highlighted the complexity involved when interpreting ensuant effects that are expected to manifest in reproductive components, it served also to illustrate the fact that negative effects of low density on visitation rate or behaviour need not necessarily signal unfavourable consequences for fecundity or fitness and therefore, the persistence of these populations. It is important that visitation measurements are followed by measurements of reproduction in systems that exhibit density-dependent visitation. Additionally, density should be incorporated into analyses that relate to reproduction; the importance of density may outweigh that of factors such as population size or connectivity.

The incorporation of three species, each at three populations, across two seasons and with contrasting breeding systems yielded several axioms of utility for researchers investigating plant reproductive ecology in fragmented systems. The use of multiple populations for each species revealed that the direction of responses can be variable among sites, demonstrating the necessity to incorporate a number of populations into study designs. Furthermore, the utility of parameters commonly used to measure plant fecundity varied among all of the study species. For all species, FR: FL ratios were an inadequate measure of realised fecundity and in some cases were entirely misleading. At the very least, it is prudent to employ both FR: FL and S: FR ratios and ultimately, full comprehension of reproductive output can only be achieved via S: FL ratios. Since each ratio represents a measure at a discrete point along the reproductive continuum, using all three allows for a more precise interpretation. In addition, indicators that are commonly used to investigate offspring fitness were also found to vary in their utility. Moreover, the effort required for *ex-situ* plant growth trials when investigating offspring fitness outweighed the benefits; time would be better invested in site replication or on investigating the utility of growth trials in the field where the natural environment may serve to better elucidate fitness responses.

Compared with the facultative outcrossing *T. australe* and *W. luteola*, the obligate outcrossing *D. sieberi* showed the greatest utility for measuring density-related visitation and subsequent reproductive responses. This is because it was amenable to a wide range of practicable manipulation and data collection techniques, but more importantly, its self-incompatibility meant that factors that may confound investigations using selfing species were not manifest. For example, one can safely assume that all fruit and seed arise from outcross pollination events that are facilitated by pollinators, that reproduction is not supplemented by

selfing, and the differences between self- and outcross-pollen germination and growth are highly tractable.

Although it is important to understand how density-related processes may affect reproduction in species that reside across the breeding system spectrum, the results of this study have shown that clarification of general trends occurring in fragmented systems may warrant using the simplest model possible, i.e. species that exhibit strong self-incompatibility. These species should allow the basic processes underlying reproductive responses to visitation to be elucidated and, coupled with the recognition that performance indicators can vary in their utility across all stages of the reproductive process, other variables concomitant with breeding systems that allow for selfing can be integrated as necessary.

Finally, the choice of 'indicator' species in studies of fragmented ecosystems is in itself potentially confounded by the time elapsed since fragmentation. Presumably, the species that we study are the species that have survived the actual process of fragmentation and those that survive may therefore exhibit, to varying degrees, resilience to perturbation. It is the study of the variation then that many of us are measuring and we need to be mindful that a benefit of this is the detection of species that can survive the hostilities of fragmentation. *Wahlenbergia luteola* and *D. sieberi* may be two such species however, given the findings of this study, *T. australe*'s ability to persist long-term on the fragmented New England Tablelands remains dubious. Yet, there is little doubt that in a system void of (or severely lacking in) pollinators, the breeding systems of both *W. luteola* and *D. sieberi* would render them particularly susceptible to reproductive failure; yet even species that maintain a strong capacity for autogamy, as exemplified by *T. australe*, can also be ultimately vulnerable.