THE ECOLOGY OF REPRODUCTION IN A RARE PLANT -
TETRATHECA JUNCEA

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This thesis is submitted for the degree of
Doctor of Philosophy
In the School of Environmental and Life Sciences
The University of Newcastle
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STATEMENT OF ORIGINALITY

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

Colin Driscoll
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First things first! An eight year part-time PhD program combined with full-time work leaves little time for anything else. So, much gratitude from me to my wife Linda for tolerating my chronic absence.

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**Disclaimer:** this research program was fully self-funded other than for several of my consultancy client’s unwitting facilitation by trusting me with their projects, often providing valuable access to otherwise restricted habitat.

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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AOO</td>
<td>Area of Occupancy</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>BBCH</td>
<td>Biologische Bundesanstalt, Bundessortenamt, and CHemical industry</td>
</tr>
<tr>
<td>CC</td>
<td>Central Coast</td>
</tr>
<tr>
<td>DTM</td>
<td>Digital Terrain Model</td>
</tr>
<tr>
<td>ENFA</td>
<td>Environmental Niche Factor Analysis</td>
</tr>
<tr>
<td>ENM</td>
<td>Environmental Niche Model</td>
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<tr>
<td>EOO</td>
<td>Extent of Occurrence</td>
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<td>Fruit</td>
</tr>
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<td>Genetic Algorithm for Rule-set Production</td>
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<td>General Linear Model</td>
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<td>Global Positioning System</td>
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<tr>
<td>HS</td>
<td>Habitat Suitability</td>
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<td>International Union for Conservation of Nature</td>
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<td>Local Government Area</td>
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ABSTRACT

Rare species hold a particular fascination for amateur naturalists and professionals alike and a real thrill is experienced when one is encountered in its natural habitat. As a component of overall biodiversity, rare species have received legal protection that requires careful management of these species and their habitat so as to avoid or minimise loss as a consequence of human activity. It is however, inevitable that a continually expanding human populace will result in some loss of diversity, including rare species. The key here is to manage these losses so that they do not lead to extinction and this can only be achieved through a detailed understanding of rare species life-cycle and habitat requirements.

This thesis deals with aspects of the reproductive biology of rare plants through the lens of a detailed investigation into *Tetratheca juncea*, a cryptic, low sub-shrub restricted to a small area of the NSW central and lower north coast of Australia. The currently known distribution of the species divides naturally into two regional populations designated as Central Coast and North Coast. The species propagates through both clonal spread and sexual reproduction from primarily xenogamous hermaphrodite flowers. Clonality poses a problem for determining population density and an arbitrary convention is used defining an individual plant as a clump if separated from the next nearest by 30 cm or more, irrespective of whether it would be a genet or ramet. In 2002, prior to the start of this study in 2003, *Tetratheca juncea* was reported to consist of no more than 10,000 individual clumps existing in small, highly fragmented subpopulations. A critical weakness in the reproductive cycle of the species was considered to be low levels of pollination inferred from reported low levels of fruit being set, and the fact that no pollinators had been confirmed despite intensive investigation. A literature investigation revealed considerable scope for detailed study to be conducted into phenology, pollination and distribution of this species.

The reproductive phenology of *Tetratheca juncea* was investigated in detail for the majority of one flowering season. Four phenophases: budding, flowering, fruiting and seed release were documented monthly for eight months (June to January) over 12 sites in the Central Coast regional population, covering approximately 30 km north to south.
Budding commenced in June and daylight/darkness hours and temperature analysis pointed to photoperiod being the primary initiator. The flowering curve showed a strong positive skew, rising sharply from September, peaking in October and falling slowly, with the suggestion of a second but lower peak in November. Detailed bud length analysis indicated that a second phase of budding in September contributed to the second peak in flowering and extended the overall flowering period. Fruit development commenced in September and seed release in October with both these events being concurrent with budding and flowering past the time that data collection was stopped in January.

The flower structure of *Tetratheca juncea* with poricidal anthers suggested that pollinators would be specialised native bees capable of extracting pollen by buzz-pollination. Six buzz-pollinating native bee species have been confirmed collecting pollen from *Tetratheca juncea* flowers. These bees are small; visits to flowers are infrequent and even the act of closely observing has been shown to alter pollinator behaviour which makes the task of directly documenting pollinator visits impractical. However, abiotic pollination in the wild appears to be a rare occurrence so the presence of developing fruit can be used as a quantitative surrogate for pollinator activity. Two indices used were Fruit Flower Ratio (FFR) and total fruit per clump, both providing information on different aspects of pollinator activity, flowering and fruiting patterns.

Pollinator activity was investigated at both regional population and subpopulation levels. The regional population study was conducted over six years and covered a major portion of the Central Coast regional population. The aim of this study was to determine levels of pollinator activity over time and geographic distribution. Pollinator activity was shown to be highly variable at many levels yet overall FFR values were consistent with those from other studies of hermaphrodite species. There was no indication of pollinator deficit.

The subpopulation study was conducted in a group of approximately 500 clumps over six years, mostly overlapping the time span of the regional population study. The subpopulation study provided detail that was not available from the regional population study with its broader scale and opportunistic approach. Again pollinator activity as
measured by FFR was found to be within the previously reported range. Mean flowers per clump increased significantly over the study period and mean FFR fell. Analysis of mean fruit per clump showed that pollinator levels remained the same which pointed to falling FFR being a consequence of increased flower numbers. Clump attrition analysis showed a decline in clump numbers of 27% over the six years and habitat data analysis suggested a cause as being increased competition from ground species.

Finally, environmental niche modelling was conducted, at a grid size of 100 m, with the aim of understanding the probable distribution and abundance of the species and its broad habitat requirements. Modelling was conducted using the maximum entropy method provided in the program Maxent. Presence only data for the target species are used as input for model development. For various reasons most raw presence data are spatially biased which, if left unchecked, results in a weakened model. A novel method was used to select an unbiased set of *Tetratheca juncea* presence points that retained the spread of data across the known distribution. Maxent provides several output options and in this case a threshold was chosen that resulted in a binary model showing suitable and unsuitable habitat. The final model was submitted to, and passed, a reasonableness test by comparing niche overlap with a model of a habitat type in which *Tetratheca juncea* does not occur.

Separate models were prepared for the Central Coast and North Coast regional populations. For the Central Coast model, the entire area of interest had been surveyed for the species at varying levels of intensity so the model was run for the whole area. Only a small number of presence data were available for the North Coast area of interest so this model was run in two stages: a buffered common enclosing rectangle around the presence data followed by projection from that model across the remainder of the area of interest.

For the Central Coast regional population, the model allowed estimation of the amount of habitat loss since European settlement, habitat fragmentation and total abundance. The North Coast regional population model revealed the intriguing possibility of that population exceeding the better-known Central Coast regional population.
This study commenced with *Tetratheca juncea* considered to be in low numbers in a highly fragmented distribution with low seed production, most probably as a consequence of low numbers of unidentified pollinators. Now, from this study, it can be concluded that the species is widely distributed in two regional populations, albeit over a relatively small geographic range. Actual population numbers could be over two orders of magnitude greater than the 10,000 originally claimed. The species shows few of the attributes of a rare species, being a generalist in its habitat requirements. The species has attributes of being well adapted to its place in a competitive environment. It flowers for longer than pollinators are seasonally available thus maximising pollination opportunities apparently offsetting the disadvantage of having no nectar as an attractant. Specialised buzz-pollinators limit the chances of stigma clogging by pollen from co-flowering species by transferring pollen from their ventral surface. Nectariferous co-flowering species are essential for *Tetratheca juncea* pollinators and there is a possibility that the presence of both facilitates increased pollinator visits to each species.
CHAPTER 1  GENERAL INTRODUCTION
1.1 BACKGROUND

Biodiversity is protected or preserved from anthropogenic actions in a variety of ways. These include a network of conservation reserves (Common and Norton 1993; Margules and Pressey 2000; Pressey and Bottrill 2008); *ex situ* collections (Li and Pritchard 2009); and legislation targeted at species and ecosystem protection (Kennedy *et al.* 2001; Farrier and Whelan 2004). The aim of biodiversity legislation is that the planning approval process controls impacts on threatened species and their habitat.

In the state of New South Wales, legislation aimed at biodiversity conservation at the development approval stage is embodied in the *Environmental Planning and Assessment Act 1979* (EP&A Act), and the *Threatened Species Conservation Act 1995* (TSC Act) and subsequent amendments. At the Commonwealth level, it is enshrined in the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act). The TSC Act and the EPBC Act have schedules listing threatened species, endangered communities and populations. The development approval process requires that the impact of any proposal on species and communities in the schedules of these Acts be assessed and avoided, minimised or offset. Impact assessment is formally conducted through the application of seven factors (commonly referred to as the 7-part test) listed in the TSC Act or the Significant Impact Criteria of the EPBC Act.

The seven factors of the TSC Act 7-part test include the following:

"(a) in the case of a threatened species, whether the action proposed is likely to have an adverse effect on the life cycle of the species such that a viable local population of the species is likely to be placed at risk of extinction,

(d) in relation to the habitat of a threatened species, population or ecological community:

(i) the extent to which habitat is likely to be removed or modified as a result of the action proposed, and

(ii) whether an area of habitat is likely to become fragmented or isolated from other areas of habitat as a result of the proposed action, and

(iii) the importance of the habitat to be removed, modified, fragmented or isolated to the long-term survival of the species, population or ecological community in the locality," (TSC Act Section 94)
The EPBC Act provides Significant Impact Criteria for critically endangered and endangered species, vulnerable species and critically endangered and endangered ecological communities. For example, the criteria for a vulnerable species state that:

"An action is likely to have a significant impact on a vulnerable species if there is a real chance or possibility that it will:

- lead to a long-term decrease in the size of an important population of a species;
- reduce the area of occupancy of an important population;
- fragment an existing important population into two or more populations;
- adversely affect habitat critical to the survival of a species;
- disrupt the breeding cycle of an important population;
- modify, destroy, remove or isolate or decrease the availability or quality of habitat to the extent that the species is likely to decline;
- result in invasive species that are harmful to a vulnerable species becoming established in the vulnerable species’ habitat;
- introduce disease that may cause the species to decline; or
- interfere substantially with the recovery of the species.” (EPBC Act Policy Statement 1.1.2006)

Thus, at either the NSW State or Commonwealth level, in order to determine whether a development proposal would have a significant impact on threatened plant species, the following information is needed about that species: the make-up of a viable or important population; the life cycle and the critical elements in that life cycle; the preferred habitat attributes; abundance and distribution.

A recent addition to the development approval process in NSW is BioBanking. The guiding principle of BioBanking (Department of Environment and Conservation 2006; Burgin 2008) is that a development outcome should result in no reduction in, and preferably improved, biodiversity; there should be no net loss. This is achieved through an assessment that assigns threatened species and ecosystem credits to the area of habitat to be modified or destroyed. For development to proceed, in order to meet the
maintain or improve principle, habitat must be obtained and set aside in perpetuity that has higher credit value than that which would be modified or destroyed. The credit value of the land to be used as an offset is enhanced if it is not in pristine condition so that, under management, its biodiversity values will increase over time. For example, if a development were to destroy 500 individual threatened plants, sufficient habitat would be required that, when suitably managed, would result in an existing population of that species increasing by at least, and preferably more than, 500 individuals.

A software tool, the Credit Calculator, is used to determine the credit values of the development and offset habitat and uses an underlying database of species’ life cycle and habitat attributes. The author was a member of an expert panel that was tasked with providing these data for threatened plant species from within the NSW Lower Hunter Valley and Central Coast. Details were required of aspects of a species life cycle. For example, reproductive strategy, age at first significant flowering, the number of viable seeds produced annually by a mature individual, seedbank persistence, seed dispersal distance and life-span. This exercise highlighted the paucity of information available from published literature.

As part of the investigative process leading up to an impact assessment, the habitat in question needs to be surveyed to determine if threatened species are present or likely to be present given suitable circumstances. It is a requirement that surveys be conducted at a time and in a manner that maximises the opportunity for discovering threatened species (Department of Environment and Conservation 2004). This requires published information detailing the ecology of target species; for example, habitat requirements, reproductive cycle and distribution.

1.2 Rarity Concepts

The distribution of species can be broadly described as common, uncommon, rare or endemic. Unfortunately, these conditions are not clearly definable with considerable overlap and relativity. For example, species can be locally common but rare at a larger scale, locally rare but common at a larger scale, uncommon but not rare, locally common, endemic and rare at a larger scale. Within any of these circumstances there
can be occasions of temporal rarity (Harper 1981) with population density falling according to seasonal conditions or stochastic climate extremes. Examples of temporal rarity, where population densities vary over time, are prevalent in arid Australia (Silcock et al. 2011).

Related but different is the level of threat to species survival. Related because species included in threatened categories of vulnerable, endangered or critically endangered have one thing in common; they have been classified as rare. Different because not all rare species are classed as threatened. Darwin (1872) considered rarity to be a precursor to extinction. Thus, the condition of rarity for a species should not be taken lightly or imposed lightly either.

There are usually few occurrence records for rare species. However, the quality of these records has a lot to do with the robustness of the classification of the species as rare. Contributing factors could be: rarely recorded cryptic species; species occurring in rugged habitat and rarely encountered; large areas of suitable habitat in private ownership and not searched; *ad hoc* occurrence data rather than from systematic survey; or the ‘species’ may be a taxonomic blunder unnecessarily split from a widespread genus.

The ‘appeal’ of certain plants will also contribute to the number of records. For example, orchids capture the imagination of amateurs and professionals alike whereas the unremarkable herb *Thesium australis* R.Br. (Santalaceae) will attract little attention. Guralnick and Van Cleve (2005) noted the potential for museum data sets to be biased by collector interest towards under-represented species. Moerman and Estabrook (2006) identified what they termed the ‘botanist effect’ demonstrated by greater species richness reported from counties in the USA that had universities than from adjoining counties not having universities.

Rarity would seem to be a fairly straightforward concept. If the species has low numbers, and/or a small geographic range, it is likely to be rare (Gaston 1994). However, geographic scale introduces a complexity into defining rarity (Hartley and Kunin 2003). For example, at a local level a species may be abundant and common. In
contrast, if the distribution of the species in question is constrained to a small geographical range, at larger geographical scales it could be classified as rare.

The seminal treatment of rarity was put forward by Rabinowitz (1981) describing rarity as multifaceted. An eight cell matrix was derived from three main attributes of any species in a landscape. Four columns were created where geographic range both small and large were each subdivided into wide and narrow habitat specificity. Two rows were created, representing large and small local population size. The conditions in one cell, of species with a wide geographic range, present in a number of different habitats (wide habitat specificity), and having a large local population size describe commonly occurring plants. The remaining seven describe the variety of circumstances in which uncommon or rare plants are found and are referred to as the seven forms of rarity.

The distinction between uncommon and rare is not clear in the literature. The two terms are often used in ways that imply that there is a difference e.g. “rare and uncommon plants”. From usage context, uncommon plants appear to be those that meet the Rabinowitz (1981) condition of “Constantly sparse over a large range in several habitats”. Uncommon species are unlikely to be at risk and would not be classified as threatened. Murray et al. (1999) catalogued species that were at the tail end of rank-abundance curves (visually assessed) for dry sclerophyll woodland and temperate rain forest. They found that, out of 55 species in dry sclerophyll woodland and 116 species in temperate rain forest, 5% and 9% respectively had low abundance across their full range of habitats. Conversely, they found that 91% and 95% respectively of species that appeared in the tail at a location were abundant elsewhere. The two groups were categorised as “everywhere-sparse” and “somewhere-abundant”. This example demonstrates that low abundance of itself is not sufficient evidence of rarity. Rarity is an outcome of the same evolutionary processes that all species are subject to (Gaston 1994). Given that natural selection cannot select for rarity as an adaptive strategy (Rabinowitz 1981) its origins must lie elsewhere and the search for these origins has motivated much theoretical discussion and research.

As an extension of the work by Fiedler (1986), Fiedler and Ahouse (1992) developed a hierarchical structure of the probable causes of rarity. Four main categories were: Short Persistence/Wide Distribution; Long Persistence/Wide Distribution; Short
Persistence/Narrow Distribution; and Short Persistence/Wide Distribution. Within these categories, a variety of possible factors were suggested as contributing to the species condition. Major factors were: stochasticity; taxon age, ecology or genetics; evolutionary history; co-evolution; reproductive biology; population ecology and dynamics.

Rarity can be *intrinsic* or *extrinsic* (Fiedler and Ahouse 1992). Intrinsic rarity has come about through the interaction of species and their environment over evolutionary time scales. Extrinsic rarity is primarily a result of human influence interrupting these long-term processes. There is no reason for human intervention to preserve intrinsically rare species in wilderness habitat. There is, however, a case for protecting species from human activities that either raise the extinction risk level of intrinsically rare species or turn formerly common species into extrinsically rare species (Huenneke 1991; Brigham 2003).

### 1.3 Rarity and Threat Status

Rarity and threat are intertwined to the point of being circular. Measures used for threat classification are frequently used to determine rarity status following which threatened status is conferred. At a global scale, the International Union for Conservation of Nature and Natural Resources (IUCN 2001) sets out criteria based around the extent of occurrence, area of occupancy, past and future loss including interactions between these parameters. For Australia, a comprehensive assessment of the status of rare plants known as the Rare or Threatened Australian Plants (ROTAP) list (Leigh *et al*. 1981; Briggs and Leigh 1995) uses extent of occurrence, level of threat (referred to as conservation status) and the numbers of individuals known to exist in conservation reserves. Comparing these two schemas, the problem of scale becomes apparent with the IUCN using both extent of occurrence and area of occupancy in square kilometres with varying cutoff levels for different levels of threat and ROTAP using linear geographic range only, with a cutoff of 100 km.

Crain and White (2011) have devised a ranking scheme intended to identify rarity at a local level that falls inside the resolution of the IUCN criteria. The measures are based
on the number of 1 km$^2$ grid cells that lie within the boundary of interest and are occupied by the target species. Suggested cut-offs are, <10 km$^2$ or 10 grid cells, <50 km$^2$ or 50 grid cells or <250 km$^2$ or 25 grid cells. The appeal of this method is that it can be applied at different scales: local government, state and national. For example, a species that might not be classified as threatened at the state or national level could well need careful management at a local level. Management at the local level could then prevent the species from becoming threatened at a state or national level through reducing the impact of ‘death by a thousand cuts’ or the ‘tyranny of small decisions’ (Odum 1982).

1.4 RARITY AND ENDEMISM

Endemism is the state where organisms (can be family, genus or species) are restricted to discreet areas. For a comprehensive review of endemism in higher plants see Kruckeberg and Rabinowitz (1985). The areas occupied by endemics may be limited to features in the landscape (geographic endemism) or specialised ecosystems (ecological endemism) and can be a combination of the two. An endemic species can be a single continuous population or it can be two or more disjunct populations. These populations can cover areas of a few square metres to many hectares and can be varying shapes governed by geographic and/or ecological constraints. Endemics can be relatively isolated amongst widely distributed species, or regions can have an abundance of endemic species. In fact, endemism as an areal concept has been difficult to define (Anderson 1994; Peterson and Watson 1998). Endemics can be anywhere along the scale of common to rare. They can be endemic to a small region, a state or a country (Stebbins and Major 1965; Shmida 1984; Crisp et al. 2002).

Vicarious species are a particular case of endemism that arise when the area of occupancy by a species or biota is split, either geographically or ecologically, resulting in two closely related species (Löve 1954). Generally, vicarious species will hybridise if brought back together. Some vicarious species can take on quite different forms, like forest trees becoming small shrubs in a different environment. The relationship between Australian, New Zealand and South American species having their origin in a common landmass (McCarthy et al. 2007) is an example of vicariance biogeography (Hausdorf
2002). However, some apparently vicariant species could instead be the product of long-distance dispersal (Crayn et al. 2006).

Microclimate, mutualisms and edaphic conditions are some of the elements that an endemic species is restricted by and is a reflection of a lack of genetic capacity to adapt to a wider range of conditions. Cain (1944) pioneered the study of plant geography and recognised that endemic species can be subdivided by their evolutionary age. Expanding on this, Stebbins and Major (1965) described two broad categories: neoendemics being of recent origin and paleoendemics being ancient and relictual species. This separation is by no means clear cut, but some general trends are thought to be valid. A single restricted population could be either neo- or paleoendemic while disjunct occurrences are probably paleoendemics. The ploidal level of endemic species can also be a guide to the type of endemic, with paleoendemics diploid or high polyploid and neoendemics low polyploid. Low polyploids, derived from diploids, are the more recent amongst related species.

1.5 RARITY AND ECOSYSTEM FUNCTION

It is taken for granted that rare species should be protected and conserved by virtue of their rarity. The questions here are, why? what is the role of rare species in an ecosystem? and should conservation be directed at individual populations or habitat (Boyd et al. 2008)? It is well documented that the floristic content of any sampled area of vegetation is dominated by a few species with the majority of species in low numbers. Smith and Knapp (2003) experimentally modified sample areas by reducing the numbers of rare and uncommon species and the abundance of the dominant species. They found that overall vegetative growth was unaffected by a threefold loss of the rare or uncommon species but declined with the loss of the abundant species.

Edwards and Westoby (2000) compared the incidence of rarity in families of flowering plants between the floras of Australia, New Zealand and North America. They found no indication that a family had a pre-disposition towards rarity and concluded that rarity had multiple origins that included family traits, geographic and environmental factors. Schwartz and Simberloff (2001) and Lozano and Schwartz (2005) looked for
relationships between taxon size and incidence of rarity and found that species-rich taxa had a higher incidence of rarity than species-poor taxa. There was also a positive relationship between overall regional flora species richness and the proportion of rarities. Ricotta et al. (2008) conducted a similar study in the vegetation throughout urban Brussels and Rome. They also found that the accumulated rare species had much greater taxonomic diversity than the accumulated common species. This condition has been attributed to environmental filtering whereby common species are more general and adaptive in their environmental requirements. On the other hand, the requirements of rare species are narrower, and microhabitat variation provides more sites for occupation, albeit at lower densities.

There are few examples of the role of rare species in ecosystems (Lyons et al. 2004). Severns and Moldenke (2010) provide an account from Oregon, USA. The managers of a wildlife refuge elected to remove a small population of a natural hybrid, *Grindelia integrifolia* X *Grindelia nana* (Asteraceae), aiming to prevent contamination of genetically ‘pure’ populations of *Grindelia integrifolia* elsewhere in the refuge. Unfortunately, the hybrids were the sole source of pollen for the only known and small population (a few hundred individuals) of a solitary, locally endemic, native bee species. Since that time, approximately 20 individuals have been found elsewhere, again dependent on the hybrid *Grindelia*. There are two issues here: the importance of taking mutualisms into account and that rarity can also extend to hybrid species.

The study of keystone species attempts to identify species whose contribution to overall ecosystem function and services is far reaching, and disproportionate to their low abundance, to the extent that if they were removed, there would be a significant negative impact (Mills et al. 1993; Power et al. 1996).

### 1.6 Rarity and Reproduction

Most organisms live for sex. It is sexual reproduction (aside from the possibility of beneficial mutations) that provides genetic heterogeneity that can ensure the species’ fitness by endowing its members with variable levels of tolerance to changing conditions. This is evolution at work with a variety of selection forces demanding an
adaptive response. While evolutionary time scales are generally long, stochastic events may not permit a measured response over many generations. Sudden change can result in contraction of a species, leading to rarity or endemism, or the species can actually respond in kind, quickly. An example of a quick response (in less than ten years) is the capacity of some grasses to adapt to heavy metals in soil (Krukeberg and Rabinowitz 1985).

Comparisons of reproductive processes between common and rare species from various parts of the world have concluded that certain reproductive traits are over-represented in rare species (Harper 1979; Kunin and Shmida 1997; reviewed by Gaston and Kunin 1997).

Fragmented or dispersed populations are a characteristic of rare species. and this has been shown to contribute to reduced pollinator visitation rates and consequently lower seed set (Schmitt 1983; Jennersten 1988; Kunin 1992; Morgan 1995; reviewed by Ghazoul 2005). A significant proportion of rare species are self-compatible and tend toward vegetative reproduction (Harper 1979; Kunin and Gaston 1993; Kunin and Shmida 1997; Honnay et al. 2007). On the other hand, Murray et al. (2002), examining eastern Australian plants, found no relationship between growth form, pollination or dispersal mode and the threat of extinction. They acknowledged that this was at odds with similar research from other continents. Their view was that geographic context and the type of flora could be confounding factors when attempting to generalise the relationship between rarity and lifeform. Luzuriaga et al. (2006) studied the effect of population structure on reproduction in the rare Spanish Centaurea hyssopifolia Vahl. (Asteraceae) and found that plant aggregation, density and size had a significant effect. More aggregated plants produced more seed as did larger individual plants; however dense patches of plants produced less seed.

Zygomorphic flowers (bilaterally symmetrical) have been shown to be over-represented in rare taxa (Harper 1979). Related to this was the higher number of species with specialised access requirements for pollen collection. Both conditions are thought to enhance fidelity between pollinator and flower thus maximising the opportunities for fertilisation (Neal et al. 1998). By contrast in a review of pollen limitation research
Knight et al. (2005) found no difference in pollen limitation between zygomorphic and actinomorphic (radially symmetrical) flowers.

Very few rare species are wind pollinated or are descended from wind pollinated ancestors (Harper 1979). This is understandable since wind pollinated species rely on adequate population density for the pollen to be successfully received by another stigma.

1.7 SUMMARY OF RARITY

Rare plant species capture the imagination of field botanists who target them and get a real buzz out of discovering them. Rarities are frequently classified as threatened and given the benefit of the legal protection this classification affords. In order to conserve these species that are potentially on the brink of existence through anthropological causes, managers need to ‘know’ them (Kruckeberg and Rabinowitz 1985). There is, however, little information available as to the importance of most rare species and their function in their host ecosystems. Are any of them keystone species with influence far greater than their numbers suggest? Would they not be missed were they to disappear? Would the accumulation of apparently insignificant losses ultimately lead to cascading loss? Species have come and gone throughout evolutionary history and it is hard to say whether past losses mean that we are worse off today. Answers to these questions can only come from diligent investigation into the lives of individual species and it is the intention of this project to contribute through research into Tetratheca juncea.

1.8 TETRATHECA JUNCEA SMITH (ELAEOCARPACEAE)

The subject of this research project is Tetratheca juncea. This species is listed as vulnerable in both the New South Wales Threatened Species Conservation Act 1995 and the Commonwealth Environment Protection and Biodiversity Conservation Act 1999. Its prominence in development applications from the NSW Central Coast and Lower North Coast, and paucity of ecological information available to enable informed decisions, meant that it was an ideal study subject.
The family Tremandraceae, containing *Platytheca*, *Tetratheca* and *Tremandra*, had long been described as distinct and endemic to Australia. However, recent genetic studies have placed the family as either a part of Elaeocarpaceae (Savolainen *et al.* 2000; Bradford and Barnes 2001; Crayn *et al.* 2006) or completely absorbed into Elaeocarpaceae (APG 2003). This has been supported by comparative morphology analysis (Matthews and Endress 2002).

Records extracted from the Australian Virtual Herbarium (http://www.chah.gov.au/avh/) and the Australian Plant Census (http://www.chah.gov.au/chah/apc/) show that there are 63 species of *Tetratheca* in Australia. The genus occurs in all States other than the Northern Territory. Western Australia (http://florabase.dec.wa.gov.au) has the greatest number of species with 40 species, all endemic to that State. Eleven *Tetratheca* species are listed as threatened at State level and seven at Commonwealth level; in addition, several species not formally listed are rated as conservation significant.

*Tetratheca juncea* is a terrestrial, herbaceous plant endemic to NSW. The species was first described by the renowned English botanist and founder of the Linnean Society, James Edward Smith (Smith 1793). Smith also named the genus *Tetratheca* on account of the “curious structure of its antherae, each of which consists of four cells”. Smith gave the common name of Rushy Tetratheca. However, Black-eyed Susan, named after the appearance of the dark anthers at the centre of pink petals, is now the common vernacular.

### 1.8.1 Taxonomic Description

This description is summarised from the revision of the *Tetratheca* genus by Thompson (1976). A generally leafless shrub, prostrate, with stems to 1 m in length. The stems branch above a woody stock that is often horizontal and rhizomatous. The stems are 0.8–1.5 mm wide, winged, glabrous and with alternate branching. Leaves are sessile, alternate, narrow elliptical and up to 20 mm long and 5 mm wide, although mostly reduced to narrow scales 3 mm in length.
Flowers occur singly or in pairs in the leaf axils on peduncles 5–10 mm long. Floral structure (Figure 1.1) is comprised of 4 deciduous, generally orbicular, calyx segments 1-1.5 mm in length. Four petals are dark pink or rarely white, obovate to linguiform in shape and 7–11 mm long. Eight stamens 3-3.5 mm long are comprised of tubular anthers with a narrow orifice all supported on short (0.5 mm) filaments.

The ovary is glabrous and on a broad base with the top tapering to a slender style 1.75-2 mm long. There are 2 locules with 2 ovules in each. The fruit is obovate 6-8 mm long and ½-⅔ as wide. Seeds are approximately 4 mm long, obovoid-cylindrical with fine brown appressed hairs and have an irregularly twisted, hairy appendage. Figure 1.2 shows the flowers, stems and leaves and Figure 1.3 shows seed about to be released from the capsule.

Figure 1.1 Showing the parts of a *Tetratheca juncea* flower
Two petals and two anthers have been removed to expose the gynoecium.
Figure 1.2 *Tetratheca juncea* flowers, stems and leaves.
This shows the typical 4-petalled pendant flowers with the central cluster of dark poricidal anthers as well as the narrow stems and small scattered leaves.

Figure 1.3 *Tetratheca juncea* seed about to be released from the capsule.
A maximum of four seeds can be in a capsule. The creamy chalazal appendage (elaiosome) can be seen attached to the end of the seed.
1.8.2 Ecology and Biology

Knowledge of the ecology and biology of *Tetratheca juncea* is essential for its management as a rare species. This involves understanding where the species grows and why, population structure and size, the dynamics of flowering, fruiting, seed dispersal and germination, and response to stochastic events. As already noted, the fact that much of this information has not been available was the motivation for this research project. What follows is a review and critique of the current state of knowledge of the species’ ecology and biology.

1.8.3 Growth Form

An individual *Tetratheca juncea* plant is commonly referred to as a ‘clump’ and a clump has been arbitrarily defined as a group of stems separated from the next nearest group by at least 30 cm (Payne *et al.* unpub.). This convention was developed in order to be able to quantify populations that may consist of isolated single stems, multiple stems like a grass tussock or multiple stems continuous over a few square metres. This variable form comes about from the species’ rhizomatous habit with aerial stems sprouting from the underground rhizome (Figure 1.4).

In flower, the plant is clearly visible. However, when not in flower it is decidedly cryptic with narrow, mostly leafless stems blending with surrounding grasses, herbs and shrubs. This feature could have contributed to the species listing as threatened.

1.8.3.1 Geographic Distribution, Population Size and Structure

As far back as the late 1800’s to the mid 1900’s there were records of *Tetratheca juncea* from Sydney, Newcastle, Port Stephens and Bulahdelah regions and the only records added since then have been within those bounds (Atlas of NSW Wildlife 2011).

Figure 1.5 shows the distribution of Atlas of NSW Wildlife records of *Tetratheca juncea* as at the start of this research project (end of 2003). As can be seen, there are a number of records from metropolitan Sydney where the species is now considered to be extinct.
Figure 1.4 A single *Tetratheca juncea* rhizome with three aerial stems.

The species is now known to exist only from the Wyong area to Bulahdelah, from the coast and inland to the edge of the main ranges; an overall range of approximately 120 km north-south and 20 km east-west. The greatest concentration of records is from the Lake Macquarie local government area.

Payne (2000) described *Tetratheca juncea* that occurred within the currently known range of Wyong to Bulahdelah as a single *metapopulation*; the historical Sydney records were excluded. *Populations* were defined as occurring within an arbitrary division of the Lake Macquarie LGA component of the metapopulation into four quadrants centred on the intermediate cardinal points of NE, SE, NW and SW. *Subpopulations* were any discrete grouping within a population. Probably these divisions were selected for management purposes rather than for ecological reasons.

1.8.3.2 Habitat Association
The earliest attempt at relating occurrence of *Tetratheca juncea* to geographic and environmental conditions, as well as associated species, was by Payne (1993).
Figure 1.5 The distribution of *Tetratheca juncea*.
The total historic and current recorded distribution of *Tetratheca juncea* as at March 2003. The distribution has been divided into regional populations on the assumption that the distance between them is sufficient to prevent natural transfer of genetic material. The South Coast regional population is presumed extinct following the development and spread of the Sydney urban area. Data source Atlas of NSW Wildlife (2003). Inset shows the location within Australia.
The analysis was subjective/empirical, concluding that the species preferred flat ridge-tops on shallow skeletal sand, with *Angophora costata* as a dominant canopy species, and close proximity to the sea. While presented as predictive, this report was simply a summary of the conditions in which the species occurred in the sampled area.

Driscoll (2003) conducted a Geographic Information System (GIS) assessment with 400 *Tetratheca juncea* occurrence records overlaid on a regional vegetation map (National Parks and Wildlife Service 2000). Based on a count of records within each community the occurrences were 62% in Coastal Plains Smooth-barked Apple Woodland, 14% in Coastal Plains Scribbly Gum Woodland and 10% in Coastal Foothills Spotted Gum – Ironbark Forest. The remaining 14% of occurrences were divided between 13 different vegetation communities. A limitation of this approach is that the National Parks and Wildlife Service (2000) vegetation map is a model of the association of vegetation communities with geographic, environmental and climate variables. A review of the National Parks and Wildlife Service (2000) vegetation map by Nicholls et al. (2002) found that the model was not adequately ground-truthed and was unsuitable for use at scales of less than 1:25,000. Using the same GIS method, Driscoll (2003) found that the species was recorded from locations with the following underlying geologies: Quaternary Sands; Triassic Sandstones; Triassic Shales; Permian Coal Measures and Carboniferous Volcanics.

What is the local population of *Tetratheca juncea*? A local population would be one where genetic material can move freely between the members of that population, a classic Mendelian population:

“A Mendelian population is a reproductive community of sexual and cross-fertilizing individuals which share in a common gene pool . . . The smallest Mendelian populations are panmictic units (Wright, 1943), which are groups of individuals any two of which have equal probability of mating and producing offspring.” (Dobzhansky 1950).

A local population also undergoes the processes of birth, death and dispersal and is somehow spatially segregated from other local populations; it is often considered as occupying a suitable, discrete habitat patch (Freckleton and Watkinson 2002).
The metapopulation concept originated with Levins (1969); for reviews see Hanski and Gilpin (1991) and Hanski (1998) and is a subset of the regional distribution. A useful definition is that a metapopulation is "a population of populations more or less connected by gene flow and characterized by colonization–extinction dynamics" (Harder and Barrett 2006 p 349). By inference, individual metapopulations of the same species are somehow separated so that no gene flow in any form occurs between them. Hanski (1997) nominated four conditions under which a group of local populations would constitute a metapopulation, namely, when: (i) a suitable habitat occurs in discrete patches that may be occupied by local reproducing populations; (ii) even the largest local populations have a measurable risk of extinction (unless the largest population is the source of a source-sink system); (iii) habitat patches are not too isolated to prevent recolonization following local extinctions; and (iv) local populations do not have completely synchronous dynamics (or the dynamics of the global population will not be much longer than that of the local populations).

Strictly speaking, there is no information available that would allow division of the distribution of Tetratheca juncea into true metapopulations. However, for convenience, distribution has been subdivided into three regional ‘populations of populations’ (Figure 1.4). It is assumed as unlikely that there would be any natural transfer of genetic material between these regional populations. These are the South Coast where the species is presumed extinct; the Central Coast from Wyong to Beresfield and the North Coast from Karuah to Bulahdelah. Any further division within the Central Coast and North Coast regional populations would not be possible without additional data, particularly information about dispersal distances of genetic material. It is conceivable that these regional populations could contain more than one true metapopulation.

Overall population numbers of Tetratheca juncea have been thought to be small and distribution fragmented as a result of clearing of habitat for urban, rural and industrial purposes. Hogbin (2002a) reviewed the conservation status of the species as part of an overall review (Hogbin 2002b) of the status of all flora species listed as threatened in the schedules of the NSW TSC Act.

Actual population numbers have been quoted as: within the Lake Macquarie LGA, 9645 clumps (Payne 2000) made up of 231 sub-populations of which >60% had 20 clumps or
less; or, a total extant population of 9881 – 11893 clumps (Hogbin 2002a) with 83% of sub-populations of <50 clumps. In 2006, a property developer was fined $43000 for destroying 2400 clumps that the court proceedings (NSW Land and Environment Court 2006) noted was 20% “of the total population of the species in the world” based on the assessment by Hogbin (2002a).

The methods used by Payne (2000), to arrive at the census figure, do not appear to have been systematic and were possibly to some extent biased by applying the habitat association information from Payne (1993) as a predictive tool. Both the NSW and Commonwealth Scientific Committees relied heavily on Hogbin (2000a) in making their determinations.

1.8.3.3 Flowering and Pollination

The flowering period is reported to be from mid to late winter through to late summer (Gardner and Murray 1992). There has been no detailed study of *Tetratheca juncea* flowering and fruiting phenology, and this leaves a significant information gap for the management of the species. Surveys for the species should be undertaken at peak flowering which is when it is most visible. This would maximise the chances of recording any or all of a population depending on the survey aims. Lack of data defining the reproductive phenology of *Tetratheca juncea* has meant that Lake Macquarie City Council Flora and Fauna Survey Guidelines (Murray *et al.* 2001) require surveys to be replicated 2 – 3 times during the flowering period. Knowing the most opportune time for a survey would improve the quality of the result and reduce cost and time required for surveys.

The flowers of *Tetratheca juncea*, as with other members of the *Tetratheca* genus produce no nectar (Gross *et al.* 2003) that could serve as a pollinator attractor. Thus, it would appear that pollen is the sole reward available.

The *Tetratheca* flower structure with poricidal anthers resembles what has been termed a Solanum-type (Vogel 1978), and later Solanoid (Faegri 1986), after the dominance of these structural characteristics in *Solanum* (Solanaceae). The pollination vectors of flowers with poricidal/porose anthers are bees capable of buzz pollination also termed vibratory pollination or sonication. Pollen extraction is achieved by the bee grasping the
androecium with the anthers against the venter and transferring vibrations to the anthers from their body by means of rapid contraction of their indirect flight muscles (Buchmann 1983; Buchmann and Cane 1989; Harder and Barclay 1994). The audible component of the vibratory process is a useful detectable artefact to the energetics applied to the flower parts by the bee in order to extract the pollen (Buchmann 1983). The poricidal anthers of *Tetratheca juncea* contain the pollen within locules in tapetal fluid (Bartier et al. 2003). In common with other buzz-pollinated plants (King and Lengoc 1993; King and Buchmann 1996), *Tetratheca juncea* anthers appear to dispense pollen, probably through the gradual drying of the tapetal fluid. Dissection of a number of *Tetratheca juncea* anthers showed that, at any time, only a fraction of the total pollen content was dry, just inside the disseminating pore (Driscoll 2003).

Gross et al. (2003) proposed that the most likely pollinators would be native bees capable of buzz-pollination and spent over 100 h observing for pollinators with no result. The first confirmed pollinators, reported by Driscoll (2003), were two native bee species, *Lasioglossum (Chilalictus) convexum* Smith 1879, and *Exoneura* sp. captured while they were collecting *Tetratheca juncea* pollen. The apparent lack of pollinators has led to considerable conjecture (Gross et al. 2003) about the level of extinction risk faced by *Tetratheca juncea*. Habitat fragmentation, habitat degradation and low levels of seed set have been suggested as contributing to low pollinator numbers. More pollinator data is needed, both in terms of the species involved and their activity across a regional population.

Using hand pollination, Gross et al. (2003) demonstrated that *Tetratheca juncea* was capable of self-fertilisation but concluded that the species was predominantly xenogamous (outcrossing). Clonality and self-incompatibility has been associated with pollinator limitation (Bond 1994; Vallejo-Marin and O’Brien 2007) whereas Larson and Barrett (2000) found that selfing species were not associated with pollen limitation. *Tetratheca juncea* could be described as having a ‘mixed mating’ system (Goodwillie et al. 2005). That is, where hermaphrodite plants reproduce by both selfing and outcrossing. This versatility could well provide some insurance against problems associated with the fragmentation of purely outcrossing clonal plant populations. Given the negative consequences of inbreeding (inbreeding depression, Charlesworth and Charlesworth [1987]; Husband and Schemske [1996]; Crnokrak and Roff [1999]),
selection should be away from mixed mating systems. However, self-fertilization can result in the purging of inbreeding depression (Goodwillie et al. 2005). Honnay and Jacquemyn (2007) found that reduced population size had less impact on genetic diversity for selfing species than for species with a mixed mating system that was strongly outcrossing.

Fragmentation is expected to impact on the capacity of native bee pollinators by restricting movement between habitat patches. Any limiting of the capacity of bees to traverse gaps appears to be related to the quality of the vegetation in the gap. Jauker et al. (2009) investigated the relationship between bee species diversity, bee numbers and distance travelled from native vegetation across an agricultural landscape. They found that, provided there was sufficient native species content in the open groundcover, diversity and total numbers fell away across quite large distances of up to 2 km. Also, diversity fell more rapidly when ground cover quality was poor. Russell et al. (2005) investigated the value of powerline easements for native bees and found that there was a rich diversity of plant species that improved when slashing was kept to a minimum.

A recurring theme with the management of Tetratheca juncea has been that the species is potentially on a pathway of “insidious delayed extinction” (Driscoll 2003) as a result of existing in small populations in a fragmented landscape. Further research is needed into population size, geographic distribution and degree of fragmentation to clarify the risk status of the species.

1.8.3.4 Fruit Set, Seed Dispersal and Germination
Payne (2000) concluded that Tetratheca juncea produced low numbers of fruit from early December through to the finish of flowering in January and that fruit set peaked through February and March. Low fruit numbers were thought to be in part an artefact of the cryptic condition of the species following flowering. The conclusion appears to have been based on field impressions rather than targeted data collection. Gross et al. (2003) found that fruit contained an average of 1.65 seeds (out of a possible 4).

Seeds are shed by the capsule drying and opening with seeds dropping straight to the ground. Tetratheca juncea is a myrmecochorous plant with ants collecting the shed seed for the lipid rich elaiosome (Brew et al. 1989, Boeswinkel 1999). The ants later act as a
dispersal agent depositing the seed minus the elaiosome within a limited area around their nests. Both the Green Ant \textit{(Rhytidoponera metallica)} and a small black ant (tentatively \textit{Stigmacros} species) have been recorded collecting \textit{Tetratheca juncea} seed (C. Driscoll unpubl.).

Bellairs \textit{et al.} (2006) reported that the seed bank contained no viable seed even in February which is towards the end of the flowering season. However, Butcher \textit{et al.} (2008) studying the rare Western Australian \textit{Tetratheca paynterae} subsp. \textit{paynterae}, found that when a mature plant died, seedlings grew in the immediate vicinity. This demonstrated a viable and dormant seedbank for that species. The fact that germination occurred after the death of the adult plant also suggests the possibility of allelopathy where chemicals from a mature plant suppress germination.

The soil samples collected for the Bellairs \textit{et al.} (2006) study were taken approximately 10 cm from the base of a clump, and there are plausible explanations as to why viable seed were not found. Berg (1975) investigated the behaviour of seed-collecting ants and included \textit{Tetratheca} sp. seed in his experiments. He placed seed in a depot and found that all seed with an elaiosome were removed by ants from the depot over a period of approximately 30 min. Maybe ants are selective and only collect viable seed from around a \textit{Tetratheca juncea} clump. Brew \textit{et al.} (1989) reported on the chemical compounds found in elaiosomes that induced ants to collect seeds of \textit{Acacia myrtifolia} and \textit{Tetratheca stenocarpa}. They found that the chemical fraction that induced collection by ants was a minor component of the overall lipid content of elaiosomes. From an evolutionary standpoint, it is not unreasonable to suspect that the elaiosomes of empty or poorly developed, non-viable seed do not contain the biologically active fraction.

Under laboratory conditions, Bellairs \textit{et al.} (2006) showed that \textit{Tetratheca juncea} seed germinated in varying proportions from seed that were untreated, scarified, treated with gibberellic acid or smoke water. Smoke water treatment resulted in the highest levels of germination and of these treatments smoke would be the most likely germination trigger to be encountered in natural ecosystems.

There is scope for much more research into the fate of \textit{Tetratheca juncea} seed after they have been released.
1.9 RESEARCH AIMS AND THESIS STRUCTURE

As already described, decision makers and their advisers are routinely faced with the task of balancing development and biodiversity needs and almost invariably there is a paucity of information about species to enable informed decisions. Generally, either explicitly or implicitly, the precautionary principle (Meyers 1993; Loechler et al. 2001) is invoked resulting in an unsatisfactory outcome for all, including the subject species.

The results of detailed research into parts of the life cycle or biology of any species are of limited assistance; at least not until many projects have been completed and reviewed. As Holsinger and Gottlieb (1991) stated: “Surprisingly little is known about the overall biology of any individual plant species growing in the wild because scientists often choose plants to study in order to answer particular questions, not to find out all about them as organisms.” It has been estimated that 8000 study years would be required to conduct comprehensive life-cycle research on all of the listed Australian threatened flora species (Edwards 1996 cited in Murray et al. 2002). However, such a grandiose task is not needed with not all listed species demanding the same priority. There are a few species that are regularly under pressure as a result of their location in areas with a high level of development activity. In the NSW Central Coast, Lower Hunter and Lower North Coast *Tetratheca juncea* is one of those high profile species seemingly encountered in most development applications involving habitat clearing; so much so that it is often disparagingly referred to as a ‘weed’ that is ‘everywhere’.

A specific aim of this research project was to quantify key components of the *Tetratheca juncea* life-cycle so that its conservation and management could be better informed. A broader aim was to contribute to the body of knowledge of the reproductive ecology and biology of an individual species and its interactions with its environment. To achieve these aims data were collected from naturally growing populations across a wide geographic range. The research has covered the main life-cycle components of flowering, pollination, seed set and release, dispersal and vegetative propagation.
Specific aims of the four experimental chapters are:

**Chapter 2**
To investigate *Tetratheca juncea* reproductive phenology across a wide geographic range within the Central Coast regional population, over one season.

**Chapter 3**
To expand the list of known and presumed pollinators.
To examine flowering, pollinator activity and fruit production across a wide geographic range within the Central Coast regional population, over six years.

**Chapter 4**
To examine flowering, pollinator activity and fruit production in a subpopulation from within the Central Coast regional population, over six years.

**Chapter 5**
To model the habitat suitability of *Tetratheca juncea* for both the Central Coast and North Coast regional populations.

Finally, **Chapter 6** will present a general discussion of the results, their implication for the management of *Tetratheca juncea* and the usefulness of this research approach for studying other rare and threatened plant species. The classification of the species as rare will be reviewed.
CHAPTER 2  TETRATHECA JUNCEA REPRODUCTIVE PHENOLOGY
2.1 INTRODUCTION

Phenology is defined as “the study of the timing of recurring biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species” (Lieth 1974). These are commonly annual events: "the occurrence of certain obvious biotic and abiotic events or group of events within a definite limited period or periods of the astronomic (solar, calendar) year" (Lieth 1974).

The principle of phenology has been part of human life for millennia. For example, seasonal events have been used to determine when best to plant crops, hunt for game or move to a less hostile environment (Puppi 2007). Throughout a season, the subject species will progress through several phases or phenophases. These phases were first catalogued for cereals by Zadoks et al. (1974) and subsequently, more generally for monocots and dicots, in the BBCH (Biologische Bundesanstalt, Bundessortenamt, and Chemical industry) scale (Meier 2003) with the principal growth stages shown in Table 2.1. The BBCH scale is used to provide consistent phenological terminology, and has commonly been applied to commercial species from cereal crops to fruit trees.

Plant phenology has been researched over many years (Grainger 1939; Hulbert 1963; Kelly 1992; Schwartz 2003; Forrest and Miller-Rushing 2010). Recently, in response to global climate change concerns, phenology has attracted a rapidly increasing amount of interest. Phenological phases are considered to be potential early indicators for tracking ecological changes in response to changes in climate both regionally and globally (Menzel 2003; Cleland et al. 2007; Keatley and Hudson 2007; Hudson 2010).

Phenology is the framework on which the study of the ecology and evolution of species is built (Albert et al. 2008; Devaux and Lande 2009; Forrest and Miller-Rushing 2010) and as such is a key component of the current research project. Phenology is exhibited at the individual plant and population level. Even within the same plant there can be phenological differences such as not all flowers opening together and certainly not all flowers being pollinated and setting fruit at the same time (Rathcke and Lacey 1985). The phenological properties of a population will be similar to any statistical distribution, being the combination of the variation amongst the individuals (Kudo 2006).
Table 2.1. Biologische Bundesanstalt, Bundessortenamt, and CHemical industry (BBCH) principal growth stages of a plant species

In the full BBCH scheme, each principal growth stage is expanded to describe intermediate stages as the plant progresses from one stage to the next. It is used to provide a standardised description of the phenology for any species.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Germination / sprouting / bud development</td>
</tr>
<tr>
<td>1</td>
<td>Leaf development (main shoot)</td>
</tr>
<tr>
<td>2</td>
<td>Formation of side shoots / tillering</td>
</tr>
<tr>
<td>3</td>
<td>Stem elongation or rosette growth / shoot development (main shoot)</td>
</tr>
<tr>
<td>4</td>
<td>Development of harvestable vegetative plant parts or vegetatively propagated organs / booting (main shoot)</td>
</tr>
<tr>
<td>5</td>
<td>Inflorescence emergence (main shoot) / heading</td>
</tr>
<tr>
<td>6</td>
<td>Flowering (main shoot)</td>
</tr>
<tr>
<td>7</td>
<td>Development of fruit</td>
</tr>
<tr>
<td>8</td>
<td>Ripening or maturity of fruit and seed</td>
</tr>
<tr>
<td>9</td>
<td>Senescence, beginning of dormancy</td>
</tr>
</tbody>
</table>

Because physical changes in plant reproductive cycles are related to seasonal events, there must be conditions that act as triggers for the various phases. These conditions could be soil condition such as moisture content, ambient temperature or photoperiod (Dean et al. 1998; Wielgolaski 2001; Menzel et al. 2005; Resco and Hartwell 2009).

The earliest visible phenophase of plant reproduction is budding. However, buds originate from meristem flowering initials or primordia (flower initiation) that have themselves been triggered to develop from vegetative meristems (Lyndon and Battey 1985; Bohn-Courseau 2010). Once developed, reproductive buds may stay dormant until their further development is triggered. In annuals, flower initiation is commonly followed directly by bud and flower development; however, the long-term existence of perennials allows for a delay between initiation and development (Battey 2000).

Plants need external assistance to complete critical elements in the reproductive cycle with plant reproductive phenology interacting with other species and events (Thompson 1981; Oberrath and Bohning-Gaese 2002; Elzinga et al. 2007). Flowers need pollinating, either by biotic or abiotic means so it is ideal that the flowers are open when pollinator vectors are most available. However, competition between flowering species for pollinators means that subtle changes in timing could be either beneficial or detrimental. The entire reproductive process from budding to seed release is dependent on the plant having sufficient resources to complete. Hence, phenology should be timed...
to when metabolic processes can proceed maximally. Away from the tropics, seasonal changes from summer through to winter become significant in relation to the prime growing times and so phenology patterns reflect these changes. Seed dispersal vectors also need to be available, and they may have their own seasonal phenologies (see reviews by Kudo 2006; Elzinga et al. 2007). To maximise fitness, the reproductive phenology of flowering plants should coincide with dispersal vector phenology.

Phenophase synchrony (or asynchrony) can occur at several levels, and the most obvious is with pollinators. Flowering can synchronise with pollinator availability or pollinators can synchronise their own phenology with flowering (Bolmgren 1997). Other factors could be the need to avoid high predator activity such as seed predators or to time seed release with peak availability of dispersal vectors (Augspurger 1981). Variations in phenophase timing can have phenotypic origins (Gomez 1993), and this is where selection can theoretically play a part in modulating the phenology of a population or species through increased fitness of phenotypic variants.

Kochmer and Handel (1986) compared flowering times of biotic pollinated species at the disparate locations of the Carolinas and Japan and found no significant difference in flowering times within the same families. The fact that these two locations have been geographically separated for many millions of years, and have experienced different environmental changes, suggests that the flowering phenologies have been genetically hard-coded a long time prior.

First flowering date is a commonly used phenophase marker (Kudo 2006; Elzinga et al. 2007; Miller-Rushing et al. 2008; Tooke and Battey 2010). However, there can be ambiguity about when a flower is open (Fitter et al. 1995; Tooke and Battey 2010), particularly those with complex corollas. Fortunately the *Tetratheca juncea* flower is simple, and opening is unambiguous. In bud the anthers are enclosed in pairs by a single whorl of petals (Driscoll 2003) and so are not accessible to pollinators or the stigma until the flower is open.

The overall course of flowering, the flowering curve, is another informative phenological characteristic (Rabinowitz et al. 1981; Clark and Thompson 2011). The flowering curve can originate from longitudinal data, being a plot of the number of flowers on an individual plant at selected time intervals, or cross-sectional data, being
periodic sampling of flower numbers from a population (Clark and Thompson 2011). Among other things, flowering curves have been used to examine relationships between the flowering of biotic and abiotic pollinated species (Rabinowitz et al. 1981); plant pollinator interactions (Gross et al. 2000); within species variability (Ollerton and Lack 1998), and climate/flowering/species relationships (Tyler 2001). Clark and Thompson (2011) have developed a regression method to describe the shape of the flowering curve. The curve can also be used with partial data, to obtain dates of start, finish and maximum flowering as well as the overall shape of the curve. From the references cited in this paragraph, it is clear that the shape of the flowering curve is variable. It can be close to normal, but more commonly rises sharply and falls slowly over time (i.e. right skewed).

Referring back to Table 2.1, the four principal stages relating specifically to reproduction are budding (5), flowering (6), fruiting (7) and seed release (8). The main aim of this research into the reproductive phenology of Tetratheca juncea was to determine the starting times and site and time effects of these four phenophases. Primary matters for investigation were as follows:

1. **The flowering curve**

   Driscoll (2003) made an empirical observation that the pattern of flowering in a season had a main peak around September, fell away and then a peak around December and another around March. Data collected for this study will enable a detailed investigation of the flowering curve.

2. **Fruit development and seed release**

   It has been reported that *Tetratheca juncea* produces few fruit. Subsequently, this feature has been presented as either a demonstration of, or cause of, the rarity of the species (Payne 2000). It has also been stated that fruit development occurs at the end of the flowering season when the plant is difficult to find (Payne 2000) and so the reported low fruit levels may be an artefact.

3. **Variability across a regional population**

   What level of synchrony in flowering, fruiting, and seed release occurs across a regional population? This is of biological and management interest. If flowering were asynchronous, what local conditions would be involved? It is
possible that asynchrony in pollinator availability could be reflected in the timing of fruit production? If flowering were asynchronous, surveys for the species might need to be timed differently depending on the locality.

4. Peak flowering

Knowledge of the seasonal flowering pattern will allow surveys for this otherwise cryptic plant to be conducted at times that will maximise the opportunity for discovery.

2.2 MATERIALS AND METHODS

Twelve sites were randomly selected for sampling (Figure 2.1). These sites covered a geographic range of approximately 30 km north-south and 10 km east-west and were within the Central Coast Tetratheca juncea regional population. From general field observation over time, it was clear that floral buds appeared en masse during June each year. Some of the chosen sites were checked in May 2010, and there were no buds. Consequently, collection commenced towards the end of June 2010 and continued monthly until January 2011. See Section 2.3.6 for the reasons for the January cutoff.

Because the species grows clonally, as well as from seed germination, it was not possible to identify genetic individuals. Consequently, the study could only be conducted at the level of the average performance of each local population. At each site, stems were collected randomly from several plant clumps over an area of approximately 2000 m². To avoid collection bias, stems were picked at their base and teased out of the clump. A preliminary trial was undertaken to determine the amount of stem material needed.

The steps in capturing the targeted phenological data (Table 2.2) were:

- Scan freshly collected stems, along with a metric scale bar, using an A3 flatbed scanner.
- Import scanned images into Manifold System GIS (www.manifold.net) and set image local scale to centimetres using the scanned scale bar as reference.
- With the scaled image (Figure 2.2) as background, trace stems, peduncles, buds and fruit with a line and mark all leaf axils, open flowers and open fruit with a point, each in a separate drawing. The traced lines provided length and count data with points providing count data. Table 2.2 lists the characters and their recorded attributes.
- Collate length and count data and export for statistical analysis and interpretation.
Figure 2.1. The geographic location of sites (numbered 1-9) within the Central Coast *Tetratheca juncea* regional population from which plant material was collected for the phenology study.

Material was collected from discrete local groups of plants. The inset shows the location of the sample sites in relation to a convex hull of the known records of the Central Coast regional population.
Figure 2.2 Scan of Site 2 from 25/10/2010
1 = developing bud, 2 = open flower, 3 = developing fruit, 4 = mature fruit, 5 = open fruit. In a Geographic Information System the image was scaled to cm and stems, buds and peduncles were traced with lines for length measurement and count data. Flowers were marked with a point for counting. Lengths and counts were automatically generated by the program and exported for statistical analysis.
Table 2.2. Characters recorded from the scanned stems, and their parameters
Open fruit are those from which seed have been released.

<table>
<thead>
<tr>
<th>Character</th>
<th>Length</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stem</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Peduncle</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Bud</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Flower</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Open fruit</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

2.2.1 Statistical Analysis

The majority of statistical analysis was conducted using SPSS version 19. Analysis was by way of repeated measures MANOVA or ANOVA with appropriate post hoc tests applied where a significant effect was determined. Primer 6 (Clarke and Gorley 2006) was used to investigate synchrony across the regional population through the use of hierarchical agglomerative clustering.

2.3 RESULTS

2.3.1 Sample Size and Primary Data Treatments

To provide some insight into the amount of material sampled, Table 2.3 shows the total stem lengths collected for each site and Table 2.4 records the number of inflorescences examined. Over half a kilometre of stem was collected, and 6239 inflorescences were examined.

For analysis of each phenophase, because different amounts of stem (Table 2.3) were collected each month, raw records for budding, flowering, fruiting and seed release were normalised by converting them to their proportion of total inflorescences examined (Table 2.4). This was possible because each inflorescence consisted of one peduncle and one bud or later phase. Of course, there is almost invariably an exception to any rule in nature, and in rare cases (two in 6239 inflorescences) there were two buds
at the end of one peduncle. Total number of inflorescences was the sum of the individual phase values. In addition, flowering intensity was calculated as the number of open flowers per centimetre of stem.

**Table 2.3. Total stem length (cm) of collected samples from each specified site (Fig 2.1) across 2010/2011.**

<table>
<thead>
<tr>
<th>Site</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>678</td>
<td>583</td>
<td>434</td>
<td>466</td>
<td>696</td>
<td>541</td>
<td>673</td>
<td>620</td>
</tr>
<tr>
<td>2</td>
<td>850</td>
<td>655</td>
<td>457</td>
<td>628</td>
<td>759</td>
<td>717</td>
<td>827</td>
<td>598</td>
</tr>
<tr>
<td>3</td>
<td>537</td>
<td>621</td>
<td>465</td>
<td>621</td>
<td>726</td>
<td>648</td>
<td>540</td>
<td>538</td>
</tr>
<tr>
<td>4</td>
<td>294</td>
<td>140</td>
<td>351</td>
<td>346</td>
<td>391</td>
<td>420</td>
<td>408</td>
<td>389</td>
</tr>
<tr>
<td>5</td>
<td>688</td>
<td>559</td>
<td>597</td>
<td>708</td>
<td>642</td>
<td>556</td>
<td>643</td>
<td>709</td>
</tr>
<tr>
<td>6</td>
<td>569</td>
<td>590</td>
<td>445</td>
<td>782</td>
<td>723</td>
<td>673</td>
<td>677</td>
<td>672</td>
</tr>
<tr>
<td>7</td>
<td>576</td>
<td>467</td>
<td>426</td>
<td>676</td>
<td>694</td>
<td>746</td>
<td>606</td>
<td>714</td>
</tr>
<tr>
<td>8</td>
<td>561</td>
<td>569</td>
<td>411</td>
<td>679</td>
<td>646</td>
<td>585</td>
<td>670</td>
<td>699</td>
</tr>
<tr>
<td>9</td>
<td>281</td>
<td>493</td>
<td>390</td>
<td>547</td>
<td>618</td>
<td>512</td>
<td>582</td>
<td>754</td>
</tr>
<tr>
<td>10</td>
<td>740</td>
<td>682</td>
<td>582</td>
<td>723</td>
<td>623</td>
<td>741</td>
<td>672</td>
<td>686</td>
</tr>
<tr>
<td>11</td>
<td>525</td>
<td>706</td>
<td>649</td>
<td>547</td>
<td>619</td>
<td>664</td>
<td>671</td>
<td>572</td>
</tr>
<tr>
<td>12</td>
<td>838</td>
<td>729</td>
<td>641</td>
<td>739</td>
<td>524</td>
<td>747</td>
<td>656</td>
<td>790</td>
</tr>
</tbody>
</table>

**Table 2.4. Total number of inflorescences examined on samples collected from each specified site (Fig. 2.1) across 2010/2011.**

<table>
<thead>
<tr>
<th>Site</th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>116</td>
<td>85</td>
<td>58</td>
<td>51</td>
<td>89</td>
<td>63</td>
<td>91</td>
<td>68</td>
</tr>
<tr>
<td>2</td>
<td>85</td>
<td>95</td>
<td>44</td>
<td>66</td>
<td>66</td>
<td>69</td>
<td>74</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>51</td>
<td>68</td>
<td>53</td>
<td>69</td>
<td>92</td>
<td>73</td>
<td>55</td>
<td>23</td>
</tr>
<tr>
<td>4</td>
<td>49</td>
<td>27</td>
<td>53</td>
<td>55</td>
<td>67</td>
<td>54</td>
<td>49</td>
<td>7</td>
</tr>
<tr>
<td>5</td>
<td>57</td>
<td>52</td>
<td>65</td>
<td>71</td>
<td>61</td>
<td>54</td>
<td>45</td>
<td>13</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>74</td>
<td>48</td>
<td>90</td>
<td>81</td>
<td>59</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>7</td>
<td>68</td>
<td>48</td>
<td>41</td>
<td>109</td>
<td>77</td>
<td>101</td>
<td>78</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>73</td>
<td>84</td>
<td>50</td>
<td>76</td>
<td>66</td>
<td>60</td>
<td>55</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>34</td>
<td>109</td>
<td>61</td>
<td>46</td>
<td>47</td>
<td>33</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>74</td>
<td>70</td>
<td>81</td>
<td>82</td>
<td>69</td>
<td>88</td>
<td>85</td>
<td>55</td>
</tr>
<tr>
<td>11</td>
<td>90</td>
<td>121</td>
<td>77</td>
<td>84</td>
<td>86</td>
<td>83</td>
<td>50</td>
<td>26</td>
</tr>
<tr>
<td>12</td>
<td>124</td>
<td>115</td>
<td>109</td>
<td>87</td>
<td>86</td>
<td>92</td>
<td>72</td>
<td>46</td>
</tr>
</tbody>
</table>

**Appendix 1** shows plots of the progression from budding to seed release for the individual sites and **Figure 2.3** shows the means of the 12 sites. The temporal pattern of each phenophase was similar for the twelve sites. Budding commenced in June, flowering in August, fruiting in September/October and seed release in December/January. On close inspection, budding and flowering, after initial peaks, did not decline monotonically.
Chapter 2  Tetratheca juncea reproductive phenology

Figure 2.3. The progression of the four phenophases over the eight months. This plot shows the means for the 12 sites of the proportion of inflorescences bearing buds, open flowers, fruit or released seed (SR).

2.3.2 Inflorescence Changes Across Time

Inflorescence count data allowed analysis of the twelve sites as monthly replicates from within the Central Coast regional population. There was no replicate data from within each site to permit analysis of differences between plant clumps, sites and time.

Repeated-measures multiple analysis of variance (rm MANOVA) was used to determine whether there was a significant difference between months for each of the four phenophases, budding, flowering and fruiting as well as flowering intensity. This method was used because (a) there were multiple inter-correlated outcome variables, and (b) each of those dependent variables was measured multiple times at each site. Table 2.5 shows the extent of correlation between the dependent variables. The use of MANOVA is considered appropriate when the correlation between dependent variables lies between 0.3 and 0.7 (Maxwell 2001) and power is increased when they are negatively correlated (Cole et al. 1994).
Table 2.5. Pearson correlation among the dependent variables.

<table>
<thead>
<tr>
<th></th>
<th>Buds</th>
<th>Flowers</th>
<th>Fruit</th>
<th>SR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flowers</td>
<td>-0.516</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruit</td>
<td>0.005</td>
<td>-0.694</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>SR</td>
<td>-0.178</td>
<td>-0.478</td>
<td>0.592</td>
<td>1</td>
</tr>
<tr>
<td>Flowering Intensity</td>
<td>-0.022</td>
<td>0.575</td>
<td>-0.295</td>
<td>-0.403</td>
</tr>
</tbody>
</table>

The alternative was to conduct an rm ANOVA where the dependent variable was the phenophase of flowers and the independent variable was time in months. This analysis was run but not reported here because the outcome was similar to the following analysis using rm MANOVA.

Analysis was conducted over the six months, August to January inclusive, because, other than for Site 11 (July, 1 flower), all inflorescences contained only buds in June and July.

Table 2.6 shows the multivariate statistics for the effect of time on all dependent variables simultaneously. The effect of time was significant ($p < 0.001$) for all of the tests used and indicates that the changes in the dependent variables across time were statistically significant.

Table 2.6. Multivariate statistics for the effect of time on all dependent variables simultaneously.

The dependent variables were the phenophases of buds, flowers, fruit, seed release and flowering intensity. The independent variable was time in months.

<table>
<thead>
<tr>
<th>Within Subjects Effect</th>
<th>Value</th>
<th>F</th>
<th>Hypothesis df</th>
<th>Error df</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pillai's Trace</td>
<td>1.849</td>
<td>6.456</td>
<td>25.000</td>
<td>275.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Wilks' Lambda</td>
<td>0.013</td>
<td>16.827</td>
<td>25.000</td>
<td>190.959</td>
<td>0.000</td>
</tr>
<tr>
<td>Hotelling's Trace</td>
<td>18.216</td>
<td>35.994</td>
<td>25.000</td>
<td>247.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Roy's Largest Root</td>
<td>15.099</td>
<td>5.000</td>
<td>55.000</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

Because changes in the dependent variables across time were statistically significant, for each variable the significance of changes across time was examined. Table 2.7 presents these results, and it can be seen that the changes across time were significant for all variables. Even the most conservative ‘Lower-bound’ test statistic was significant at $p < 0.05$. 


Because the changes across time were significant for each variable, it was appropriate to investigate the significance of the pairwise differences for the six months and the five variables. Tukey’s post hoc test was used, and because of the large number of comparisons a Bonferroni adjustment was used to avoid Type 1 errors in the determination of significance. The full set of results is provided in Appendix 2 and is referred to where relevant throughout the chapter.

Table 2.7. The statistical significance of changes across time for each individual variable. The variables are the proportion of total inflorescences containing buds, flowers, fruit, released seed (SR) and flowering intensity (inflorescence number/cm stem).

<table>
<thead>
<tr>
<th>Measure</th>
<th>Type III</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sum of Squares</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buds</td>
<td>Sphericity Assumed</td>
<td>4.042</td>
<td>5</td>
<td>0.808</td>
<td>108.021</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>4.042</td>
<td>3.476</td>
<td>1.163</td>
<td>108.021</td>
</tr>
<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>4.042</td>
<td>5.000</td>
<td>0.808</td>
<td>108.021</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>4.042</td>
<td>1.000</td>
<td>4.042</td>
<td>108.021</td>
</tr>
<tr>
<td>Flowers</td>
<td>Sphericity Assumed</td>
<td>1.172</td>
<td>5</td>
<td>0.234</td>
<td>25.168</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>1.172</td>
<td>2.919</td>
<td>0.401</td>
<td>25.168</td>
</tr>
<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>1.172</td>
<td>4.088</td>
<td>0.287</td>
<td>25.168</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>1.172</td>
<td>1.000</td>
<td>1.172</td>
<td>25.168</td>
</tr>
<tr>
<td>Fruit</td>
<td>Sphericity Assumed</td>
<td>2.491</td>
<td>5</td>
<td>0.498</td>
<td>52.685</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>2.491</td>
<td>1.702</td>
<td>1.464</td>
<td>52.685</td>
</tr>
<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>2.491</td>
<td>1.981</td>
<td>1.257</td>
<td>52.685</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>2.491</td>
<td>1.000</td>
<td>2.491</td>
<td>52.685</td>
</tr>
<tr>
<td>SR</td>
<td>Sphericity Assumed</td>
<td>.062</td>
<td>5</td>
<td>0.012</td>
<td>9.468</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>.062</td>
<td>1.565</td>
<td>0.039</td>
<td>9.468</td>
</tr>
<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>.062</td>
<td>1.779</td>
<td>0.035</td>
<td>9.468</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>.062</td>
<td>1.000</td>
<td>0.062</td>
<td>9.468</td>
</tr>
<tr>
<td>Flowering intensity</td>
<td>Sphericity Assumed</td>
<td>.020</td>
<td>5</td>
<td>0.004</td>
<td>27.829</td>
</tr>
<tr>
<td></td>
<td>Greenhouse-Geisser</td>
<td>.020</td>
<td>3.532</td>
<td>0.006</td>
<td>27.829</td>
</tr>
<tr>
<td></td>
<td>Huynh-Feldt</td>
<td>.020</td>
<td>5.000</td>
<td>0.004</td>
<td>27.829</td>
</tr>
<tr>
<td></td>
<td>Lower-bound</td>
<td>.020</td>
<td>1.000</td>
<td>0.020</td>
<td>27.829</td>
</tr>
</tbody>
</table>

The conclusion of this analysis was that there was a significant time effect for each of the four phenophases over the study period.

2.3.3 The Flowering Curve

Figure 2.3 shows that the greatest number of inflorescences bearing open flowers was around September. The flowering curve was examined in detail to determine whether significant change occurred between peak flowering and the end of data collection in January, and the probable cause of such a change. Figure 2.4 shows the flowering curve with an early peak and subsequent lesser peak.
The origin and significance of the apparent second peak in the flowering curve was then investigated.

![Figure 2.4. The *Tetratheca juncea* flowering curve.](image)
The means for the 12 sites of the proportion of inflorescences having open flowers. Bars represent SE of the means, N = 12 sites each month

### 2.3.3.1 Budding and Flowering Inflorescences
The pre-cursor to flowers is of course buds so their progress over time was examined (Figure 2.5).

A cursory look at Figures 2.4 and 2.5 suggests that the decline in flowering and budding was not monotonic. There was an increase in flowers from October to November and an increase in buds from September to October. However, the Tukey post hoc test (Appendix 2) from the rm MANOVA (Section 2.3.2) shows that the apparent increase in flowers or buds was not significant (Table 2.8).

### 2.3.3.2 Bud and Peduncle Length
An alternative was to investigate changes in bud and peduncle length over time and between sites. New buds and their peduncles are of course shorter than when mature. A batch of new buds should then result in a reduction in overall mean bud and peduncle length. The independent variables were time (month) and site, and the dependent variable was bud or peduncle length. Over a period of eight months across the 12 sites...
3689 buds and 6424 peduncles were measured. **Figure 2.6** shows the variation of mean bud length over time.

**Table 2.8. Data from the Tukey post hoc test for buds and flowers (Appendix 2).**
The change in the proportion of inflorescences having buds and flowers between the months shown and the significance of that change.

<table>
<thead>
<tr>
<th>Period</th>
<th>Change in buds</th>
<th>Change in flowers</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>September to October</td>
<td>0.15</td>
<td>0.007</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>October to November</td>
<td>0.007</td>
<td>0.03</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

**Figure 2.5. The means of the proportion of budding inflorescences of *Tetratheca juncea* over time.**
Bars represent SE of the means, N = 12 sites each month

Given that time is a repeated measures independent variable and location is a between groups independent variable, a mixed ANOVA would be appropriate for analysis of these data. However, this was not possible because the same clumps were not sampled each month. The method of removing stems for digital counting and measuring meant that no individual clump could provide sufficient material over eight months of sampling. Consequently, a post hoc test was used following a Bonferroni adjusted 1-way ANOVA to investigate the interaction effect between time and sampling site. Dunnett C pairwise comparison with heterogeneity assumption was conducted as a follow-up test.
Figure 2.6. The variation of mean bud length of *Tetratheca juncea* over time.
Bars represent SE of the means, N = 12 sites each month.

The results (Table 2.9) showed that there was a significant site effect for bud length (p <0.001) for the period June to August after which there was no significant difference between sites (p ≥0.05/8 = 0.006). The fact that site rankings were not the same for each of the eight months indicated that there was an interaction effect on bud length between site and time.

An rm ANOVA, under sphericity assumption (W=34.8, df=27, p =0.212), was then conducted to determine whether there were differences in bud length across the eight months. This showed that bud length significantly varied with time as indicated by F(7, 63) = 13, p <0.001.

Because there was a significant time effect, Fisher’s Least Significant Difference (LSD) post hoc test was conducted to determine which months had significantly different bud lengths compared to others. June significantly differed from all other months (p <0.05) other than January (p = 0.180 >0.05). September was significantly different to November (p = 0.003 <0.05) indicating that bud length increased from September to November. At the 90% confidence level, September was significantly different from August (p =0.099<0.100). There was insufficient evidence to support the claim that the rest of the pairs were significantly different.
Table 2.9. ANOVA results for *Tetratheca juncea* bud length and month.
Effects are significant where p < 0.05.

<table>
<thead>
<tr>
<th>Month</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>10.345 (11, 844)</td>
<td>0.000</td>
</tr>
<tr>
<td>July</td>
<td>17.193 (11, 935)</td>
<td>0.000</td>
</tr>
<tr>
<td>August</td>
<td>6.55 (11, 609)</td>
<td>0.000</td>
</tr>
<tr>
<td>September</td>
<td>1.751 (11, 389)</td>
<td>0.061</td>
</tr>
<tr>
<td>October</td>
<td>2.427 (11, 403)</td>
<td>0.006</td>
</tr>
<tr>
<td>November</td>
<td>1.43 (11, 248)</td>
<td>0.160</td>
</tr>
<tr>
<td>December</td>
<td>0.744 (11, 125)</td>
<td>0.694</td>
</tr>
<tr>
<td>January</td>
<td>0.671 (9, 42)</td>
<td>0.730</td>
</tr>
</tbody>
</table>

Budding is the pre-cursor to flowering so this evidence that a new crop of buds was injected between August and October meant that, at least, the rate of decline in flowering was subsequently reduced as illustrated in Figure 2.5 above. This is sufficient evidence to support the proposition that *Tetratheca juncea* flowering is boosted around November by a new batch of buds in September.

The mean peduncle length increased across the eight months (Figure 2.7).

![Figure 2.7](image-url)

*Figure 2.7. The variation of peduncle length of *Tetratheca juncea* buds/flowers over time.*
Bars represent SE of the means, N = 12 sites each month.
An rm ANOVA would normally be used to determine whether the differences in the mean peduncle lengths were significant. However, recorded data for peduncle lengths
has the same properties as recorded data for bud lengths viz. that different clumps were sampled at each site each month. Accordingly a Bonferroni adjusted 1-way ANOVA was conducted to determine the significance of the differences in peduncle lengths measured among different sites for each month. Tukey pairwise comparison was conducted as a follow up test under homogeneity assumption and Dunnett C pairwise comparison under heterogeneity assumption

The ANOVA results showed that peduncle length varied significantly with site (p < 0.001) for the eight months (Table 2.10). Individual site peduncle lengths did not have the same ranking for each of the eight months. This suggested that there was an interaction effect between location and time on peduncle lengths.

Table 2.10. ANOVA results for *Tetratheca juncea* peduncle length and month. All months are significant at p <0.05

<table>
<thead>
<tr>
<th>Month</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>6.374 (11, 865)</td>
<td>0.000</td>
</tr>
<tr>
<td>July</td>
<td>14.739 (11, 977)</td>
<td>0.000</td>
</tr>
<tr>
<td>August</td>
<td>3.098 (10, 763)</td>
<td>0.000</td>
</tr>
<tr>
<td>September</td>
<td>4.002 (11, 910)</td>
<td>0.000</td>
</tr>
<tr>
<td>October</td>
<td>6.199 (11, 889)</td>
<td>0.000</td>
</tr>
<tr>
<td>November</td>
<td>3.147 (11, 821)</td>
<td>0.000</td>
</tr>
<tr>
<td>December</td>
<td>3.424 (11, 736)</td>
<td>0.000</td>
</tr>
<tr>
<td>January</td>
<td>3.499 (10, 368)</td>
<td>0.000</td>
</tr>
</tbody>
</table>

An rm ANOVA, under sphericity assumption (W=37.5, df=27, p =0.124), showed that peduncle lengths varied significantly with time as indicated by F(7, 70) =125, p <0.001. Determining which months had significantly higher mean peduncle lengths was achieved through the LSD post hoc test.

This shows that peduncle length increased significantly (p <0.05) across June to December with the change from December to January not being significant (p >0.05).

If peduncle length approached a maximum in the way that bud length does prior to flower opening one would have expected their mean lengths to have decreased with the
introduction of new buds (on short peduncles) in September/October; the shape of the plot of means in Figure 2.7 hints at this. The fact that peduncle lengths steadily increased indicates that they continually lengthen through to seed release and that the rate of lengthening masks the introduction of new buds. Peduncles lengthening in this manner appears to be fairly common. A search of data for the flora of New South Wales via PlantNET (http://plantnet.rbgsyd.nsw.gov.au/) shows this to occur in at least 10 species including Tetratheca thymifolia which is reported as having peduncles lengthening as the flower develops.

This analysis has shown that the shape of the Tetratheca juncea flowering curve, being heavily right-skewed (Figure 2.4), is the result of a second phase of budding. This serves to boost the number of flowers towards the middle of flowering and thus extend the flowering phase.

2.3.4 The Timing of Fruit Development and Seed Release

The aim here was to document the times at which fruit development and subsequent seed release commenced. Summary data are shown in Figure 2.3 above and detailed in Appendix 1. For convenience, Table 2.11 lists the phenophases by site and shows that fruit first appeared in September and seed release first occurred in October.

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buds</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Flowers</td>
<td>1</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Fruit</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Seed Released</td>
<td>2</td>
<td>2</td>
<td>7</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

These results at least partly explain reports that Tetratheca juncea sets low numbers of fruit (Gross et al. 2003, and references therein) with associated implications of low pollination/pollinator levels. At any point in time, the number of fruit present will only be a small portion of the total production for a season.
2.3.5 Synchrony across the regional population

The research aim here was to discover whether or not there was synchrony in reproductive phenology between local sites within the Central Coast regional population.

Augspurger (1983) described a method for quantifying synchrony of phenophases for individuals and populations. This method has been applied by Gomez (1983) and a variation has been described by Keatley et al. (2004). Because the phenology of specific individuals was not recorded in the current study, the Augspurger (1983) method could not be used.

However, it was possible to examine the similarity of the overall reproductive phenology between sites. This was done in Primer 6 (Clark and Gorley 2006) using hierarchical agglomerative clustering with similarity profile permutation testing (SIMPROF).

For this analysis, the raw count data for individual phenophases was used and was treated as abundance data. A matrix was prepared for each phase that consisted of 12 sites (columns) by eight months (rows). The raw count data were standardised by total and a square root transform applied to down weight the higher count values. An input matrix of Bray Curtis similarities was then prepared (termed resemblance). The Primer cluster analysis was run using the SIMPROF option that examines the data for statistically significantly different clusters. Significance level was set at 5% ($p = 0.05$) and results are summarised in Table 2.12 and the dendrograms are provided in Appendix 3.

Table 2.12. Results from hierarchical agglomerative clustering of each phenophase and all sampling sites, applying the SIMPROF significance test.
Brackets enclose sites that were not significantly different.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Significantly different sites ($p &lt; 0.05$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buds</td>
<td>9 (1,2,3,4,5,6,7,8,10,11,12)</td>
</tr>
<tr>
<td>Flowers</td>
<td>No significant difference between sites</td>
</tr>
<tr>
<td>Fruit</td>
<td>9 (1,2,5,6,7,10,11,12) (3,4,8)</td>
</tr>
<tr>
<td>Seed Release</td>
<td>No significant difference between sites</td>
</tr>
</tbody>
</table>
Referring to the summary histograms in Appendix 1, these results make sense: Site 9 had no inflorescences by January and Sites 3, 4 and 8 had the highest fruit numbers. Even though Site 9 had no inflorescences in January it did not split out on flowers or seed release because other sites also had no flowers (Site 8) or seed release (Site 11) in January.

These results show minor significant differences in overall flowering and fruiting phenology between individual sites within the Central Coast regional population. The conclusion is that overall reproductive phenology was synchronised across the sampled range of the regional population.

2.3.6 New Growth

A phenophase that had not been anticipated at the start of this investigation was the development of new stems. These new stems sprouted in empty leaf axils as well as axils already bearing an inflorescence. The month in which the first new stem sprouts appeared varied between sites from September to December (Figure 2.8). As noted in Section 2.2, by January these new stems had matured to the point that it was becoming difficult to distinguish them from old stems. Up to and including January, new stems could be distinguished from old by the long, soft growing tip that was a different green compared to the tips of old stems. After January it was not possible to reliably distinguish between new and old stems so no more data were collected.

2.3.7 Attrition

Phenophase attrition was determined as the proportion of inflorescences that had failed at some stage as evidenced by a truncated peduncle. Figure 2.9 shows the mean attrition rate for each site over 8 months from June to January, and it was surprisingly low. The mean attrition over the entire 12 sites and 8 months was 3.093% ± 0.004.

A potentially significant source of attrition would be herbivory at the level of individual inflorescences (insect larvae or herbivorous arthropods for example). It would appear from these data that *Tetratheca juncea* is not vulnerable to this type of attack.
Figure 2.8. The new *Tetratheca juncea* stem growth period for each site. New stems started growing at the start of the solid bar and continued thereafter.

2.3.8 Peak Flowering

The final research question posed was whether there was a peak in flowering during which opportunities would be maximised to find the species during a survey.

Figure 2.9. Phenophase attrition rate at the 12 sites. Bars represent SE of the means, N = 8 months sampled.
As noted earlier, Driscoll (2003) observed that *Tetratheca juncea* flowering levels fluctuated throughout the season. The current data suggest the same with the means of flowering intensity (flowers/cm stem) peaking in September (Figure 2.10) and declining thereafter. Referring to the monthly pairs comparison in Appendix 2, it can be seen that the increase in flowering intensity from August to September of 0.043 was significant ($p < 0.001$), and the decrease from September to October of 0.021 was also significant ($p < 0.05$).

This evidence suggests that the most opportune time for surveying for *Tetratheca juncea* would be late September through early October, bearing in mind that the sampling was conducted between the 20th and the 25th of each month.

![Figure 2.10. The means of flowering intensity over time for Tetratheca juncea. Flowering intensity is the number of flowers per cm of stem. Bars represent SE of the means, N = 12 sites each month.](image)

### 2.4 DISCUSSION

By December, prolific new stem growth, which is quite obvious in the field, was recorded at all sites (Section 2.3.6). This raises the question as to whether the flowering initials are set in the axillary meristem tissue of these new stems but remain dormant until the following June. Empirical evidence suggests that this would not be the case. *Tetratheca juncea* clumps are frequently grazed by mammal herbivores and are also routinely slashed short when growing in cleared powerline easements. Under either
circumstance, at the next flowering season flowers develop from leaf axils in the cropped stems. It is also apparent that flowering along uncropped stems occurs much further up the stem than where flowers appear on cropped stems.

Several environmental factors, in isolation or combination, have been shown to trigger flowering (Yanovsky and Kay 2003). For example, photoperiod, temperature and rainfall have been demonstrated to be involved, separately or in combination. King et al. (2008) reviewed the published information on the environmental control of flowering in Australian plants.

They found a variety of, sometimes complex, combinations of photoperiod (long or short days, or integral of day length) and temperature (rising or falling, high or low) triggered bud initiation. Furthermore, species within the same genus could have differing controls, as could variants of the same species (King et al. 1992; King et al. 1996; Law et al. 2000; Hudson et al. 2003).

The current study has shown that *Tetratheca juncea* budding commences early in June (Section 2.3.2). As explained above, empirical evidence from flowering of grazed stems indicates that, as with many species, budding commences in response to recent environmental conditions. Figure 2.11 shows a plot of the mean monthly temperatures for the Australian Bureau of Meteorology weather stations, having long-term records, nearest to the most southern and most northern study site. The southern station was Narara, approximately 37 km south of the most southern study site while the northern station was Maitland, approximately 9 km north of the most northern study site. Figure 2.11 also shows the hours of daylight and darkness on the last day of each month.

Probably the earliest Spring flowering species growing sympatrically with *Tetratheca juncea* is the terrestrial climber *Hardenbergia violacea* Schneev. (Stern) (Fabaceae: Faboideae). King (1998) showed that flower initiation in this species (using cultivar Mini haha) was triggered by a combination of short-day photoperiods (<12.5 h light daily) and mean daily cool temperatures of 15-18°C over a 12-week period. Furthermore, the primordia only developed into flowers when held at an average temperature of 15°C in combination with short days. When exposed to higher
Figure 2.11. The pattern of minimum temperature and hours of daylight and darkness in relation to *Tetratheca juncea* budding.

---
*seasonal commencement of budding*

---
*approximate time of second budding*

\[\text{mmT} = \text{mean monthly temperature}\]

Temperatures abortion of buds occurred followed by vegetative growth in the previously flowering axil.

The *Hardenbergia violacea* study by King (1998) provides an example of a species where flowering is in part controlled by low temperatures higher than those involved in vernalisation. Looking at Figure 2.11, at no time does the mean temperature fall into the vernalisation range of 1 – 10°C (Simpson and Dean 2002).

It also appears unlikely that low temperature above the vernalisation level is involved in *Tetratheca juncea* bud initiation because, using the King (1998) *Hardenbergia violacea* flowering initiation temperatures as a guide, there are no more than 2 months of average temperatures below 18°C before buds are visible to the naked eye.

Initial budding occurs as the day/night cycle approaches the Winter solstice (10 hours of daylight and 14 hours of darkness). The second budding episode occurs towards the
Spring equinox (equal hours of daylight and darkness). The evidence, therefore, suggests that budding in *Tetratheca juncea* is initiated by photoperiod. The complexity of controls at the molecular level (reviewed in Yanovsky and Kay 2003) is such that it is quite conceivable that two different combinations of photoperiod could initiate budding in *Tetratheca juncea*. However, the role of temperature cannot be excluded without more detailed study using controlled laboratory experiments. Clearly, flowering is not sensitive to temperature increases since budding and flowering continue through Summer into Autumn (Driscoll 2003 and Section 2.3.3 of this study).

*Tetratheca juncea* is distributed over a latitudinal range of 0.8° (from Wyong north to Bulahdelah). Such a narrow range means that there would be little likelihood of any local adaptation to small differences in day length at the same time of the year (King et al. 1996). It is, therefore, probable that *Tetratheca juncea* reproductive phenology follows the same course across the entire distributional range. Species with phenology timed by photoperiod alone would not be expected to exhibit phenological changes as a direct response to a warming climate. Indirectly however, earlier spring warming could result in earlier pollinator activity which in turn could have a positive fitness effect on early flowering phenotypes and ultimately shift the species’ flowering phenology.

As noted in Section 2.1, investigation into flowering curves has shown a predominance of curves that rise sharply and fall slowly over time i.e. right skewed. However, rarely has the physical origin of the shape of the curve been investigated in detail. Unpublished data show that the right skew in the flowering curve of *Crocosmia x crocosmiiflora* has its origins in new flowering stems developing toward the end of the flowering season as well as individual flowers lasting longer later in the season (R. Thompson pers. com.). The current study has shown that the extended flowering curve for *Tetratheca juncea* originates with a second budding/flowering phase. The effect of this extended flowering curve is to expose more flowers to pollinators. Thomson (1980) considered flowering curves to be resource utilisation curves with the resource being pollinators. He hypothesised that positive skew was an expression of the plant competing for pollinators and demonstrated this in the flowering curves of species in Colorado subalpine meadows. In the case of *Tetratheca juncea*, it is difficult to see the skewed flowering curve having a similar function given that flowering starts earlier, and continues for longer, than most co-flowering species. A plausible explanation for the lengthy flowering season lies with the nectarless flowers. The pollinators also need
nectar from other flowering species, so the long flowering maximises the chances of a pollinator visit.

Since flowering is about reproduction, and consequently pollinators, the phenology of flowering needs to be selected so as to maximise pollination opportunities. Pollination and successful fertilisation needs to occur with sufficient time and resources available for fruit to develop to maturity and seed release and dispersal. Furthermore, there is competition with other flowering species for pollinators that imposes another constraint on the most opportune time for flowering.

Chapter 1, Section 1.8.3.3 described the specialised native bee pollinators of *Tetratheca juncea* that collect pollen from the poricidal anthers by way of thoracic vibration. In common with all flying insects, flight in bees is only possibly within thoracic temperature ranges (Chown and Nicolson 2004) that are in part dependent on the size and body colour of the insect (Willmer 1983). Bees are capable of raising their internal temperature above ambient (endothermy) although there is a threshold ambient temperature (different for each species) at or below which flight temperatures cannot be achieved and they are functionally ectothermic. Studying *Anthophora plumipes* (Hymenoptera; Anthophoridae), a Spring-flying solitary bee found from the UK to Israel, Stone (1993) showed that this species could fly with thoracic temperatures as low as 25°C in ambient temperatures of 5°C. *A. plumipes* is approximately double the size of the bees identified as pollinators of *Tetratheca juncea* so it would be expected that the smaller bees would have a higher ambient temperature limit below which they could not fly. Some indication of the temperatures at which *Tetratheca juncea* pollinators become active can be inferred from the timing of the fruiting phenophase and mean ambient temperatures. The first flowers open in August and first fruit are present in September. Driscoll (2003) recorded the first pollinator on 2 September 2002 and fruit approximately two weeks after that. The temperature data from Figure 2.11 shows the mean temperature range for August (over 32 years) was 10.7°C – 13.7°C and for September 12.4°C – 16.5°C. This indicates that the minimum voluntary flight ambient temperature for the *Tetratheca juncea* pollinators would be around 15°C. The earliest that 15°C was achieved was in late August/early September and the latest was in October.
Chapter 2 Tetratheca juncea reproductive phenology

Contrast *Tetratheca juncea* flowering and pollinator phenology with that of the Western Australian *Tetratheca paynterae* subsp. *paynterae*. This species grows in an hostile habitat, in rock crevices of banded ironstone ranges, an area of high summer temperatures (up to $50^\circ$C on the rock face) and low annual rainfall (<300 mm pa) (Butcher *et al.* 2011). The species has adapted to this environment by being leafless, stems dying back in response to high temperatures and low rainfall, then regrowth following rain. Flowering is sporadic throughout the year in response to sufficient rainfall (Butcher *et al.* 2008) although the primary flowering period is August to October (R. Howard pers. com.). Similar to *Tetratheca juncea*, buzz-pollinators collect pollen from *Tetratheca paynterae* subsp. *paynterae* and these have been shown to only be active in the temperature range of 20-30$^\circ$C (Butcher *et al.* 2008).

As is characteristic for flowers having poricidal anthers (Harder and Barclay 1994), *Tetratheca juncea* flowers have no nectar, only pollen, as a food resource for its native bee pollinators. However, bees also need nectar (Michener 1965; Buchmann 2000). From a detailed study of the interaction between pollinators and flowering species Hingston (1999) concluded that it was probable that the presence of nectariferous flowers enhanced pollination in nectarless flowers. This means that, rather than flowering in a time window that minimises competition for pollinators with other flowering species, *Tetratheca juncea* needs to flower along with nectariferous species. Driscoll (2003) listed 28 flora species from 11 families that flowered at some time during the *Tetratheca juncea* flowering period. On one occasion, in the process of capturing native bee pollinators described in the following Chapter 3, bees were observed to collect pollen from *Tetratheca juncea* and then move directly to collect nectar from *Goodenia heterophylla* Sm. subsp. *heterophylla* (Goodeniaceae). While this was only an empirical observation it is interesting to note that the flowering period for *Goodenia heterophylla* subsp. *heterophylla* is reported to be from August to May (Carolin 1992), longer than for *Tetratheca juncea*. Its distribution coincides with that of *Tetratheca juncea* and so it may be an important co-flowering species for *Tetratheca juncea* pollinators. Such co-flowering of a nectariferous species and a nectarless species might be an example of facilitative flowering where two species flowering together attract more pollinators than either would flowering separately (Rathcke 1983). A further use for nectar by foraging bees is to assist with maintaining thoracic temperature...
at the level needed for flight. It has been shown that nectar can be several degrees warmer than ambient temperature (Whiney and Chittka 2007; Norgate et al. 2010).

One problem arising from multiple species flowering together is that of stigma clogging where the stigma is clogged by pollen from other species. This problem is minimised for *Tetratheca juncea* because the specialised buzz pollinating bees carry the pollen on a different part of their body to where the pollen from other flowers is carried. The pendant presentation of the flowers (See Chapter 1 Figure 1.1) has the stigma and the surrounding tubular anthers pointing downward so that the pollen-collecting bee grasps the anthers and the vibrated pollen falls onto the venter (Figure 2.12) which is a general term for the ventral (or underneath) surface. The ‘targeting’ of specific body locations for carrying pollen is known to occur in epiphytic orchids for example. The orchid flower throat architecture is designed to force its euglossyne bee pollinators to rotate so that the pollinia become attached to where they will only become detached when entering a flower of the same species (Buchmann and Nabhan 1997). There are other buzz-pollinated plants such as *Dianella caerulea* Sims (Phormiaceae) (Bernhardt 1995) that flower during *Tetratheca juncea* flowering (Driscoll 2003) so the possibility does exist for some foreign pollen to be transferred. However, as described above, the flowering period for *Dianella* species is much shorter than that of *Tetratheca juncea*.

Of course stigma clogging is not the only potential problem arising from multiple flowering species sharing the same pollinators. With so many flowers from several species available it is possible that pollinator visits to a particular species are sufficiently infrequent that fecundity is reduced. Can this be the case for *Tetratheca juncea*? That problem is addressed in Chapter 3.

At first it would appear that having no nectar would place *Tetratheca juncea* at a competitive disadvantage. However when the overall reproductive phenology is considered in a mutualistic context the species has evolved a very effective niche. The open flowering phenophase could not be wider. It starts in late Winter with flowers already open when it is warm enough for pollinators to become active and continues into Autumn past the time that pollinators are available (Driscoll 2003). Fruit development immediately follows fertilisation and continues to maturity and seed release. From October all four phenophases of budding, flowering, fruiting and seed
release are concurrent. The combination of downward anther presentation and specialised buzz pollinator minimises the risk of stigma clogging and obviates the need to flower in a narrow time window to minimise competition for pollinators from other flowering species.

Figure 2.12. An *Exoneura* sp. bee with freshly collected *Tetratheca juncea* pollen on the ventral surface or venter.

It is an interesting question as to what the selection forces were that resulted in the species developing such a long flowering period i.e. eight months covering four seasons, August to March. *Tetratheca juncea* is not the only nectarless buzz pollinated species found on the east coast of Australia; the *Dianella* (Phormiaceae), *Hibbertia* (Dilleniaceae) and *Solanum* (Solanaceae) families for example. Duncan (2004) found that *Dianella revoluta* R.Br. flowered for about the three months of Spring and the sympatrically growing *Dianella longifolia* R.Br. flowered after *D. revoluta*. Here there is an interesting contrast with *Tetratheca juncea* that at many locations grows mixed with its congener *Tetratheca thymifolia* Sm. with both species flowering at the same time (Driscoll 2003). Bernhardt (1995) studied *Dianella caerulea* var. *assera* R.J.F.Hend. over two years and found that it flowered from late October to the end of
November. Bernhardt (1984) found that the flowering period for *Hibbertia stricta* (R.Br. ex DC.) ex F.Muell. (now *H. riparia* (R.Br. ex DC.) Hoogland) was from early August to mid-December. Information collated from Conn (1992) shows that there are 11 *Solanum* species whose geographic range includes *Tetratheca juncea*. Of those 11 species, four are reported as flowering sporadically throughout a year with the remainder flowering over two or three seasons. In Tasmania, Johnson and McQuillan (2011) found that two species of *Sprengelia* (Ericaceae), *S. incarnata* Sm. and *S. propinqua* A.Cunn. ex DC. were nectarless, buzz-pollinated and flowered September to October and October to November respectively. From field observation, *Tetratheca juncea* appears to be a very slow growing plant. This attribute might necessitate early starting and a long reproductive phenology. By comparison Fabaceous species in the Sydney region flower over Winter into Spring and have released their seed by December or earlier (Auld 1996).

Three main factors considered to be involved in the evolution of flowering phenology are pollinator availability and competition for pollinators from other flowering species, pre-dispersal seed herbivores and phenotypic plasticity in flowering time having a genetic origin (Widen 1991; Brody 1997; Elzinga *et al.* 2007). These three factors have been investigated in this *Tetratheca juncea* reproductive phenology study in the form of pollinator activity, co-flowering species, attrition and synchrony.

The timing of fruit development shows that *Tetratheca juncea* pollinators are active in September (Section 2.3.4), up to a month after the first flowers open (Section 2.3.3). Synchrony analysis (Section 2.3.5) showed no significant variation in the timing of any of the phenophases across the regional population. Attrition (Section 2.3.7) of inflorescences was shown to be negligible. These results together make it difficult to be conclusive about the evolutionary origin of *Tetratheca juncea* flowering phenology. In part this could be a consequence of the fairly coarse resolution of this study with data having been collected monthly and without replication at individual sites.

Whatever the evolutionary origin of the flowering phenology of *Tetratheca juncea*, the end result is that the species flowers over the entire period in which its pollinators are seasonally available, thus maximising pollination opportunity. The comparison by Kochmer and Handel (1986) of flowering phenologies of flora from the Carolinas and
Japan was described in the introduction to this chapter. They found that there was no difference in phenology despite millions of years of geographic separation with its associated climate and habitat changes. This suggested that flowering phenology can have been genetically fixed long in the past and this could be the case for *Tetratheca juncea*. There are however examples that demonstrate that flowering phenology can evolve over a short time-span (reviewed in Elzinga *et al.* 2007).

This investigation did not follow the entire *Tetratheca juncea* flowering season. Driscoll (2003) reported that some flowers could be found somewhere in the regional population, albeit in very low numbers outside of the recognised flowering season, at any month of the year. Also, data collection was stopped while fruit development and seed release frequency were increasing. The understanding of *Tetratheca juncea* reproductive phenology can be expanded by collecting data through to at least March or longer. As noted in Section 2.3.6, data collection was stopped in January because maturing new stem growth was becoming difficult to distinguish from the older stems and stem length was one of the parameters being recorded for the purpose of determining flowering intensity (Section 2.3.8). Full year phenology could be determined by counts of inflorescences at the budding, flowering, fruiting and seed release phenophases. This method, conducted in situ, would permit replicate data to be collected from the same clumps at a variety of sites.

Further informative work could be conducted to determine whether the rate of bud development to flower opening was in any way mediated by temperature.

For the management of *Tetratheca juncea*, this research has shown that the prime time to survey for an otherwise cryptic species is at peak flowering in late September/early October. Budding commenced at all sites in June and fell away as flowers opened in August (**Appendix 1**). Even though flower numbers were boosted in November/December as a result of additional budding in October, flowering intensity did not achieve the earlier peak (Section 2.3.8). Furthermore, the species’ reproductive phenology was synchronised across the 12 study sites drawn from the Central Coast regional population (Section 2.3.5).
CHAPTER 3 A SPATIOTEMPORAL STUDY OF POLLINATION ACROSS A TETRATHECA JUNCEA REGIONAL POPULATION
3.1 INTRODUCTION

The transfer of genetic material from male to female is the critical step in sexual reproduction. Without this step, the phenological pathway described in Chapter 2 for *Tetratheca juncea* would grind to a halt. Buds might form and flowers bloom, but it would all end there: no fruit, no seed, no dispersal. In fact for some plants, this is exactly what happens, at least to some degree (Eckert 2002; Honnay and Bossuyt 2005). This does not (necessarily) mean that the plant cannot propagate and is destined for extinction. Vegetative spread, or clonality, becomes the primary or only means of dispersal. As described in Chapter 1, *Tetratheca juncea* is one of those plants that exhibits both sexual and asexual reproduction. Chapter 2 demonstrated that the sexual reproductive process was carried through to completion with the release of seed for dispersal.

Pollen transfer is a crucial step in the reproductive process that is outside of the plant’s immediate control and Buchmann and Nabhan (1996) propose that knowledge of pollination ecology is critically lacking in our understanding of how to manage threatened plants. This Chapter 3 investigates pollinator activity during the *Tetratheca juncea* flowering season over time, and across a large part of the Central Coast regional population.

A plant lives in an environment in which, as a whole organism, it competes for its share of finite resources. The acquirement of resources is limited by competition with conspecifics, with other plant species and the restrictions imposed by the processes of resource extraction (Pugnaire and Valladares 2007). Within the life cycle of the plant there are functions that compete for the resources that are obtained, and when one life cycle component dominates resources another component is likely to be sacrificed to some degree. Three main components compete for resources in a plant: somatic growth, defence and reproduction. Without reproduction the whole plant, and by extension the species, ceases to exist in a short time, so the reproductive processes can have a level of precedence over other processes. However, as already noted, there is likely to be a cost to allocating resources to reproduction (see review by Obeso 2002) being a concomitant reduction elsewhere in the plant. A high sexual reproductive investment in one good
season might also result in reduced reproduction in subsequent seasons as the balance between life-cycle components and resources is re-established (Janzen et al. 1981). This has been termed a fecundity cost.

Resource allocation can be demonstrated through morphological comparison between self-fertilising and regularly outcrossing species (reviewed in Lloyd 1987). In self-fertilising species, structures such as the corolla are much reduced, and there are less pollinator attractants, such as nectar or scent. Also, the commitment of resources to pollen production and dispersal are lower in self-fertilising species than in outcrossing species.

Bateman’s Principle, initially described by Bateman (1948) but re-discovered by Trivers (1972), even though originating from work with *Drosophila melanogaster*, has played a large part in developing an understanding of sexual selection in both animals and plants. This principle has a variety of expressions (Arnold 1994; Wilson et al. 1994) of which the one most frequently applied to plants is that the female component of sexual reproduction is most limiting because it is more resource intensive than the male component. Darwin (1874) decided that plants could not select for reproductive traits because they had no conscious sensory perception, so secondary sex displays would be wasted. This view has held until recently when plant reproductive processes, and in particular those of hermaphrodites, were examined more closely from both theoretical and empirical points of view. Arnold (1994) condensed Bateman’s principle into a staged process showing mating combinations, male and female fecundity and overall longevity implying that selection can act on any of these stages and impact on fitness.

Pollinators play an integral role in the survival of most plant species, and pollen is an essential source of nutrient for the pollinators themselves. Buchmann and Nabhan (1996) estimated the number of animal species dependent on floral resources to be over 300,000. A recent global analysis has shown that 78% of temperate zone plant species and 94% of tropical species are biotically pollinated (Ollerton et al. 2011). Any major decline in pollinator diversity and/or abundance is viewed with concern for both the security of human food crop production and overall biodiversity (Kluzer and Peduzzi 2007; Potts et al. 2010). The ecology of native bee pollinators is complex. While loss of
habitat through vegetation clearing may result in pollinator loss, it is not clear that there is a decline in relatively undisturbed habitat or even to some degree in fragmented habitat (Cane 2001; Roubik 2001; Ghazoul 2005). In some circumstances cleared habitat can result in more suitable soil nesting locations and an increase in those native bee pollinators (Cane 2001). This could be used as an argument in the discussion for increased pollination after fire?

Pollen and pollinator limitation have been the subject of much research as potential indicators of low pollinator numbers and in understanding one of the selective forces involved in the evolution of plant reproductive ecology (Garwood and Horvitz 1985). The meaning of pollinator limitation is obvious and has been distinguished from pollen limitation being an inadequate supply of suitable pollen to the stigmas. An abundance of pollinators might still result in pollen limitation under circumstances such as stigma clogging by pollen. Pollen from co-flowering species or too much self pollen provided to an outcrossing species can reduce fertilisation. Either condition is demonstrated when increased pollen supply to flowers, either by experimental manipulation or natural events, results in increased seed production.

The term ‘limitation’ implies that these lower levels of pollination are in some way sub-optimal for the plant (Calvo and Horvitz 1990). However, more is not necessarily better. From a resource allocation viewpoint, an increase in reproductive output in one season may consume resources and place the plant at later risk or at least result in reduced output for a time (Janzen et al. 1980). It can also be demonstrated that pollen limitation is the normal condition for some species when tested over several seasons and locations (Wilson et al. 1994). In fact, pollen limitation has been reported across a wide range of species (Bierzychudek 1981; Burd 1994; Knight et al. 2005).

Haig and Westoby (1988) developed a model describing how competition over time between the allocation of limited resources to the components of the reproductive process might have resulted in a preferred equilibrium. Similar to Bateman’s Principle, the Haig and Westoby model requires that the acquisition of pollen and the production of seed have a maternal cost such that an increase in either will have a fitness impact elsewhere in the process. In practise, there have been many apparent exceptions to the
Haig and Westoby model (Burd 1994; Ashman et al. 2004). Ashman et al. (2004) and Knight et al. (2005) provide thorough reviews of the issues and offer an expanded view. It is possible, for example, that the Haig and Westoby equilibrium is broader than first presented as a consequence of environmental stochasticity. Depending on the evolutionary age of the species or recent, persistent environmental changes, the species might not have achieved, or no longer be at, equilibrium.

In hermaphrodite flowers such as *Tetratheca juncea*, female resources are initially committed to floral attractiveness to pollinators and male resources to pollen production. The overall success of this commitment can be measured by the Fruit:Flower ratio (Sutherland 1986; Morgan 1993). In its simplest expression, Fruit:Flower ratio is the proportion of flowers that produce fruit: Fruit:Flower ratio = Total Fruit/Total Flowers.

Fruit:Flower ratio levels have been used to investigate the contribution of compatibility, breeding system, female and male function to fitness in plants (Sutherland and Delph 1984; Sutherland 1986). High flower numbers and low Fruit:Flower ratio appear to be the norm for hermaphrodites. If the purpose of producing large numbers of flowers is to attract more pollinators, then Fruit:Flower ratio values should increase with increasing floral display (Holland et al. 2004). However, this does not seem to be the case and the question arises as to the purpose of substantial, apparently excess, resource commitment. Ayre and Whelan (1989) proposed proximate (ecological) and ultimate (evolutionary) causes of low Fruit:Flower ratio. Pollen quality, pollen limitation and low resources were proximate causes known to reduce fruit levels. Ultimate causes might consist of selective forces resulting in large floral displays for the purpose of attracting more pollinators, bet hedging against variable pollination services, or pollen donation (to flowers of congener plants).

Pollinator activity is commonly monitored by direct observation (e.g. Horvitz and Schemske 1990; Robertson and Lloyd 1993; Ghazoul 2004; Mitchell et al. 2004). However, this is either unreliable or excessively time-consuming when the pollinators are extremely small. Driscoll (2003) reported that the very act of observing altered the behaviour of *Tetratheca juncea* native bee pollinators. Furthermore, just because an
insect is observed visiting flowers doesn’t mean it is successfully pollinating even if pollen is being deposited. Sánchez-Lafuente *et al.* (2012) demonstrated that pollinator visits cannot necessarily be used as a surrogate for seed production. The only evidence of successful pollination is the production of fruit.

The use of fruit production to infer pollinator activity for this investigation was carefully considered. As mentioned earlier, while Gross *et al.* (2003) found that *Tetratheca juncea* was capable of selfing they concluded that it was facultatively xenogamous. However, in the greenhouse they recorded abiotic autogamous pollination (expressed in fruit production) at the rate of 1 in 50 flowers. Driscoll (2003), who while in search of the then unknown *Tetratheca juncea* pollinators, closely observed over 200 plant clumps from initial flowering in early August through to November. This involved two to three-hour sessions three times per week in 2001, a total of approximately 100 hours of observation. The first native bee was observed collecting pollen on 2 September 2001, approximately one month after the start of flowering. It was not until 16 September 2001 that seed capsules were recorded. Thus there was at least a month of flowering during which no pollinators were observed and no evidence that fertilisation had occurred in that time. This initial finding has been supported by the Chapter 2 phenology study (Section 2.3.4) showing that flowering commenced in late July but fruit were not recorded until late September. While there was no attempt to relate this to the presence of pollinators, these results were consistent with those of Driscoll (2003), again showing a one month period during which no fertilisation had occurred.

It was, therefore, considered reasonable to use fruit production as an indicator of pollinator activity in *Tetratheca juncea*. Other examples of the use of fruit (or seed) production to infer pollinator activity levels can be found in Inoue (1986), Eguiarte and Bujrquez (1987) and Hiers *et al.* (2000).

Fruit production at least provides qualitative evidence that pollinators have attended the plant. Flowering and fruitting data must be analysed several ways to determine quantitative pollinator activity, particularly for population-wide studies over several flowering seasons. For example, Fruit:Flower ratio levels could change because pollinator activity has changed, or because the number of flowers has changed.
Similarly, the number of fruit produced between plants and seasons can only be compared if the data can be standardised in some way.

As described in Chapter 1, published information regarding the distribution and reproductive ecology of *Tetratheca juncea* (Payne 2000; Gross *et al.* 2003) described the distribution of the species as highly fragmented with low numbers. Also, there were no known pollinators and fruit set was reported to be low, probably a consequence of the lack of pollinators. These factors were considered to be significant contributors to the rarity and threatened status of the species.

The broad aim of this Chapter was to determine whether *Tetratheca juncea* reproductive ecology is limited by insufficient pollinator species and/or pollinator activity.

### 3.2 METHODS

#### 3.2.1 Pollinator Identification

For this project, the work of Driscoll (2003) was continued mostly at the same sites with the same mix of lengthy observation periods (2 – 3 h) either at a fixed location or moving around through a flowering population. Opportunistic captures were made at two other locations.

Any bees found collecting pollen from *Tetratheca juncea*, or other flowering species in the immediate vicinity, were captured using a small hand net. The captured bees were then transferred to a vial containing denatured ethanol (100% grade). Later, the vial was shaken to flush off any carried pollen and the bee was transferred to a vial of fresh ethanol ready for dispatch for identification. The flushed pollen was retained for later processing and identification of the plant species represented in the carried pollen (data not reported here). All bee specimens were sent to native bee taxonomist Dr. Michael Batley for identification and vouchering.
3.2.2 Pollinator Activity

To gain an understanding of the levels and pattern of pollinator activity across the Central Coast *Tetratheca juncea* regional population, flowering and fruiting data were collected over six flowering seasons, 2003-2004 to 2008-2009 inclusive. Flowering season rather than calendar year was used because the species flowers from August through to March. Data were collected from 11 sites (Figure 3.1) mostly in the months of October to December with a few records from January. The sites covered approximately 35 km north-south and 8 km east-west, a substantial part of the overall regional population. Some locations were targeted for repeat data collection over different seasons while data were collected opportunistically from other locations. per clump, and a variant of Fruit:Flower ratio referred to by the acronym FFR to differentiate it from conventional usage.

\[ FFR = \frac{Total\ Fruit}{(Total\ Flowers + Total\ Fruit)} \]

FFR was determined from the fruiting and flowering status of a clump *at a point in time* and was thus an index of pollinator success up to that time (see Section 3.4.7 and Section 4.4 for further discussion).

For each clump, data collected were the number of open flowers and fruit (at any stage of development including those from which seed had been released). Two indices of pollinator activity were then available. Total fruit, converted to mean number of fruit per clump, and a variant of Fruit:Flower ratio referred to by the acronym FFR to differentiate it from conventional usage.

3.2.3 Statistical analysis

Analysis was conducted using SPSS (Version 19). Relationships between variables were examined using 1-way ANOVA, General Linear Model, regression or correlation. Prior to running a model, data were examined for normality and an appropriate transform applied where applicable.
Figure 3.1. The location of sites sampled for Fruit/Flowering Ratio data. Inset shows the sites in relation to the boundary of the Central Coast regional population. The red patches are the geographic area from which samples were collected.
3.3 RESULTS - POLLINATOR IDENTIFICATION

To date six native bee species have been captured while they were collecting pollen from *Tetraphaca juncea* flowers (Table 3.1). Two species are from Driscoll (2003) and an additional four are from this study. A further five species, capable of buzz-pollination (pers. com. Dr. Michael Batley), were collected in this study from flowers of other species within a *Tetraphaca juncea* population. Bees captured on species other than *Tetraphaca juncea* can be considered likely pollinators because, as described in Chapter 2, all of these buzz-pollinators are polylectic needing nectar that *Tetraphaca juncea* does not supply. The species listed in Table 3.1, other than the two from Driscoll (2003), are from a total of 21 individuals captured over the flowering seasons 2003 – 2004 and 2004 – 2005.

Table 3.1. Potential native bee pollinators of *Tetraphaca juncea*.

<table>
<thead>
<tr>
<th>Confirmed collecting pollen from <em>Tetraphaca juncea.</em></th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Exoneura</em> sp.*</td>
<td>6, 8</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) convexum</em> (Smith, 1879)*</td>
<td>6, 8</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) erythrurum</em> (Cockerell, 1914)</td>
<td>8</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) gilesi</em> (Cockerell, 1905)</td>
<td>2</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) hemichalceum</em> (Cockerell, 1923)</td>
<td>6, 8</td>
</tr>
<tr>
<td><em>Lasioglossum (Parasphecodes) carbonarium</em> (Smith, 1853)</td>
<td>6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Captured within <em>Tetraphaca juncea</em> population on other plant species and capable of buzz-pollination.</th>
<th>Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Homalictus (Homalictus) holochlorus</em> (Cockerell, 1914)</td>
<td>6</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) imitans</em> (Cockerell, 1914)</td>
<td>6, 8</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) instabilis</em> (Cockerell, 1914)</td>
<td>5</td>
</tr>
<tr>
<td><em>Lasioglossum (Chilalictus) sculpturatum</em> (Cockerell, 1930)</td>
<td>8</td>
</tr>
<tr>
<td><em>Lipotriches (Austronomia) australica</em> (Smith, 1875)</td>
<td>8</td>
</tr>
</tbody>
</table>
3.4 RESULTS – INFERRED POLLINATOR ACTIVITY

The aim of this quantitative analysis was to determine whether there was a significant difference in pollinator activity, measured as FFR between flowering sites as well as different flowering seasons. In addition, the relationship between total flowers and FFR was investigated to determine whether pollinators were significantly more attracted to larger floral displays.

3.4.1 Descriptive Statistics

FFR data were collected from a total of 528 clumps from 11 flowering sites over six flowering seasons (Table 3.2). The flowering seasons ranged from season 2003-2004 to season 2008-2009 inclusive. The majority of records were from seasons 2004-2005 ($n = 173, 32.8\%$) and 2003-2004 ($n = 136, 25.8\%$) while the least number of records were from season 2005-2006 ($n = 43, 8.1\%$). For the flowering site, the majority of the clumps were from Site 7 ($n = 99, 18.8\%$) and Site 8 ($n = 79, 15.0\%$) while the least number of plants were observed for Site 5 ($n = 8, 1.5\%$).

3.4.2 Relationship Between FFR and Flowering Site

The relationship between FFR and flowering sites was explored in order to gain an understanding of the availability of pollinators across the regional population. Descriptive statistics of mean FFR values according to flowering site are presented in Table 3.4.

The significant difference in mean FFR across sites was tested using a 1-way ANOVA. The first step in the analysis was to determine whether the raw data met the necessary ANOVA assumption of being normally distributed. A histogram of FFR across all sites showed positive skewness and a Shapiro Wilk’s test for the goodness of fit of the data to a normal distribution reported a p value of $<0.05$, confirming that the data were not normally distributed.
Table 3.2. The number of individual clumps sampled in each flowering season and at each site and percent contributing to the total sampled.
A flowering season is from late winter to early autumn the following year so seasons are from the end of one year to the start of the next e.g. season 2003-2004. The sites are those shown in Figure 3.1 above.

<table>
<thead>
<tr>
<th>Season</th>
<th>Clumps</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003-2004</td>
<td>136</td>
<td>25.8</td>
</tr>
<tr>
<td>2004-2005</td>
<td>173</td>
<td>32.8</td>
</tr>
<tr>
<td>2005-2006</td>
<td>43</td>
<td>8.1</td>
</tr>
<tr>
<td>2006-2007</td>
<td>80</td>
<td>15.2</td>
</tr>
<tr>
<td>2007-2008</td>
<td>32</td>
<td>6.1</td>
</tr>
<tr>
<td>2008-2009</td>
<td>64</td>
<td>12.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>528</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>

Table 3.3 presents the descriptive statistics for the dependent variables.

Table 3.3. Descriptive statistics of total fruit, total flowers, and FFR.
These are the total data collected for the whole study.

<table>
<thead>
<tr>
<th></th>
<th>N*</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Fruit</td>
<td>528</td>
<td>0</td>
<td>121.00</td>
<td>5.22</td>
<td>0.430</td>
</tr>
<tr>
<td>Total Flowers</td>
<td>528</td>
<td>0</td>
<td>238.00</td>
<td>16.21</td>
<td>0.990</td>
</tr>
<tr>
<td>FFR</td>
<td>516</td>
<td>0</td>
<td>1.00</td>
<td>0.35</td>
<td>0.015</td>
</tr>
</tbody>
</table>

*The value of N (total clumps from which data were collected) for FFR is less than that for total fruit and total flowers because a small number of clumps had no flowers or fruit.
Table 3.4. Descriptive statistics of FFR according to flowering site.
These are the total number of clumps from which flower and fruit data were collected at each site. The mean and range of FFR values for each site are shown.

<table>
<thead>
<tr>
<th>Site</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>28</td>
<td>0.37</td>
<td>0.05</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site 2</td>
<td>32</td>
<td>0.26</td>
<td>0.04</td>
<td>0</td>
<td>0.92</td>
</tr>
<tr>
<td>Site 3</td>
<td>141</td>
<td>0.25</td>
<td>0.03</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site 4</td>
<td>11</td>
<td>0.14</td>
<td>0.04</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Site 5</td>
<td>8</td>
<td>0.70</td>
<td>0.10</td>
<td>0.25</td>
<td>1</td>
</tr>
<tr>
<td>Site 6</td>
<td>64</td>
<td>0.35</td>
<td>0.03</td>
<td>0</td>
<td>0.97</td>
</tr>
<tr>
<td>Site 7</td>
<td>99</td>
<td>0.35</td>
<td>0.04</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site 8</td>
<td>79</td>
<td>0.67</td>
<td>0.04</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site 9</td>
<td>20</td>
<td>0.30</td>
<td>0.08</td>
<td>0</td>
<td>0.89</td>
</tr>
<tr>
<td>Site 10</td>
<td>12</td>
<td>0.36</td>
<td>0.10</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Site 11</td>
<td>22</td>
<td>0.01</td>
<td>0.01</td>
<td>0</td>
<td>0.19</td>
</tr>
<tr>
<td>Total</td>
<td>516</td>
<td>0.35</td>
<td>0.02</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

The dependent variable FFR was made normal through a logarithmic transformation. This procedure is valid as the logarithm of the data increases monotonically and so does not distort the original relationships in the data. The histogram and Shapiro Wilk’s test results (p >0.05) confirmed that the log transformed data were normally distributed.

An ANOVA was conducted with log (FFR) as the dependent variable and the categorical variable ‘sites’ as the independent variable. There was a significant difference in the mean log (FFR) according to flowering site with F(9, 333) = 9.937, p <0.001.

A Tukey post hoc test was used to determine which flowering sites differed significantly in their FFR values (Table 3.5). Sites 1, 6, 9 and 10 were not significantly different between themselves or the other sites. The conclusion at this stage of the analysis was that there was a significant amount of variability in pollinator activity across the majority of the 11 sites. This is consistent with several reports (reviewed in Devaux and Lande 2009) of high variability in pollinator abundance during a flowering season.
3.4.3 Relationship Between FFR and Flowering Season

The purpose of this analysis was to assess the activity of pollinators over the six seasons in which data were collected and to investigate probable sources of any substantial variation. To achieve this, FFR values for each flowering season (Figure 3.2) were investigated.

Table 3.5. Tukey Post Hoc Test for comparison of mean log FFR according to flowering site.

<table>
<thead>
<tr>
<th>(I) Site No</th>
<th>(J) Site No</th>
<th>Mean Difference*</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval Lower Bound</th>
<th>95% Confidence Interval Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4</td>
<td>-0.37913</td>
<td>0.11856</td>
<td>0.048</td>
<td>-0.7568 -0.0015</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>-0.28429</td>
<td>0.05111</td>
<td>0.000</td>
<td>-0.4471 -0.1215</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>-0.37432</td>
<td>0.05032</td>
<td>0.000</td>
<td>-0.5346 -0.2140</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>-0.55936</td>
<td>0.16639</td>
<td>0.029</td>
<td>-1.0894 -0.0294</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>-0.46452</td>
<td>0.12745</td>
<td>0.011</td>
<td>-0.8705 -0.0586</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>-0.55455</td>
<td>0.12713</td>
<td>0.001</td>
<td>-0.9595 -0.1496</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>0.37913</td>
<td>0.11856</td>
<td>0.048</td>
<td>0.0015 0.7568</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>0.55936</td>
<td>0.16639</td>
<td>0.029</td>
<td>0.0294 1.0894</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>-0.20501</td>
<td>0.05854</td>
<td>0.019</td>
<td>-0.3915 -0.0185</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>-0.29503</td>
<td>0.05785</td>
<td>0.000</td>
<td>-0.4793 -0.1108</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>0.28429</td>
<td>0.05111</td>
<td>0.000</td>
<td>0.1215 0.4471</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>0.46452</td>
<td>0.12745</td>
<td>0.011</td>
<td>0.0586 0.8705</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>0.20501</td>
<td>0.05854</td>
<td>0.019</td>
<td>0.0185 0.3915</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>0.37432</td>
<td>0.05032</td>
<td>0.000</td>
<td>0.2140 0.5346</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>0.55455</td>
<td>0.12713</td>
<td>0.001</td>
<td>0.1496 0.9595</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0.29503</td>
<td>0.05785</td>
<td>0.000</td>
<td>0.1108 0.4793</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>0.77408</td>
<td>0.23038</td>
<td>0.029</td>
<td>0.0402 1.5079</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>-0.77408</td>
<td>0.23038</td>
<td>0.029</td>
<td>-1.5079 -0.0402</td>
<td></td>
</tr>
</tbody>
</table>

*All mean differences were significant at the 0.05 level.

A 1-way ANOVA was used to determine whether there were significant differences in FFR between seasons, again using log (FFR) to satisfy normality assumptions. The ANOVA results show that there was a significant difference in mean log (FFR) according to flowering season with $F(5, 338) = 21.372, p <0.001$. 
Table 3.6 presents the results of the Tukey post hoc tests from which it can be concluded that starting in 2003-2004, for each passing season, pollinator activity was either the same or significantly less than the previous season.

![Graph showing FFR and flowering season](image)

**Figure 3.2 A plot of fruit flower ratio (FFR) and flowering season.**
The seasons are truncated years e.g. 0304 = season 2003-2004. Bars are ± 1se, N 0304 = 136, 0405 = 173, 0506 = 43, 0607 = 80, 0708 = 32, 0809 = 64.

The preceding analysis has shown that the FFR values for season 2003-2004 were significantly higher than for any other seasons (Table 3.6), and this warranted further investigation. Examination of the raw data shows that of the 136 records for that season, 93 (68%) were from Sites 7 and 8 and the mean FFR (Table 3.7) for these two sites was 0.70, double the mean for the overall study. The combined mean of the other 43 records for that season was 0.14.

Sites 7 and 8 were where the author first captured native bees collecting pollen from *Tetratheca juncea* flowers in 2001, reported in Driscoll (2003). At that time pollinator visits were infrequent (2 – 4 hourly or none) with low fruit set observed but not quantified. In November 2002 a severe bushfire swept through the area leaving a charred landscape. Over the next few months the *Tetratheca juncea* clumps resprouted from rootstock, a fire response that had previously been documented by Norton (unpub.). Season 2003-2004 was the first flowering following the fire and was prolific. During late September 2003 native bees, mostly *Lasioglossum convexum*, were observed in high numbers with visits to *Tetratheca juncea* flowers at 10 – 15 min
... intervals. Subsequently fruit set levels were high, resulting in high FFR values. In one respect, the high 2003-2004 FFR value could be considered an aberration for this study because the fire created an abnormal condition at Sites 7 and 8 compared with the other sites. However, it also highlights a role of fire in the *Tetratheca juncea* life cycle and this is discussed in Chapter 6.

Table 3.6. Tukey Post Hoc Test for comparison of mean log FFR according to flowering season.

<table>
<thead>
<tr>
<th>(I) Season</th>
<th>(J) Season</th>
<th>Mean Difference* (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003-2004</td>
<td>0405</td>
<td>0.138</td>
<td>0.045</td>
<td>0.025</td>
<td>0.011</td>
<td>0.266</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0607</td>
<td>0.436</td>
<td>0.056</td>
<td>0.000</td>
<td>0.275</td>
<td>0.597</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0708</td>
<td>0.368</td>
<td>0.072</td>
<td>0.000</td>
<td>0.162</td>
<td>0.573</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0809</td>
<td>0.456</td>
<td>0.056</td>
<td>0.000</td>
<td>0.295</td>
<td>0.617</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0304</td>
<td>-0.138</td>
<td>0.045</td>
<td>0.025</td>
<td>-0.266</td>
<td>-0.011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0607</td>
<td>0.298</td>
<td>0.055</td>
<td>0.000</td>
<td>0.140</td>
<td>0.455</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0708</td>
<td>0.229</td>
<td>0.071</td>
<td>0.017</td>
<td>0.026</td>
<td>0.433</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0809</td>
<td>0.318</td>
<td>0.055</td>
<td>0.000</td>
<td>0.160</td>
<td>0.475</td>
<td></td>
</tr>
<tr>
<td>2004-2005</td>
<td>0607</td>
<td>0.252</td>
<td>0.076</td>
<td>0.012</td>
<td>0.035</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0809</td>
<td>0.272</td>
<td>0.076</td>
<td>0.005</td>
<td>0.055</td>
<td>0.489</td>
<td></td>
</tr>
<tr>
<td>2005-2006</td>
<td>0304</td>
<td>-0.436</td>
<td>0.056</td>
<td>0.000</td>
<td>-0.597</td>
<td>-0.275</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0405</td>
<td>-0.298</td>
<td>0.055</td>
<td>0.000</td>
<td>-0.455</td>
<td>-0.140</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0506</td>
<td>-0.252</td>
<td>0.076</td>
<td>0.012</td>
<td>-0.469</td>
<td>-0.035</td>
<td></td>
</tr>
<tr>
<td>2006-2007</td>
<td>0304</td>
<td>-0.368</td>
<td>0.072</td>
<td>0.000</td>
<td>-0.573</td>
<td>-0.162</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0405</td>
<td>-0.229</td>
<td>0.071</td>
<td>0.017</td>
<td>-0.433</td>
<td>-0.026</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0506</td>
<td>-0.272</td>
<td>0.076</td>
<td>0.005</td>
<td>-0.489</td>
<td>-0.055</td>
<td></td>
</tr>
</tbody>
</table>

*All mean differences were significant at the 0.05 level.

Comparison of Site 7 FFR values at close to the same date in the 2003-2004 and 2004-2005 seasons is also informative. On 6 October 2003 the mean FFR value was 0.775±0.48SE. On 15 October 2004 there was no fruit (43 clumps, 587 flowers). Just over one month later on 27 November 2004 the mean FFR value was 0.462±0.14SE (4 clumps, 56 flowers).
Table 3.7. Mean FFR values by season and site.

<table>
<thead>
<tr>
<th>Season</th>
<th>Site</th>
<th>Mean FFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0304</td>
<td>3</td>
<td>0.14</td>
</tr>
<tr>
<td>0304</td>
<td>7</td>
<td>0.63</td>
</tr>
<tr>
<td>0304</td>
<td>8</td>
<td>0.78</td>
</tr>
<tr>
<td>0304</td>
<td>9</td>
<td>0.28</td>
</tr>
<tr>
<td>0304</td>
<td>11</td>
<td>0.01</td>
</tr>
<tr>
<td>0405</td>
<td>1</td>
<td>0.37</td>
</tr>
<tr>
<td>0405</td>
<td>5</td>
<td>0.43</td>
</tr>
<tr>
<td>0405</td>
<td>7</td>
<td>0.05</td>
</tr>
<tr>
<td>0405</td>
<td>8</td>
<td>0.55</td>
</tr>
<tr>
<td>0405</td>
<td>9</td>
<td>0.32</td>
</tr>
<tr>
<td>0405</td>
<td>10</td>
<td>0.36</td>
</tr>
<tr>
<td>0506</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>0607</td>
<td>2</td>
<td>0.25</td>
</tr>
<tr>
<td>0607</td>
<td>5</td>
<td>0.23</td>
</tr>
<tr>
<td>0708</td>
<td>7</td>
<td>0.26</td>
</tr>
<tr>
<td>0809</td>
<td>2</td>
<td>0.19</td>
</tr>
<tr>
<td>0809</td>
<td>4</td>
<td>0.70</td>
</tr>
<tr>
<td>Overall mean FFR</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

The absence of pollinators up to 15 October 2004 at Site 7 could be because the local bees were foraging elsewhere within the foraging range from their brood sites; site or flower constancy by native bees is well documented (Goulson 1994; Osborne and Williams 2001; Willmer and Stone 2004). These data provide insights into an ephemeral aspect of native bee pollinator activity and shows that samples should be collected from a wide area for meaningful results.

3.4.4 Relationship between FFR, Flowering Season and Flowering Site

This analysis tested whether there was any interaction between season and site on FFR.

Flowering season and flowering site are categorical variables and log (FFR) is an interval variable. A General Linear Model (GLM) was used with log (FFR) as the dependent variable and sites and seasons as predictor factors. Results of the GLM are shown in Table 3.8. The differences in the log (FFR) means were significant for both sites (F (9, 326) = 8.433, p < 0.001) and seasons (F (5, 326) = 15.623, p < 0.001).
FFR is shown here to vary significantly with season and site, consistent with the isolated ANOVA analyses as reported above. However the interaction effect between site and season was not significant (F(2, 326) = 0.325, p =0.723).

Table 3.8. Results of GLM analysis for log FFR vs Site and Season

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>16.936</td>
<td>16</td>
<td>1.059</td>
<td>12.916</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>26.790</td>
<td>1</td>
<td>26.790</td>
<td>326.894</td>
<td>0.000</td>
</tr>
<tr>
<td>Season</td>
<td>6.402</td>
<td>5</td>
<td>1.280</td>
<td>15.623</td>
<td>0.000</td>
</tr>
<tr>
<td>Site</td>
<td>6.220</td>
<td>9</td>
<td>0.691</td>
<td>8.433</td>
<td>0.000</td>
</tr>
<tr>
<td>Season * Site</td>
<td>0.053</td>
<td>2</td>
<td>0.027</td>
<td>0.325</td>
<td>0.723</td>
</tr>
<tr>
<td>Error</td>
<td>26.717</td>
<td>326</td>
<td>0.082</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94.703</td>
<td>343</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.4.5 Relationship between FFR and Size of Floral Display

The purpose of this analysis was to determine whether pollinator activity was proportionately greater as floral display size increased. The result of a regression of the means of total fruit and total flowers for each season was a significant positive linear relation with F(1,5) 15.795 p <0.05 and $r^2 = 0.798$. The regression equation was:

$$\text{Mean Total Fruit} = -1.39 + 0.397 \times \text{Mean Total Flowers}$$

This does not however imply that pollinators have a preference for larger floral displays. It simply confirms the intuitively obvious, that more flowers provide more pollen sources for pollinators, hence more fruit. If pollinators were more attracted to larger floral displays, FFR would be expected to increase with floral display size.

The relationship between FFR and Total Flowers was examined using the combined data for the six years. There were 510 records in which Total Flowers ranged from 1 to 238 (mean 16.8, SD 22.83) and FFR ranged from 0 to 1.00 (mean 0.357, SD 0.352). A histogram of FFR showed that the data were not normally distributed, being positively skewed and bimodal. The data also contained extreme values.

An ordinary least square (OLS) regression model with FFR as the dependent variable and Total Flowers as the independent variable was found to not be significant.
(F(1, 508) = 2.649, p=0.104). This meant that Total Flowers was not a significant direct predictor of FFR.

Residuals analysis revealed possible violation of assumptions of linearity and constant error variance. To account for this, the response variable (FFR) was transformed using a square root transformation. A quadratic component was also included to account for the probable non-linear form of the relationship. Also, extreme values (zero) were excluded to reduce the effect of outliers.

The final fitted model was:

\[ \sqrt{FFR} = 0.782 + (-0.006) \text{Total Flowers} + 0.00003 \text{(Total Flowers)}^2 \]

The coefficient of determination R^2 was 0.117 and the model was significant (F (2, 341) = 22.541, p < 0.001) indicating that at least one independent variable is a significant predictor of FFR. The fitted model indicates that for a unit increase in Total Flowers, the square root of FFR increases by an average -0.006 units. A test of significance of this estimate revealed that the null hypothesis can be rejected (t(1) = 5.256, p < 0.001).

The residuals histogram for this model showed a normal distribution indicating that the assumptions of the model were satisfied.

In summary, this analysis has demonstrated that over the six years of this investigation pollinator activity (as indicated by FFR) reduced by a small and accelerating amount with increasing flowers per clump. This points to the possibility that there was a limited number of available pollinators across the survey area and that more flowers per clump becomes increasingly ineffective in attracting pollinators.

### 3.4.6 Relationship between Total Flowers per Clump and Flowering Season

The final research question to be addressed was whether there was a significant difference in total flowers per clump between flowering seasons. The descriptive statistics of mean total flower values according to flowering season are presented in Table 3.91.
Table 3.9. Descriptive statistics for total flowers per clump by flowering season.

<table>
<thead>
<tr>
<th>Season</th>
<th>N</th>
<th>Mean</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003-2004</td>
<td>136</td>
<td>20.64</td>
<td>2.94</td>
<td>0.00</td>
<td>238.00</td>
</tr>
<tr>
<td>2004-2005</td>
<td>173</td>
<td>14.38</td>
<td>1.05</td>
<td>0.00</td>
<td>63.00</td>
</tr>
<tr>
<td>2005-2006</td>
<td>43</td>
<td>6.93</td>
<td>1.25</td>
<td>0.00</td>
<td>45.00</td>
</tr>
<tr>
<td>2006-2007</td>
<td>80</td>
<td>13.26</td>
<td>1.84</td>
<td>0.00</td>
<td>78.00</td>
</tr>
<tr>
<td>2007-2008</td>
<td>32</td>
<td>24.41</td>
<td>5.13</td>
<td>1.00</td>
<td>108.00</td>
</tr>
<tr>
<td>2008-2009</td>
<td>65</td>
<td>17.45</td>
<td>2.13</td>
<td>1.00</td>
<td>68.00</td>
</tr>
<tr>
<td>Total</td>
<td>529</td>
<td>16.20</td>
<td>0.98</td>
<td>0.00</td>
<td>238.00</td>
</tr>
</tbody>
</table>

A 1-way ANOVA was used to investigate these data. Again, the distribution of the variable total flowers deviated from normality as shown by the histogram and the results of the Shapiro-Wilk’s test (p <0.05). Consequently, the logarithm of total flowers was taken as the dependent variable.

The ANOVA results show there was a significant difference between mean log (total flowers per clump) and flowering seasons ($F(2.39, 716) = 2.397, p$-value $= 0.02$).

Multiple comparison Tukey post hoc test was used to determine which seasons had significantly different flowers per clump (summarised in Table 3.10). The Tukey results show that flowers per clump decreased significantly from season 2003-2004 to season 2005-2006, then increased in 2008-2009, recovering to the 2003-2004 levels.

### 3.4.7 A Closer Look at FFR and *Tetratheca juncea*

As previously described, FFR values were calculated from the number of flowers and fruit present on a clump *at a point in time* and could only be considered as indicative of a true Fruit:Flower ratio, designated here as FFRi.

Using data from the Chapter 2 phenology study, the relationship between FFRi and a true Fruit:Flower ratio, designated FFRt, can be examined. By way of summary, the reproductive phenophases of budding, flowering, fruiting and seed release were documented monthly at 12 sites from June 2010 to January 2011. **Figure 3.3** shows plots of the FFRi values for each month that flowers were open and fruits were present. Site 9 was aberrant and is plotted separately from the means of the other 11 sites. It was excluded from further analysis.
Table 3.10. Tukey post hoc test results for significantly different means of log total flowers per clump and flowering season
Seasons have been truncated e.g. 0304 = 2003 to 2004

<table>
<thead>
<tr>
<th>(I) Season</th>
<th>(J) Season</th>
<th>Mean Difference (I-J)*</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
<th>Upper Bound</th>
<th>Lower Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>0304</td>
<td>0506</td>
<td>0.288</td>
<td>0.090</td>
<td>0.019</td>
<td>0.030</td>
<td>0.547</td>
<td>0.030</td>
</tr>
<tr>
<td>0405</td>
<td>0506</td>
<td>0.256</td>
<td>0.088</td>
<td>0.045</td>
<td>0.004</td>
<td>0.508</td>
<td>0.508</td>
</tr>
<tr>
<td>0506</td>
<td>0304</td>
<td>-0.288</td>
<td>0.090</td>
<td>0.019</td>
<td>-0.547</td>
<td>-0.030</td>
<td>-0.547</td>
</tr>
<tr>
<td>0405</td>
<td>0304</td>
<td>-0.256</td>
<td>0.088</td>
<td>0.045</td>
<td>-0.508</td>
<td>-0.004</td>
<td>-0.004</td>
</tr>
<tr>
<td>0708</td>
<td>0405</td>
<td>-0.466</td>
<td>0.119</td>
<td>0.001</td>
<td>-0.805</td>
<td>-0.127</td>
<td>-0.127</td>
</tr>
<tr>
<td>0809</td>
<td>0708</td>
<td>-0.316</td>
<td>0.101</td>
<td>0.022</td>
<td>-0.604</td>
<td>-0.028</td>
<td>-0.028</td>
</tr>
<tr>
<td>0708</td>
<td>0506</td>
<td>0.466</td>
<td>0.119</td>
<td>0.001</td>
<td>0.127</td>
<td>0.805</td>
<td>0.805</td>
</tr>
<tr>
<td>0809</td>
<td>0506</td>
<td>0.316</td>
<td>0.101</td>
<td>0.022</td>
<td>0.028</td>
<td>0.604</td>
<td>0.604</td>
</tr>
</tbody>
</table>

* The mean differences were significant at the 0.05 level.

The plot in Figure 3.3 is empirically sensible where the FFRi values would be expected to approach unity as the number of open flowers decreases to zero while fruit ripening and seed release continue to the end of the reproductive season. If this trend exists in the data for the current study it would add a bias to the data that would potentially make the between sites and months analysis unreliable.

An ANOVA showed that there was a significant difference between the means of the 11 sites ($F(4,54) = 53.315$, $p$-value <0.001). A Tukey Post Hoc test (Table 3.11) showed that there was a significant difference between all pairs of months other than November and December. A Pearson correlation analysis showed a significant positive correlation between the FFRi means and months (Pearson $r = 0.882$, $p$ <0.001, $N = 55$).

To determine whether a similar trend was apparent in the seasonal analysis that is the subject of this chapter, the FFRi values were plotted against the months in which they were collected (Figure 3.4). The same trend was not present.

An ANOVA showed that there was a significant difference between the means from the 4 months ($F(3,515) = 19.61$, $p$ <0.001). A Tukey Post Hoc test (Table 3.12) showed that there was a significant difference between the November mean and the other three months that were not significantly different from each other. However a Pearson
correlation analysis showed no correlation between the FFRi means and months (Pearson $r = .003 \ p > 0.05$).

![Figure 3.3. A plot of the monthly mean FFRi values for 11 sites and the individual values for Site 9.](image)

This is a plot of the FFRi values from the Chapter 2 phenology study and starts in September when fruit first appeared and finishes in January. The values are the combined means for all sites for each month. Bars are ± 1se, N = 11 sites.

**Table 3.11. Tukey post hoc test homogeneous subsets for monthly FFRi values**

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th>Subset for alpha = .05</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Sept</td>
<td>11</td>
<td>0.054335</td>
</tr>
<tr>
<td>Oct</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Nov</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Dec</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Jan</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>1.000</td>
</tr>
</tbody>
</table>

So far this comparison suggests that the monthly trend in FFRi values found in the recent phenology study is not apparent in the current study. Variation in the data could completely obscure this trend as illustrated by the overall mean FFRi being 0.352 and the standard deviation also 0.352.
Figure 3.4. A plot of mean FFRi values for the months in which they were collected. This plot shows the means of FFRi data by the month in which the data were collected irrespective of year. Bars are ± 1se, N = 154, 154, 182, 26 for each sequential month.

Table 3.12. Tukey post hoc homogeneous subsets for FFRi and month

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th>Subset for alpha</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>= 0.05</td>
</tr>
<tr>
<td>Sep</td>
<td>154</td>
<td>0.2621</td>
</tr>
<tr>
<td>Dec</td>
<td>182</td>
<td>0.2864</td>
</tr>
<tr>
<td>Jan</td>
<td>26</td>
<td>0.3246</td>
</tr>
<tr>
<td>Nov</td>
<td>154</td>
<td>0.5240</td>
</tr>
<tr>
<td>Sig.</td>
<td></td>
<td>0.687</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.000</td>
</tr>
</tbody>
</table>

To approximate a true Fruit:Flower ratio, FFRt, data can be extracted from the Chapter 2 phenology data from the increase in flowers and fruit for each month over a selected series of months. Thus FFRt can be expressed by the following equation:

$$FFRt = \frac{\sum_{i=1}^{n} \Delta \text{Fruit}}{\Delta \text{Flowers} + \Delta \text{Fruit}}$$

Where \(\Delta \text{Fruit}\) is the change in fruit numbers over a month, \(\Delta \text{Flowers}\) is the change in flower numbers over the same month and n is the number of months. This relationship is only valid for sequential months in which flowers and fruit are increasing so that the changes are always positive.
This calculation is only possible because of the negligible attrition rate of inflorescence development in the phenology study (Section 2.3.7). FFRt was calculated over the period from the start of flowering to the point at which fruiting was at its maximum, being September to December for sites 1, 2, 6, 7, 10, 11, 12 and September to January for sites 3, 4, 5, 8. Because a different amount of material was collected each month the flowering and fruiting values were standardised as per centimetre of stem. FFRi was calculated from the raw fruit and flower data for the same periods.

The values calculated were:
FFRt = 0.45±0.073SE
FFRi = 0.39±0.034SE

This value of FFRi compares well with the overall value for the current study of 0.352±0.016SE. This small and independent comparison indicates that FFRi underestimates FFRt by approximately 15% over the months September to January.

3.5 DISCUSSION

The broad aims of this study have been met. There are now six confirmed, and five potential, native bee pollinators of *Tetratheca juncea* (Table 3.1) and FFR values indicate that pollinator activity is widespread but highly variable across the Central Coast regional population.

From data collected for this Chapter there was further evidence of the lack of abiotic pollination in the wild (see Section 3.1 for earlier evidence). A site with 43 clumps that had abundant fruit in October 2003 had no fruit at around the same time in October 2004. The first fruit for 2004 were recorded in November, about three months with no fruit (see details in Section 3.4.3).

Comparison of data from the current study with the Chapter 2 phenology study showed that FFR data collected at a single point in time was indicative of pollinator activity but understated the true level of activity across a flowering season. The level of pollinator activity across individual sites was variable (Section 3.4.2). While it appears that
pollinator activity, as indicated by FFR values, declined over the six-season study period (Section 3.4.3) the majority of this effect was shown to be due to a response to fire at two sites where pollinator activity was double the study mean early in the time series.

Sutherland and Delph (1984) compiled fruit set records from published sources and found that for hermaphrodite species (such as *Tetratheca juncea*), overall mean Fruit:Flower ratio could be subdivided into self-incompatible species (here quoted as percentages), 22.1% and self-compatible species, 72.5%. While technically self-compatible (as shown by hand pollination experiments) *Tetratheca juncea* is primarily a xenogamous species (Gross et al. 2003). The mean FFRi for the current study was 35% with FFRt probably around 40%. Gross et al. (2003) reported *Tetratheca juncea* Fruit:Flower ratio values ranging from 0 – 0.65 for 32 clumps from nine populations. Compared with the Fruit:Flower ratios reported by Sutherland and Delph (1984) Fruit:Flower ratio and FFR values for *Tetratheca juncea* do not appear to be abnormally low.

These numbers raise the question as to the impact of pollinator limitation on *Tetratheca juncea*. It is clear that more pollinator visits will result in more fruit being set, as demonstrated by Sites 7 and 8 in the flowering following a bushfire (Table 3.7). Hand pollination experiments by Gross et al. (2003) also resulted in increased fruit set. However, just because an organism has the capacity to respond positively to an increase in resources doesn’t mean that continuous higher levels are necessary or even beneficial for survival.

In fact there could well be a price to pay for the sort of flowering and fruiting extravagance as seen at Sites 7 and 8. Following the fire there was an increase in resources for both the female and male reproduction component. The increased soil nutrients resulted in a noticeable increase in vegetative growth and flowering intensity and for an unknown reason there was an increase in pollinator numbers. The majority of potential pollinators (Table 3.1) use tunnels in soil for breeding, so it can be speculated that fire provides more open ground. The outcome was FFRi levels over double the regional long-term mean. However observation at the two sites over subsequent years
showed that the immediate post fire vegetative growth levels, flowering intensity and fruit set were not maintained. It is possible that the pressure of excess pollinators depleted resources for other reproductive or somatic needs. On the other hand it is also possible that the post-fire nutrient boost was depleted and the plants returned to pre-fire growth and flowering patterns. However empirical evidence was that the plants looked poorer in the season following the post-fire prolific growth and flowering. There is scope for more research here.

The higher levels of fruit, if matched by higher levels of seed production, could also have a positive impact on the local population. Theoretically, there would be a substantial boost to the seed bank but the value of this would depend on the validity of the report by Bellairs et al. (2006) that the *Tetrathea juncea* seed bank is short-lived; as noted in Chapter 1, there are some plausible alternate explanations for that finding. By comparison, Butcher et al. (2011) found that the seed of the Western Australian *Tetrathea paynterae* Alford subsp. *paynterae* were viable for 3 years. Speculating, a short-lived seed bank would seem inconsistent with other aspects of the species’ evolution. It has adapted to having no nectar by having a long and prolific flowering season and by having specialist pollinators. As shown by Bellairs et al. (2006) it has adapted to fire by using heat and smoke as triggers to break seed dormancy. Norton (unpub.) showed that the species has adapted to fire by being able to resprout from its underground rhizomes. Given these adaptations it would be surprising if abundant seed were produced following fire, only to be wasted.

However, FFR alone is not informative as to the ultimate response of the plant to either pollinator limitation or abundance. It is the number and quality of seed produced that matters (Burd 1994). Gross et al. (2003) determined that the mean number of seeds per fruit was 1.64 out of a possible 4. If the Haig and Westoby (1998) resource allocation model applied to *Tetrathea juncea* then the response to a significant increase in pollinators could well result in less seed per fruit and the increased FFR could be misleading. Conversely, where there is a paucity of pollinators, maybe more seed per fruit are produced. This is a rich area for further research.
CHAPTER 4  A SPATIOTEMPORAL STUDY OF POLLINATION IN A TETRATHECA JUNCEA SUBPOPULATION
Chapter 4  A Spatiotemporal Study of Pollination in A Tetratheca juncea Subpopulation

4.1 INTRODUCTION

Chapter 3 showed that pollinator activity in *Tetratheca juncea* was highly variable between sites across a regional population. Over the study period, there was a steady decline in pollinator activity while total flowers per clump fell and then returned to starting levels. This chapter provides a report of a study, conducted concurrently with the Chapter 3 study, of flowering and pollination in a single subpopulation over six years. Studying a single subpopulation allowed investigation of flowering and pollination in more detail than was possible in the broader regional population study. The pattern of flowering and pollination for any species is the outcome of many years of selection. Documenting these patterns over long periods can provide insights into some of the selection pressures faced and responses that have evolved.

There are few reported long-term spatiotemporal studies of flowering and pollination, especially for individual plant species (Table 4.1). A common feature of these studies was considerable variability in pollinator type and/or abundance over the study period.

### Table 4.1 Examples of long-term spatiotemporal studies of pollination and pollinators.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Study Period</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire pollinator assemblage for <em>Lavandula latifolia.</em></td>
<td>6 years</td>
<td>Herrera (1988)</td>
</tr>
<tr>
<td>Pollinator and antguards of <em>Calathea ovandensis.</em></td>
<td>3 – 4 years</td>
<td>Horvitz and Schemske (1990)</td>
</tr>
<tr>
<td>Pollinators of <em>Silene virginica</em> using caged and control plants.</td>
<td>5 years</td>
<td>Fenster and Dudash (2001)</td>
</tr>
<tr>
<td>Pollination of <em>Ipomopsis aggregata.</em></td>
<td>7 years</td>
<td>Price <em>et al.</em> (2005)</td>
</tr>
</tbody>
</table>

Pollination is often viewed as one-dimensional, being the act of transferring pollen to the stigma to enable seed development. However, ecosystems are dynamic.

Accounting for these dynamics can expand understanding of the selection pressures at work and the time span over which they might act on the ecology of a species (Herrera 1988; Price *et al.* 2005).
In general terms, this study aimed to test the hypothesis that flowering and pollinator activity in a *Tetratheca juncea* subpopulation were not significantly different from year to year. Considering that the hypothesis might be rejected, data were collected on population attrition, habitat conditions and local weather variables so that causes of any variation could be investigated.

### 4.2 METHODS

#### 4.2.1 Subpopulation Site and Data Collection

Data were collected from a 504 clump *Tetratheca juncea* subpopulation located at the northern limits of the Central Coast regional population. Figure 4.1 shows the geographic location of the site and the individual clump distribution. A professional surveyor was engaged to determine the geographic coordinates (datum, Geocentric Datum of Australia 1994; projection, Map Grid of Australia Zone 56) of all clumps and other features used in this investigation.

To sample from the subpopulation, 100 randomly located clumps (Figure 4.1) were marked with a small galvanised metal stake located approximately 20 cm south of the clump. It was from these 100 clumps that data were collected as a representative sample of the entire subpopulation. In mid-December, each year from 2005 to 2010 inclusive, the data collected per clump were presence or absence of the clump, clump size (< or > than 10 stems), total open flowers and total fruit. Collecting data at the same time each year minimised potential confounding effects from changes in the measured variables that occur progressively through the reproductive season. Also, as shown in Chapter 2 Figure 2.3, in December all components of the reproductive process are present.

Structure and floristic content of the vegetative habitat was quantified in an attempt to detect habitat changes over the survey period. These data were collected in 2002 and 2008 in the form of cover-abundance data from ten permanently pegged 10 m square quadrats (Figure 4.1) using the Braun-Blanquet (Poore 1955) system with scores as shown in Table 4.2.
Table 4.2. The Braun-Blanquet cover-abundance scores.

<table>
<thead>
<tr>
<th>Cover range</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5% few individuals</td>
<td>1</td>
</tr>
<tr>
<td>&lt;5% many individuals</td>
<td>2</td>
</tr>
<tr>
<td>5% - &lt;25%</td>
<td>3</td>
</tr>
<tr>
<td>25% - &lt;50%</td>
<td>4</td>
</tr>
<tr>
<td>50% - &lt;75%</td>
<td>5</td>
</tr>
<tr>
<td>75% - 100%</td>
<td>6</td>
</tr>
</tbody>
</table>

Within each quadrat, all flora species were listed. Each species was given a cover-abundance score, being an estimate of the percentage of the total quadrat area that the foliage of the species would cover were all individuals aggregated. Species were assigned to three broad structural layers, canopy, woody shrubs to 2 m tall and ground cover. Overlap between structural layers means that total cover by all species in all layers exceeds the quadrat area.

The spatial relationship between monitored indices of year, total flowers per clump, attrition and pollinator activity as measured by FFR was explored. The number of clumps (including the monitored clump) in a 5x5 metre grid cell (Figure 4.1) was used to create a density matrix.

Rainfall and temperature have been shown to have a variety of impacts on flowering phenology (Spano et al. 1999; Tyler 2001; Inouye et al. 2003; Peñuelas et al. 2004).

Water is an essential resource for plants, particularly during flowering when adequate amounts are needed to maintain turgor in the floral display (Galen et al. 1999; Galen 2005). The study subpopulation was situated approximately 1 km west of an automatic weather station located at an operating open-cut coal mine.

This provided a rare opportunity to investigate the impact of rainfall on flowering and fruiting in *Tetratheca juncea* using data from the immediate vicinity of the sampled subpopulation. For each year in which flowering and fruiting data were collected, total rainfall for the following four time periods was prepared for the analysis:

- A. Six months up to data collection;
- B. Three months up to data collection;
Figure 4.1. Location of *Tetrathec juncea* clumps and vegetation sampling quadrats within the *Tetrathec juncea* subpopulation used for this study. Inset shows the site in relation to the boundary of the Central Coast *Tetrathec juncea* regional population. The grid cell size is 5 m from which a clump density value was determined for spatial analysis.
C. 30 days up to data collection; and
D. The penultimate 30 days prior to data collection.

Unfortunately, temperature data from the nearby coalmine were incomplete. So data from the nearest Australian Government Bureau of Meteorology reporting station at Maitland (Station 22008, 9 km north west of the sampling site) were used (http://reg.bom.gov.au/climate/data/index.shtml) to assess the effect of temperature over time.

4.2.2 Statistical Analysis

Standard statistical analysis was conducted using SPSS version 19. Methods used were ordinary least squares (OLS) regression, linear regression, Chi-Square, and 1-way ANOVA.

The ANOSIM module in Primer 6 (Clarke and Gorley 2006) was used to investigate changes in habitat over time.

The spatial relationship between the monitored indices of year, total flowers per clump, attrition, and pollinator activity, was examined using R version 2.13.1 (R Development Core Team 2010). Several statistical analysis techniques were used including the t-test, Likelihood Ratio test, Cochran Q test, F-test, Pearson correlation, Moran I test, Lagrange test, Studentized Breusch-Pagan test, multiple linear regression and spatial regression model. Backward-elimination procedure was used to identify the prognostic factors affecting dependent variables. Backward elimination regression starts with all potential dependent variables and successively removes those that make an insignificant contribution i.e. whose p-value is greater than $\alpha$ (generally 0.05). The procedure terminates when there is no variable having a p-value $> \alpha$. 

4.3 RESULTS

4.3.1 Descriptive Statistics

Data were collected over six years, 2005 to 2010. Table 4.3 presents the frequency and percentages of FFR data collected for each year. Although there were a total of 100 clumps pegged for sampling each year, not all were sampled each year. Not all pegged clumps could be found in some dense areas of scrub even though they had been marked with flagging tape. As better quality hand held Global Positioning System (GPS) units became available along with GIS software capable of uploading points to the GPS, it became possible to find most clumps. Furthermore, as will be shown, not all of the original 100 clumps survived.

This analysis investigates the relationship between the independent variable time (years) and the dependent variables FFR, Total Flowers (FLT) and Total Fruit (FR). Both main and interaction effects were examined.

4.3.2 Main Effects

Main effects were examined through the use of an ordinary least squares (OLS) regression model. An OLS model was first run using untransformed variables with the hypothesised form of the model being \( y = \beta_0 + \beta_1 \text{Time} + \epsilon \).

The residual plot was examined for possible violation of assumptions of normality of residuals and constant error variance. In each case (FFR, FLT and FR) the residual plots were not normally distributed. To account for the non-normality of residuals, a logarithmic transformation was applied to each of the three dependent variables. Extreme values (zero) were excluded from the analyses to nullify the effect of outliers. The revised form of the model then became \( \log y = \beta_0 + \beta_1 \text{Time} + \epsilon \).

4.3.2.1 The Effect of Time on FFR

Figure 4.2 summarises the change in FFR over the sampling years and shows a 65% reduction.
Table 4.3. The number and percentage of *Tetratheca juncea* clumps from which fruit flower ratio (FFR) data were collected.
This table shows the number of the permanently pegged clumps that were sampled each year along with the proportion of the total population represented by the sampled clumps.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of the 100 pegged clumps sampled</th>
<th>Percentage of total population sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005</td>
<td>51</td>
<td>9.9%</td>
</tr>
<tr>
<td>2006</td>
<td>69</td>
<td>13.4%</td>
</tr>
<tr>
<td>2007</td>
<td>98</td>
<td>19.1%</td>
</tr>
<tr>
<td>2008</td>
<td>99</td>
<td>19.3%</td>
</tr>
<tr>
<td>2009</td>
<td>99</td>
<td>19.3%</td>
</tr>
<tr>
<td>2010</td>
<td>98</td>
<td>19.1%</td>
</tr>
<tr>
<td>Total unsampled</td>
<td>52</td>
<td>10.1%</td>
</tr>
<tr>
<td>Total</td>
<td>514</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 4.2. Annual changes in mean fruit flower ratio (FFR) per clump in the subject *Tetratheca juncea* subpopulation sampled from 2005 to 2010 inclusive.
Bars are ± 1 s.e. For 2005, N = 51; for 2006, N = 69; for 2007, N = 98; for 2008, N = 99; for 2009, N = 99; for 2010 N = 98.
Table 4.4 presents descriptive statistics of the three dependent variables considered in this analysis.

### Table 4.4. Descriptive statistics of *Tetratheca juncea* clump data collected.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
<th>Std. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Fruit</td>
<td>299</td>
<td>.00</td>
<td>32.00</td>
<td>2.24</td>
<td>0.21</td>
</tr>
<tr>
<td>Total Flower</td>
<td>365</td>
<td>.00</td>
<td>96.00</td>
<td>12.13</td>
<td>0.87</td>
</tr>
<tr>
<td>FFR</td>
<td>299</td>
<td>.02</td>
<td>1.00</td>
<td>0.20</td>
<td>0.02</td>
</tr>
</tbody>
</table>

The fitted OLS model was \( \text{Log FFR} = -0.800 + (-0.197) \text{Time} \). Residual analysis showed all standardised residuals ranged between -3.01 to +2.52 with the histogram clearly showing a normal distribution. Thus the assumptions of the regression model were satisfied.

The model was overall significant \( (F (1, 178) = 24.883, p < 0.001) \), coefficient of determination \( R^2 = 0.123 \), meaning that time was a significant predictor of FFR. Estimate of the regression coefficient for time was \(-0.197\) meaning that for a unit increase in the time (one year) the corresponding log FFR decreased on an average by \(-0.197\) units. A test for significance of this estimate \((\text{Ho}: \beta_1 = 0 \text{ Vs } \text{H1}: \beta_1 \neq 0)\) indicated that null hypothesis was rejected \((t (1) = 4.988, p<.001)\) confirming that the average change in FFR for a unit change in time was significant. In other words, on average, in every passing year, FFR decreased by 19.7%.

#### 4.3.2.2 The Effect of Time on Fruit

Mean fruit per clump (FR) values over the six years are shown in **Figure 4.3**. The fitted model was \( \text{Log FR} = 1.022 + (-0.028) \text{Time} \). Residual analysis showed all standardised residuals ranged between -1.17 to +2.99 with the histogram clearly showing a normal distribution. Thus the assumptions of the regression model were satisfied.

The model was not overall significant \( (F (1, 178) = 0.525, p = .470) \), coefficient of determination \( R^2 = 0.003 \), meaning that time was not a significant predictor of FR.
Figure 4.3. Annual changes in mean fruit per clump in the subject *Tetratheca juncea* subpopulation sampled from 2005 to 2010 inclusive. Bars are ± 1 s.e. For 2005, N = 51; for 2006, N = 69; for 2007, N = 98; for 2008, N = 99; for 2009, N = 99; for 2010 N = 98.

4.3.2.3 The Effect of Time on Total Flowers

A plot of the mean number of flowers per clump (FLT) (Figure 4.4) over the sampling years shows a steady increase with the greatest rate of increase appearing to have occurred from 2006 to 2008 (ca 40%).

The fitted OLS model was \( \log \text{FLT} = 1.646 + (0.097) \text{Time} \). Residual analysis showed all standardised residuals ranged between -1.803 to +1.984 with the histogram clearly showing a normal distribution. Thus the assumptions of the regression model were satisfied. The model was overall significant \( (F (1, 297) = 4.967, p = 0.027) \), coefficient of determination \( R^2 = 0.016 \), meaning that time was a significant predictor of FLT.

Estimate of the regression coefficient for time was 0.097 meaning that for a unit increase in the time (one year) the corresponding log FLT decreased on an average by 0.097 units. A test for significance of this estimate (Ho: \( \beta_1 = 0 \) Vs H1: \( \beta_1 \neq 0 \)) indicated that null hypothesis was rejected \( (t (1) = 2.229, p = .027) \) confirming that the average change in FLT for a unit change in time was significant. In other words, on average, for every passing year, FLT decreased by 9.7%. 

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**Figure 4.4** Graph showing annual changes in mean fruit per clump for the *Tetratheca juncea* subpopulation from 2005 to 2010.
4.3.3 Interaction Effects

Pearson’s correlation was used on the logarithmically transformed variables FFR, FR and FLT to test for possible interactions. Table 4.5 provides the correlation matrix of all variables along with tests for their significance.

In confirmation of the preceding independent effects analysis, Time was significantly negatively correlated with FFR, positively correlated with FLT and not correlated with FR. As would be expected, FFR and FR were positively correlated. Consistent with the regional analysis (Chapter 3) FLT and FR were also positively correlated (Table 4.5).

Overall, this analysis has shown that FFR values fell significantly over the six years, suggesting that pollinator activity had reduced in that time. However, total flowers per clump (FLT) increased significantly over the six years while total fruit per clump (FR) remained unchanged. Thus, it appears that pollinator numbers remained static over the six years and that their resource needs were satiated by a much lower level of flowering than was provided. In other words, the falling FFR values were the result of increasing flower numbers, not decreasing pollinator numbers.
Table 4.5 Correlation Matrix of Time, FFR, FR and FLT

<table>
<thead>
<tr>
<th></th>
<th>Time</th>
<th>logFFR</th>
<th>logFR</th>
<th>logFLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>-.350**</td>
<td>-.054</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.000</td>
<td>.470</td>
<td>.027</td>
</tr>
<tr>
<td>logPI</td>
<td>Pearson Correlation</td>
<td>-.350**</td>
<td>1</td>
<td>.192**</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.000</td>
<td>.010</td>
<td>.000</td>
</tr>
<tr>
<td>logFR</td>
<td>Pearson Correlation</td>
<td>-.054</td>
<td>.192**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.470</td>
<td>.010</td>
<td>.000</td>
</tr>
<tr>
<td>logFLT</td>
<td>Pearson Correlation</td>
<td>.128*</td>
<td>-.682**</td>
<td>.587**</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>.027</td>
<td>.000</td>
<td>.000</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4.3.3.1 Effect of Rainfall and Temperature on Flowering

A resource that could impact on flower numbers is available water (Galen et al. 1999). Empirical observation of *Tetratheca juncea* indicates that there is a response to soil moisture levels with smaller flowers (corolla width) produced when soil moisture is abnormally low, such as during drought. As described in Section 4.2 total rainfall for four different periods, before and up to sampling, were chosen for this investigation. Before testing for the effect of rainfall on flower numbers over time regressions were conducted to determine whether there was a significant trend in total rainfall for each period over the six years (Table 4.6). The result was no significant trend for each period over the six sampling years ($p >0.05$) therefore rainfall from any of these four periods could have had no effect on the increase in total flowers per clump over time.

4.3.4 Clump Attrition

As data were collected each year, it became apparent that there was a steady loss of clumps (Figure 4.5). A Chi-Square analysis was conducted to determine the significance of these losses over time. Table 4.7 shows the annual attrition/survival data. The Chi-Square analysis showed that there was a significant relationship ($p <0.05$) between the proportion of surviving clumps and the sampling year. Conversely, there was a significant decline (27.3%) in clump numbers from 2006 to 2010.

From this analysis, it can be concluded that the overall *Tetratheca juncea* subpopulation population suffered a >25% decline in clump numbers over the five years sampled.
4.3.4.1 Effect of Attrition on Total Flowers per Clump

In Section 4.3.2.3, it was shown that total flowers per clump increased significantly over the study period.

Table 4.6. Regression analysis of rainfall period and year.

Testing whether there was a significant trend in rainfall vs year for each of the four selected periods. A = total rainfall in the six months up to sampling, B = total rainfall in the three months up to sampling, C = total rainfall in the 30 days up to sampling, D = total rainfall in the penultimate 30 days before sampling.

<table>
<thead>
<tr>
<th>Rainfall period</th>
<th>$R^2$</th>
<th>$F$ values and significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.170</td>
<td>$F(1, 5) = 0.82, p = 0.416$</td>
</tr>
<tr>
<td>B</td>
<td>0.489</td>
<td>$F(1, 5) = 3.83, p = 0.122$</td>
</tr>
<tr>
<td>C</td>
<td>0.116</td>
<td>$F(1, 5) = 0.53, p = 0.508$</td>
</tr>
<tr>
<td>D</td>
<td>0.510</td>
<td>$F(1, 5) = 4.16, p = 0.111$</td>
</tr>
</tbody>
</table>

The potential for mean annual temperature to impact on total flowers was also investigated. A linear regression of mean annual temperature and years showed no significant trend ($r^2 = 0.001, F (1, 71) = 0.039, p >0.05$). This being the case no further analysis was appropriate, and it was concluded that changes in annual temperature could not have contributed to the increase in flowers per clump.

Table 4.7. Chi-Square analysis of survival of Tetrapheca juncea clumps over the sampling years 2005 to 2010.

$N1 =$ cumulative number of clumps lost year-on-year; $N2 =$ clumps surviving each year; $N3 =$ total clumps sampled each year.

<table>
<thead>
<tr>
<th>Year</th>
<th>$N1$</th>
<th>$%$</th>
<th>$N2$</th>
<th>$%$</th>
<th>$N3$</th>
<th>$%$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005 - 2006</td>
<td>11</td>
<td>2.40%</td>
<td>58</td>
<td>12.60%</td>
<td>69</td>
<td>14.90%</td>
</tr>
<tr>
<td>2006 - 2007</td>
<td>19</td>
<td>4.10%</td>
<td>78</td>
<td>16.90%</td>
<td>97</td>
<td>21.00%</td>
</tr>
<tr>
<td>2007 - 2008</td>
<td>26</td>
<td>5.60%</td>
<td>73</td>
<td>15.80%</td>
<td>99</td>
<td>21.40%</td>
</tr>
<tr>
<td>2008 - 2009</td>
<td>31</td>
<td>6.70%</td>
<td>68</td>
<td>14.70%</td>
<td>99</td>
<td>21.40%</td>
</tr>
<tr>
<td>2009 - 2010</td>
<td>39</td>
<td>8.40%</td>
<td>59</td>
<td>12.80%</td>
<td>98</td>
<td>21.20%</td>
</tr>
<tr>
<td>Total</td>
<td>126</td>
<td>27.30%</td>
<td>336</td>
<td>72.70%</td>
<td>462</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

$\chi^2(462,4) = 15.969, p = .003$

Section 4.3.3.1 demonstrated that neither rainfall nor temperature could have had an effect on total flowers over time. If attrition was biased towards the loss of small clumps, then the average size (i.e. more stems) of remaining clumps would increase over time with the number of flowers carried also increasing.
Chapter 4  A Spatiotemporal Study of Pollination in A Tetratheca juncea Subpopulation

The size of surviving clumps each year was recorded using a broad condition index of 1 = clumps having <10 stems and 2 = clumps having >10 stems. One-way ANOVA was used to determine whether there was a significant change in mean clump size over the study period of 2006 to 2010, five years. There was not a significant difference with F(4,321) = 0.097 p = 0.983.

While the condition index was coarse, it would have been adequate to detect the change in size needed to have resulted in flowers per clump more than doubling over the study period. It can be concluded that clump size did not contribute to the increase in flowers per clump.

4.3.5 Habitat Changes Over Time

Habitat floristic content and structure data were collected in 2002 and 2009 from ten 10 m x 10 m quadrats (see details in Section 4.2). Analysis was directed at ground habitat to determine whether any significant change had occurred that might explain the reduction of the Tetratheca juncea population over time. The means of cover abundance scores for ground species increased from 2002 (0.392±0.32SE) to 2009 (0.481±0.038SE). Examination of these data revealed a large number of zero values, non-normal distribution and unequal variances. No transformation could rectify these data problems so parametric tests (such as t-tests) were inappropriate. The non-parametric ANOSIM (Analysis of Similarities) module in Primer 6 (Clarke and Gorley 2006) operates on a distance matrix in a similar manner to a standard 1-way ANOVA, with the null hypothesis being that there is no significant difference between groups (in this case ground species abundance scores for years 2002 and 2009). ANOSIM (using a Bray-Curtis similarity resemblance matrix) showed that the global statistic R = 0.120 was marginally significantly different from zero (p = 0.051).

This analysis suggests that there was a real increase in the amount of competing ground species over those seven years. This increased competition could be related to the reduction in Tetratheca juncea reported in Section 4.3.4.
4.3.6 Spatial Analysis

Tobler’s first law of geography (Tobler 1970) states: "Everything is related to everything else, but near things are more related than distant things." One of these relationships is termed spatial autocorrelation where two or more attributes vary systematically with spatial location.

Referring to Figure 4.5, it is clear that the overall population was not evenly distributed. This analysis investigates whether there was spatial autocorrelation in FFR or total flowers per clump across the subpopulation. Nine variables were used in the analysis (Table 4.8).

Table 4.8. Variables used in the analysis to determine whether fruit flower ratio (FFR) and/or total flowers per clump (FLTotal) were spatially autocorrelated.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description and Codes defined</th>
</tr>
</thead>
<tbody>
<tr>
<td>PlantID</td>
<td>Just for reference</td>
</tr>
<tr>
<td>Year</td>
<td>Actual year</td>
</tr>
<tr>
<td>Density</td>
<td>The number of plants in 5x5 meters including the monitored plant</td>
</tr>
<tr>
<td>FLT</td>
<td>Total flowers on the clump</td>
</tr>
<tr>
<td>FFR</td>
<td>Fruit flower ratio</td>
</tr>
<tr>
<td>x</td>
<td>Geographic coordinate</td>
</tr>
<tr>
<td>y</td>
<td>Geographic coordinate</td>
</tr>
<tr>
<td>Attrition</td>
<td>1 = Loss, 0 = Non-loss</td>
</tr>
</tbody>
</table>

The analysis was conducted in three stages: (1) homogeneity analysis on attrition data (attrition versus time); (2) spatial regression analysis on the Density variable, where it was hypothesized that there was a relationship between clump density and the monitored clump’s parameters. Therefore, Spatial Lag Model was considered appropriate. Residual analysis using Lagrange multiplier diagnostics for spatial dependence may indicate that Spatial Error Model should also be considered.

Since Density did not change during the studied period and spatial analysis does not require the same location for different observations, then average FFR, average FLT and 2010 Attrition were used. Hence, time was not required for spatial analysis; (3) spatial regression analysis on pollinator activity using the same dataset. This analysis was dependent on the previous one, where if spatial analysis showed that there was no spatial effect then multiple linear regression would be required. The original data would then be used with time included.
Figure 4.5 The *Tetratheca juncea* study population and the pattern of attrition over time. The annual losses are cumulative of the previous year’s losses. The grid cell size is 5 m and used to determine a density value for spatial analysis (see Section 4.3.6).
Spatial regression analysis was based on several steps:

1. Moran I test on the dependent variable;
2. estimate OLS regression and associated residuals;
3. examine residuals for spatial autocorrelation using Moran I;

Finally, if the first test was significant i.e. there was spatial autocorrelation, then Spatial Lag Model would be required, otherwise, if the third test was significant i.e. there was spatial autocorrelation, then Spatial Error Model is required and, if both, then mixture model is required.

A t-test and z-test were used to assess the significance of the resulting models (OLS regression and spatial regression, respectively), where, if there was a non-significant relationship between exploratory fixed-effect variables and outcomes, they would be eliminated until the model became meaningful.

For spatial analysis and the Moran I test, a matrix of inverse distance weights was generated based on x and y coordinates.

For this analysis, the Density parameter was held constant across the years despite the analysis in Section 4.3.4 showing an overall decline of 27.3%. This assumption was made based on observation of Figure 4.5 above indicating that attrition was spread evenly across the population. Holding density constant enabled the hypothesis to be tested that at 2010 attrition was not spatially autocorrelated.

**Homogeneity analysis for attrition data**

The Cochran Q test was used to investigate whether the ratio of loss was different across years. Results show that $\chi^2(5) = 55.45$ and $p <0.001$, which was sufficient evidence to support the claim that the amount of loss was significantly different across years at any reasonable level of significance.

**Pearson correlation**

Preliminary analysis showed that Density had a weak to no relationship with other variables $r <0.05$. However, it seemed that FFR and FLT were weakly and negatively associated with $r = -0.133$ (Table 4.9).
Table 4.9. Pearson $r$ values showing correlation between variables and clump density across the *Tetratheca juncea* subpopulation (See Table 4.7 for a description of the variables).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Density</th>
<th>FLTotal</th>
<th>FFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>1</td>
<td>-0.003</td>
<td>0.067</td>
</tr>
<tr>
<td>FLT</td>
<td>-0.003</td>
<td>1</td>
<td>-0.133</td>
</tr>
<tr>
<td>FFR</td>
<td>0.067</td>
<td>-0.133</td>
<td>1</td>
</tr>
</tbody>
</table>

The full spatial analysis results are provided in Appendix 4 and have confirmed that the population distribution shown in Figure 4.5 was significantly spatially autocorrelated. However, there was no significant relationship between the spatial distribution of the population and the other variables. In other words, pollinator activity was no different between the more and less densely populated areas. The same was shown for the increase in total flowers per clump.

While the distribution of FFR was not spatially autocorrelated, it was affected to a small degree by Time and FLT. This was consistent with the analyses (Sections 4.3.2 and 4.3.3) that showed a decline in FFR and an increase in total flowers per clump over time.

### 4.4 DISCUSSION

This research project has delivered a range of new information on the overall aim of providing further insights into the relationship between *Tetratheca juncea* and its pollinators and habitat over time. Results can be summarised as follows:

- The distribution of the overall population was significantly spatially autocorrelated;
- The distribution of total flowers and pollinator activity as indicated by FFR, over time, was not significantly spatially autocorrelated;
- There was a significant decline in FFR of 66%;
- The mean number of flowers per clump increased by over 100%;
- Pollinator activity was not significantly different over the six years meaning that the fall in FFR was due to increased floral display rather than lower pollinator activity;
- There was a significant decline of 27% in clump numbers.
Chapter 4  A Spatiotemporal Study of Pollination in A Tetratheca juncea Subpopulation

*Tetratheca juncea* exists in the ground structural layer, and changes in that layer should have greater potential to impact the species through direct competition than either shrubs or canopy. The analysis conducted (Section 4.3.5) showed a marginally significant increase in ground species cover from 2002 to 2009. Examples of heterospecific competition observed were clumps overgrown by *Entolasia stricta* (R.Br.) Hughes (Poaceae), *Patersonia sericea* R.Br. (Iridaceae) and *Xanthorrhoea latifolia* subsp. *latifolia* (A.T.Lee) D.J.Bedford (Xanthorrhoeaceae).

The literature offers no explanation for the increase in flowers per clump over the study period. Rainfall and temperature were shown to have no effect (Section 4.3.3.1). Resource allocation theory (Haig and Westoby 1988) suggests that increased flower numbers could be a response to reduced pollinator activity resulting in less fruit production. However, analysis of total fruit per clump over time (Section 4.3.3.2) implied that pollinator activity did not change significantly. Was the increase in flowers a response to an overall population attrition of 27%? Unlikely in one respect, because attrition and total flowers were not spatially correlated (Section 4.3.6). If the increase in flowers was a response to population reduction, this would have more impact where clumps were closer than further apart. However, if increased competition by heterospecifics had also created stress in the surviving clumps then attempting to increase reproductive output would be a probable response. Of course, the success of this strategy would be entirely dependent on sufficient pollinators for pollinating the extra flowers. It was demonstrated by Gross *et al.* (2003), with further confirmation shown in Chapter 3 Section 3.4.3, that *Tetratheca juncea* fruit production is pollinator limited. In the case of the subpopulation under present discussion, it was clear that there were insufficient pollinators to meet the demand of increased flowering. In fact at no time throughout the monitored period did pollinator activity come close to saturation.

Has this apparent incapacity to cope with heterospecific competition revealed a weakness in the *Tetratheca juncea* life cycle? Not necessarily so. It may be that as competition starts to become overpowering, the species changes strategies from direct competition to preservation. Novoplansky (2009), borrowing from neuroscience, proposed the term *metaplasticity* to describe higher order responses to competition in plants than those of phenotypic plasticity. Increasing floral display size might be one
metaplastic response; another might occur underground. As noted in Chapter 1, the species is not entirely reliant on sexual reproduction but is also clonal, with new vegetative shoots developing from underground rhizomes. Studies of plant root behaviour have shown that, in a competitive environment, plants will develop greater root mass than when growing alone (reviewed in Laird and Aarssen 2005; Novoplansky 2009). Norton (unpub.) showed that *Tetratheca juncea* resprouted following fire. Driscoll (unpub.) found that, even when the main clump was killed by fire, resprouting occurred from rhizomes away from the original clump. It is therefore, possible that the species protects its future in the face of increasing heterospecific competition by increasing its below-ground biomass in preparedness for the inevitable fire.

This speculation leads to consideration of the place of fire in the *Tetratheca juncea* life cycle. Referring back to Chapter 3 Section 3.4.3, increased pollinator numbers and fruit set were reported for the flowering season following a bushfire. It appears likely that fire provides more suitable habitat, probably with more open ground, for pollinator nesting. Fire also initiates sprouting from rhizomes. Seed remain dormant until triggered by fire to germinate (Bellairs *et al.* 2006), and this is likely an adaptation that avoids exposing seedlings to the competitive environment that increases with time since fire (Novoplansky 2009). At no time during eight years of close investigation of *Tetratheca juncea* populations did the author find seedling recruitment, other than after fire. ‘High frequency fire resulting in the disruption of life cycle processes in plants and animals and loss of vegetation structure and composition’ is listed as a Key Threatening Process in the NSW *Threatened Species Conservation Act 1995*. An over-reaction to this listing has the potential to result in too infrequent fire intervals for species such as *Tetratheca juncea*.

The research in Chapters 2 and 3, and now this chapter, has demonstrated that FFR should be used with care when examining reproductive success in *Tetratheca juncea*; and by extension, any plants with similar prolonged reproductive seasons. True Fruit:Flower ratio is the ratio of total fruit and total flowers produced in an entire reproductive season for whatever the chosen sampling unit e.g. single inflorescence, single plant or several plants sampled from a population. This is readily achieved for plants with a short flowering period followed by fruit set. However, as illustrated with
*Tetrapheca juncea*, for plants with long reproductive seasons having concurrent budding, flowering, fruit set and seed release, FFR values taken at one time point within the reproductive season will at best be indicative of reproductive success up to that point (FFRi). At worst they can be outright misleading as was illustrated in Chapter 3 Section 3.4.7, with FFRi approaching 100% as flowering draws to a close (Figure 3.3 and Table 3.17); an artefact of the circumstance when only fruit are present. However, if used judiciously, FFR can still provide useful information on both reproductive yield and pollinator activity. Comprehensive reviews of Fruit:Flower ratio literature by Sutherland and Delph (1984) and Sutherland (1986) did not include information about how Fruit:Flower ratio data were collected and it is beyond the scope of this study to research this.

The current chapter has shown that treating FFRi values, in isolation, as a quantitative indicator of pollinator activity can also be misleading. FFRi values were shown to have declined over the study period (Section 4.3.2.1). However, the investigation of total fruit per clump showed that pollinator activity was constant, and decreasing FFRi was a result of increasing flower numbers (Section 4.3.2.2).

An unwritten implication of low FFR values is that they could represent less than adequate reproductive output. What then is the total seed output of *Tetrapheca juncea*? In the current study, there was a mean of two fruit per clump and Gross et al. (2003) reported a mean of 1.61±0.91 seed per fruit (out of a possible four). This equals 3.2 seed per clump or 1600 seed per season for the total subpopulation of approximately 500 clumps. Whether or not this number of seeds is adequate to maintain subpopulation viability depends on several unknowns amongst which are, life of the soil seedbank (including predation levels) and germination triggers.
CHAPTER 5

MODELLING THE HABITAT OF TETRATHECA JUNCEA
5.1 INTRODUCTION

It is a near impossible task to survey, in detail, large geographic regions for the presence of rare plants to determine their distribution, abundance and habitat preferences. However, valuable information can be obtained from a well-constructed habitat suitability model. For example: potential versus actual distribution of a species; amount of habitat loss and reduction in abundance that has occurred over time; fragmentation of populations; amount of habitat in reserve and under future threat; overall population size; insights into dispersal efficiency and history; associations with other species and vegetation communities; environmental preferences.

A variety of methods have been used over many years for predicting species presence or habitat suitability. For example: Mahalanobis distance (Mahalanobis 1936); generalised linear model (Nelder and Wedderburn 1972); generalised additive model (Hastie and Tibshirani 1990) and variants such as logistic or Gaussian logistic regression; Genetic Algorithm for Rule-set Production (GARP, Stockwell and Peters 1999); Ecological Niche Factor Analysis (ENFA, Hirzel et al. 2001); Discriminant analysis (Friedman 1989); Artificial neural networks (Gurney 1997).

When applied to species modelling these methods all have in common analysis of species presence only or presence/absence data (dependent variable) against a set of environmental variables (independent variable) referred to from hereon as EV. Most methods are developed around the Hutchinsonian concept of the environmental niche-the hypervolume in the multidimensional space of ecological variables in which a viable population can be maintained (Hutchinson 1957).

The environmental niche was then split into fundamental niche being the entire area of suitable habitat and realised niche, commonly considered a subset of the fundamental niche that the species occupies. A model based on presence only data will be primarily an approximation of the realised niche given that the model receives no input on the conditions of the fundamental niche (Phillips et al. 2006). However, an unknown but increasing amount of the fundamental niche will be included as presence data becomes a more representative sample from the species’ full distribution. Furthermore, realised niche need not always be a subset of fundamental niche with source/sink dynamics for
highly dispersed organisms sometimes resulting in presence being maintained in marginal habitat (Pulliam 1988, 2000). These models are referred to as Habitat Models, Species Distribution Models (SDM) or Environmental Niche Models (ENM).

Elith et al. (2006) evaluated 16 modelling methods using 226 species drawn from sources in 6 regions of the world, and using both presence/absence and presence only data. The predictive capability of the models was assessed using area under the Receiver Operating Characteristic curve (AUC), correlation and Kappa. One of the top performing models was Maxent (Phillips et al. 2004; Phillips et al. 2006; Phillips and Dudik 2008) and this was the method selected for this study.

Since its introduction, Maxent has been used to model habitat suitability for a variety of species or groups in many locations around the world (See Table 5.1 for several examples).

Table 5.1. Examples where Maxent has been used in habitat suitability modelling.

<table>
<thead>
<tr>
<th>Location</th>
<th>Subject</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa and Asia</td>
<td>Viverid mammals</td>
<td>Papes and Gaubert 2007</td>
</tr>
<tr>
<td>Amazon and Andean regions</td>
<td>Several fauna species</td>
<td>Buerrmann et al. 2008</td>
</tr>
<tr>
<td>Brazil</td>
<td>Amazonian tree species</td>
<td>Saatchi et al. 2008</td>
</tr>
<tr>
<td>California</td>
<td>Four rare plant species</td>
<td>Gogol-Prokurat 2011</td>
</tr>
<tr>
<td>China</td>
<td>Nematode distribution</td>
<td>Yun-Sheng et al. 2007</td>
</tr>
<tr>
<td>Europe</td>
<td>Bryophytes</td>
<td>Sergio et al. 2007</td>
</tr>
<tr>
<td>Madagascar</td>
<td>Gekkos</td>
<td>Pearson et al. 2007</td>
</tr>
<tr>
<td>New Zealand</td>
<td>Invasive ants</td>
<td>Ward 2007</td>
</tr>
<tr>
<td>Oregon</td>
<td>Sage Grouse nesting habitat</td>
<td>Yost et al. 2008</td>
</tr>
<tr>
<td>South America</td>
<td>Wild Tomatoes</td>
<td>Nakazato et al. 2010</td>
</tr>
<tr>
<td>Spain</td>
<td>Little Bustard distribution</td>
<td>Suárez-Seoane et al. 2008</td>
</tr>
<tr>
<td>Texas</td>
<td>Invasive species</td>
<td>Young et al. 2007</td>
</tr>
</tbody>
</table>

In addition to rating well in the performance comparison, Maxent has two useful attributes: the ability to accept both continuous and categorical EV; and, to model using target species presence only data. True absence data can be difficult to obtain, particularly for a rare plant that only occurs sporadically across a landscape. Without conducting an exhaustive survey at a fine scale, the field researcher can never be sure that the species was not present a short distance from where it was absent. In particular, if that distance is less than the model resolution, the false negative can result in a misleading model. Also, absences do not necessarily mean that the species might not be
present at a location sometime in the future, or have been there in the past. It could be that the species has not yet dispersed to that location, has temporarily been out-competed or is yet to recover from a stochastic event such as fire.

Maxent operates on the principle of maximum entropy introduced by E.T. Jaynes (Jaynes 1957) described as providing a means to obtain least-biased statistical inference when insufficient information is available and only using available information. Entropy is a measure of randomness, and the state of maximum entropy is that which is most random given any prevailing constraints. Translating this into environmental niche terms, constraints are the combination of EV at each of the species presence locations. Maxent does not treat each EV independently but in keeping with ‘real life’, uses interactions between the variables termed features which are the EV or a function of the EV. Features used are: Linear, the variable itself; Quadratic, variable squared; Product, the product of two variables; Binary, categorical; Threshold, proportion above a threshold; and Hinge, mean above/below a threshold.

The area to be modelled is divided into a grid of suitable cell size, and the state of maximum entropy is arrived at when each cell has been assigned a value proportionate to that of the combined average of the features at presence sites. To avoid over fitting, where the modelled distribution is tightly associated with the presence records, a process called regularisation is applied. Regularisation expands feature means by bounds so that the model fits on environmental conditions ‘like’, rather than identical to, those prevailing at presence points. The level of regularisation can be altered by the modeller. Because the environmental conditions at suitable habitat locations across a region are never identical, Maxent assigns values to cells according to their degree of similarity to that combined feature average. The process also uses background data, being a large enough sample of the cells not occupied by the target species to be representative of the whole; these are pseudo absences. The modelling process itself is derived from machine learning using the process of training. A probability distribution is determined across the background data (default around 10,000 cells) such that the cumulative value of all cells sums to 1 and is extended to the overall model area with the sum of all cells exceeding 1. This is referred to as the raw Maxent output (which is exponential) with the value of each cell being extremely small and difficult to interpret.
in terms of habitat suitability or probability of occurrence. The raw output can be transformed into more useful cumulative or logistic formats.

The default logistic format is an expression of the probability of occurrence of the subject species given the EV and features on which the model was built. However, it is not a true probability because no absence data has been provided, and the field sampling methodology is unknown. Its interpretation is that a value of 0.5 represents the average probability of occurrence given the environmental conditions with >0.5 having a better than average probability of occurrence, and <0.5 a worse than average probability of occurrence. Because these probabilities have been generated from the relationship of known occurrences to the provided EV, the logistic output can be used as a surrogate for habitat suitability.

Collection bias (a form of spatial autocorrelation) has the potential to distort or misdirect a model. Distortion can come about from model training presence records drawn from a small part of the overall distribution. Misdirection can be the consequence of records being from geographically biased locations within the overall distribution. A remedy for a locally distorted model could be to engage in an iterative process whereby the model is used to suggest areas for further survey. New records are added, the model re-run, and surveys repeated until a more representative training data set is obtained (Pearson et al. 2007; Wisz et al. 2008). A nice example of this is in de Siqueira et al. (2009) where they started with only one record of a rare plant and expanded that to seven, across a wide geographic range.

Geographic bias is a common feature of herbarium records arising from collections from easily accessible areas such as near roads. Roads are generally located on flat terrain such as along ridges, and even though the records might cover the distribution of the species a model developed from these records inadvertently becomes a model of the location of roads. Such bias is not easy to correct: Dudik et al. (2005) proposed various ways of manipulating the presence data to reduce the impact of bias; and, Phillips et al. (2009) examined the effectiveness of restricting background data (pseudo absences) to the locality of the biased presence records.

It is important to assess how well the geographic distribution of records covers the available environmental conditions of the modelled area. It can be that even though
Chapter 5 Modelling the Habitat of Tetratheca juncea

records might be correlated with accessibility, the access network and sampling is sufficiently extensive to be environmentally unbiased.

The aims of modelling *Tetratheca juncea* habitat suitability were:

1. To estimate habitat lost since European settlement. In addressing this question, a convention is followed that prior to 1750 there was no European impact in Australia (Burgman *et al.* 2001), referred to as *pre-1750*. Remnant habitat at any point in time after 1750 is referred to as *extant*.
2. To compare the degree of habitat fragmentation between *pre-1750* and *extant* habitat.
3. To estimate *pre-1750* and *extant* population size.
4. To estimate the amount of *extant* habitat in conservation reserves.
5. To gain insight into habitat requirements.
6. To compare potential extent of occurrence with current known area of occupancy.

**Figure 5.1** shows the boundaries of the Central Coast and North Coast models. Apart from covering the species’ distribution, the areas were sized to incorporate the extent of the coastal Local Government Areas.

### 5.2 METHODS AND MATERIALS

#### 5.2.1 Modelling Tools and Scope

Models were prepared using Maxent version 3.3.3e ([www.cs.princeton.edu/~schapire/maxent/](http://www.cs.princeton.edu/~schapire/maxent/)) with settings being default other than for using 1000 iterations. ENMTools version 1.3 ([Warren *et al.* 2010](http://www.sagagis.org)) was used to determine correlation between environmental variables, niche overlap and for model selection. Preparation of environmental variables and spatial analysis was conducted in Manifold System 8 GIS ([www.manifold.net](http://www.manifold.net)), SAGA 2.0 ([www.sagagis.org](http://www.sagagis.org)) and Surfer 9 ([www.goldensoftware.com](http://www.goldensoftware.com)). IBM SPSS Statistics 19 was used for statistical analysis.

The scope of the models was the entire area including cleared and developed land so that *pre-1750* and *extant* habitat, and habitat loss could be estimated. A vector drawing of *extant* vegetated habitat was prepared for each model area by tracing from recent
Figure 5.1. *Tetrapheca juncea* habitat suitability model boundaries. The boundaries were established around each regional population and sized to incorporate the extent of the coastal Local Government Areas.
orthorectified aerial imagery. Final models were cropped to the vegetation drawing to show extant habitat.

### 5.2.2 Samples

_Tetratheca juncea_ records were collated from the Atlas of NSW Wildlife (referred to as Atlas data), unpublished data and records acquired as part of this research project. Final models were prepared using data available up to and including March 2011.

Presence records were assessed for collection bias which had two main sources: accessibility bias from opportunistic sightings along vehicular tracks; and, locally concentrated records where population density assessments had been conducted. There was also a more subtle potential for bias resulting from surveys related to development applications with data being from the fringes of urban areas; a significant motivator for threatened species surveys came with the introduction of the NSW Threatened Species Conservation Act 1995 (see Chapter 6 for more on this).

Following collation from all data sources the following steps were taken to prepare a set of samples suitable for model input:

1. Atlas data is provided with levels of accuracy from a few metres up to 10,000 m and all records with accuracy >100 m were removed.
2. Removed duplicates.
3. The model grid size was 100 m square and many cells contained multiple samples. To eliminate a potential source of bias in the modelling process samples were reduced to one per occupied grid cell. The centre point (centroid) of the occupied cell was used as a surrogate record.
4. To reduce sampling bias the set of centroids (from Step 3) was overlaid in Manifold GIS on a 1 km square grid (Central Coast) or a 500 m square grid (North Coast). These grid sizes were arbitrarily selected to suit the spread and density of the centroids. Using some tailored code one centroid was selected at random from within each grid cell containing occurrence centroids. One hundred sets of random samples were made. This procedure of using the coarse grid overlay has not previously been reported and was used in order to retain the known spread of samples across geographic and environmental space while reducing bias.
5. ENM’s were then created in Maxent using the raw output option and 1000 iterations for each of the 100 random sample sets with all other Maxent settings being defaults.
6. Using ENMTools (Warren et al. 2010), the resulting 100 models were then each tested for degree of fit against the full set of centroid samples data.
created in Step 3. The Akaike Information Criteria metric constrained for small samples (AICc, Akaike 1974) was computed for each model. The set of centroid samples that resulted in the lowest AICc value (i.e. which best predicted the larger centroids samples set) was selected as training input for the final models.

7. Centroid samples from the set having the lowest AICc were subtracted from the set having the highest AICc with the balance being used to test the final Maxent model. This was done on the assumption that these were from the poorest fit out of the 100 replicates.

5.2.3 Environmental Variables

EV were selected for biological or ecological relevance. The resolution of the source data and whether it could reasonably be used as a 100 m grid was also a selection consideration. For example, there is a large amount of gridded climate data available (www.worldclim.org) but the grid size is 1 km and so cannot be usefully used in a 100 m grid model. Also, with respect to climate data, the restricted geographic range of Tetratheca juncea, meant that it was more likely that local environmental factors would be the main drivers of the species’ distribution (Guisan and Thuiller 2005).

Categorical grids originated from primary data sources that were in vector polygon format e.g. soil landscape type. To develop categorical grids the various attributes (e.g. soil type) were given a sequential number and this number was transferred to each overlying cell of a 100m vector grid. Continuous grids were prepared from raster grids such as a Digital Terrain Model (DTM) with the values of each grid cell being passed to the master vector grid. The final input grids were created by converting the 100m vector cells into raster grids without interpolation. Table 5.2 lists the EV prepared and their origin.

Inclusion of potential biotic interactions in the model variables, such as competitors, pollinators and similar mutualisms could be informative and was considered. The main difficulty was the lack of sufficient data covering the geographic range of the models.

Probably, rather than attempt to include biotic factors in the model variables, tests of model overlap of separate target species and biotic factor models would be more appropriate (e.g. Trethowan at al. 2011).
Table 5.2. Environmental variables prepared for the *Tetratheca juncea* habitat suitability model, and their origin.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Code</th>
<th>Format</th>
<th>Data type</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elevation</td>
<td>ELEV</td>
<td>Raster</td>
<td>Continuous</td>
<td>A 25 m DTM (NSW Digital Topographic Database) was resampled in GIS to 100 m resolution using bicubic interpolation.</td>
</tr>
<tr>
<td>Aspect</td>
<td>ASPECT</td>
<td>Raster</td>
<td>Categorical</td>
<td>Extracted in Manifold GIS from the 100m DTM then partitioned into 8 categories 22.5° either side of N, NE, E etc. An additional category 9, of flat was created for all areas where slope &lt;1° .</td>
</tr>
<tr>
<td>Slope</td>
<td>SLOPE</td>
<td>Raster</td>
<td>Continuous</td>
<td>Extracted in GIS from the 100 m DTM.</td>
</tr>
<tr>
<td>Terrain roughness index</td>
<td>TRI</td>
<td>Raster</td>
<td>Continuous</td>
<td>Computed in Manifold GIS from the 100 m DTM after Riley et al. (1999).</td>
</tr>
<tr>
<td>Wetness index</td>
<td>WI</td>
<td>Raster</td>
<td>Continuous</td>
<td>Created in SAGA.</td>
</tr>
<tr>
<td>Geological Period and Lithology</td>
<td>GEOPER</td>
<td>Vector</td>
<td>Categorical</td>
<td>Data from the NSW Department of Mineral Resources (DMR 1999).</td>
</tr>
<tr>
<td>Rainfall</td>
<td>RAIN</td>
<td>Raster</td>
<td>Continuous</td>
<td>A rainfall raster was prepared by Kriging in Surfer using historical rainfall station data supplied by the Australian Bureau of Meteorology.</td>
</tr>
<tr>
<td>Summer and winter insolation</td>
<td>INSDEC</td>
<td>Raster</td>
<td>Continuous</td>
<td>Prepared in SAGA for the longest (December 21) and shortest (June 21) days from the 100 m DTM.</td>
</tr>
<tr>
<td>Summer and winter solar radiation</td>
<td>SRDEC</td>
<td>Raster</td>
<td>Continuous</td>
<td>Prepared in SAGA for the longest (December 21) and shortest (June 21) days from the 100 m DTM.</td>
</tr>
<tr>
<td>Summer and winter hillshading</td>
<td>AHSDEC</td>
<td>Raster</td>
<td>Continuous</td>
<td>Prepared in SAGA for the longest (December 21) and shortest (June 21) days from the 100 m DTM.</td>
</tr>
<tr>
<td>Distance to nearest stream</td>
<td>STRDIST</td>
<td>Raster</td>
<td>Continuous</td>
<td>Computed in Manifold GIS. The distance of each grid cell from the nearest stream using Lands NSW streamline data.</td>
</tr>
</tbody>
</table>

Chapter 3 raised the possibility that *Tetratheca juncea* populations are vulnerable to interspecific competition with the increase of time since fire. An attempt was made to include fire frequency as a variable. Past records were obtained from the New South Wales Rural Fire Service but an audit of these records against known fires revealed that they were not sufficiently complete to be useful.
While Maxent is tolerant of some degree of correlation between EV it is better to avoid using those that are clearly correlated. Correlated EV exist where one variable has some form of relationship with another. For example, Wetness Index and Slope could be negatively correlated (flat ground being likely to have a high wetness index) meaning that the presence of one could be a surrogate for the other. Using correlated variables in Maxent might not have a significant impact on the final geographic model, but makes for difficulty interpreting which of the correlated EV are significant for the subject species. ENMTools was used to determine the amount of correlation between each EV pair. The output matrix was examined and pairs having $r^2 \geq 0.8$ were assessed to determine which EV would best not be run together in a model.

5.2.4 Choosing a Threshold

The degrees of habitat suitability provided in the Maxent logistic output are potentially useful for additional targeted searches for the species. However, a binary distribution map is required for determining overall habitat extent, threat and loss as well as facilitating estimates of total population size and conservation levels. To create a binary map a threshold needs to be determined above which the species is considered to be present. Several (up to 12) methods have been proposed for setting a threshold (Anderson et al. 2003; Liu et al. 2005; Jimenez-Valverde and Lobo 2007; Freeman and Moisen 2008) with no one method standing out as preferable for all models or species.

In principle, thresholds are determined based on the relationship between specificity and sensitivity, omission and commission, lowest known presence probability or simply an arbitrary probability cut-off. One point of agreement was that the frequently used method of 50% predicted presence/suitability as a cut-off was one of the worst.

An important consideration in choosing a cut-off is that one would not necessarily expect the model to predict all known presence locations. From a niche consideration and taking dispersal into account, not all recorded occurrences will be in preferred habitat.

If a plant species has an active dispersal mechanism, then seed can be deposited and germinate in a variety of habitat types. An individual or small sub-population of the
species might even become established for a while in marginal habitat (Pulliam 1988 and 2000). However, the continued presence of the species in these habitats is entirely dependent on migration from source habitats because environmental conditions are such that death rate exceeds birth rate. Conversely, species having dispersal limitations will probably only occupy a fraction of their fundamental niche.

The threshold condition of *equal training sensitivity and specificity* was chosen because the resulting model was not smeared across habitat known not to support *Tetratheca juncea*.

### 5.2.5 Model Validation

The validity of an ENM can be measured by the degree of success for correctly predicting known presences (and absences). For binary models built on presence/absence data, an assessment can start with a confusion matrix created from the true and false presences and absences (reviewed in Fielding and Bell 1997). To achieve the same from a model of continuous suitability values a threshold must be set above which the habitat is considered suitable (or the species is considered to be present). These are *threshold dependent* methods.

Maxent provides a *threshold independent* version of area under the curve (AUC) of the receiver-operating characteristic (ROC, Fielding and Bell 1997). The curve is the plot of sensitivity (proportion of presences correctly predicted; omission error) and 1-specificity (proportion of correctly predicted absences; commission error) across the full range of possible thresholds. In other words, it is a plot of true positives vs false positives across the threshold range of 0 to 1 where, at a threshold of 0 all of the model area is predicted as suitable. The AUC statistic is best obtained using independent test data. Partitioning species presence data is a commonly used method whereby the model is trained on a proportion of the data then tested on the withheld balance. The amount withheld for testing depends on the total number of presence points available but is commonly 10 – 25% of the total. The option is also available to provide an independent set of data for testing.
However, as a measure of accuracy of ENM, the AUC statistic should be used with caution, if at all (Lobo *et al.* 2008). Among other things, it is not a measure of goodness-of-fit of the model and does not incorporate the predictions, and its value artificially increases as the proportion of pseudo absences to presence records increases. Thus a model created from presence records contained within a fraction of the overall geographic area will result in an unrealistically high AUC as a consequence of the preponderance of correctly predicted absences.

Warren and Seifert (2011) show how information criteria can be applied to model selection. ENMTools (*Warren et al.* 2010) provides the means to select models based on the Akaike Information Criteria (AIC), the small samples corrected form (AICc) or the Bayesian Information Criteria (BIC). For a comprehensive explanation of these information theoretic model selection methods see Burnham and Anderson (2004); these authors conclude that AICc is the better of the three alternatives.

AICc values themselves are not interpretable, however the model with the lowest AICc values out of the full set of models is considered to be the best approximation to the unknown true model. The relative probability that the model with the lowest AICc value was the best approximation out of the full set tested was determined by calculating Akaike weights $w_i$ as follows:

$$w_i = \exp \left(-\frac{\Delta_i}{2}\right) / \sum_{r=1}^{R} \exp \left(-\frac{\Delta_r}{2}\right)$$

where $\Delta_i = \text{AICc}_i - \text{AICc}_{\text{min}}$ and $R$ is the full set of individual models $r$.

As can be seen from the above equation, over the full set of models $w_i$ sums to 1 providing an expression of the relative probability that a model is the best model in the full set.

In addition to assessing the predictive ability of individual models, measures for comparing model coverage are useful. Degree of overlap indices can be used to compare models for the same species using different modelling settings or to evaluate niche similarity between species. Rodder and Engler (2011) evaluated five indices and
found that Schoener’s D and Bray Curtis were the best performers; Schoener’s D, available in ENMTools, was selected for this project.

5.2.5.1 A two-edged validation

Reliable absence data were not available for *Tetratheca juncea*. However, field experience shows that the species will never be found in habitat that is subject to periodic inundation. A tree canopy indicator for such habitat is *Eucalyptus robusta* (Swamp Mahogany). Consequently, a model of *Eucalyptus robusta* habitat should have little overlap with a model of *Tetratheca juncea* habitat.

Samples data for *Eucalyptus robusta* from within the Central Coast model area (Bell and Driscoll unpub.) were prepared in the same manner as for *Tetratheca juncea* (Section 5.2.2). A Maxent model for *Eucalyptus robusta* was prepared using the same parameters as were used for *Tetratheca juncea* including a threshold of equal training sensitivity and specificity.

Thirty models were run for each species using 30 sets of samples drawn randomly from the 100 sets of samples described above in the samples preparation section.

Two tests were applied to the final binary models:

1. The 30 models were randomly paired and tested for overlap using Schoener’s D (ENMTools) which is scaled from 0 for no overlap to 1 for complete overlap. Rodder and Engler (2011) make the point that it is better to use a binary model for overlap testing than a continuous model, particularly when there are large areas having low probability as was the case with the models for *Tetratheca juncea* and *Eucalyptus robusta*.
2. Using the best performing set of samples from the original randomly selected 100, the proportion of total samples was determined for each species occurring in the following habitat categories: suitable and unsuitable, and the overlap of the suitable areas for the two species. The significance of the result was tested using paired t-tests.

5.2.6 Abundance Estimate

Because they model probability of presence, ENM’s cannot of themselves describe abundance. However, it is possible to estimate abundance by using field-collected abundance data extrapolated across the area modelled as suitable habitat after applying a suitable threshold. To this end, for this project the number of *Tetratheca juncea* clumps
were recorded at ten locations throughout the Central Coast area; two unpublished data sets were also obtained.

5.2.7 Models

5.2.7.1 Central Coast

The Central Coast is an area that has been extensively surveyed for *Tetratheca juncea* as a consequence of significant development pressures which is reflected in the high number of records. A large amount of the western part of the model area has been State Forest (some of which is now conservation reserves), and targeted *Tetratheca juncea* surveys were conducted through these areas (Adam Fawcett pers. com.). Consequently it was appropriate to model the entire area allowing the model to extrapolate from the area containing the samples into areas with no samples coverage.

5.2.7.2 North Coast

There are few *Tetratheca juncea* records for the North Coast. A contributing factor would be that the incentive to survey for the species is low because of low development pressure. For this reason, the North Coast model was run in two stages. First an arbitrary common enclosing rectangle boundary was placed around the samples and extended by 2 km. The model was run on this constrained area and then projected into the remaining area. For comparison purposes, the model was also run on the entire North Coast area.

5.3 RESULTS

5.3.1 Environmental Variables

Correlation testing between all possible pairs of EV for the Central Coast and North Coast model areas resulted in SLOPE and AHSJUN being excluded, leaving 14 EV for modelling (Table 5.2 above).

5.3.2 Samples Preparation

The results of the samples preparation steps described in Section 5.2.2 above are shown in Table 5.3.
Table 5.3. Sample numbers based on the stages of samples preparation.
Samples were prepared as described in section 5.2.2.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Central Coast</th>
<th>North Coast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw data</td>
<td>7451</td>
<td>77</td>
</tr>
<tr>
<td>Occupied 100 m grid cells</td>
<td>1633</td>
<td>67</td>
</tr>
<tr>
<td>Randomly selected from 1 km (CC) or 500 m (NC) grid cells and used in the models</td>
<td>338</td>
<td>53</td>
</tr>
</tbody>
</table>

5.3.3 Central Coast Regional Population

5.3.3.1 Selecting the best set of samples for model training and testing

As described in section 5.2.2 samples for use as training data were selected from 100 replicates randomly selected from the samples within 1 km grid cells. Using ENMTools models were tested for fit against the 1633 centroids that represented the 100 m grid cells occupied by the original samples data points. The metric used was AICc and Replicate 58 had the lowest AICc of 36145.17 and Replicate 84 the highest of 37483.07. The Akaike weight \( w_i \) (Section 5.2.5) of Replicate 58 was 0.94 and that of the next nearest replicate was 0.03 meaning that Replicate 58 was clearly the model with the best fit to the data.

By comparison, the AUC for Replicate 58 was 0.901 and for Replicate 84 was 0.895. However, these were neither the lowest (0.894) nor highest (0.904) AUC values for the 100 replicates. AUC and AICc were not significantly correlated with Pearson \( r = -0.144, p > 0.05 \).

The 100 replicate models were tested for niche overlap using Schoener’s D as calculated in ENMTools with overlap being determined for each possible pair; a value of 1 means complete overlap and 0 means no overlap. The value of D for the overlap of Replicate 84 with Replicate 58 was 0.655 with the range for all pairs being 0.646 to 0.912. Replicate 58 was used to train the model.
5.3.3.2 Model validation

One method for model testing is to withhold a randomly selected fixed percentage of samples and test the model using these data. This procedure can be replicated any number of times using a randomly selected different set of test samples in order to achieve the best model or the average model. In this case, the replication has been carried out in the samples selection process. Rather than reduce the samples that had already been shown to produce the best model another set of samples was created. Samples were extracted from Replicate 84 that were not contained in Replicate 58 giving 189 test samples. This method retained the distribution of training samples across geographic and environmental space.

Maxent provides two graphs that show the quality of the model. Figure 5.2 is a plot of omission vs predicted area for training and test samples. The omission value is the false negative error, and the threshold is the proportion of the model area predicted as suitable. As the threshold increases from zero where the whole area is predicted as suitable, the omission errors increase. The fewer omission errors there are the closer the training and test lines come to the ideal predicted omission line.

Figure 5.3 is a plot of the receiver operating curve (ROC) for training and test samples and includes the area under the curve (AUC) value which is an index of how different from random the model is. The ROC curve and associated AUC value are normally used for validation of models using presence-absence data. In Maxent, this has been developed as a threshold-independent method using fractional predicted area instead of commission rate (fraction of absences predicted as present).

5.3.3.3 Tetratheca juncea and Eucalyptus robusta

The research question here was: knowing that Tetratheca juncea is not found growing in habitat subject to periodic inundation and using Eucalyptus robusta as an indicator species for that habitat, will a Maxent model of both species using the same methods clearly discriminate between the habitat of the two species?
Figure 5.2. A plot of omission vs predicted *Tetratheca juncea* suitable habitat area for the Central Coast model. This plot shows that there were very low omission rates for both the training and test data.

Figure 5.3. A plot of the ROC curve for the *Tetratheca juncea* Central Coast habitat suitability model. This plot shows AUC values well above those of a random prediction.
Figure 5.4 shows the two models together with the areas that overlap. Consistent with the preferred habitat of *Eucalyptus robusta* the model for that species is aligned with the main drainage lines and flood plains.

**Overlap test**
Mean overlap value of the 30 models was (Schoener’s D) 0.244±0.005SE, meaning that the modelling process was able to discriminate clearly between the two habitat types.

**5.3.3.4 Habitat occupancy test**
This test compared the proportions of samples for each species that occurred in 30 replicates of modelled suitable habitat, overlapping habitat and unsuitable habitat. Paired t-tests were used to determine the significance of the difference between the proportion of each species occurring in *Tetratheca juncea* and *Eucalyptus robusta* habitat, overlapping habitat and unsuitable habitat.

The distribution of *Tetratheca juncea* and *Eucalyptus robusta* samples in each habitat category is shown in Figure 5.5.

Differences for each species in each habitat were normally distributed (Shapiro-Wilk significance >0.05). Correlation between the pairs was not significant.

The paired t-tests (Table 5.4) showed that the differences between the proportion of samples in each habitat were significant ($p <0.001$).

Ecologically one would expect that there would be some overlap between the two models because in reality habitat boundaries are fuzzy rather than discrete (Leung 1987). The area of overlap could be properly described as the ecotone (Kolasa and Zalewski 1995) where one habitat merges into the other.

**5.3.4 North Coast Regional Population**

**5.3.4.1 Selecting the best set of samples for model training and testing**
As described in Section 5.2.2, samples for use as training data were selected from 100 replicates randomly selected from the samples within 500 m grid cells.
Figure 5.4. Central Coast model of *Tetrapheca juncea* and *Eucalyptus robusta* habitat.
Figure 5.5 The proportion of samples of each species present in each habitat type with SE bars.
Key: TJ = *Tetrateca juncea*, ER = *Eucalyptus robusta*, TJH = *Tetrateca juncea* habitat, ERH = *Eucalyptus robusta* habitat, OLH = overlapping habitat, USH = unsuitable habitat.

Table 5.4 Paired t-test results for the proportion of samples occurring in the different *Tetrateca juncea* and *Eucalyptus robusta* habitat categories.
Key: TJ = *Tetrateca juncea*, ER = *Eucalyptus robusta*, TJH = *Tetrateca juncea* habitat, ERH = *Eucalyptus robusta* habitat, OLH = overlapping habitat, USH = unsuitable habitat.

<table>
<thead>
<tr>
<th>Pair</th>
<th>Paired Differences</th>
<th>Mean</th>
<th>StDev</th>
<th>StErr</th>
<th>95% Confidence Interval of the Difference</th>
<th>t</th>
<th>df</th>
<th>Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TJ_TJH - ER_TJH</td>
<td>0.562</td>
<td>0.021</td>
<td>0.004</td>
<td>0.554 - 0.570</td>
<td>145.468</td>
<td>29</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>ER_ERH - TJ_ERH</td>
<td>0.366</td>
<td>0.051</td>
<td>0.010</td>
<td>0.347 - 0.384</td>
<td>39.426</td>
<td>29</td>
<td>0.000</td>
</tr>
<tr>
<td>3</td>
<td>ER_OLH - TJ_OLH</td>
<td>0.306</td>
<td>0.053</td>
<td>0.010</td>
<td>0.286 - 0.325</td>
<td>31.461</td>
<td>29</td>
<td>0.000</td>
</tr>
<tr>
<td>4</td>
<td>TJ_USH - ER_USH</td>
<td>0.110</td>
<td>0.018</td>
<td>0.003</td>
<td>0.103 - 0.116</td>
<td>33.601</td>
<td>29</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Models were tested for fit against the 77 centroids that represented the 100 m grid cells occupied by the original samples data points. The metric used was AICc and Replicate 43 had the lowest AICc of 1452.51 and Replicate 39 the highest of 1701.13.
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The Akaike weight $w_i$ (Section 5.2.5) of Replicate 43 was 0.43 and that of the next nearest replicate was 0.35 meaning that Replicate 43 was marginally the model with the best fit to the data.

By comparison, the AUC for Replicate 43 was 0.958 and for Replicate 39 was 0.957. However, these were neither the lowest (0.953) nor highest (0.959) AUC values for the 100 replicates. AUC and AICc were not significantly correlated with Pearson $r = -0.013$, $p >0.05$.

The 100 replicate models were tested for niche overlap using Schoener’s D as described in Section 5.3.3.1. The value of D for the overlap of Replicate 43 with Replicate 39 was 0.967 with the range for all pairs being 0.885-0.994. Replicate 43 was used to train the model.

5.3.4.2  Model validation

Using the same procedure as for the Central Coast (Section 5.3.3.2), the test samples used were those in Replicate 39 that were not contained in Replicate 43 resulting in 9 test samples. There were no available data for the North Coast to conduct a validation test based on *Eucalyptus robusta*, so the two validation measures provided by Maxent were relied upon. The plot of omission vs predicted area (Figure 5.6) shows that both training and test data sets lie close to the predicted omission rate. The training and test AUC values (Figure 5.7) are high and well above that of a random prediction. These results are from the model that was projected from the area constrained by known samples into the remaining habitat.

**Figure 5.8** shows a composite of the North Coast models. The constrained and then projected model is quite different to the unconstrained model with a substantial amount of habitat predicted as suitable in the projected area.

When projecting, Maxent compares the environmental conditions in the projected area against those predicted as suitable in the constrained area. A further assessment is made of the individual EV and values in the projected area that are outside of their
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Figure 5.6. Omission vs predicted area for the North Coast *Tetratheca juncea* habitat suitability model.
This plot shows that there were very low omission rates for both the training and test data.

Figure 5.7. The ROC curve for the North Coast *Tetratheca juncea* habitat suitability model.
This plot shows AUC values well above those of a random prediction.
Figure 5.8. The North Coast *Tetratheca juncea* habitat suitability models. The first model was constrained within the oblique rectangle and then projected to the environmental conditions in the remaining area. The second model was unconstrained and modelled the entire area.
range in the constrained area model are mapped (Elith et al. 2010). These areas are the novel limiting areas shown in Figure 5.8 and are removed from the final model. The EV contributing to these areas were primarily elevation and soil.

By comparison, the unconstrained model hardly extends outside of the area bounded by the training samples.

5.3.5 Habitat Attributes

5.3.5.1 Habitat fragmentation
Only the Central Coast model was used for this analysis because of the ambiguity of the North Coast model. The impact of clearing on habitat fragment sizes is shown in Figure 5.9, and Table 5.5 shows the size and distribution of the major fragments.

---

**Figure 5.9.** Individual values plot of the habitat fragments in the *Tetratheca juncea* Central Coast habitat suitability model for pre-1750 and extant habitat.
Table 5.5. Distribution of major habitat fragments in the *Tetratheca juncea* Central Coast habitat suitability model for pre-1750 and extant habitat.

<table>
<thead>
<tr>
<th>Fragment size</th>
<th>pre-1750</th>
<th>extant</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000ha</td>
<td>79.20</td>
<td>29.00</td>
</tr>
<tr>
<td>&gt;100-1000ha</td>
<td>4.69</td>
<td>34.80</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>83.89</strong></td>
<td><strong>63.80</strong></td>
</tr>
</tbody>
</table>

The distribution of fragment sizes in both the pre-1750 and extant habitats were not normally distributed (Figure 5.9) (Shapiro-Wilk *p* <0.001). To determine the significance of the differences in size and distribution of fragments between the two periods a series of non-parametric tests were conducted.

The results of significance testing (Table 5.6) indicate that European activity has had no impact on the distribution of fragment size but has significantly reduced the median and range of fragment sizes.

**Table 5.6. Results of non-parametric tests of the significance of size and distribution of *Tetratheca juncea* suitable habitat fragment sizes for the Central Coast model.**

<table>
<thead>
<tr>
<th>Null Hypothesis</th>
<th>Test</th>
<th>Sig.</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>The distribution of Fragments is the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Wald-Wolfowitz Runs Test</td>
<td>1.000</td>
<td>Retain the null hypothesis.</td>
</tr>
<tr>
<td>The distribution of Fragments is the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Mann-Whitney U Test</td>
<td>0.061</td>
<td>Retain the null hypothesis.</td>
</tr>
<tr>
<td>The distribution of Fragments is the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Kolmogorov-Smirnov Test</td>
<td>0.268</td>
<td>Retain the null hypothesis.</td>
</tr>
<tr>
<td>The distribution of Fragments is the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Kruskal-Wallis Test</td>
<td>0.061</td>
<td>Retain the null hypothesis.</td>
</tr>
<tr>
<td>The medians of Fragments are the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Median Test</td>
<td>0.043</td>
<td>Reject the null hypothesis.</td>
</tr>
<tr>
<td>The range of Fragments is the same across pre-1750 and extant habitat.</td>
<td>Independent-Samples Moses Test of Extreme Reaction</td>
<td>0.000</td>
<td>Reject the null hypothesis.</td>
</tr>
</tbody>
</table>

**Figure 5.10** shows the final pre-1750 and extant models for both areas. The model for the North Coast is complex because of the small number of samples available from a
Figure 5.10. The final pre-1750 and extant *Tetratheca juncea* habitat suitability models for both areas.

Pre-1750 habitat underlies extant habitat so only shows in areas that have been cleared of vegetation. The North Coast model shows the result of modelling initially constrained within the oblique rectangle then projected from that model to the remainder of the area. By comparison, the entire North Coast area was modelled (the full model) directly.
limited geographic range. Table 5.7 provides a breakdown of the pre-1750 and extant areas of suitable habitat modelled for the North Coast.

**Table 5.7. Pre-1750 and extant habitat in the North Coast Tetratheca juncea habitat suitability models.**
Constrained = suitable habitat modelled within and limited to the constraining rectangle.
Unconstrained 1 = the amount of suitable habitat from the full model that lies within the constraining rectangle.
Projected = the amount of suitable habitat outside of the constraining rectangle projected from the constrained model result.
Unconstrained 2 = the amount of suitable habitat from the full model that lies outside of the constraining rectangle.

<table>
<thead>
<tr>
<th>Model</th>
<th>pre-1750 area (ha)</th>
<th>extant area (ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constrained</td>
<td>8120</td>
<td>4664</td>
</tr>
<tr>
<td>Unconstrained 1</td>
<td>5538</td>
<td>3555</td>
</tr>
<tr>
<td>Projected</td>
<td>70747</td>
<td>42719</td>
</tr>
<tr>
<td>Unconstrained 2</td>
<td>23520</td>
<td>14199</td>
</tr>
</tbody>
</table>

By comparison, there was 40241 ha of pre-1750 habitat and 25716 ha of extant habitat in the Central Coast.

At this point, it is worth noting that as recently as 1989 there were only 39 records of *Tetratheca juncea* in the Central Coast model area, many of which had a precision of 10 km. As mentioned previously, the North Coast has not been subjected to the level of development pressure that the Central Coast has experienced, a consequence being that there has been little motivation to survey for the species. There is scope for further investigation by field-testing the North Coast model in areas away from known occurrences.

### 5.3.5.2 Available habitat and abundance estimate

An estimate of abundance was made for the Central Coast; no abundance data were available for the North Coast. Data from 12 sites was available where a full count of clumps had been conducted (**Figure 5.11**). The 12 sites were effectively a random selection in that they were on parcels of land subject to development applications, so the selection process was independent of the probability of *Tetratheca juncea* occurring. The counts were restricted to within the land parcel boundaries rather than a hull drawn around the perimeter of the local population. No adjustment of the parcel
Figure 5.11. Location of the 12 sites where *Tetratheca juncea* total clump counts were conducted.
Sites are shown as red diamonds and the inset shows locations within the Central Coast model area.
boundaries was made for *Tetratheca juncea* habitat suitability. Table 5.8 shows the data.

**Table 5.8. *Tetratheca juncea* clump count and density data from 12 locations in the Central Coast habitat suitability model area.**

<table>
<thead>
<tr>
<th>Total clumps</th>
<th>Site area (ha)</th>
<th>Density (clumps/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>377</td>
<td>16.71</td>
<td>22.57</td>
</tr>
<tr>
<td>813</td>
<td>7.56</td>
<td>107.50</td>
</tr>
<tr>
<td>14822</td>
<td>52.39</td>
<td>282.92</td>
</tr>
<tr>
<td>2712</td>
<td>47.16</td>
<td>57.50</td>
</tr>
<tr>
<td>34</td>
<td>28.62</td>
<td>1.19</td>
</tr>
<tr>
<td>512</td>
<td>19.67</td>
<td>26.02</td>
</tr>
<tr>
<td>2294</td>
<td>38.97</td>
<td>58.87</td>
</tr>
<tr>
<td>990</td>
<td>18.71</td>
<td>52.91</td>
</tr>
<tr>
<td>642</td>
<td>6.51</td>
<td>98.54</td>
</tr>
<tr>
<td>300</td>
<td>7.02</td>
<td>42.75</td>
</tr>
<tr>
<td>917</td>
<td>60.06</td>
<td>15.27</td>
</tr>
<tr>
<td>150</td>
<td>5.28</td>
<td>28.42</td>
</tr>
</tbody>
</table>

The density data were tested for normality and found not to be normally distributed but skewed right with a Shapiro-Wilk significance of $p < 0.05$. The median and its standard error were calculated in SPSS using the bootstrap function, and these values were used to estimate population numbers across the modelled area. The median density was determined to be $47.83 \pm 14.16$ clumps per hectare. The lower 95% confidence interval for the median was 24.29 clumps per hectare.

**Table 5.9** shows the modelled pre-1750 and extant suitable habitat areas and estimated abundance along with amounts lost and the extant habitat in conservation reserves. These figures show that approximately 35% of the *Tetratheca juncea* population has been lost since European settlement through clearing of habitat, and that only 14% of the extant population is held in conservation reserves.

**Table 5.9. Available *Tetratheca juncea* habitat from the Central Coast model and abundance estimates calculated using the median density derived from 12 sample locations.**

<table>
<thead>
<tr>
<th>Habitat (ha)</th>
<th>Clumps estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-1750</td>
<td>1924727(\pm 569813)</td>
</tr>
<tr>
<td>Extant</td>
<td>1229996(\pm 364139)</td>
</tr>
<tr>
<td>Loss</td>
<td>694731(\pm 205674)</td>
</tr>
<tr>
<td>Reserved</td>
<td>127419(\pm 37722)</td>
</tr>
</tbody>
</table>
5.3.6 Generalist or Specialist?

Maxent provides information about the primary environmental drivers of the model which in turn assists in understanding the constraints on the species’ distribution, and the factors contributing to the species being classed as rare. Two broad classes of rarity (Brigham 2003) are: (i) formerly common species that have recently become rare and, (ii) historically rare species. Most species falling into the first category are those with fairly generalist habitat requirements while those in the second category will require specialised habitat. As the model is developed Maxent examines the influence of each environmental variable for both training and test data. A jackknife procedure is used where each variable is left out in turn, then the effect of the variable by itself and the effect of all variables together is tested. The effect is measured as the gain (goodness of fit) as the model iterates from the starting condition of all cells having an equal presence probability to the end point of maximum entropy given the constraints provided by the regularised average of the environmental conditions at the presence points. Bar charts are provided that show the gain for each EV under the different conditions.

Figures 5.12 and 5.13 show the Central Coast model jackknife results and Figures 5.14 and 5.15 show the North Coast model jackknife results. For both models, SOIL was the primary driver having the greatest impact both when used in isolation and when left out for both training and test data. For the Central Coast ELEV (elevation), RAIN and STRDIST (distance from nearest stream), and for the North Coast RAIN and STRDIST, also influence the outcome while the remainder of EVs have little or no discernible impact.

Further detail is available showing the contribution from the parameters within each EV in the form of response curves. For example, Figures 5.16 and 5.17 show the data for SOIL for the Central and North Coast respectively. These data show that Tetratheca juncea has a preference (with >50% probability of presence) for 16 of 63 soil types in the Central Coast and 8 of 126 soil types in the North Coast. Table 5.10 lists the soil landscape types and processes for both models. There is also >50% probability of Tetratheca juncea being present in 8 of 18 soil processes.
Figure 5.12. Results of the jackknife test of EV importance for the *Tetrapheca juncea* Central Coast training model.

Figure 5.13. Results of the jackknife test of EV importance for the Central Coast *Tetrapheca juncea* test model.
Figure 5.14. Results of the jackknife test of EV importance for the *Tetratheca juncea* North Coast training model.

Figure 5.15. Results of the jackknife test of EV importance for the *Tetratheca juncea* North Coast test model.
Figure 5.16. Detail of the contribution of the different soil types to the *Tetratheca juncea* Central Coast model.
See Table 5.9 for key to soil codes.

Figure 5.17. Detail of the contribution of the different soil types to the North Coast model.
See Table 5.9 for key to soil codes.
The models indicate that *Tetratheca juncea* can be present in almost the full range of Aspect, Geology, Hillshade, Insolation, Solar Radiation, Terrain Ruggedness and Wetness Index conditions. While soil type is the primary driver of the model, the species can be found in a wide range of types. Furthermore, the geology of the Central Coast is sedimentary, and that of the North Coast is volcanic. Annual rainfall tolerance in the Central Coast is 950 to 1178 mm whereas in the North Coast it is from 1135 to 1245 mm. In both models, the response to distance from streams is a monotonic decline as distance increases.

**Table 5.10.** Soil types contributing to >50% probability of presence in the *Tetratheca juncea* Central Coast and North Coast models (Kovak and Lawrie 1991; Matthie 1995; Bulahdelah Soil Landscape unpub.).

The categorical code is that used to identify soil landscape types in the Maxent model.

<table>
<thead>
<tr>
<th>Model</th>
<th>Landscape</th>
<th>Process</th>
<th>Categorical Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>Awaba</td>
<td>Associated</td>
<td>2</td>
</tr>
<tr>
<td>CC</td>
<td>Cockle Creek</td>
<td>Alluvial</td>
<td>11</td>
</tr>
<tr>
<td>CC</td>
<td>Cedar Hill</td>
<td>Colluvial</td>
<td>12</td>
</tr>
<tr>
<td>CC</td>
<td>Doyalson</td>
<td>Erosional</td>
<td>13</td>
</tr>
<tr>
<td>CC</td>
<td>Gateshead</td>
<td>Erosional</td>
<td>16</td>
</tr>
<tr>
<td>CC</td>
<td>Gorokan</td>
<td>Erosional</td>
<td>17</td>
</tr>
<tr>
<td>CC</td>
<td>Killingworth</td>
<td>Erosional</td>
<td>27</td>
</tr>
<tr>
<td>CC</td>
<td>Norah Head</td>
<td>Aeolian</td>
<td>38</td>
</tr>
<tr>
<td>CC</td>
<td>Shamrock Hill</td>
<td>Erosional</td>
<td>46</td>
</tr>
<tr>
<td>CC</td>
<td>Stockrington</td>
<td>Colluvial</td>
<td>48</td>
</tr>
<tr>
<td>CC</td>
<td>Sugarloaf</td>
<td>Colluvial</td>
<td>51</td>
</tr>
<tr>
<td>CC</td>
<td>Tuggerah</td>
<td>Associated</td>
<td>52</td>
</tr>
<tr>
<td>CC</td>
<td>Tacoma Swamp</td>
<td>Associated</td>
<td>54</td>
</tr>
<tr>
<td>CC</td>
<td>Warners Bay</td>
<td>Residual</td>
<td>56</td>
</tr>
<tr>
<td>CC</td>
<td>Woy Woy</td>
<td>Beach</td>
<td>61</td>
</tr>
<tr>
<td>CC</td>
<td>Wyong</td>
<td>Alluvial</td>
<td>62</td>
</tr>
<tr>
<td>NC</td>
<td>Alum Mountain</td>
<td>Colluvial</td>
<td>1</td>
</tr>
<tr>
<td>NC</td>
<td>Gan Gan</td>
<td>Colluvial</td>
<td>46</td>
</tr>
<tr>
<td>NC</td>
<td>Gilmore Hill</td>
<td>Colluvial</td>
<td>48</td>
</tr>
<tr>
<td>NC</td>
<td>North Arm Cove</td>
<td>Residual</td>
<td>85</td>
</tr>
<tr>
<td>NC</td>
<td>Nungra</td>
<td>Transferral</td>
<td>87</td>
</tr>
<tr>
<td>NC</td>
<td>River Road</td>
<td>Erosional</td>
<td>95</td>
</tr>
<tr>
<td>NC</td>
<td>Shamrock Hill</td>
<td>Erosional</td>
<td>100</td>
</tr>
</tbody>
</table>

From the preceding EV analysis, *Tetratheca juncea* appears to be a generalist in its habitat requirements. If the species is indeed now rare, this has come about recently through habitat loss as a consequence of European settlement rather than it having
been historically rare due to habitat restriction. The geographic spread of the models supports this with the pre-1750 models consisting of large continuous areas of suitable habitat (Section 5.3.5.1).

5.3.7 *Tetratheca juncea* in Conservation Reserves

Since its introduction in the early 1980’s (Soule 1985) conservation biology has become a major field of study (Heywood and Iriondo 2003). It is axiomatic now that an important measure to mitigate against the many anthropogenic threats to individual species and diversity, is the establishment of conservation reserves.

A detailed examination of *Tetratheca juncea* suitable habitat modelled in reserves in the Central Coast (Table 5.11) shows the majority in Sugarloaf and Munmorah State Conservation Areas, with the remainder divided across 13 reserves. Overall, 14% of modelled suitable habitat occurs in reserves, the adequacy of which is yet to be assessed.

![Table 5.11. Modelled *Tetratheca juncea* suitable habitat in conservation reserves.](image)

<table>
<thead>
<tr>
<th>Reserve</th>
<th>Area (ha)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Awabakal NR*</td>
<td>100</td>
<td>3.75</td>
</tr>
<tr>
<td>Blue Gum Hills RP</td>
<td>31</td>
<td>1.16</td>
</tr>
<tr>
<td>Brisbane Water NP</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Colongra Swamp NR</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>Glenrock SCA*</td>
<td>194</td>
<td>7.28</td>
</tr>
<tr>
<td>Jilliby SCA</td>
<td>42</td>
<td>1.58</td>
</tr>
<tr>
<td>Lake Macquarie SCA*</td>
<td>280</td>
<td>10.51</td>
</tr>
<tr>
<td>Munmorah SCA*</td>
<td>756</td>
<td>28.38</td>
</tr>
<tr>
<td>Pulbah Island NR</td>
<td>27</td>
<td>1.01</td>
</tr>
<tr>
<td>Sugarloaf SCA*</td>
<td>1108</td>
<td>41.59</td>
</tr>
<tr>
<td>Tingira Heights NR*</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>Tuggerah NR</td>
<td>1</td>
<td>0.04</td>
</tr>
<tr>
<td>Wallarah NP*</td>
<td>84</td>
<td>3.15</td>
</tr>
<tr>
<td>Watagans NP</td>
<td>29</td>
<td>1.09</td>
</tr>
<tr>
<td>Wyrrabalong NP</td>
<td>7</td>
<td>0.26</td>
</tr>
</tbody>
</table>
5.4 DISCUSSION

5.4.1 Modelling Process

Four novel methods were used in developing the final ENM for *Tetratheca juncea*.

5.4.1.1 Minimising bias in samples

As introduced in Section 5.3.2, most target species records collections contain geographic bias (spatially autocorrelated) from one or more sources, and if left would reduce the quality of a model (Veloz 2009). Samples bias affects a presence only type model through the process of selecting pseudo absences from background data. While biased samples are concentrated in certain areas, the random selection of background data has the potential to result in a lot of atypical habitat being selected for comparison with that in the sample sites.

Methods for correcting for sample bias have been directed at resolving the problem of biased background sample selection rather than removing the bias in the samples set. So, Dudik *et al.* (2005) and Phillips *et al.* (2009) proposed modifying background selection to reflect bias in the samples.

The approach used in developing the *Tetratheca juncea* models was to minimise any bias in the samples. The process of selecting one sample randomly from each of the occupied cells of an appropriately sized grid (Section 5.3.2) minimised bias while retaining the geographic spread of the data. This then allowed random selection of background from across the entire modelled area.

5.4.1.2 Model selection

The most common published method for model selection has been the AUC$_{\text{test}}$ statistic (for example Sergio *et al.* 2007; Urbina-Cardona and Flores-Villela 2010; York *et al.* 2011). Gogol-Prokurat (2011) acknowledged the potential weakness in the use of AUC as described in Lobo *et al.* (2008) and developed an independent test AUC (AUC$_{\text{ind}}$) using presence/absence data and environmental data different to that from which the model was created. Pearson *et al.* (2007) worked with models having very low numbers of samples and used a jackknife technique whereby several models were created using n-1 samples with a different sample removed for each model. Final
models were tested on their ability to predict the removed sample. Raes and ter Steege (2007) developed a null model method for model testing.

To select the best models for *Tetratheca juncea*, this investigation used information criteria, specifically AICc, in an *a priori* manner. The usual process would involve developing a number of models from randomly selected training and test samples. The best fitting model would then be selected, generally based on the highest AUC value. Instead, models were created from 100 different sets of randomly selected samples using the Maxent raw output. The set of samples that resulted in the model with the lowest AICc was then used to create the final model.

5.4.1.3 Model validation
In general terms model validation and model selection can be interrelated to the point of being the same. However, in the case of the *Tetratheca juncea* Central Coast model, an ecologically based reasonableness test was applied as a validation that was independent of the *Tetratheca juncea* samples data (Sections 5.2.5.1 and 5.3.3.3). Comparison of the degree of overlap of models for *Tetratheca juncea* and *Eucalyptus robusta*, habitat in which *Tetratheca juncea* is known not to occur, showed good discrimination.

5.4.1.4 Modelling across cleared and urban areas
In developing management strategies for rare plants in a highly anthropogenically modified environment, knowledge of the species prior distribution, and estimates of the amount of habitat lost/remaining, is essential. Running a model over cleared habitat, as though it were uncleared, will not always be ecologically sound, particularly for some fauna. However, in the case of *Tetratheca juncea* it was appropriate and allowed estimation of habitat loss since European settlement.

5.4.2 Utility of the models

“Essentially, all models are wrong, but some are useful” (Box and Draper 1987) because they are approximations of an unknown and generally unknowable truth, such as the true distribution and niche requirements of a species.
There is an underlying assumption that ENM represent some part of the niche of the target species. This is a primary point of difference between models developed using relationships between a species and environmental variables and models developed by spatial interpolation of known occurrences. However, this concept needs to be viewed with a degree of caution because a model is only as good as the data from which it is constructed. It has been argued that models developed from occurrence records and environmental variables can be simply the expression of spatial autocorrelation between variables and occurrences (Bahn and McGill 2007). In Maxent, this risk is reduced, if not eliminated, through the use of features (various relationships between environmental variables) and averaging conditions prevailing between groups of presence locations. The latter means that the modelling process searches for areas having conditions ‘like’, rather than the same, as those at known sites. There is generally no direct ecological evidence that all or any of the chosen environmental variables either entirely or partially influence the species niche.

A model is based on a particular set of known occurrence records (the samples). What is not known is what proportion of these samples are from different theoretical classes of the species niche (Pulliam 2000). Expanding on this, Guisan and Thuiller (2005) note that models should be built on samples drawn from populations known to be reproducing successfully, thus having a positive growth rate. The work on pollinators and sexual reproduction reported in Chapters 3 and 4 provide some assurance in this regard for the *Tetrapheca juncea* models.

The foregoing comments serve to underline the need for caution in the use of models in developing management plans for threatened species. In the case of the *Tetrapheca juncea* models, validation (Sections 5.2.5, 5.3.3.2 and 5.3.4.2) shows that they are reliable in their prediction of habitat suitability at and around known occurrences of the species. Thus, the models are a reasonable starting point for framing management plans for the species but should be subject to review as more location and ecological data become available.

### 5.4.3 A Dispersal quandary

It is apparent when looking at the distribution of known records in relation to modelled habitat for the Central Coast that *Tetrapheca juncea* has dispersed to the
extent of its preferred habitat, and into marginal habitat. The species can be found in, and the model covers, habitat from coastal dunes to the foothills of the main ranges. There appears to have been few barriers to dispersal.

What then, are the dispersal vectors for the species? It is known that seed are collected by ants attracted to the lipid rich elaiosome (Brew et al. 1989; Boeswinkel 1989) that is a common feature of all *Tetratheca* seed. However, myrmecochorous dispersal alone could not have resulted in the wide geographic spread achieved by the species. This could be an example of Reid’s Paradox (Clark et al. 1998), that describes the discrepancy between currently known dispersal mechanisms for tree species, and migration distances by those species from early Holocene paleorecords being considerably larger. Pakeman (2001) has proposed that this paradox extends to herbaceous species and that endozoochorous (through the mammal gut) dispersal could be an explanation.

It is common to see *Tetratheca juncea* clumps that have been heavily grazed. The prominent native terrestrial herbivores sharing the species’ habitat are macropods that have been shown to disperse viable seed through their gut (Calvino-Cancela 2011). Grazing macropods could ingest *Tetratheca juncea* seeds which pass through the gut and are deposited some distance from the grazed clump. As a pilot exercise, a game camera (Bushnell Trophy Cam 119456C) was placed near a large *Tetratheca juncea* clump for several weeks. Figure 5.18 shows a Swamp Wallaby (*Wallabia bicolor*) browsing on the clump, with a *Tetratheca juncea* stem in its mouth.

Following this, the next obvious task was to find *Tetratheca juncea* seeds in wallaby scats and successfully germinate them. Again, simply as a pilot, scats were collected from within and near large *Tetratheca juncea* populations at a time when the plants were carrying fruit. The scats were broken down and passed through a series of screens. No *Tetratheca juncea* seeds were found. However, there were abundant grass seeds of several species.

On the other hand, *Tetratheca juncea* might be a paleoendemic species that was once essentially continuous across its range, with its preferred habitat having fragmented over time. Kruckeberg and Rabinowitz (1985) describe paleoendemics as having
more than one disjunct population. As Figure 1.4 shows, the historic distribution of *Tetratheca juncea* was in three disjunct regional populations. It is interesting to observe that these three regional populations are separated by two major drainage systems viz. the Sydney Basin in the south and the Hunter Valley in the north. The erosion of these systems could have fragmented a once continuous *Tetratheca juncea* distribution. If this were the case, then the two remaining regional populations might be vicariant endemic populations. It is possible that detailed ploidy analysis of *Tetratheca juncea* would provide some insight into the age of the species. Stebbins and Major (1965) propose that paleoendemics are likely to be diploid or high polyploid.

Figure 5.18. A Swamp Wallaby (*Wallabia bicolor*) grazing a *Tetratheca juncea* clump.
The wallaby is standing on the grass-like *Tetratheca juncea* stems and is chewing a *Tetratheca juncea* stem.
CHAPTER 6  GENERAL DISCUSSION
6.1 KEY RESEARCH OUTCOMES

This research project has provided the first comprehensive investigation into the reproductive ecology of *Tetratheca juncea*, a plant listed as threatened with extinction in both NSW State and Australian Commonwealth legislation. Others have reported on aspects of the species life cycle (Gross *et al.* 2003; Driscoll 2003; Bellairs *et al.* 2006) and this research built on those reports, developing a holistic view of the species reproductive ecology.

Chapter 2 examined *Tetratheca juncea* reproductive phenology. Data were collected monthly from 12 sites across a large part of the Central Coast regional population, from June 2010 to January 2012 (Section 2.2, Figure 2.1). Budding commenced in early June and evidence suggests that the primary initiator is photoperiod (Section 2.4). Flowering starts in August, however evidence suggests that fruit-set does not begin until pollinators are active in late September. Fruit maturation and seed release occur from November, after which all four phenophases of budding, flowering, fruiting and seed release are concurrent (Section 2.3.2) for a time, before they diminish in the reverse order that they started in. In common with several studies of other species (Rabinowitz *et al.* 1981; Ollerton and Lack 1988; Clark and Thompson 2011) the overall flowering curve is heavily right skewed, to the point of being bimodal (Section 2.3.3). The underlying cause of this skewed shape has not been reported in previous flowering curve studies. In the case of *Tetratheca juncea*, bud length analysis showed that the right skew was the result of a batch of new buds developing around September followed by increased flowering. The effect of this was to extend flowering, thus increasing pollination opportunities in a plant whose flowers offer no nectar.

Chapters 3 and 4 document aspects of pollination in *Tetratheca juncea*, both regionally and in a subpopulation. Adding to the introductory work by Driscoll (2003), four new native bee pollinators were recorded collecting pollen from *Tetratheca juncea* flowers, making a total of six known pollinator species with at least four others probable (Section 3.3). FFR values were found to be comparable with Fruit:Flower ratios of other species (Sutherland and Delph 1984; Sutherland 1986). A high degree of variability in *Tetratheca juncea* FFR values across both region and
time was found inferring variable pollinator activity. Again this is consistent with reports for other species (Devaux and Lande 2009). Close examination of FFR, also drawing from Chapter 2 data, revealed that this index should be interpreted carefully (Sections 3.4.7 and 4.4), particularly with respect to levels of pollinator activity. In Chapter 3, a fall in FFR over the six-year study was found to be a consequence of increasing flower numbers rather than decreasing pollinator activity (Section 4.3.2 and 4.3.6).

Chapter 5 provides results from modelling *Tetratheca juncea* habitat suitability for both Central Coast and North Coast regional populations using presence only data, selected environmental variables and the maximum entropy software package Maxent (Phillips et al. 2004). A novel method was used to select a set of input presence points that retained their geographic and environmental spread and maximised model quality (Section 5.2.2). The resulting binary model (100 m grid resolution) of suitable/unsuitable habitat was tested for overlap with a model of a habitat type in which *Tetratheca juncea* is known not to occur. The small amount of overlap was consistent with an ecotonal transition between both habitats (Section 5.2.5).

The final model allowed estimation of:

- habitat loss since European settlement, approximately 30%;
- extant population size in the Central Coast regional population, 1229996 ± 364139 clumps;
- amount of suitable habitat in conservation reserves, 2664 ha in 15 reserves, approximately 14% of extant habitat;
- degree of fragmentation in extant habitat, 29% in fragments >1000 ha and 24% in fragments 100-1000 ha.

Fire appears to have a prominent role in the *Tetratheca juncea* long-term life cycle, as opposed to the annual reproductive cycle. Chapter 3 Section 3.4.3 describes increased flowering, pollinator numbers and FFR in response to fire. A similar response has been described for the NSW threatened tree, *Angophora inopina* Hill (Myrtaceae) (Tierney 2004). Increased growth rates, flowering and seed production following fire were reported. The investigation of a subpopulation reported in Chapter 4 found that *Tetratheca juncea* numbers reduced by >25% over six years, most likely as a consequence of the species being outcompeted by other ground species. It was concluded that fire would be an essential factor in the species life cycle, as has been
reported for other Australian species. In the rare QLD grassland herb *Trioncinia retroflexa* (F.Muell.) Veldkamp (Asteraceae), inflorescence numbers were shown to increase following fire (Fensham et al. 2002). In a study of nine grassland herbs, Lunt (1994) found that plant numbers peaked in the six months after fire and then fell as time since fire increased. In contrast to *Tetratheca juncea*, flower numbers also fell with time since fire. In Victorian native grasslands, Stuw and Parsons (1977) showed that fire was needed to maintain diversity in the face of competition from dominant grass species. There was no evidence of seedling recruitment in the *Tetratheca juncea* subpopulation investigated in Chapter 4 over the six years. Bellairs et al. (2006) showed that fire was the most likely seed germination trigger for the species, which also highlights the importance of fire in the species’ life-cycle. The author has observed, but not documented, the response of *Tetratheca juncea* to three fires. In one instance, no germination occurred following a severe fire (presumably seed were killed) while the other two instances resulted in seed germination.

Reference was made in Chapters 2, 3 and 5 to the possible role of selection in shaping components of the reproductive process in *Tetratheca juncea*. Whether sexual selection occurs in plants, particularly in hermaphrodites, has been argued since the late 1970’s (reviewed in Willson 1994; Skogsmyr and Lankinen 2002; Delph and Ashman 2006). The two components of sexual selection are male-male competition and female choice. Within either of these, selection can act only on variation that originates in the genotype and must lead to increased fitness. Natural selection of phenotypes cannot produce cumulative change in a species (Williams 1996).

In flowering plants, male-male competition, both within populations of the same species and between species, comes about through variation in attractiveness of flowers to pollinators, pollen accessibility and availability, and timing of flowering. In Chapter 1 Section 1.6 it was noted that bilaterally symmetrical (zygomorphic) flower shape was over-represented in rare taxa. Flower symmetry also appears to play a role in selection (Sargent 2004). It is proposed that the selective function of zygomorphic flowers lies with the flower architecture forcing pollinators to collect pollen on a specific part of the body; successful transfer of pollen to a stigma of the same species is then more likely. The flowers of *Tetratheca juncea* are actinomorphic, but pollen is collected via specialised buzz pollinators onto their ventral surface (Chapter 2 Figure
2.12). This then reduces the possibilities for *Tetratheca juncea* pollen to be wasted on other species and lowers the potential for stigma clogging by foreign pollen. King and Buchman (1996) demonstrated that the rate of pollen release in Solanum is mediated by the drying of tapetal fluid in the poricidal anther. The poricidal anthers of *Tetratheca juncea* also hold pollen in tapetal fluid (Gross *et al.* 2003) and it is expected that pollen will be similarly gradually dispersed. This is not the case for all species having poricidal anthers. Larson and Barrett (1999) describe the Canadian wetland herb *Rexia virginica* (Melastomataceae) as having poricidal anthers that, when buzzed by bumble bees, release a dry pollen cloud, a lot of which does not fall on the bee. On the assumption that *Tetratheca juncea* fecundity was low, Gross *et al.* (2003) surmised that poricidal anthers were not a successful trait. This research has shown that fecundity is about average for a functionally self-incompatible hermaphrodite (Chapter 3 Section 3.5). A more positive view would be that dispensing pollen gradually was an adaptation allowing pollen from any individual flower to be widely dispersed.

### 6.2 REASSESSING THE RARITY OF *TETRATHECA JUNCEA*

Species become listed as threatened in NSW by the Scientific Committee which is governed by the TSC Act. Similarly for national listings, the Australian Commonwealth Scientific Committee is governed by the EPBC Act. Submissions to the scientific committees come from a variety of sources, including public nominations. If a submission is deemed to have merit, preliminary determinations are exhibited for public comment before a final decision is made. As thorough as this process might be, it is dependent on the quality of the information about the subject species that is available at that time. Information about the subject species at the time of listing is not always comprehensive. Hogbin (2002b) reassessed the rarity of all threatened plant species listed in the schedules of the TSC Act. The reassessment considered distribution, population size, threats, rates of decline, population structure and response to disturbance. Hogbin (2002b) concluded that the entire *Tetratheca juncea* population consisted of approximately 10,000 individuals. Distribution was described as made up of 240 highly fragmented groups, most with fewer than 50 plants. Only one population was conserved.
The results presented in this thesis highlight risks associated with reliance on \textit{ad hoc} occurrence data when assessing the level of threat faced by a species. This is demonstrated in the records history of \textit{Tetratheca juncea} for the Central Coast regional population (Figure 6.1). Records from the Atlas of NSW Wildlife were collated in 10-year intervals up to 2009 followed by one year to 2010. Median geographic distance between records, an indicator of records density, was determined using the spanning tree algorithm in Manifold GIS. The plot shows the acceleration in records following the introduction of the TSC Act in 1995. Increased survey effort was driven by a requirement that threatened species be documented and managed as part of development proposal assessments.

![Figure 6.1 The history of \textit{Tetratheca juncea} records for the Central Coast regional population.](image)

Records from the Atlas of NSW Wildlife at ten year intervals up to 2009. Median separation is the median distance in meters between records connected by a spanning tree.

While the increase in records shown in Figure 6.1 is impressive, they do not provide sufficient information on which rarity and level of threat can be based. Chapter 5 has shown the value of using records in association with a properly constructed habitat model. This is illustrated by the model of the North Coast regional population. Development pressures are substantially lower in that area; consequently there are fewer \textit{Tetratheca juncea} records. The projected model (Chapter 5, Figure 5.8) shows large areas of potentially suitable habitat for the species, most of which are well outside the bounds of available occurrence records.
An unresolved quandary, when assessing rarity, is how to describe and quantify distribution (Gaston 1991). Rare plants, by definition, have a sporadic distribution across a landscape, but so do many species that are considered common (Murray et al. 2002a). Many different metrics have been devised to describe rare plant distribution. Area of occupancy (AOO) and extent of occurrence (EOO) are two common indices (Hartley and Kunin 2003). Other examples are: latitudinal range; ratio of east-west and north-south distribution; number of administrative areas (e.g. counties); number of grid cells occupied, of a wide range of sizes; or biogeographic regions containing the species (Kelly et al. 1996; Murray et al. 2002a; Hartley and Kunin 2003; Crain and White 2011).

In considering *Tetratheca juncea* rarity, how the species is placed in a selection of schemes is examined, comparing information about the species from this research project with that prior. Rabinowitz (1981) was the first to propose a scheme for describing plant species status based on geographic range, habitat requirements, population size and distribution (Table 6.1). Benayas et al. (1999) built on the Rabinowitz (1981) scheme by including habitat occupancy (Table 6.2).

A difficulty with both the Rabinowitz (1981) and Benayas et al. (1999) models is that geographic range is not quantified. Responding to this problem, Crain and White (2011) developed a scale for evaluating rarity at a local level (L-rank) based on the number of one square kilometre grid cells (km$^2$) known to be occupied by the species. The cutoff for *vulnerable to threat or extinction* (L-rank 3) was proposed to be an area of occupation <250 km$^2$.

Applying this to the Lake Macquarie Local Government Area (LGA), to date being the administrative area with the majority of *Tetratheca juncea* records (Chapter 5, Figure 5.1). Based on actual occurrences the species is now known to occupy >280 km$^2$ whereas when Hogbin (2002b) completed her re-evaluation, only 112 km$^2$ were known to be occupied. From the Central Coast extant regional population model (Chapter 5 Section 5.3.3) suitable habitat occupies >600 km$^2$ in the LGA. Therefore, in the Crain and White (2011) L-rank scheme, *Tetratheca juncea* would not be considered threatened with extinction in the Lake Macquarie LGA.
Chapter 6  General Discussion

Table 6.1 Species distribution categorised according to geographic range, habitat specificity and local population size.
From Rabinowitz (1981). Common species have a large geographic range, wide habitat specificity and large somewhere dominant local population size. The remaining combinations comprise what are described as the seven forms of rarity. The combination that best describes the status of *Tetratheca juncea* prior to this research is shaded grey and status as an outcome of this research is bolded.

<table>
<thead>
<tr>
<th>Geographic range</th>
<th>Large</th>
<th>Small</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat specificity</td>
<td>Wide</td>
<td>Narrow</td>
</tr>
<tr>
<td>Local population size: large, dominant somewhere</td>
<td>Locally abundant over a large range in several habitats</td>
<td>Locally abundant over a large range in a specific habitat</td>
</tr>
<tr>
<td>Local population size: small, non-dominant</td>
<td>Constantly sparse over a large range and in several habitats</td>
<td>Constantly sparse in a specific habitat but over a large range</td>
</tr>
</tbody>
</table>

Table 6.2 Species distribution categorised according to geographic range, habitat specificity, local population size and habitat occupancy
From Benayas *et al.* (1999), the seven forms of rarity are expanded to include a habitat occupancy assessment. The combination that best describes the status of *Tetratheca juncea* prior to this research is shaded grey and status as an outcome of this research is bolded.

<table>
<thead>
<tr>
<th>Geographic range</th>
<th>Wide</th>
<th>Restricted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Habitat specificity</td>
<td>Broad</td>
<td>Small</td>
</tr>
<tr>
<td>Abundance</td>
<td>Large</td>
<td>Small</td>
</tr>
<tr>
<td>Habitat occupancy high</td>
<td>Common</td>
<td>Widespread</td>
</tr>
<tr>
<td>Habitat occupancy low</td>
<td>Highly dispersed</td>
<td>Sparse</td>
</tr>
<tr>
<td>Habitat occupancy low</td>
<td>Locally endangered</td>
<td>Potentially endangered</td>
</tr>
</tbody>
</table>

Finally, a formal assessment is conducted by applying the International Union for Conservation of Nature (2001) Red List conditions for listing as a vulnerable species (*Table 6.3*). Criterion A has an option of assessing loss over 10 years or three generations, whichever is the longest. Generation length for *Tetratheca juncea* is unknown. The species is clonal, and clonality can confer long generation times (Watkinson and White 1985; Callaghan *et al.* 1992). However, a recent genetic study (Jones 2011) of a small *Tetratheca juncea* subpopulation (104 clumps) identified an unexpected high number of genets (clonal diversity 0.85). Genets were also identified between individuals separated by less than the 30 cm conventionally used to identify an individual clump. While a much more comprehensive genetic study is needed,
Table 6.3 The International Union for Conservation of Nature Red List conditions for classification as Vulnerable (Version 3.1, 2001).

A taxon is Vulnerable when the best available evidence indicates that it meets any of the criteria A to E and it is therefore considered to be facing a high risk of extinction in the wild. Criterion A has been assessed based on the 10-year interval but this is debatable (see main text).

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Description</th>
<th>Conditions</th>
<th>Condition met?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Reduction in population size</td>
<td>≥50% over last 10 years or 3 generations (whichever is longest)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥30% over last 10 years or 3 generations</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥30% projected/suspected over next 10 years or 3 generations</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>≥30% over any 10 year period or three generations (including past and future)</td>
<td>No</td>
</tr>
<tr>
<td>B</td>
<td>Geographic range</td>
<td>Extent of occurrence &lt;20,000 km$^2$ and 2 of the following</td>
<td>Yes, ~1100 km$^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Severely fragmented or 10 or less known locations</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Continuing to decline (occurrence, area, subpopulations, individuals)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Extreme fluctuations (occurrence, area, subpopulations, individuals)</td>
<td>No</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Area of occupancy &lt;2000 km$^2$ and 2 of the following</td>
<td>Yes, ~400 km$^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Severely fragmented or 10 or less known locations</td>
<td>No</td>
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<tr>
<td></td>
<td></td>
<td>• Continuing to decline (occurrence, area, subpopulations, individuals)</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Extreme fluctuations (occurrence, area, subpopulations, individuals)</td>
<td>No</td>
</tr>
<tr>
<td>C</td>
<td>Population size &lt;10,000 mature individuals</td>
<td>Estimated decline of ≥10% 10 years or 3 generations (whichever is longest)</td>
<td>No</td>
</tr>
<tr>
<td>D</td>
<td>Population very small or restricted</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>E</td>
<td>Quantitative analysis showing probability of extinction ≥10% within 100 years.</td>
<td></td>
<td>Data deficient</td>
</tr>
</tbody>
</table>

these results suggest the possibility of variable generation times for individuals within a subpopulation. *Tetratheca juncea* does not meet the conditions of Criteria B, C and D with E being unknown. Classification as vulnerable, therefore, hinges on whether...
three generations span more than 10 years. If so, then Criterion A would be the only one by which the species could now be classed as vulnerable.

Summarising, *Tetratheca juncea* could best be described as rare, and endemic to the Central and North Coasts of New South Wales, Australia. It is common within the Lake Macquarie LGA, and this explains the high profile of the species in the development approval process in that jurisdiction. Using the International Union for Conservation of Nature (2001) guidelines, Near Threatened would be a more appropriate classification based on species’ numbers and distribution as presented in this thesis.

However, Near Threatened is not a class of threat available to Scientific Committees under the NSW TSC Act or Commonwealth EPBC Act; it is used in Queensland, Victoria and Western Australia. In 2009 an independent review of the EPBC Act was published (http://www.environment.gov.au/epbc/review/publications/final report.html) in which it was stated:

"The addition to the Act of a ‘near threatened’ category, similar to that available under the IUCN framework, where a species is close to qualifying for or is likely to qualify for a threatened category in the near future, is not likely to provide significant conservation outcomes. The move towards broad-scale landscape approaches to biodiversity protection and conservation, such as regional planning, should be a more efficient way of protecting species, habitats and ecological communities that may not qualify for listing under existing categories."

In the case of *Tetratheca juncea*, it is difficult to see how broad-scale biodiversity protection measures could protect the species without quarantining over 40% of the unreserved vegetated area of Lake Macquarie LGA for example.

This study of *Tetratheca juncea* has arrived at similar conclusions to those from a study by Garcia *et al.* (2002) that investigated the status of five threatened Pyrenean species. The aim of these authors was to assess “the real vulnerability of these species”. They investigated death and recruitment within populations, population structure, life span of individuals, reproduction, herbivory and attrition of the
components of reproduction. Their study was conducted over four to six years. The outcome was that all five species appeared to be well adapted to their environment and had no reproductive problems. As has been shown here with *Tetratheca juncea*, fruit production levels (measured using FFR) were within the range shown by other studies of plants with similar reproductive processes.

Population numbers were found to be larger than was previously thought. The outcome was that, at a regional scale, one species could be down-listed while, at a continental scale, three of the species could be down-listed.

The current *Tetratheca juncea* study and that of Garcia et al. (2002) demonstrate that the wrong question(s) can be asked based on the assumption that a species is rare and threatened because it is listed. This certainly was the case for *Tetratheca juncea*.

Listing was based on sporadic reports from *ad hoc* sources, and confirmation bias appears to have had a part in conclusions drawn from early investigations. Thus, Driscoll (2003) wrote that the species was probably in a state of insidious delayed extinction based on assumed low population numbers, high levels of fragmentation and low pollinator numbers; Gross et al. (2003) concluded that the species was poorly fecund and that having poricidal anthers was probably an unsuccessful reproductive trait. Burgman (2002) provides an extensive analysis showing that lists of threatened species are rarely based on systematic and targeted investigations, and can be subject to a variety of biases. Auld (2001) reviewed the known ecology of members of the family Rutaceae occurring in the Sydney, Australia region, a large number of which are listed as threatened. He concluded that there was insufficient information to inform management of the rare and threatened members of the family.

Habitat suitability modelling has matured considerably over recent years and results from a properly constructed fine scale model should be a useful addition to the decision making process of Scientific Committees.

The foregoing is not intended to imply that *Tetratheca juncea* should be delisted as a vulnerable species. Without alternate protection status of Near Threatened, if delisted
the species would most likely be relisted within a few years given its known and predicted concentration in Lake Macquarie LGA alone.

6.3 **FUTURE RESEARCH**

This research provides information that can better inform management of *Tetratheca juncea* as a legally listed threatened species. A key outcome is that the species is unlikely to be, as was previously thought, on the brink of extinction through being poorly adapted to its environment. It is a species that is well adapted to its environment and, in fact, can be described as common within the Lake Macquarie LGA. Suitable habitat (Chapter 5) occurs in over 40% of the uncleared Lake Macquarie LGA landmass that lies outside of reserves. The effective proportion would be larger once the area of clearly unsuitable habitat types like wetlands and swamps was removed.

An inevitable consequence of this wide distribution is that losses will occur under pressure from an expanding human population. The task of managing these losses is to minimise the impact on the long-term survival of the species, avoiding extinction. To this end, the critically missing information lies in understanding the genetics of the species across its range. Detailed genetic studies are needed to resolve issues like the separation distance between subpopulations across which genetic material cannot be transferred through seed dispersal or pollen transfer, whether there are locally adapted genotypes, the level of clonality and effective population size. Without this information, it is a matter of guessing what losses can be sustained.

In the background to this thesis the NSW TSC Act 7-part test of the impact of a development proposal on a species (or community) was described (Section 1.1). The first factor considered in this test is:

"(a) in the case of a threatened species, whether the action proposed is likely to have an adverse effect on the life cycle of the species such that a viable local population of the species is likely to be placed at risk of extinction,"

Known occurrences and modelled distribution presented in this thesis indicate that the Central Coast regional population of *Tetratheca juncea* is widespread. Applying that first factor of the 7-part test highlights an underlying assumption that threatened
species exist in a fragmented condition, either naturally, as a result of anthropogenic disturbance or a combination of both.

It is conceivable that a patch of *Tetratheca juncea* under threat from a proposed development could be genetically connected across a wide geographic area, thus forming a large local population. The response to factor (a) of the 7-part test would be that the proposed loss would be a negligible part of the local population such that its loss would not place the local population at risk of extinction. It is clear that the 7-part test was not written to assess the impact of loss on a widespread genetically connected species.

At present, the only means of protecting a listed threatened species having a wide connected distribution is to invoke the uncertainty principle. While it is not an unreasonable assumption that local patches of *Tetratheca juncea* are genetically connected over a wide area, the actual conditions of such a connection are unknown. This further highlights the need for genetic research to determine maximum transfer distances for genetic material, both pollen and seed.

A fine example of the benefits of genetic research can be found in studies into the Western Australian species *Tetratheca paynterae* subsp. *paynterae* (Butcher *et al.* 2009; Butcher *et al.* 2011). This species is restricted to four small populations in one km of the Windarling Range ironstone outcrops in south-western Western Australia. From a total known population of 7,700 plants, 1,900 were removed through mining. Genetic analysis of the remainder has shown that any further losses would be detrimental to the survival of the species, and no further mining has occurred in their habitat. Monitoring has not revealed any impact from the loss on the remainder of the population (R. Howard pers. com.). Other examples are, conservation genetics of two rare eucalypts (Prober *et al.* 1990) and *Zieria prostrata* (Hogbin and Peakall 1999).

What impact does sexual interference (Barrett 2002) have on the efficiency of reproduction in *Tetratheca juncea*? It is unknown whether flowers are protandrous or protogynous, or whether the species is a simultaneous hermaphrodite. Having no nectar means that any temporal differential between anthesis and stigma receptivity (dichogamy) could not deter pollinators. Gross *et al.* (2003) noted that the style
continued to lengthen after flower opening. Perhaps herkogamy plays a part in reducing sexual interference.

Across its range, *Tetratheca juncea* is occasionally found growing in proximity with its far more common congener, *Tetratheca thymifolia* a species with a range from Victoria through NSW to Queensland. In fact, they can be so close that they most likely share the same root space (Driscoll 2003). Many rare plant studies have sought insights into causes and attributes of rarity by comparing and contrasting rare and common congeners (Hodgson 1986; Bevill and Louda 1999; reviewed in Murray et al. 2002b and 2002b). Similar work comparing *Tetratheca juncea* and *Tetratheca thymifolia* would be fruitful.

While conducting habitat suitability modelling (Chapter 5), it became frustratingly obvious that the key environmental drivers of habitat suitability were not available. Soil type was the primary driver of the model, but it was clear that there were other drivers. Thus, the model was unable to show, for example, that soil derived from Permian geology was equally suitable for the species as soil derived from Carboniferous geology (as is observed in the field). Comparative research into these two soil types would be a start towards greater insight into edaphic requirements.

Habitat suitability modelling suggests that the North Coast regional population might be larger than the better-known Central Coast regional population. Targeted surveys are needed during peak flowering (from Chapter 2, late September/early October) to determine the validity of this prediction.

So far emphasis has been on direct conservation of *Tetratheca juncea* and its preferred habitat. Pollinators also need consideration for, without them, there is no sexual reproduction. What is it about *Tetratheca juncea* pollen that pollinators value? Interesting studies have been conducted on native bees and their pollen preferences (Roulston et al. 2000; Muller et al. 2006; Praz et al. 2008). Muller et al. 2006 reported that from 7 to 1100 flowers were needed to provide enough pollen to raise a single larva. Which relationship has priority: pollinators to *Tetratheca juncea* or *Tetratheca juncea* to pollinators? Or is it a true mutualism? More research is needed here.
6.4 FINAL REMARKS

From this research, *Tetratheca juncea* can be best described as a restricted endemic possibly Near Threatened, but common across its range, and does not appear to be on the brink of extinction. Based on currently used tools for assessing threat level, whether the species is justifiably listed as threatened is arguable. However, its listing provides the opportunity for management of the species to avoid it becoming genuinely threatened with extinction; in practise, a *de facto* Near Threatened listing.

In fact, this research project has demonstrated that the species is well adapted to its environment, and if left undisturbed in its preferred habitat it would be unlikely to decline. However, for a future in which losses are inevitable, it is imperative that plans are made to minimise the impact of these losses and avoid the threat of extinction. Understanding the genetics of the species across its range is critical for determining sustainable loss levels.

Proper management of extant populations is also necessary. Pressure points in the species’ life-cycle, like habitat fragmentation, pollinator abundance or seed dispersal vectors and fire management would rank highly in management consideration. As noted in Chapter 4, ‘High frequency fire resulting in the disruption of life cycle processes in plants and animals and loss of vegetation structure and composition’ is listed as a Key Threatening Process in the NSW Threatened Species Conservation Act 1995. This listing has potential for an overreaction with areas being protected from fire to the detriment of species that depend on a regular fire cycle. From the known history of that location, the subpopulation studied in Chapter 4 had not been burnt for at least 10 years, the last six of which were the monitoring period. Evidence indicated that the reported population reduction of >25% was most likely a result of the species being outcompeted by heterospecifics in the ground vegetation layer, a situation that periodic fire would ameliorate.

This research project commenced with the author having observed that there were discrepancies between available published information and field experience with *Tetratheca juncea*. At least three other listed threatened species come to mind that would benefit from a similar examination as has been conducted here: *Acacia bynoeana* Benth. (Fabaceae: Mimosoideae), *Grevillea parviflora* R.Br. subsp. *parviflora* (Proteaceae) and *Rutidosis heterogama* Philipson (Asteraceae).


References


Department of Mineral Resources (1999) Lower North East Region 1:250,000 scale equivalent geology (lner5ge_p (polygons) and lner5ge_l (lines or arcs) geological coverage comprising the area covered by parts of the Dorrigo, Tamworth, Hastings, Singleton and Newcastle 1:250 000 sheet areas, Hunter Coalfield and Newcastle.
Coalfield Regional Geology 1:100 000 sheet areas and part Sydney 1:250 000 sheet area. CRA project Lower North East. NSW Department of Mineral Resources.


References


Central Coast Regional Biodiversity Conservation Strategy. CSIRO Sustainable Ecosystems.


APPENDIX 1: THE PROGRESSION OF EACH PHENOPHASE AT EACH SITE OVER EIGHT MONTHS

Showing the proportion of each phenophase in inflorescences for each month

Key: Buds Flowers Fruit Seed Release

![Bar charts showing the proportion of each phenophase for different sites from May to January.](chart1.png)
APPENDIX 2: TUKEY POST HOC TEST FOR EACH PHENOPHASE AND EACH MONTH

<table>
<thead>
<tr>
<th>Measure</th>
<th>(I) Time</th>
<th>(J) Time</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buds</td>
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<td>Aug</td>
<td>0.402</td>
<td>0.042</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Sep</td>
<td>Sep</td>
<td>-0.402</td>
<td>0.042</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Oct</td>
<td>Oct</td>
<td>0.387</td>
<td>0.046</td>
<td>0.000</td>
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<td>0.537</td>
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<td></td>
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<td>Dec</td>
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<td>0.000</td>
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<tr>
<td></td>
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APPENDIX 3: DENDROGRAMS FROM THE PHENOLOGY SYNCHRONY ANALYSIS

The solid black lines lead to individual sites or groups of sites that are significantly different. For example in the Budding dendrogram Site 9 is significantly different from all other sites that are not significantly different from each other.
APPENDIX 4: BACKWARD-ELIMINATION SPATIAL REGRESSION MODEL

Backward-elimination procedure for spatial regression model (Density)

Even though the preliminary analysis showed that Density was not associated with any variable (Section 4.3.6), this may have been due to spatial effects. Therefore, the Moran I test was required. The Moran I statistic was 0.156, *p*-value < 0.001 indicating that the null hypothesis, the Density variable was not significantly spatially autocorrelated, can be rejected. Next, the Global Moran I test for residuals of the OLS regression model (Predicted Density = 2.17 + 0.005 FLTotal + 0.926 FFR + 0.298 Attrition) showed that the Moran I statistic was 0.153, *p*-value < 0.001. This indicated that a Spatial Error Model was required including a spatially lagged density variable. Lagrange tests confirmed the previous result as indicated by LMerr(1) = 34.9808 and LMLag(1) = 35.9388, with *p*-value <0.001.

The full model showed no significant factor with *p*>0.5. Moreover, it seemed that Density did significantly participate in the model, which confirmed the above results.

Coefficients: (asymptotic standard errors)

|                | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | -2.208479| 0.719479   | -3.0696 | 0.002144 |
| FLTotal        | -0.011543| 0.020156   | -0.5727 | 0.566874 |
| FFR            | 0.693102 | 1.131505   | 0.6125  | 0.540175 |
| Attrition      | -0.032104| 0.468397   | -0.0685 | 0.945356 |
| Density        | 1.723379 | 0.235652   | 7.3132  | 2.607e-13|

Based on the backward-elimination method, **attrition** was the first required variable to be removed because it had the highest *p*-value (*p* = 0.945). After reduction, it appeared that the remaining factors (FLTotal and FFR) did not significantly affect Density with *p*-value >0.5, which was greater than any reasonable significance level.
Coefficients: (asymptotic standard errors)

|                | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | -2.218053| 0.704129   | -3.1501 | 0.001632 |
| FLTotal        | -0.011154| 0.019297   | -0.5780 | 0.563260 |
| FFR            | 0.702815 | 1.121678   | 0.6266  | 0.530938 |
| Density        | 1.720739 | 0.233501   | 7.3693  | 1.716e-13 |

Hence, **FLTotal** was the second required variable to be removed because it had the highest $p$-value = 0.563. The reduced model appeared to have no significant parameters because FFR did not significantly affect Density with $Z = 0.707$, $p$-value = 0.48 greater than any reasonable significance level. Hence, Density had no significant relationship with the other parameters.

Coefficients: (asymptotic standard errors)

|                | Estimate | Std. Error | z value | Pr(>|z|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | -2.31386 | 0.67966    | -3.4044 | 0.000663 |
| FFR            | 0.78761  | 1.11414    | 0.7069  | 0.479615 |
| Density        | 1.70295  | 0.22801    | 7.4688  | 8.105e-14 |

**Spatial regression model (FFR)**

The Moran I statistic for FFR was 0.002, $p$-value = 0.244, which was greater than any reasonable significance level. Thus the null hypothesis that there was no spatial autocorrelation present in the FFR variable cannot be rejected. Secondly, the Global Moran I test for residuals of the OLS regression model (Predicted FFR = 0.233 - 0.003 FLTotal - 0.051 Attrition) showed that the Moran I statistic was 0.005, $p$-value = 0.208, again greater than any reasonable significance level. This indicated that a multiple linear regression model was appropriate. The Lagrange tests confirm these results as indicated by $LMerr(1) = 0.0336$, $p$-value = 0.8546 and $LMlag(1) = 0.0195$, $p$-value = 0.889.
Backward-elimination procedure for linear regression model (FFR)

Based on previous results, it was necessary to investigate the required regression model on the original data. The full model showed that none of the parameters were significant with p-value > 0.1, which was greater than any reasonable significance level, except for YearID (Time) (t = -3.19, p-value = 0.0016) which negatively affected FFR.

|             | Estimate  | Std. Error | t value | Pr(>|t|)     |
|-------------|-----------|------------|---------|-------------|
| (Intercept) | 0.3236057 | 0.0393194  | 8.230   | 6.08e-15 ***|
| YearID      | -0.0304325| 0.0095449  | -3.188  | 0.00158 **  |
| FLTotal     | -0.0013843| 0.0008812  | -1.571  | 0.11726     |
| Attrition   | 0.0710092 | 0.0763678  | 0.930   | 0.35322     |

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Signif. codes: p-value 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Since Attrition had the highest p-value of 0.353, it was the first parameter eliminated. The reduced model was significant with F(2, 296) = 8.97 and p-value < 0.001. The estimated equation was:

\[
\text{Predicted FFR} = 0.336 - 0.03\text{YearID} - 0.0015\text{FLTotal}
\]

Time significantly affected FFR as indicated by t = -3.536 and p-value <0.001. Moreover, Time appeared to negatively affect FFR where each year the FFR reduced by 3%. FLTotal was also significantly involved in the model’s estimation with t = -1.67 and p-value = 0.096, less than 0.1 level of significance. Adjusted R-square was 0.051 indicating that 5.1% of data were explained by the model.

Coefficients:

|             | Estimate  | Std. Error | t value | Pr(>|t|)     |
|-------------|-----------|------------|---------|-------------|
| (Intercept) | 0.335807  | 0.037057   | 9.062   | < 2e-16 *** |
| YearID      | -0.032616 | 0.009249   | -3.526  | 0.000488 ***|
| FLTotal     | -0.001462 | 0.000877   | -1.667  | 0.096599    |

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Signif. codes: p-value 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1