Conservation genetics and evolutionary systematics of the genus

Tetratheca (Tremandraceae)

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Abstract

Conservation status was examined in 28 populations of seven selected species of *Tetratheca*. Allozyme variation at 5 polymorphic loci was examined using allozyme electrophoresis. This technique and multivariate morphometrics were used to assess systematic and evolutionary relationships.

Tetratheca species are woody perennnial shrubs and occur in small disjunct populations in the South-West and transitional botanical districts of Western Australia. The genetic diversity maintained within *Tetratheca* was high, comparable to that of long lived perennial plants. The mean number of alleles per locus was 2.3 and the expected panmictic heterozygosity (genetic diversity) was 0.342. A considerable deficit in heterozygotes was observed for all populations except for a 'granite' form of *T. hirsuta* Heterozygote deficiency may indicate underlying sub-population structure or inbreeding. No relationship was found between population size and genetic diversity. Widespread species maintained higher levels of genetic diversity than restricted species.

The average interspecific pairwise comparison of genetic similarity (range 0.130-0.874) are similar to intraspecific values (range 0.302-0.959). The mean number of alleles per locus and the mean percentage of polymorphic loci were high in all populations except *Tetratheca paynteri*. Allozyme electrophoresis and morphometric analysis of floral characters validated the status of the newly described *Tetratheca paynteri*. and provided evidence that at there are at least four forms of *Tetratheca hirsuta*.

Tetratheca species show uniformly low interspecific genetic similarity estimates (average I=0.32), a large number of different alleles per population, pronounced morphological differences and allopatric distributions all support recognised species definitions and suggest that that restricted *Tetratheca* species are relictual endemics whose populations have been reproductively isolated for a long period of time.

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CHAPTER ONE

GENERAL INTRODUCTION

Tetratheca is one of three genera in the small Australian family Tremandraceae. Twenty one species are endemic to Western Australia and these extend from Geraldton in the north, to Albany in the south, and to Coolgardie in the east. Eighteen species are distributed in the eastern states and these range from Kangaroo Island, to southern Queensland, and also occur in Tasmania (Thompson, 1976).

Tetratheca species occupy a range of habitats from clay swamps in the karri forest *(Tetratheca filiformis)*, lateritic loams in the jarrah forest *(T. hirsuta)*, banded ironstone hills *(T. harperi* and *T. aphylla)*, granite monoliths *(T. deltoidea)* and sandplain *(T. efoliata)* (Thompson, 1976). Population sizes and records of species associated with *Tetratheca* are generally not available.

The systematic relationships between Western Australian *Tetratheca* species are obscure. Many *Tetratheca* species have been poorly collected and provide an inadequate database on which to base taxonomic revisions. Thompson (1976) noted the general lack of data for Western Australian species in a taxonomic monograph of the genus. Peripheral taxonomic research has investigated the wood morphology of *Tetratheca retrorsa* (Carlquist, 1977), floral anatomy of *T. efoliata* (Suvartha, 1984) and pollen morphology of *T. affinis* (Erdtman, 1986). However, none of this information has been used for systematic analyses.

Conservation strategies for *Tetratheca* species have by necessity, been based primarily on their geographic rarity and have been implemented, at least in the short-term by the preservation of habitat. Of the 21 recognised species of *Tetratheca*, two are gazetted as rare flora under the Western Australian Wildlife Conservation Act 1950-1979; six are considered priority species for conservation, based on guidelines established by the Department of Conservation and Land Management; two are believed to be extinct after not being observed in the wild in the last 50 years and the remaining 11 species are presumed to be widespread (from herbarium records) but require taxonomic revision.

Legal protection of *Tetratheca* species and their associated habitat represents only the first step towards their conservation. Long-term survival, the ability to adapt to environmental changes, and continued evolution and reproduction are dependent on the

maintenance of their genetic diversity (Frankel, 1982). Conservation genetics aims to assess levels and distribution of genetic diversity in order to define parameters which will provide the greatest opportunity for species to survive and evolve. (Ledig, 1987; Moran and Hopper, 1987; Soule and Simberloff, 1986).

The population, defined as "individuals in an area experiencing at least 1% pollen or seed movement across its width" (Loveless and Hamrick, 1987) is the focal point of studies on genetic diversity. If gene flow is restricted by limited seed and pollen dispersal, small and or isolated conspecific populations may lose genetic variation and diverge as genes become fixed due to the effects of random drift, founder effects and selection. The reproductive strategy of plants, especially whether a plant is primarily selfing or outcrossing, may operate to counteract or compound the effects of limited gene flow. Other biological attributes that shape population genetic structure are the population demography (spatial and temporal contribution of parental genotypes), fecundity and the habitat. The relative effects of these 'life history' attributes on levels of genetic variation in plants have been reviewed by Hamrick (1979) and Brown (1978).

Methods for assessing levels of genetic variation range from morphological comparisons to DNA techniques (Stace, 1989). Morphological characters may be indicative of different genotypes or may reflect phenotypic plasticity. Convergence, the possession of similar characteristics in two or more groups without an immediate ancestor is a major problem in systematics when using morphological characters (Stace, 1989). Convergence is highly unlikely as the amount of evolutionary and taxonomic information increases from secondary compounds, to proteins and nucleic acids (Takhtajan, 1973; Gottlieb, 1977). DNA sequence data provides the most basic genetic information for systematics, however routine techniques are not widely available (Brown, 1990).

The technique of allozyme electrophoresis has been recognised as one of the fastest and most economical ways of assessing genetic structure and microevolutionary processes such as migration, population differentiation, mating systems and hybridization (Brown, 1989). Its widespread use has resulted in a wealth of literature on which to compare and understand levels of variation. One advantage of using allozyme electrophoresis is that the amount of material required to survey many enzyme systems is minimal, an important factor when assessing genetic diversity in rare species. A

survey of the literature indicates that allozyme electrophoresis has never been applied to the study of genetic variation in *Tetratheca* species.

Allozyme electrophoresis generally fulfills the criteria first established by Hubby and Lewontin (1966). These are:

"1. Allelic expression should be distinguishable in individuals;
2. The effect of each allelic substitution should be locus-specific;
3. All base substitutions should be detectable and;
4. Loci should be sampled at random, irrespective of their function or likely level of polymorphism."

Another advantage of allozyme analysis is that homozygous and heterozygous individuals can be differentiated by their allozymes which are inherited as co-dominants (Crawford, 1983). The specificity of enzymes allows the assignment of alleles to loci and comparison of loci between species and populations (Brown and Weir, 1983). However, there are several inherent disadvantages.with allozyme electrophoresis and these have been reviewed by Brown and Weir (1983) and Gottlieb (1977). These are listed below;

1. post-translational modification of mobility (genetic or environmental) may occur;
2. enzymes may vary in specificity making it difficult to ascribe variants to loci;
3. 30% of substitutions in nucleotides do not result in substitution of amino acids;
4. substitutions in non-translated regions of the genome cannot be detected;
5. only a limited class of enzymes are sampled;
6. the adaptive significance of most allozyme diversity is not known;
7. genetic differences between taxa may be underestimated by electrophoresis

Tetratheca species represent a mere 0.6% of Western Australia's rare and endangered plants. The resources available for the development of conservation strategies for endangered flora are dwindling and so few rare species can be investigated in detail (Hopper et al., 1990). However, species within the genus *Tetratheca* exhibit a wide range of biological and demographic qualities and so an investigation of the biogeography, biology, systematics and population genetics of the widespread *T.*

hirsuta, T. efoliata and *T. affinis* , in comparison with the restricted *T. aphylla, T. paynteri, T. harperi* and *T. deltoidea,* could potentially provide data on which to formulate conservation strategies for species sharing similar biological and biogeographic attributes.

The aim of this thesis is to clarify taxonomic relationships and to assess the conservation status of *Tetratheca* species. In order to achieve this aim it is necessary to establish the biogeographic distribution of *Tetratheca* species by extensive survey (Chapter 2). An understanding of their spatial distribution and biology are also central to taxonomic and evolutionary theory. The technique of allozyme electrophoresis using cellulose acetate gels is employed (Chapter 3) to ascertain the distribution and levels of genetic diversity within populations. Systematic and evolutionary relationships are assessed based on the results of allozyme electrophoresis and morphometric analyses (Chapter 4). The comparison of patterns of genetic diversity between species possessing different distributions and biology expedite recognition of systematic relationships (Soule, 1976) and may elucidate why some species are restricted and others widespread. Finally, the results are summarised and discussed with the aim of developing conservation strategies for Western Australian *Tetratheca* species (Chapter 5).

The hypotheses tested were as follows:

1. Widespread species are more genetically diverse than restricted species;

2. *Tetratheca hirsuta* is divisible into taxonomically meaningful groups;

3. Species occurring on the banded ironstone hills are relics of a more mesic past.

CHAPTER TWO

BIOGEOGRAPHY AND BIOLOGY

2.1 INTRODUCTION

The precise mapping of *Tetratheca* distributions, and the simultaneous recording of population sizes and habitats are the first steps in formulating a conservation strategy. The majority of *Tetratheca* species have been inadequately collected, despite their showy appearance when in flower. After flowers abscisse the shrubs become highly inconspicuous and consequently many species have never been collected in fruit. Collectors' notes accompanying herbarium specimens have provided the primary reference source for previously published maps and biological accounts (Thompson, 1976; Keighery, 1979). Many distribution records are very old (1914), are difficult to relocate and can not be assumed to reflect extant populations of even those species believed to be widespread. The recognition of spatial distributions of *Tetratheca* taxa enables the detection of hybrid zones, which is important in the understanding of evolutionary relationships and patterns of speciation (Stace, 1990). Genetic and morphological variation are best interpreted in conjunction with population demography and biological data (Hopper and Coates, 1990).

Legal protection afforded to individual *Tetratheca* species was assigned by the Department of Conservation and Land Management after consideration of all available biogeographic and biological data. A species is generally considered "rare" if there are less than several thousand individuals in the wild and/or there are substantial potential threats to its survival (Hopper, et al., 1990). Several *Tetratheca* species have not been collected for over 80 years and are presumed extinct (Briggs et al., 1988). The gazetted rare taxa *T. harperi* and *T. aphylla*, were only recollected within the last 10 years (Keighery, 1979). *Tetratheca deltoidea* was presumed extinct until recollected in 1988 (Hopper, pers comm., 1988), and is currently considered a "taxon with few poorly known populations on conservation lands" (Hopper et al., 1990). The true rarity of these *Tetratheca* species could only be ascertained by extensive survey.

This chapter investigates the distribution, habitat, population size and general biology of *Tetratheca aphylla, T. harperi, T. deltoidea, T. hirsuta, T. efoliata, T. affinis* and *T. paynteri*, a newly described species (Appendix 4). This data will provide the foundation on which to formulate conservation strategies and interpret evolutionary

processes within the genus. Failure to understand these attributes can diminish interpretation of biochemical and molecular information.

2.2. MATERIALS AND METHODS

Specimens of *Tetratheca aphylla, T. harperi, T. deltoidea, T. hirsuta, T. affinis* and *T. efoliata,* housed in the Western Australian Herbarium (PERTH) and the CALM regional herbaria at Manjimup and Albany were examined and label information on the distribution, habitat and flowering phenology was recorded.

Herbarium records that could be located on a map were visited to see if *Tetratheca* populations were still extant. Once acquainted with the habitat type of each species, topographic and soil maps were consulted to facilitate the selection of areas likely to support new populations.

Populations once located, were surveyed. A record was made of the location, species, population size (number of individuals), approximate age, reproductive stage (bud, full bloom, fruit, vegetative), topography and soil type. The dominant associated vegetation was described. Any evidence of disturbances, such as *Phytophthorra* species, fire, grazing, clearing and weed invasion was noted. Pollinators, dispersal agents and predators were actively searched for and recorded.

A total of 28 populations, representing 7 taxa, were studied and allocated a code to simplify their discussion in the following chapters. Biogeographic and biological information was recorded for all populations of the restricted species *Tetratheca aphylla, T. harperi, T. deltoidea* and *T. paynteri.* Only eleven populations representative of the geographic range of *Tetratheca hirsuta* were investigated due to limitations in time and resources. Detailed information was recorded for only two populations of *T. affinis* and one population of *T. efoliata* to provide baseline information for the systematic analyses in Chapter 4. The distributions of *Tetratheca aphylla, T. harperi, T. deltoidea and T. paynteri* (sp.nov. in.edit) were plotted onto 1:100 000 topographic maps. *Tetratheca hirsuta,T. efoliata* and *T. affinis* were plotted on 1:1 000 000 scale cadastral maps.

2.3 RESULTS

The distributions of the *Tetratheca* species investigated are illustrated in Fig. 2.1. In order to interpret distribution patterns the boundaries of the Botanical Provinces and districts in South-Western Australia described by Beard (1980) are shown. The shaded areas on Fig. 2.1. refer to detailed maps which illustrate locations of populations for each taxa (Figs. 2.2-2.14).

Locations, latititude, longitude, estimated population size and codes allocated to each of the 28 populations representing *Tetratheca aphylla, T. harperi, T. deltoidea, T. hirsuta, T. affinis, T. efoliata* and *T. paynteri* are listed in Table 2.1.

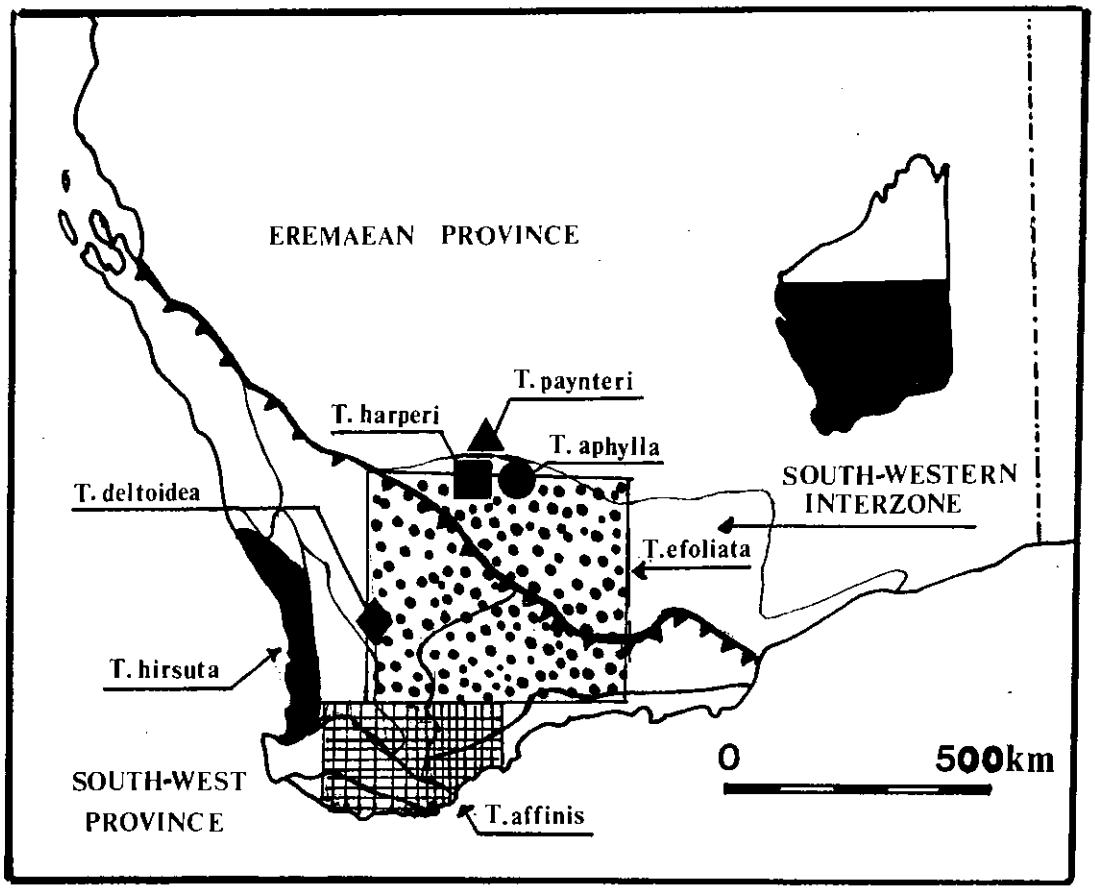

Figure 2.1 Distributions of 7*Tetratheca* species showing boundaries of the South-West Province and the Transitional botanical district (Beard, 1980).

Table 2.1. Estimated population size and locations for each of the 7 taxa of *Tetratheca*

	N=estimated population size			
TAXON	Latitude	Longitude	Location	N
CODE				
T.aphylla				
APH1	30°21'51"	119°41'52"	West of saddle, Aurora ranges	75
APH2	30°21'57"	119°41'58"	East side of saddle, Aurora ranges	150
APH3	30°23'40"	119°37'40"	Bungalbin peak	200
APH4	30°22'29"	119°37'47"	diggings, N.W. Bungalbin hill	100
APH5	30°21'11"	119°42'18"	7.5 km N.E. Bungalbin Hill	100
APH6	30°21'11"	119°42'18"	Northern most access Aurora ranges	200
APH7	30°23'40"	119°37'47"	Gully just N.E. of Bungalbin	200
T. paynteri				
PAY1	30°00'40"	119°09'17"	11 km S. of Pigeon Rocks	1000
T. harperi				
HAR1	30°15'	119°16'00"	near peak of Mt. Jackson	150
HAR2	30°15'26"	119°17'17"	N.W. end of Muddarning Hill	250
HAR3	30°15'00"	119°16'58"	S. side of Muddarning Hill	100
HAR4	30°15'00"	119°16'00"	gallery below Mt. Jackson peak	60
HAR5	30°15'00"	119°16'00"	rock to east of main Mt. Jackson popn.	100
T. deltoidea				
DEL1	31°48'00"	117°38'00"	Mt. Caroline, S. of Kellerberrin	160
T. hirsuta				
HIR1	30°01'37"	116°03'00"	end of Gilchrist Road, Lesmurdie	150
HIR2	32°30'33"	115°59'23"	Hines Road, North Dandalup	100
HIR3	32°20'00"	116°04'00"	Jarrahdale, Robinswood Follow	1000
HIR4	32°43'00"	116°05'51"	1.7 km E. of Dwellingup	400
HIR5	32°39'00"	116°02'00"	Torrens Road, N. of Dwellingup	50
HIR6	31°32'45"	116°06'10"	Muchea Rd. east, nr. Lower Chittering	200
HIR7	31°10'27"	115°50'58"	Wannamal West Rd., Gingin	500
HIR8	32°02'12"	116°01'51"	Gosnells Road East	1000
HIR9	*32 °01'06"*	*116 °01'51"*	Zig Zag Scenic Drive, Gooseberry Hill	400
HIR10	32°01'06"	116°02'28"	Ozone Terrace, Kalamunda	300
HIR11	32°02'14"	116°03'00"	Bickley Brook Reservoir	450
T. efoliata				
EFO1	31°16'55"	119°37'01"	28.3 km East of Southern Cross	100
T. affinis				
AFF1	34°40'55"	117°54'47"	Foot of Porongorups	60
AFF2	34°50'43"	117°25'46"	Sheepwash State Forest, Denmark	100

2.3.1. *Tetratheca aphylla*

This species is geographically quite restricted. Seven distinct populations have been located (Table 2.1.) between the summit of Bungalbin Hill, and 9 km north-east to the eastern end of the Aurora Ranges (Fig. 2.1.). The erect, leafless shrubs to 50 cm in height (Fig. 2.3a.) are usually found growing in the upper contours of the range, often on cliff faces, directly out of small soil pockets and crevices in massive banded ironstone and jasperlite. Two populations were located in the lower landscape, APH4 and APH2, and occasionally plants were found growing on the sides of tracks in the north east end of the range. The associated vegetation is sparse and consists mainly of low shrubs and mallees (Fig. 2.3b.). The number of individuals in the known populations was conservatively estimated to exceed 800, however there could be several thousand individuals inhabiting the inaccesible, extensive, massive ironstone detected from the main tracks.

Tetratheca aphylla appears to flower opportunistically. Unseasonal rain may initiate growth of buds that may not be destined to survive. Flowering usually occurs between September and October, but has been recorded after rains in April and May. Plants growing in more sheltered or better watered microsites tend to produce more flowers for longer periods; one such plant flowered continuously from April to December. *Tetratheca aphylla* flowers are vivid pink and have a sweet musk scent and so are potentially highly attractive to pollinators. Despite this only *Syrphid* hover flies were found near the plants. Although prolific fruit was set on most shrubs, the fecundity of the seeds is not known. The longevity of the plants was difficult to assess and only one seedling was found. The threat of fire and dieback are probably minimal due to the sparseness of the vegetation amongst the ironstone rocks. Individuals in the populations near the northern end of the Aurora Range are often damaged or destroyed by grading and trampling. Rabbits, which are abundant near some populations, may decimate the highly palatable seedlings.

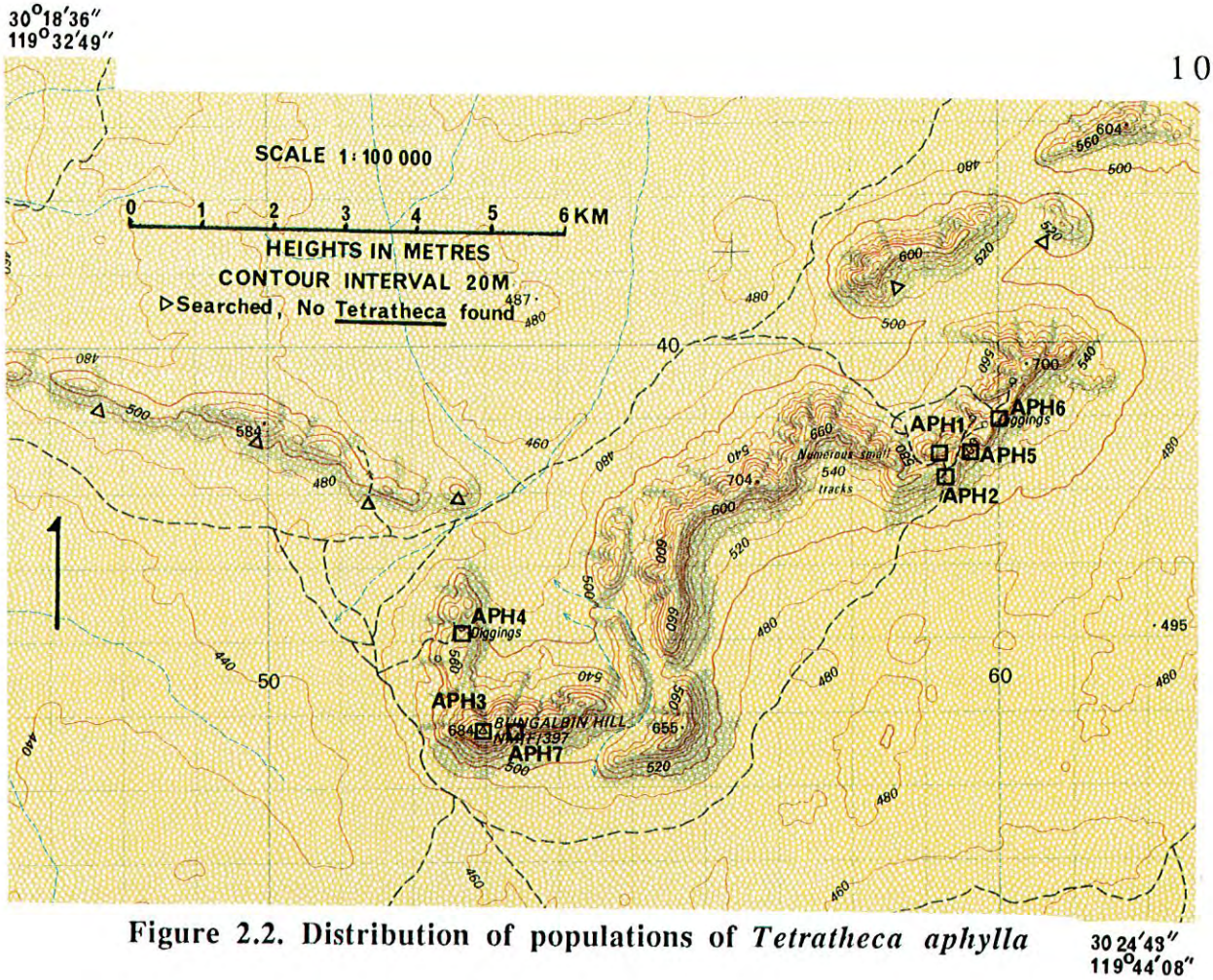

Figure 2.2. Distribution of populations of *Tetratheca aphylla*

Figure 2.3. a) habit and b) habitat of *Tetratheca aphylla*

2.3.2. *Tetratheca paynteri* (sp. nov. in edit.)

One population of less than 1000 individuals, of what was thought to be an outlier form of *T. aphylla,* was first discovered in 1988 (Rae Paynter, pers. comm.), in a small range of unnamed hills, 11 km south of Pigeon Rocks and 124 km north of Bullfinch (Fig. 2.4.). Morphological comparisons of floral and later, fruiting material of the putative *T. aphylla* "form" with *T. aphylla* established that this population represented a new species (described in Appendix 4). No other populations of *T. paynteri* were discovered, despite extensive surveying of similar habitats during this study.

Tetratheca paynteri is an erect to weeping, leafless shrub to 40 cm tall (Fig. 2.5a.). It grows directly out of small pockets of rich, moist, red, loam soil in between massive black and red ironstone rock, primarily on the northerly aspects (Fig. 2.5b.). Two sub-populations were found lower in the landscape on breakaways. Several plants in the more protected microsites facing N.N.E. appeared to be very old, judged on their size and the quantity of dead growth from previous seasons. The root system of this species is extensive, permeating large volumes of soil between the ironstone rocks. Some very large plants which appeared to have completely died retained live rootstocks or had sprouted new shoots with leaves similar in form to those of *T. harperi.* No leaves were found on adult growth.

Flowering appears to be opportunistic, depending on rainfall and was recorded in April, August and November. The flowers have a sweet, musk scent. No pollination events were recorded and few potential pollinators were seen. Ants were observed removing *Tetratheca paynteri* seeds.

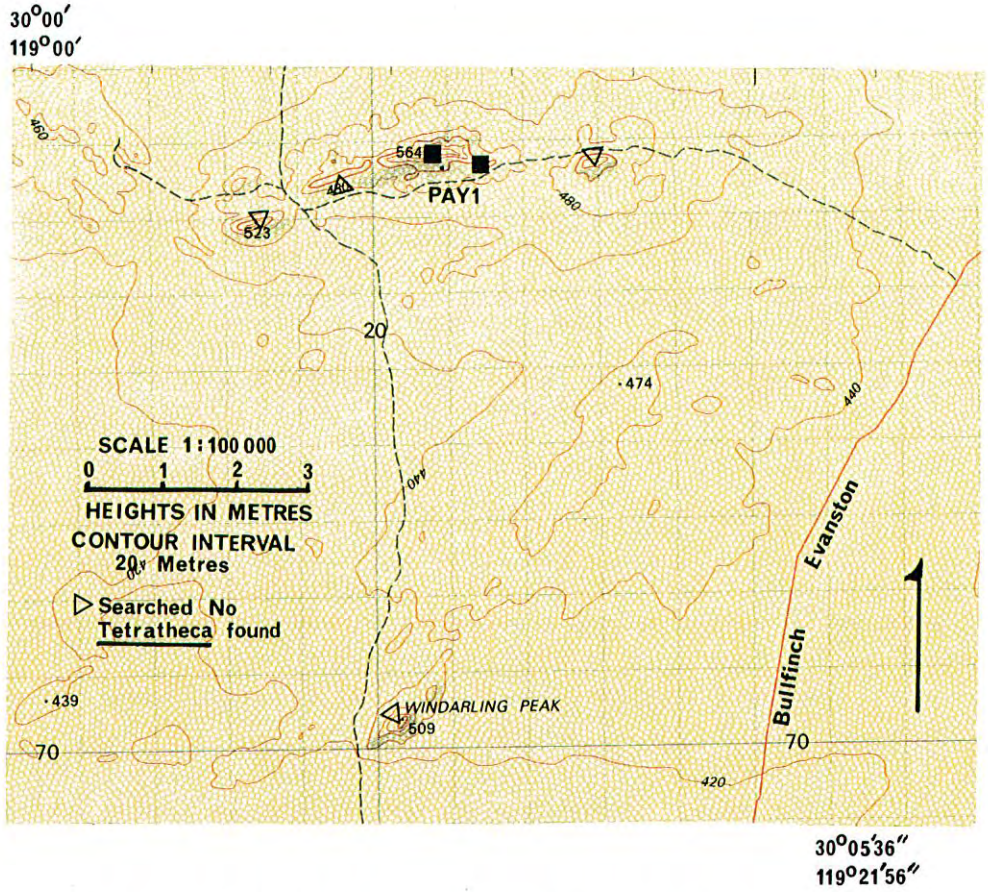

Figure 2.4. The distribution of *Tetratheca paynteri*

Figure 2.5. a) habit and b) habitat of *Tetratheca paynteri*

2.3. *Tetratheca harperi*

This species is restricted to massive ironstone hills between Mt. Jackson and the adjacent Muddarning Hill, 90 km North of Bullfinch. Five populations of between 60 and 250 individuals (conservative estimates), a total of 660, were located within a 3.4 km area (Fig. 2.6.). The spiny shrubs to 40 cm (Fig.2.7a.) are extremely selective in habitat preference growing in crevices of rich, loamy, brown soil on or close to the base of massive ironstone outcrops (Fig. 2.7b.). Populations were usually orientated south to south-east, with one sheltered population on a north-west cliff face (HAR2). Although much of the more inaccessible parts of the ranges were not surveyed for *Tetratheca* it appears that suitable habitat is limited.

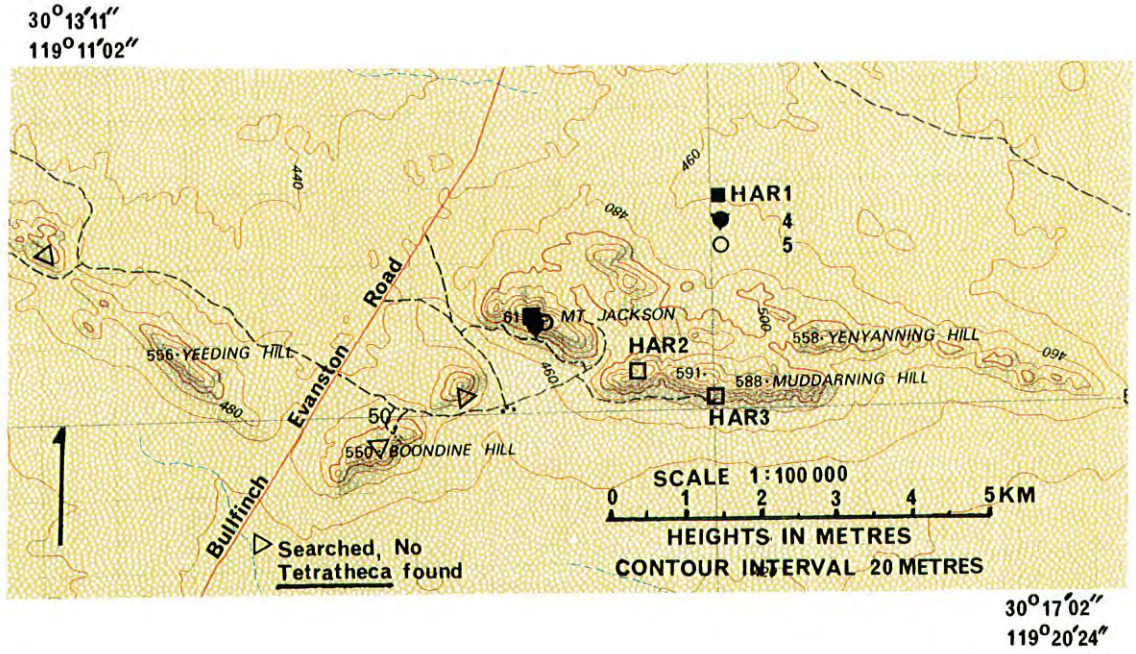

Figure 2.6. Distribution of *Tetratheca harperi* populations

The sweetly scented flowers appear from August to November. Some plants growing in wetter and/or more sheltered microsites flower opportunistically after unseasonal rains. Few fruits develop on *Tetratheca harperi* when compared with *T. aphylla* although this was not quantified. *Tetratheca harperi* does not appear to tolerate disturbance of any kind. No evidence of fire or disease was apparent, the aridity and sparseness of cover near plants affording them some protection. No pollination or dispersal events were evidenced although occasional hovering *Syphid* flies and native bees were seen. *Tetratheca harperi* root stocks extend way into rock crevices and are very fibrous and though. Some plants were found with large quantities of dead material supporting the flush of growth, suggesting that this is quite long lived. Leaves generally are only found on new stems or on plants growing in sheltered microsites. A grasshopper was seen predating fruits of *Tetratheca harperi*. (Fig. 2.7c).

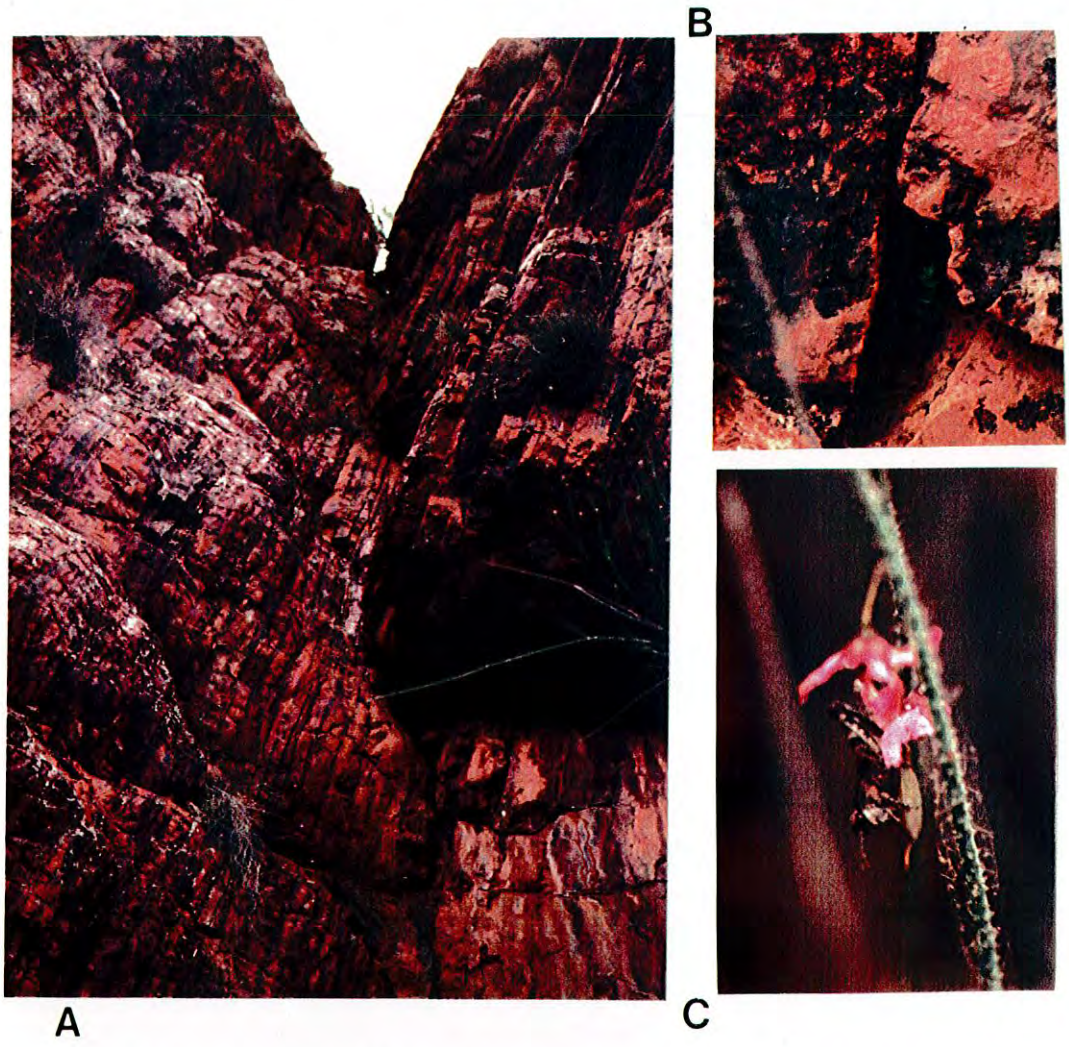

Figure 2.7. a) habit b) Seedling of *Tetratheca harperi*
c) grasshopper predating fruits of *T. harperi*

2.3.4. *Tetratheca deltoidea*

Despite extensive surveys of likely habitats, *T. deltoidea* was found to be restricted to one population of 160 individuals on the upper slopes of Mount Caroline Nature Reserve, 19 km south of Kellerberrin (Fig. 2.8.). Two sub-populations of 100 and 60 individuals are separated by only 25 metres. The leafy, trailing plants to 1 m (Fig. 2.9a.) were found growing in small pockets of rich, grey loamy humus in swales of massive granite (Fig. 2.9b.). The dominant associated plant species, *Eucalyptus caesia* and *Lepidosperma resinosum* appeared to provide dense cover and protection for the leafy, delicate *Tetratheca deltoidea*. Disturbed pockets of habitat similar to that of the main population failed to support *T.deltoidea*.

Strongly scented.flowers are produced between September and November. Seed is set in November and December. Discrimination of individual plants was difficult because of the species' habit, the density of *Lepidosperma* between plants, and because *Tetratheca deltoidea* is stoloniferous. Stolons were found a few cm underneath the loose leaf litter and nodes occurred every 50 cm. For ethical and practical reasons only a few root systems were carefully investigated. The tortuous growth pattern of the roots made it difficult to follow them without severe damage to surrounding plants. No pollination event was recorded despite the fact that "potential" pollinators such as native bees, flies and mosquitos were abundant. Evidence of disturbance was restricted to the presence of exotic grass species *(*Briza maxima, *Avena barbata)*, and rabbits, which may affect the establishment of *Tetratheca* seedlings. It is unlikely that fire or *Phytopthora* species could spread readily through this habitat type.

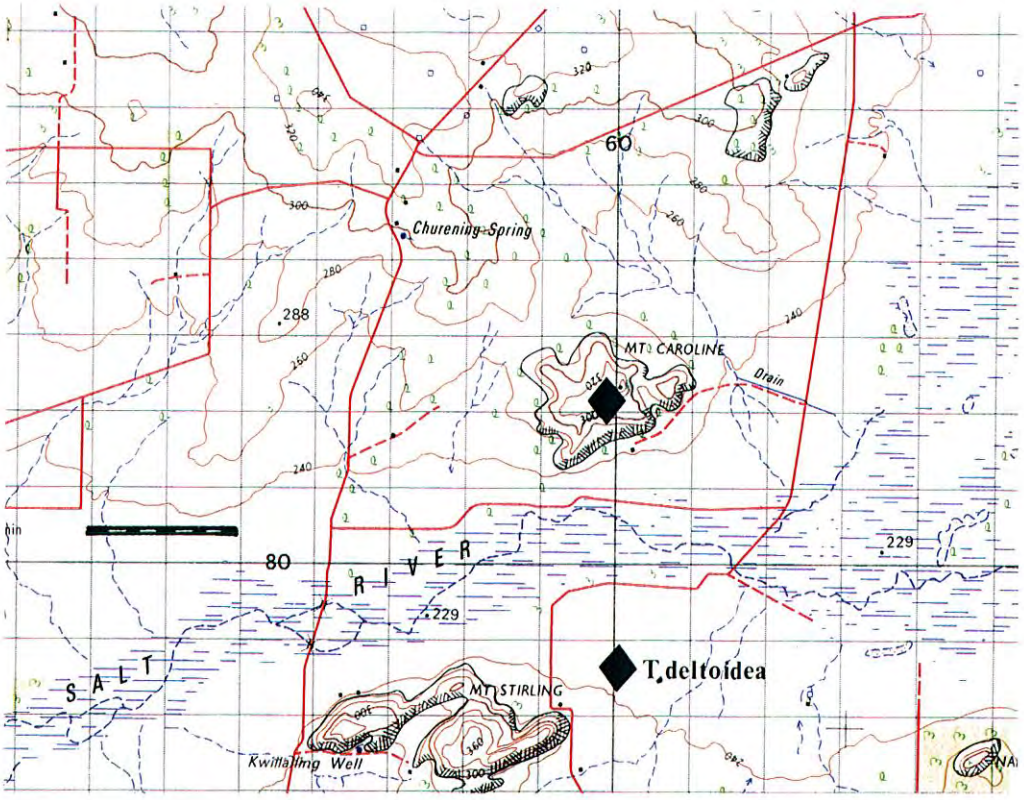

Figure 2.8. Distribution of *Tetratheca deltoidea*

Figure 2.9. a) habit and b) habitat of *Tetratheca deltoidea*

2.3.5. *Tetratheca hirsuta*

Tetratheca hirsuta has been recognised as one of the most abundant and widespread of the genus with an "historical" geographic range of 700 km from Geraldton to Albany (Fig. 2.11.). Taxonomic confusion between *T. hirsuta* and the morphologically similar species *T. setigera* and *T. hispidissima* appears to have resulted in an overestimation of the abundance of *T. hirsuta*. In this study *Tetratheca hirsuta* was recorded at 24 locations (see Fig. 2. 10) from north of Gingin, to Collie, 275 km to the south. Although geographically common, the population sizes were often small (2-1000 individuals) or persisting on increasingly degraded road reserves. Eleven of the largest populations were surveyed in detail. Searches for "historical" populations were often unsuccessful, possibly due to their being cleared or degraded and because the plants were notoriously difficult to find except when in full flower. Populations of *Tetratheca hirsuta* could be segregated into two types based on distinct differences in habit and habitat: 1. robust shrubs, granite forms and 2. small shrubs, lateritic forms. In order to evaluate the importance of these differences, the two types will be discussed separately.

2.3.5.1. Robust shrub, granite forms of T. hirsuta

Four granite populations (HIR8,9,10,11) with an estimated 300-1000 individuals were surveyed. The shrubs were generally robust and multibranched to 1.4 m. tall with two forms of leaves (Fig. 2.11a.). New seasons growth has alternate, linear leaves and the older, woody growth has whorls of 3 broad leaves. These features have caused confusion when comparing herbarium specimens. Collections of new growth (where most of the flowers develop) are difficult to discriminate from the smaller, laterite *T. hirsuta* specimens. The four *T. hirsuta* populations grow in rich, brown clay-loam, over granite; on the edge of or just upslope from watercourses. The dominant associated species are *Eucalyptus rudis, E. marginata* and *E. calophylla* over species rich heath to 1.5 m.

The granite populations of *T. hirsuta* have a lengthy flowering period, HIR8 flowered from May to October in 1990. Flowers have a heavy musky scent. The incidence of fires through the granite populations appear to be low, judging from the woodiness and size of *T. hirsuta* shrubs. Fires would probably be extinguished before reaching *T. hirsuta* granite populations because they are close to human habitation. Potential pollinators (bees, flying insects) were found in high numbers but no pollination events were recorded.

2.3.5.2. Small shrub, lateritic forms of T. hirsuta

Seven populations of this form of *T. hirsuta* were studied. Population sizes ranged from 50-500 individuals. All were either erect, multi-stemmed or somewhat lax, trailing shrubs between 25 cm and 50 cm (Fig. 2.11 b,c.). Morphological variation was observed between the populations and is discussed in Chapter 4. Habitat types ranged from gently undulating laterite gravel and loam to low, massive laterite outcrops. All populations were associated with *Eucalyptus marginata* and occasional *E. calophylla* in low forest over *Xanthorrhoea, Macrozamia* and *Banksia grandis* over low heath.

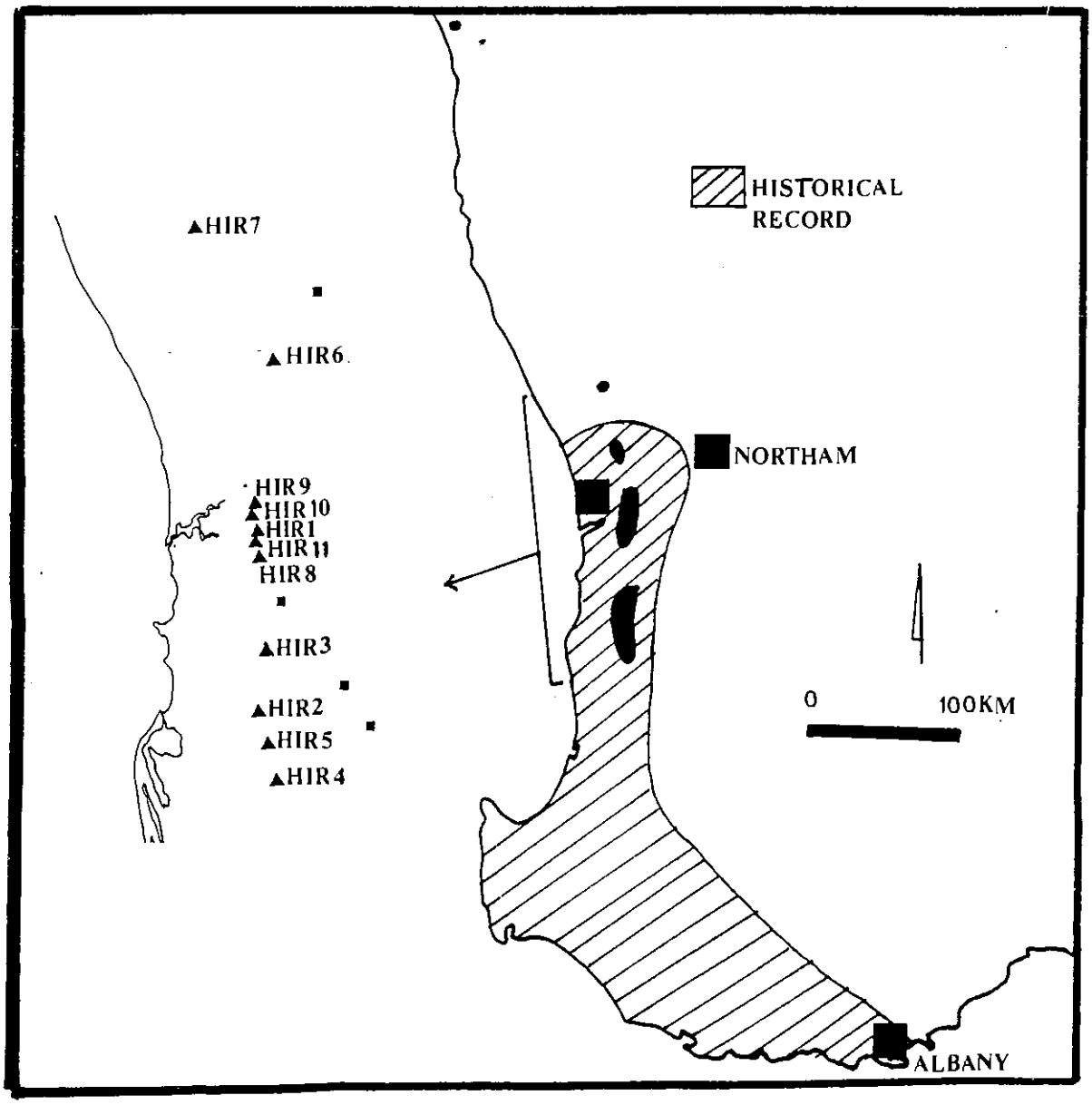

Figure 2.10. Distribution of *Tetratheca hirsuta* showing historical collection locations and the populations located in this study.

Flowering in this form of *T. hirsuta* occurred between August and November, HIR3 flowered in July. Continuous observations over a long time scale are required to ascertain if there are subtle flowering phenology differences between these populations. Flowers in populations HIR6 and HIR7 were strongly scented, all plants in other populations appeared to have no scent. In some populations numerous, small *Tetratheca hirsuta* shrubs with new growth stems arising from well developed rootstocks were found. These characteristics may be indicative of adaptations to survive fire as suggested by Keighery (1979). Indeed it appears that at some populations fires occur frequently, especially HIR3, HIR4 and HIR5.

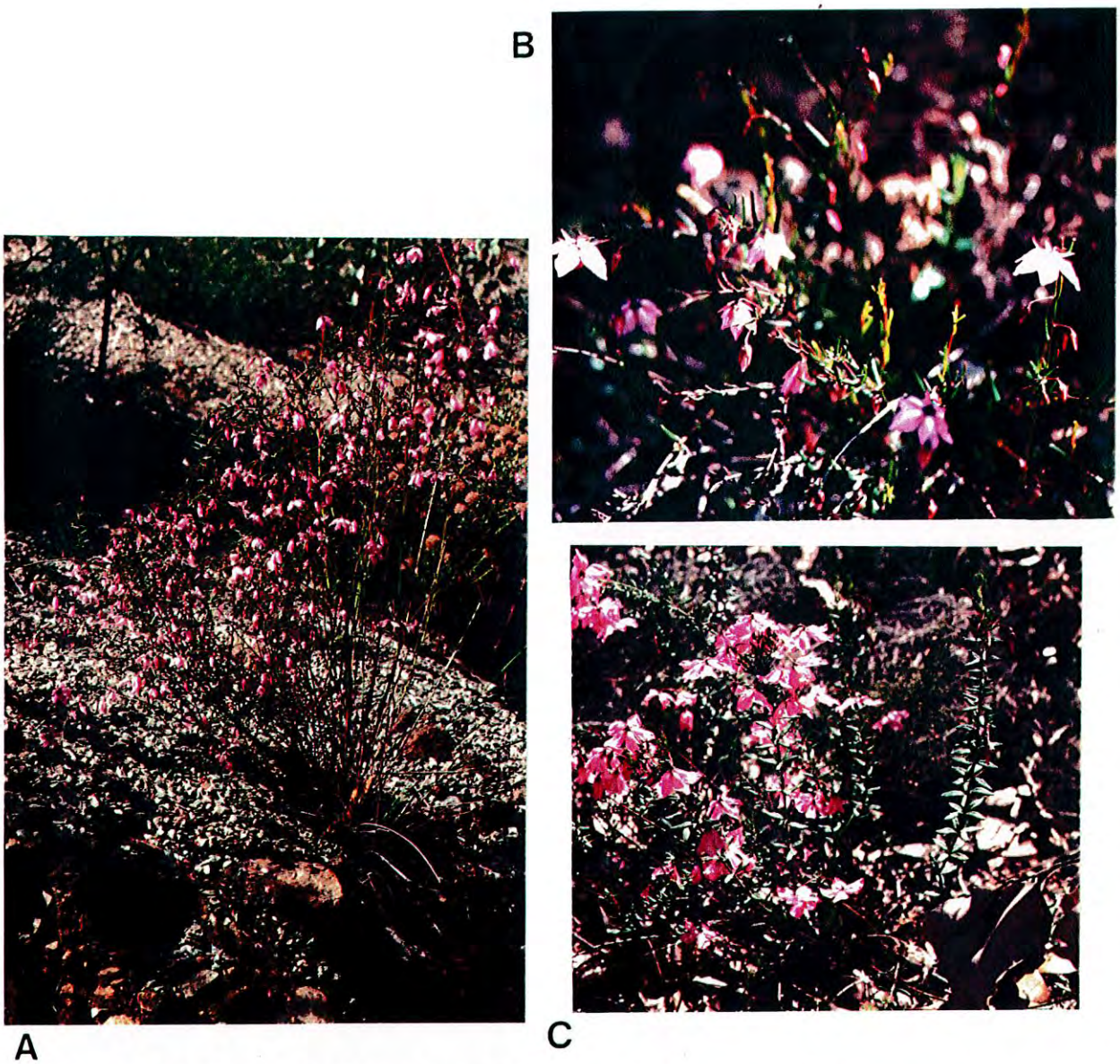

Figure 2.11. Habit of *Tetratheca hirsuta* a) HIR8 form b)HIR7 form c) HIR2 form

2.3.6. *Tetratheca efoliata*

Although this species is extremely widespread geographically, populations are surprisingly difficult to find. The historical distribution extends from Tammin in the west to Norseman in the East, south to Newdegate and to north of Bungalbin Hill (Fig. 2.12). Of several small populations of *T. efoliata* located during this study, only the largest (100 individuals), EFO1 was investigated in order to provide background data which may elucidate evolutionary relationships between this species and the most geographically close species, *T. aphylla* and *T. harperi*. EFO1 occurred on yellow sandplain country with a gravel overlay (Fig. 2.13a).

Flowering occurs in *T. efoliata* from August to December, depending upon rainfall and prolific fruits were formed (Fig. 2.13b). This species is a disturbance opportunist, often growing on regularly graded road shoulders and tracks. Plants growing in drier sites, even on a microscale, exhibited fewer leaves and were more wiry and tortuous than those growing in drainage areas or under shrubs. No pollinators were found. It is highly unlikely that *Phytopthora* species would spread or survive in this arid, well drained environment. It was difficult to assess the effects of fire, because although they carry well they are often patchy, through this habitat type. Rootstocks were extremely well developed even in very small plants, and this may reflect an adaptation to arid conditions or fire.

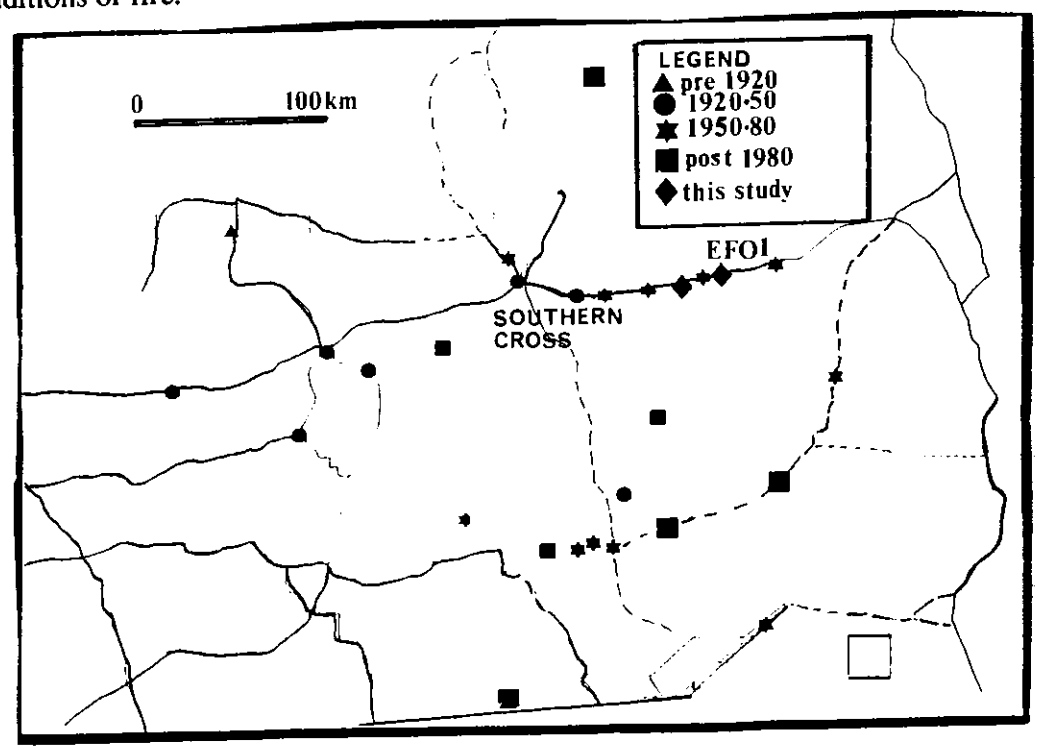

Figure 2.12. Distribution of *Tetratheca efoliata* illustrating historical records and populations located and sampled

Figure 2.13. a) habit and b) habitat of *Tetratheca efoliata*

2.3.7. Tetratheca affinis

Historical records show that geographically, *T. affinis* is an extremely widespread species, ranging from Yallingup in the West, Albany in the south to Cape Riche in the east. The populations located in this study were usually very small and disjunct, partially due to whole scale land clearance for agriculture. Intensive localised searches are needed before the distribution is completely known. Locations of *T. affinis* populations are plotted with "historical" distribution records to indicate the scale of intensive survey required (Fig. 2.14.).

Two populations of 60 (AFF1) and 100 (AFF2) individuals of T. *affinis* were investigated in this survey. AFF1 is located on a road verge at the foot of the Porongorups, north of Albany and 45 km north-east of AFF2, a population within State Forest near Denmark. *T affinis* are leafless shrubs to 40 cm, growing in laterite gravel and sand. They are associated with open, low *Eucalyptus marginata* forest over dense low shrubs. *Tetratheca affinis* appears to recover reasonably well from fires, based on the number of populations surviving in areas which are regularly burnt, however this does not imply that the populations are as viable or prolific as they were. The largest, most robust population of *T. affinis* was discovered growing with *Tetratheca setigera* amongst dense *Allocasuarina huegeliana* and *Eucalyptus marginata* to 15 m, in an area which appeared to have not been burnt for over 20 years. In the adjacent Baker's Junction Nature Reserve, *Eucalyptus marginata* woodland which has been burnt regularly, supported only scattered individuals of *T. affinis*. *Tetratheca affinis* .flowers have no detectable scent and develop between August and January. No pollination or dispersal events were recorded.

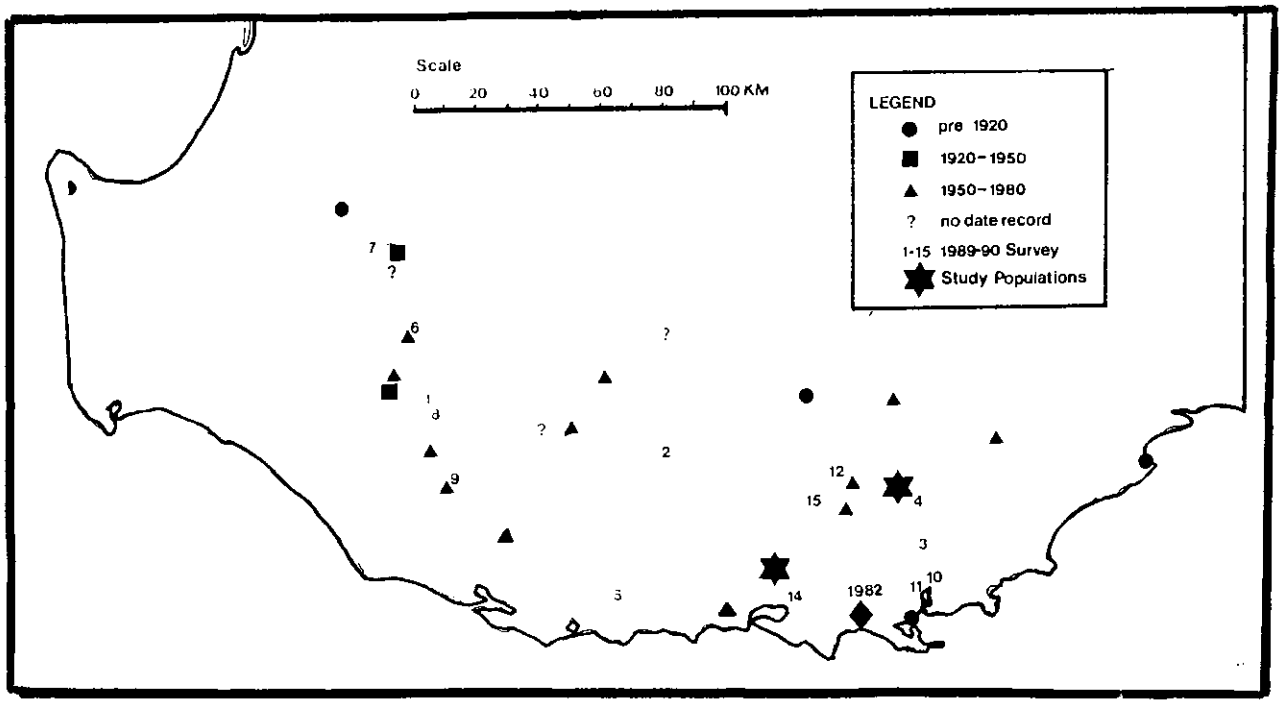

Figure 2.14. Distribution of *Tetratheca affinis*, illustrating historical records and populations located and sampled.

2.4 DISCUSSION

Tetratheca species were found to be restricted to the South-West Botanical Province and the Transitional district of Western Australia (Beard, 1980). The distribution of individual species varied from widespread, for example *T. hirsuta* with a geographic range of 275 km, to the extremely restricted species *T. deltoidea* with a range of less than 500 metres. The spatial distribution of *Tetratheca* was closely related to lithological, surface soil characteristics and the dominant plant species present. The association of *Tetratheca hirsuta* with *E. marginata* and *E. rudis*, and*T. affinis* with *E. marginata* may reflect relationships between *Tetratheca* and mycorrhiza associated with these tree species (Dixon pers. comm., 1990).

The Declared Rare Flora status of *T. aphylla* and *T. harperi* is supported by the results of this biogeographic survey. *Tetratheca. paynteri* warrants Declared Rare Flora status, because there is only one population of 1000 individuals at a location which is vulnerable to mining interests. *Tetratheca deltoidea* is probably one of the rarest species of the genus in terms of population size and geographic range. Although the only known population appears secure, changing climatic conditions, increased weed infestation and rabbits or disease could decimate the population. Without some knowledge of its population dynamics and genetic structure we cannot speculate about how viable *T. deltoidea* or any other species may be in the long term.

Tetratheca hirsuta, T. affinis and *T. efoliata* were all found to be reasonably abundant, often capitalising on disturbed sites such as gravel pits (HIR8) and regularly graded road shoulders (EFO1). Despite this the majority of populations were found to be very small (less than 100 individuals), geographically disjunct by more than 2 km and often found growing in increasingly degraded road verges or remnant bushland. *Tetratheca hirsuta* occupied two distinct habitats and expressed subtle morphological variation. In-situ recognition of hybrids between these forms is extremely difficult considering that the alpha taxonomy of the species is still fragmentary.

Flowering in *Tetratheca* species occurs between July and December, ironstone species *(T. aphylla, T. harperi, T. paynteri)* may flower opportunistically. *Tetratheca* flowers are rotate in form, lack nectar and have poricidal anthers which form a cone, connivent about the pistil. These features are recognised attributes of plants which are buzz (squeeze) pollinated by bees (Buchmann, 1983). Buzz pollination is a distinctly audible process (Keighery pers.comm., 1989) and has been observed in Western Australian Tremandraceae (Keighery, 1979). Native and feral bees were regularly seen near

populations of *T. hirsuta* (HIR6,7,8,11) and *T. deltoidea* yet no pollination events were recorded. It is unlikely, but not impossible, that the timing of visits to populations was not synchronised with optimal foraging times for pollinators, which may be dependent on diurnal microclimatic conditions such as temperature, humidity and wind velocity (Buchmann, 1983). All *Tetratheca* species are recognised as myrmecochorous (Berg, 1975). Their diaspores possess an elaiosome, a lipid rich structure which provides a food source for ants in return for seed dispersal. Myrmecochory which is observed in several Eastern States species of *Tetratheca* (Berg, 1975; Brewster, et al. 1989), was only observed for *T. paynteri* during this study. In order to observe seed dispersal in other species, it would be best to survey populations a month or so after flowers abscisse to ensure that the slow growing fruits had matured.

The effect of *Phytophthorra* species and fire on Western Australian *Tetratheca* species is difficult to assess without monitoring sites prior to disturbance. *Phytophthora* species are unlikely to be a problem in the arid, well drained Transitional botanical district. *Tetratheca ciliata*, a Victorian species which is morphologically and biologically similar to *T. hirsuta*, was found to be devastated by *Phytophthora cinnamoni* and did not recolonize diseased sites (Weste, 1986). No study of the effects of fire on *Tetratheca* species has been undertaken although it has been noted that members of the family (Tremandraceae) often possess a woody, fire resistant stock (Keighery, 1979). A comparison of the habitat and population sizes of *T. hirsuta* at HIR4 and HIR5 indicate that extensive and regular fires and the presence of *Phytophthorra* species at HIR5 have probably contributed to a reduction in population size. Most individuals in HIR5 grow on the road verge, presumably where the intensity of fires is reduced. Similarly, the largest and most vigorous population of *T. affinis* was located in habitat which had not been burnt for over 20 years. This was adjacent to depauperate populations on regularly burnt land. A major consequence of widespread land clearing and physical degradation of the environment by fire, weed infestation, pathogens (eg: *Phytophthora* species) and other disturbances has been the fragmentation of natural plant distributions. The small disjunct populations of *Tetratheca* species located in this study may have always been relatively isolated from each other or may be remnants of a much wider, more continuous distribution. Small geographic distances between conspecific *Tetratheca* populations may severely reduce gene flow (Levin and Kerster, 1974). A comparison of patterns of genetic diversity in populations of widespread and restricted species may reveal whether disjunct distributions and small population sizes are a product of geologically long-term evolutionary events or man's recent wholesale destruction of the landscape.

CHAPTER 3

CONSERVATION GENETICS IN WIDESPREAD AND ENDEMIC *TETRATHECA* SPECIES

3.1 INTRODUCTION

Conservation genetics aims to assess the levels and distribution of genetic diversity in order to define parameters which will provide the greatest opportunity for a species to survive and evolve (Ledig, 1987; Moran and Hopper, 1987, Soule and Simberloff, 1986).

Patterns of genetic diversity within *Tetratheca* species and populations may be determined by a range of biological and biogeographic attributes (Hamrick et al., 1979). *Tetratheca* species have an hermaphroditic reproduction mode, are buzz pollinated by specialised bees (Keighery, 1979; Buchmann, 1983) and they appear to be long-lived, especially in undisturbed or favourable microsites. The mating system of *Tetratheca* has not been established. Flowers produce no nectar and pollen is the only floral reward. Selfing may occur in *Tetratheca aphylla* and *T. harperi* (Appendix 1). Flowering occurs over long periods of time and flowers may last many months. Abundant intensely pink coloured, often scented rotate flowers, which have a long pedicel, long antrorse dehiscing anthers and a style that grows out between the anthers are all characteristic of outcrossing plants (Wyatt, 1983). *Tetratheca* diaspores are relatively large and robust, dispersal is primarily passive and myrmecochorous (Berg, 1972; Chapter 2). The stable environments of many *Tetratheca* species indicate that they are in late successional habitat. *Tetratheca* occupy a diverse range of unique habitats and their geographic range varies from less than 500 m (*T. deltoidea*) to almost 300 km (*T. hirsuta*).

The broad spectrum of biological attributes found in*Tetratheca* may be paralleled by their genetic diversity. The levels of genetic diversity in *Tetratheca* species were completely unknown at the commencement of this project. Widespread species of *Tetratheca* should reveal higher levels of genetic diversity than restricted species according to evolutionary theory (Karron et al., 1988; Karron, 1989). Similarly, spatially isolated populations should exhibit greater intraspecific genetic heterogeneity than continuous populations, with greater rates of genetic exchange (Endler, 1977). A comparison of widespread and restricted *Tetratheca* species and

isolated and continuous populations, may elucidate how populations function and which populations to preserve in order to maintain genetic diversity. It may become apparent that populations have suffered reductions in effective size (bottlenecks) or indicate the minimum population size likely to sustain species viability. Small heterozygote deficiencies may be observed in outbreeding plant populations due to partial selfing, population substructuring due to consanguineous matings and the Wahlund effect (Brown 1979). Principles derived from these comparisons may provide a model on which to understand genetic variation within plant taxa with similar biological traits to *Tetratheca* species, for example those that occupy the unique banded ironstone habitat.

This chapter investigates and compares the levels of genetic diversity within *T. hirsuta, T. efoliata, T. affinis, T. aphylla, T. harperi, T. deltoidea* and *T. paynteri* (sp. nov. in edit). The patterns of genetic variation will be assessed in the light of biological data derived from Chapter 2. Priority populations for conservation will discussed. The following hypotheses were tested:

1. Levels of genetic diversity are greater in the widespread species *T. hirsuta, T. efoliata* and *T. affinis* than in any of the restricted species *T. aphylla, T. harperi, T. deltoidea* and *T. paynteri*;

2. Species with small isolated populations have lower levels of genetic diversity and greater divergence between populations than species with large populations and a more continuous distribution.

3.2 MATERIALS AND METHODS.

3.2.1. Population Sampling

Open flowers and buds from populations of each species were collected between 1989 and 1990. Several buds and open flowers were randomly selected from 7-53 individual plants, placed in vials and stored in liquid nitrogen as soon as possible after collection to minimise the denaturation of enzymes. Fresh flowering stems were collected and stored, covered by a plastic bag in a refrigerator. Codes were allocated to populations and their locations are listed in Table 2.1. Distribution maps of populations surveyed within taxa are illustrated in Chapter 2.

3.2.2. Tissues

Pollen (whole stamens) produced the most consistent and scorable enzymatic results. Vegetative material (leaf and apical meristems) failed to exhibit enzyme activity. Whole seeds exhibited strong activity but could not be used due to the difficulty in obtaining sufficient material. Levels of enzyme activity were often low for material stored in liquid nitrogen, especially *T. harperi* or homogenates which expressed dark pigmentation. This may be caused by tissue damage due to cell lysis during handling and storage, or the presence of high concentrations of tannins, alkaloids and phenolics which form complexes with the enzymes. Anthocyanins have been detected in the flowers of two Eastern States species; *Tetratheca ericifolia* and *T. thymifolia* (Gascoigne, *et al.* 1948). Enzyme activity may also be reduced if stamens are collected prior to pollen maturation due to incomplete meiosis. For these reasons, fresh material was used whenever possible.

3.2.3. Electrophoresis

Whole stamens that were removed from 2 or more buds (depending on stamen size) were homogenized with 35-55 µl of a grinding buffer developed by Systma and Schaal (1985) and modified by Coates (1988) (Appendix 2). The ratio of buffer to tissue was adjusted to minimise the dilution of enzymes.

Homogenised samples were centrifuged and 11.6 µl of the supernatant was pipetted into wells of a sample plate. Cellulose acetate gels were soaked in an 80 mM Tris-EDTA-maleic acid (pH 8.2) running buffer (80 mM Tris, 1 mM Na2 EDTA, 1 mM

MgCl (2.44 mM maleic acid)) for 10-15 min and blotted dry before the samples were loaded using an applicator. Plates that were loaded with 12 samples were positioned acetate side down on the blotting paper wicks of the electrophoresis tank. The side on which samples were loaded was positioned at the cathodal end of the tank. Electrophoresis was carried out for between 25 to 40 min. at 200 volts and 4°C to optimize enzyme separation and resolution.

Immediately after electrophoresis a staining mixture of 4 ml of 3.5% agar and 4 ml of the reaction mixture for the stain were poured over the acetate plate. Full enzyme staining recipes are described in Appendix 2. The staining procedure used is as described in Richardson *et al.* (1986), except quantities of all ingredients were doubled. Gels were developed in the dark at room temperature, usually from between 5 min to 12 hrs, to optimise staining. The agar overlay was then washed off, the gel fixed in 7% acetic acid for 15 min, and then rinsed in water and air-dried.

3.2.4. Allozyme analysis

Only five of the nineteen enzyme systems tested in preliminary trials produced consistently scorable results. These were phosphoglucomutase (PGM, E.C. 2.7.5.1), leucine aminopeptidase (LAP, E.C.3.4.17.1), phosphoglucose isomerase (PGI, E.C. 5.3.1.9.), malate dehydrogenase (MDH, E.C. 1.1.1.37) and menadione reductase (MR, E.C.1.6.99.22).

Other enzyme systems tested were: aconitase (ACON, E.C. 4.2.1.3), alcohol dehydrogenase (ADH, E.C. 1.1.1.1), esterase (EST, E.C. 3.1.1.1), fumarate hydratase (FUM, E.C. 4.2.1.2), glucose-6-dehydrogenase (G6PDH, E.C. 1.1.1.49), glutamate dehydrogenase (GDH, E.C. 1.4.1.3.), glutamate-oxaloacetate transferase (GOT, E.C. 2.6.1.1), glyceraldehyde-3-phosphate dehydrogenase (G3PDH, E.C. 1.2.1.12), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42.), malate dehydrogenase (ME, E.C. 1.1.1.40), peptidase (PEP, E.C. 3.4.11), shikimate dehydrogenase (SDH, E.C. 1.1.1.25), triose phosphate isomerase (TPI, E.C. 5.3.1.1). Six-phosphogluconate dehydrogenase (6PGDH, E.C. 1.1.1.44) produced scorable but inconsistent results.

3.3.1. Interpretation

Five zones of activity representing five presumptive loci were scored for genotype. All enzymes migrated anodally. Alleles at each locus were distinguished from each

other by coding alphabetically. The most anodal was designated allele 'a' and the other alleles were lettered in order of decreasing mobility. The haploid pollen material generally expressed between one (homogygous) to two bands (heterozygous), occasionally material exhibited 3 bands (dimer), possibly as a result of high activity in the somatic tissue of the stamens.

PGM, (E.C. 2.7.5.1)

Two zones (loci) of activity were apparent but only the more anodal zone, Pgm-1, was consistently resolved and interpreted across the range of species examined. From one (*T. paynteri)* to 3 bands occurred (*T. hirsuta* and *T. deltoidea)*. Five allelic variants were scored. Typical electrophoretic banding patterns observed for PGM, and their interpretation, are illustrated in Figure 3.1.

LAP, (E.C. 3.4.17.1)

Only one zone of activity was observed and resolved. One to two bands were observed for each *Tetratheca* individual and 6 allelic variants discriminated. Figure 3.2. illustrates electrophoretic banding patterns for LAP and their interpretation.

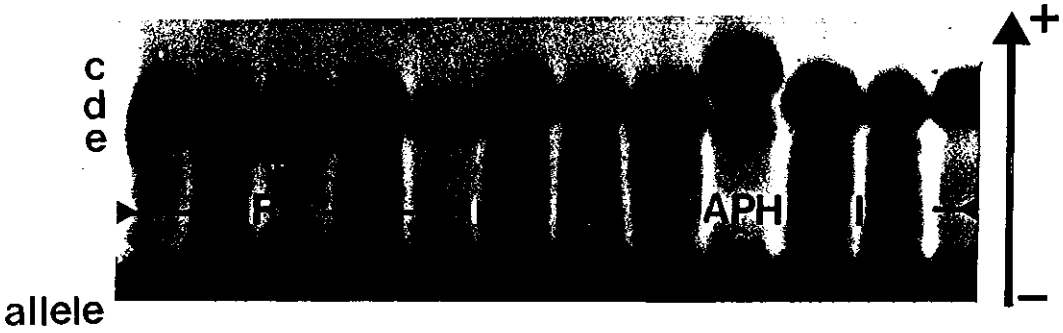

Figure 3.1. Cellulose acetate zymograms of the phosphoglucomutase (PGM) locus in *Tetratheca* species showing segregation at the Pgm-1 locus

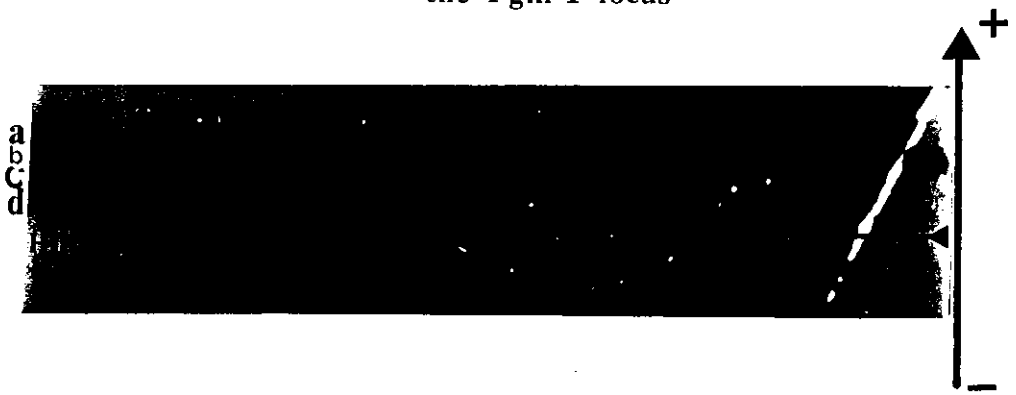

Figure 3.2. Cellulose acetate zymograms of the leucine aminopeptidase (LAP) locus in *Tetratheca* species showingsegregation at the Lap-1 locus.

PGI, (E.C. 5.3.1.9.)

Two zones of activity were evident for all species (except *T. harperi),* however only the most cathodal could be consistently interpreted and scored. Pgi-2 exhibited from one to three bands per individual and represented the most consistently of the five loci loci used. Figures 3.3. illustrates electrophoretic banding patterns observed for PGI and their interpretation.

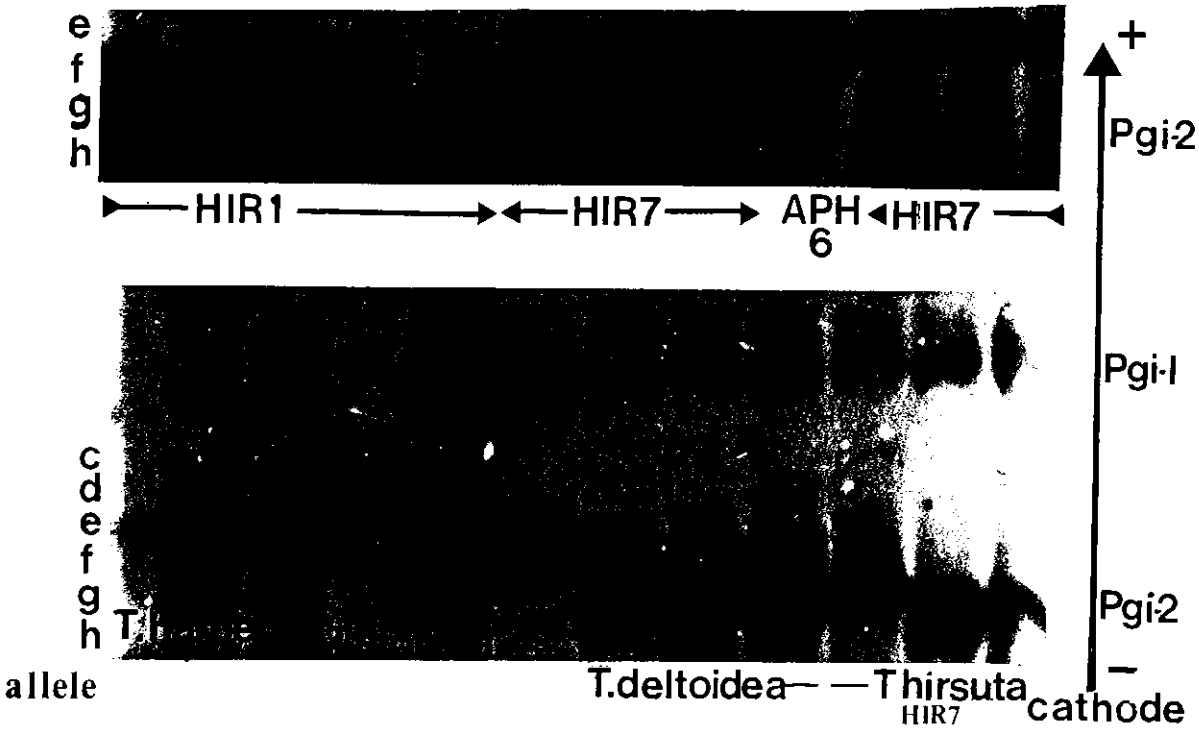

Figure 3.3. Cellulose acetate zymograms of the phosphoglucose isomerase (PGI) locus in *Tetratheca* species showing segregation at the Pgi-2 locus.

(MR, E.C.1.6.99.22) and (MDH, E.C. 1.1.1.37)

One consistently scorable zone of activity in MR and MDH was discriminated in all *Tetratheca* species. In these zones one or two bands (allelic variants) at most were expressed. Typical electrophoretic banding patterns and their interpretation are illustrated in Figure 3.4. for MR and Figure 3.4. for MDH.

Figure 3.4. Cellulose acetate zymogram of the menadione reductase (MR) locus in *Tetratheca* species showing segregation at the Mr-1 locus.

Figure 3.5. Cellulose acetate zymograms of the malate dehydrogenase, (MDH) locus in *Tetratheca* species showing segregation at the Mdh-1 locus.

3.2.5. Genetic Analyses

To provide a measure of the levels of genetic variation within a population, the following statistics were computed: A, the mean number of alleles per locus; P, the proportion of loci that are polymorphic; H_o, the observed heterozygosity (averaged over all loci); H_e, the expected panmictic heterozygosity. The expected panmictic heterozygosity (H_e) or gene diversity index (Nei, 1973) was calculated as:

$$H_e = 1 - \sum_{i=1}^{k} x^2_i$$

where x_i is the frequency of the ith allele summed over k alleles. The expected panmictic heterozygosity was then averaged over all loci for each population.

Genetic diversity can be considered to have two components, allelic richness and allelic eveness (Brown and Weir, 1983). The mean number of alleles per locus A is a measure of allelic richness whereas He is a useful measure of allelic eveness. To further investigate allelic richness of the studied *Tetratheca* species, the number of allelic variants in four different classes was determined. These classes are: (1) common, occurring in at least one population with a frequency > 10%. (2) rare, not occurring in any population with a frequency > 10%. These two categories can then be subdivided into widespread (W), when they occur in two or more populations, or localised (L), when they are only in one population (see Table 3.3).

3.3 RESULTS

Locations and sizes of the 28 populations representing seven *Tetratheca* taxa are presented in Table 3.1

3.3.1. Patterns of variation within Tetratheca populations

Mean genetic diversity measures derived from allele frequencies (Appendix 2) are given for all 28 populations in Table 3.1. The total mean values for *Tetratheca* are very high (A=2.3, P=70%, H_o=0.174 and H_e=0.3585) comparable to the mean values found for long-lived perennials (A=, P=66%, H_o=0.27, H_e=0.; Hamrick *et al.*, 1979). The average number of alleles per locus (A) ranges from a very high 3.4 for HAR1,2 and HIR2 to 1.2 for PAY1 and HAR5. The percentage of polymorphic loci (P) ranges from 100% in APH1,2,3; HAR1,2,4; HIR2 and AFF2 to 20% in PAY1 and HAR5. No relationship between mean levels of genetic diversity and population size is apparent.

A comparison of the observed and expected panmictic, heterozygosity values showed substantial deficits for all populations within the seven *Tetratheca* taxa except for *T. hirsuta* populations (HIR4,8,9,10,11). The likely causes of this deficiency are that it is an artifact of sampling error, underlying sub-population structuring or inbreeding (Brown, 1979).

Table 3.1. Mean genetic diversity estimates for populations of *Tetratheca* species.

N = No. individuals assayed, A= mean number of alleles per locus, P = mean percentage of loci polymorphic (0.99 criteria), H_o = observed heterozygosity and H_e = expected panmictic heterozygosity (Standard error)

TAXON Code	N	A	P	H_o	H_e
Tetratheca aphylla					
APH1	18	2.8(0.4)	100	0.195(0.60)	0.493(0.071)
APH2	11	2.4(0.4)	100	0.293(0.063)	0.446(0.101)
APH3	18	2.6(0.5)	80	0.082(0.043)	0.421(0.114)
APH4	19	2.8(0.4)	100	0.256(0.076)	0.502(0.042)
APH5	11	1.8(0.6)	40	0.109(0.067)	0.187(0.134)
APH6	7	1.6(0.2)	60	0.062(0.038)	0.171(0.082)
APH7	11	1.6(0.4)	40	0.00(0.00)	0.212(0.130)
Mean		2.2	74	0.142	0.347
Tetratheca paynteri					
PAY1	53	1.2(0.2)	20	0.009(0.009)	0.068(0.068)
Tetratheca harperi					
HAR1	31	3.4(0.4)	100	0.058(0.017)	0.634(0.057)
HAR2	18	3.4(0.4)	100	0.216(0.092)	0.555(0.041)
HAR3	11	2.4(0.5)	80	0.115(0.054)	0.410(0.115)
HAR4	21	2.8(0.4)	100	0.019(0.019)	0.516(0.100)
HAR5	24	1.2(0.2)	20	0.00(0.00)	0.107(0.107)
Mean		2.6	80	0.082	0.444
Tetratheca deltoidea					
DEL1	51	2.2(0.6)	60	0.173(0.107)	0.331(0.138)
Tetratheca hirsuta					
HIR1	26	2.8(0.5)	80	0.274(0.105)	0.400(0.132)
HIR2	16	3.4(1.4)	100	0.129(0.069)	0.452(0.110)
HIR3	9	2.0(0.6)	40	0.156(0.109)	0.259(0.160)
HIR4	13	2.2(0.5)	60	0.323(0.173)	0.329(0.134)
HIR5	8	1.8(0.5)	40	0.050(0.050)	0.230(0.143)
HIR6	28	2.4(0.5)	80	0.169(0.078)	0.285(0.130)
HIR7	38	3.0(0.9)	60	0.380(0.161)	0.410(0.168)
HIR8	27	2.8(0.7)	60	0.380(0.161)	0.410(0.168)
HIR9	15	2.8(0.9)	80	0.364(0.173)	0.408(0.142)
HIR10	12	2.4(0.7)	60	0.290(0.169)	0.381(0.164)
HIR11	HIR1	2.0(0.6)	40	0.261(0.163)	0.263(0.161)
Mean		2.5	64	0.239	0.3432
Tetratheca efoliata					
EFO1	17	2.4(0.5)	80	0.218(0.057)	0.438(0.140)
Tetratheca affinis					
AFF1	19	2.2(0.4)	80	0.218(0.057)	0.438(0.140)
AFF2	18	2.4(0.2)	100	0.223(0.98)	0.451(0.062)
Mean		2.3	90	0.22	0.384

3.3.2. Patterns of differentiation between populations of *Tetratheca*

Estimates of Nei's (1978) unbiased genetic similarity was used to compare populations within *Tetratheca* taxa. The calculated Nei's genetic distance are presented in Table 3.2. Nei's similarity (I) values ranged from a low 0.5948 for all *T. hirsuta* populations to a high 0.805 for *T. harperi* when compared to a mean value of 0.67 for conspecific populations of 21 plant species (Gottlieb 1977). Nei's genetic similarity is high (I=0.7595) between the 'granite' *T. hirsuta* populations (Chapter 2) when compared with other *T. hirsuta*.

Table 3.2. Values for Nei's genetic similarity (I) among populations of *Tetratheca* taxa

	I	
	Mean	Range
Among populations within taxa		
T.aphylla	0.6664	0.302-0.959
T. harperi	0.805	0.684-0.950
T. affinis	0.742	-
T. hirsuta (total)	0.5948	0.429-0.961
T. hirsuta (granite form)	0.7595	0.543-0.961
T. hirsuta (laterite form)	0.5936	0.330-0.941

The allele distributions and frequencies were variable within all populations of *Tetratheca* species. Within populations of the restricted species *T. aphylla*, 20% of the total alleles were rare. Populations APH3, 5 and 7 were homozygous at the Pgm-1 locus. In the *T. paynteri* population four loci, Pgm-1, Lap-1, Pgi-2 and Mdh-1 were fixed and homozygous in a mean sample of 43.4 individuals. Two allelic variants were detected at the Mr-1 locus. *Tetratheca harperi* had only 1 rare allele of the 20 scored. HAR5 was distinctive from all other populations by having monomorphic, homzygous loci at Pgm-1, Lap-1, Pgi-2 and Mdh-1. The sample size of HAR5 was comparable with other populations of *Tetratheca harperi* and so it would be unlikely that alleles would not have been detected. One rare allele was found in HAR2 at the Mdh-1 locus. The *T. deltoidea* population was homozygous and monomorphic at Mdh-1 and Mr-1. One third (2) of the alleles were rare, one each at the Pgm-1 and Pgi-2 loci.

Widespread Tetratheca species maintained no greater percentage of rare alleles than the restricted species. Populations HIR3,4,5 of the laterite form of T. hirsuta were fixed and homozygous at Pgm-1. HIR2 possessed a unique, common allele at Mdh-1. Rare unique alleles were detected in HIR1 at Lap-1, HIR7 at Pgi-2 and HIR2,7 at Mr-1. Populations of the granite form of T. hirsuta were monomorphic and homozygous at Mdh-1. HIR8,10,11 were monomorphic and homozygous at Pgm-1. Rare, unique alleles occurred in HIR9 at the Pgi-2 locus and in HIR8 at Mr-1. The frequency of rare alleles in all T. hirsuta populations is low (Table 3 3)

Relative frequencies of alleles between the two populations differed quite markedly. One unique rare allele was found in AFF1 at Pgm-1. Common unique, alleles were found in AFF2 at the Mdh-1, Pgi-2 and Pgm-1 loci and in AFF1 at Lap-1. The average number of alleles and polymorphic loci were high at 2.3 and 90% respectively. The observed average heterozygosity was markedly lower than expected.

3.3.2. *Genetic similarity between populations*

Values for Nei's genetic similarity (I) among populations of *Tetratheca* species are presented in Table 2.

The average Nei's similarity value among populations within the taxa ranged from 0.5936 for laterite *T. hirsuta* "forms" to 0.805 for *Tetratheca harperi* populations. The value of Nei's I for laterite populations was considerably less than for "granite form" populations of *T. hirsuta.* This may indicate that several different taxa are involved?? Despite the level of allelic variation within *T. affinis* populations the Nei's I is relatively high at 0.742. T. aphylla has quite high population differentiation ranging from 0.302 to 0.959 (Average= 0.6664).

Genetic variation within the *T. efoliata* population

No rare alleles were detected as would be expected from such a small sample. The Mdh-1 locus was unscorable. The average number of alleles, 2.4, and the percent polymorphic loci , 80%, were both very high. A large deficit was observed in heterozygosity at 0.218 compared with 0.438.

Table 3.3. Average number of alleles and their distribution between populations in *Tetratheca*

Species	Number of Popns	Loci	Allele distribution Common		Rare	
			W	L	W	L
T. aphylla	7	5	14	2	2	2
T. paynteri	1	5	-	6	-	-
T. harperi	5	5	18	1	-	1
T. deltoidea	1	4	-	7	-	2
T. hirsuta (laterite)	7	5	15	2	2	2
T. hirsuta (granite)	4	5	12	1	1	3

3.4. DISCUSSION

Tetratheca species possess a range of attributes which would suggest that the levels of genetic diversity within them are high. They are apparently long lived, fecund, primarily outcrossing and are distributed in late successional habitats. The mean measures of genetic diversity within the majority of *Tetratheca* species were found to be very high when compared to the average values of A=1.69, P=0.37, H_e=0.141 found for 113 plant taxa reviewed by Hamrick (1979). These mean parameters which define genetic diversity for *Tetratheca* species (means) and a selection of other plant taxa are summarised in Table 3.4in order to put values into perspective.

Table 3.4. A comparison of mean genetic diversity values for selected Tetratheca species and other plants with similar biological attributes

A= mean number of alleles per locus, P = mean percentage of loci polymorphic (0.99 criteria), H_0 = observed heterozygosity and H_e = expected panmictic heterozygosity

TAXON	A	P	Ho	He	SOURCE
T. hirsuta (granite)	2.5	60	0.324	0.366	
T. paynteri	1.2	20	0.009	0.068	
Fan Palm		9.8	0.009	0.0008	McClenaghan et al. 1986
113 plant taxa	1.69	37	0.156	0.141	Hamrick et al. 1979
Long lived perenn.	2.07	66		0.267	"
Endemic	1.43	0.24		0.086	"
Eremaea taxa	1.9	61	0.16	0.22	Coates, 1990

The biological attributes which promote genetic diversity within *Tetratheca* species are counteracted and adversely affected by limited population size and restricted ranges (Hamrick et. al 1979, Hamrick 1983, Loveless and Hamrick, 1984). Most populations of *Tetratheca*, even those considered to be geographically widespread, are restricted in size and isolated (disjunct) from other conspecific populations. Populations of*Tetratheca harperi, T. paynteri, T. aphylla* and *T. deltoidea* may be small and restricted as a consequence of historical, evolutionary events (see Chapter 4) and because the availability of habitat, to which they appear to have become adapted, is extremely limited. The more widespread *Tetratheca hirsuta, T. efoliata* and *T. affinis* have suffered to varying degrees from degradation of habitat since European settlement. The actual population sizes recorded for *Tetratheca* species did not reveal any relationship with the level of genetic diversity. For example, population sizes of *T. paynteri* and *T. hirsuta* (HIR8) are both 1000 and yet the levels of genetic diversity are the lowest and one of the highest respectively.

Gene flow between small, isolated conspecific populations of *Tetratheca* may be exacerbated by limited pollen and seed dispersal. It may be as low as 1% between plant populations that are only several hundred metres apart. The myrmecochorous dispersal of *Tetratheca* diaspores is unlikely to exceed 5 metres (Anderson, 1985, Berg 1985). This may affect genetic diversity levels spatially by the establishment of closely related sub-populations. Gene flow via pollen on the other hand, has been considered to be significant in shaping genetic structure; lower levels of genetic diversity were found for animal mediated dispersal compared with wind (Hamrick et. al. 1979). Dispersal of pollen may be greater than expected in small populations that are not completely isolated since the highly specialised, solitary bees implicated with *Tetratheca* pollination may need to visit all of these populations to obtain sufficient pollen, effectively increasing the dispersal distance. The actual distance between populations which effects genetic isolation is difficult to assess without fully understanding the behaviour of pollinators, though the genetic similarity between populations can give some indication of their relative isolation. Distances of 300 m have been sufficient to promote genetic differentiation between stands of the rare tree, *Eucalyptus caesia* (Hopper and Moran, 1977).

"Evolutionary theory predicts that species with small ranges and few individuals will exhibit low levels of genetic polymorphism" (Karron et. al. ,1988). The restricted species *T. paynteri, T. deltoidea, T. aphylla* and *T. harperi* exhibited lower mean levels of genetic diversity on the whole than the widespread species *Tetratheca hirsuta, T. efoliata* and *T. affinis*. Since population size alone does not account for

genetic diversity, this may reflect that populations of the widespread *Tetratheca* species are effectively more continuous in their distribution and less genetically isolated than the restricted species. The genetic similarity values for populations within widespread taxa are quite low and do not support the concept of large continuous populations. Different breeding systems or pollinators may be implicated. The rate of pollinator visits to plants in arid areas may reduce outcrossing and increase inbreeding in species with mixed mating systems (Karron, 1987).

Allelic richness (A) and mean percentage of polymorphic loci (P) varied greatly between populations within taxa of both widespread and restricted species. These values suggest that the effect of bottlenecks and founder effects have resulted in a population's loss of low frequency alleles by random genetic drift. In *T. paynteri* the very low levels of genetic diversity show that this species may have suffered severe bottlenecks that have resulted in the fixation of almost all alleles. *Tetratheca paynteri* and to a lesser extent, *T. harperi,* exhibit greatly reduced fecundity (production of flowers and fruits) relative to *T. aphylla,* which may be a consequence of a loss in heterozygosity (see Chapter 4).

Tetratheca harperi and *T. aphylla* both maintain high levels of (A) and (P) in most populations despite considerable heterozygote deficiencies. This may be the result of a mating system or population structure which favours homozygosities or may indicate that sub-populations which are primarily homozygous for unique alleles and functioning independently with little gene flow, are being sampled as if they were a continuous panmictic population(known as the Wahlund effect (Brown, 1979). In population HAR5 a dramatic reduction in genetic diversity is noted when compared with conspecific populations All individuals within this population were at the base of a large cliff-face and it may be that localised outcrossing with siblings has occurred.

Tetratheca deltoidea maintains a high average allelic richness and proportion of polymorphic loci considering that the population size is 160 isolated individuals. A large deficit in heterozygotes may reflect the genetic isolation of the population since clearing of surrounding land for farms. Populations of *T. deltoidea* may have been associated with a once extensive *Eucalyptus caesia* population, which now is represented by only a few individuals at the base of the granite monolith of Mt. Caroline. A comparison of the levels of genetic diversity between *T. deltoidea* and the associated, *Eucalyptus caesia* indicates a severe loss of diversity in the *Eucalyptus* (Moran and Hopper,1977) yet maintenance of high levels of diversity in

the *Tetratheca*. Considering the habitat requirements of the two taxa, it could be considered that the effects of land clearing in terms of population size were of an equal magnitude. It may be that *T. deltoidea*, unlike the primarily bird pollinated *Eucalyptus caesia*, never experienced high levels of gene flow. Destruction of neighbouring conspecific populations of *T. deltoidea* would not have severely affected levels of genetic diversity in the extant population. *Tetratheca deltoidea* is severely restricted by its habitat requirements and ability to disperse. Movement of seed to favourable habitats is highly unlikely to be effected by ant vectors.

The geographically widespread species *T. hirsuta, T. efoliata* and *T. affinis* maintained high levels of genetic diversity, yet nearly all expressed heterozygote deficiencies. Population substructuring, sampling error, and/or inbreeding may all result in lower than expected panmitic heterozygosity. The "granite forms" of *T. hirsuta* (HIR8,9,10,11) and HIR4 were the only populations of *Tetratheca* that have frequencies of heterozygotes in Hardy-Weinberg equilibrium; thus approximating populations with panmictic gene flow. The granite populations are all relatively large and are found nestled in the highly stable, wetter gullies of the Darling Range. It is likely that most conspecific populations are not completely genetically isolated. A wide range of microhabitats are potentially conducive to the maintenance of an equally diverse range of genotypes (Soule, 1979). The high level of genetic diversity in population HIR4 (A=2.2, P=60, Ho=0.323, He=0.329) may be attributed to its size (400), to the relatively undisturbed habitat of jarrah forest on a road verge and to gene flow between other populations in the large adjacent forest blocks. This population has also been afforded protected from fire by its proximity to human habitation The effects of disturbance on populations of *T. hirsuta* can be assessed by comparison of HIR4 and the genetically very similar (I=0.968) HIR5. Population HIR5 is located in a highly disturbed (burnt and logged) road verge in the heart of the jarrah forest and has apparently, as a consequence, suffered a reduction in population size to 50 individuals. HIR5 exhibits a very low level of genetic diversity (A=1.8, P=40, Ho=0.050, He=0.0230) with a large deficit of heterozygotes indicative of inbreeding.

In conclusion, the levels of genetic diversity within populations of *Tetratheca* are indicative of the length of time that they have been fragmented and the relative distance from conspecific populations rather than actual population sizes. Widespread species do maintain levels of genetic diversity higher than those of restricted species which suggests that *Tetratheca* species in the drier transitional zone have been isolated from each other for longer periods and have been more severely

affected by pronounced successions of aridity and lithological changes. Speciation has been greatest in the transitional rainfall areas (Hopper et al., 1990)

Despite the low levels of genetic diversity, the restricted taxa have probably survived for thousands of years in their geologically, recently stable arid environments. The ironstone species in particular, are generally leafless and waxy (*T. harperi* has spine-like setae) which are considered highly evolved adaptations for surviving arid conditions. The major threats to the survival of T. harperi, *T. paynteri* and *T. aphylla* are probably the more direct ones of habitat destruction. The presence of unique alleles and allele frequency differences between populations of *T. aphylla* and *T. harperi* suggest that it is important to preserve as many individuals and populations as possible.

The conservation of genetic diversity within geographically widespread species is often overlooked James (1982). In only two populations of *T. affinis* investigated there were 4 unique alleles found. This illustrates the importance of assessing genetic diversity in widespread and restricted populations. Limited resources available for conservation research can not usually accomodate investigation of species which are apparently widespread.

CHAPTER 4

SYSTEMATICS AND EVOLUTION

4.1 INTRODUCTION

Systematic and evolutionary relationships in the genus *Tetratheca* are obscure. Thompson (1976) revised the genus by measuring variation in gross morphological characters of dried herbarium specimens. A paucity of representative material, especially of fruiting specimens and scant general biological information limited the assessment of speciation and evolution in the genus.

Morphological approaches to plant systematics can be problematic even with comprehensive data sets. The analysis of sets of measurements usually involves mathematical reduction to a single measure of "genetic distance", the range of methods available are controversial and often biased (Diamond, 1983). Morphological characters are the manifestation of one or many different combinations of genes, parallel evolution is not uncommon (Stace, 1989).

Thompson (1976) suggests that *Tetratheca hirsuta* a widespread species with a range of variable morphological forms may be divisible into meaningful groups and that *T. efoliata* and *T. affinis* may be related. Morphological variation within species such as *T. hirsuta* may be indicative of recent genotypic divergence or may merely reflect the species ability to modify form to suit variable environmental conditions.

Plant speciation and richness in Western Australia is believed to be in part, a product of increased aridity and is most pronounced in the Transitional rainfall areas (Hopper et al., 1990). An accumulation of what may be relic*Tetratheca* taxa occured as a consequence in the wetter regions of the South-West (Keighery, 1979). On the other hand it has been proposed that *Tetratheca* and other Tremandraceae were 'secondary entrants in to the wetter forests' based on the highly xeric adapted wood morphology of a limited selection of species (Carlquist, 1977).

In evolutionary terms it it often difficult to assess the significance of the presence or absence of characters..In *Tetratheca* are the leafless species *T. aphylla* and *T. harperi* more closely related than species with highly variable leaves (*T. hirsuta, T. efoliata* and *T. deltoidea)?*.

Breeding system and cytological studies can clarify species' identities and relationships however they are often tedious and chromosome numbers may be identical, divulging little information. "The amount of evolutionary and taxonomic information highly increases from non-polymeric secondary constituents to proteins and nucleic acids." (Takhtajan, 1973). Analysis of primary and secondary metabolites have found widespread use in systematics but, like morphometric characters are often ubiquitous or the products of parallel evolution, diminishing their diagnostic value. Investigation of genotype of putative species with the now, well developed techniques using chloroplast (cpDNA), mitochondrial (mDNA) and genomic DNA is the ultimate systematic method. However, high costs restrict their widespread use.

Proteins of the same structure are highly unlikely to have evolved by convergence (Takhtajan, 1973). The investigation of enzyme proteins using allozyme electrophoresis is a relatively efficient and objective method of comparing genetic identity between putative species. Allozymes represent a measurable part of the genome and are independent of each other, unlike morphological characters. Small quantities of material are required for allozyme electrophoresis, an important consideration when investigating rare species such as *Tetratheca* deltoidea, *T. harperi* and *T. aphylla.* Allozyme electrophoresis has recently been used successfully to delineate species and assess evolutionary relationships in annual, diploid plants (Crawford and Ornduff, 1989); woody shrubs (Coates and Hnatiuk, 1990; Sytsma and Schaal, 1985) and perennial herbs (Bayer, 1988),.Australian trees, (Moran, et. al., 1990) and pine trees (Millar, et. al., 1988). Multivariate morphometric and allozymes were both used in a investigation of relationships within an Australian woody, shrub species complex (Mackay and Morrison, 1989) and in establishing hybrid origins of perennial herbs (Bayer and Crawford, 1986).The application of allozyme electrophoresis to systematics has been reviewed by Richardson et. al. (1986), Crawford (1983, 1985, 1989), Gottlieb (1977), Buth (1984) and Brown (1990). Allozyme electrophoresis has not been used to investigate systematic relationships in the genus*Tetratheca.*

The use of all available data represents the best method of understanding systematics and evolution (Takhtajan, 1973; Richardson, et al. 1986; Crawford, 1983). Methods used in plant systematics are reviewed in Stace (1989).

This chapter investigates the systematic and evolutionary relationships of *Tetratheca* species using the technique of allozyme electrophoresis and multivariate morphometric analysis of flower and leaf measurements.

The aims are:

1. To assess the level of genetic differentiation between taxa;
2. To determine whether *T. hirsuta* is divisible into meaningful taxonomic units;
3. To evaluate phylogenetic relationships between the taxa;

The following hypothesis will be tested:

Ironstone species *T. aphylla, T. harperi* and *T. paynteri* are relics of a more mesic past

4.2 MATERIALS AND METHODS

4.2.1 Allozyme electrophoresis

The application of allozyme electrophoresis data in systematics differs from that used in population genetic analyses, in that even monomorphic allele and locus data contribute to the computation of genetic identities.

4.2.1.1 Collection of material/electrophoresis methods

This is as described in Chapter 3.

4.2.1.2. Comparison of Neis genetic similarity values between taxa

Nei's interpopulation genetic identity values obtained in matrix form from the Biosys-1 (Swofford and Selander 1981) analysis in Chapter 3 were averaged to derive congeneric genetic identity values.(Table 4.1). Allele frequency data in Appendix 2 was used to acertain fixed allelic differences. Allele frequency values were used to calculate Roger's modified similarity values.(Wright, 1978) usingg Biosys-1 (Swofford and Selander, 1981).

4.2.1.3 Data analysis

Phenetic Approach using Allozyme data

The UPGMA is based on the average set of similarities in characters (operational taxonomic units or OTU's) in this case Nei's (D) (Nei, 1978) without placing emphasis

on any particular character. Similar OTU's are clustered in cycles, only one OTU can join a cluster in any one cycle. UPGMA assumes equal evolutionary rates in all taxa therefore viewed in evolutionary terms similarity is due to close ancestry.

Nei's D (1978) estimates the mean number of electrophoretically detectable nucleotide substitutions per locus that have accumulated since 2 populations diverged from their common ancestor. The probability that 2 alleles, one drawn from each population unit are the same depends on the frequency of that allele in the 2 populations.

Nei's D = -ln I

$$= -\log_e(Jab)^{0.5} (J_{aa}J_{bb})^{-0.5}$$

The UPGMA phenogram based on Nei's D must be viewed remembering that the relative distance depends on the magnitude of the values before log transformation; values have been log transformed and thus deformed (Richardson et al , 1986.)

Phylogenetic Analysis using Allozyme data

In phylogenetic analysis the taxonomic groups should consist only of monophyletic groups or clades determined by detecting the derived character states held in common with some, but not all members of a group. Diswag joins two OTU's (Rodgers' S) then tests the distance of each of remaining OTU's to that branch and repeats the procedure. To be an accurate estimate of Euclidian hyperspace (Sokal and Sneath, 1973) each axis must be independent on the same scale. In allele frequency data this can not be true because one allele frequency determines the other, but this is ignored.

Modified Rogers' (Wright, 1978) mean geometric distance between allele frequencies summarises this information across all loci.

Roger's Modified distance $R = 0.5 (P - P)^2$

n= number of alleles, P= frequency of ith allele in population A For several alleles R Total= .When R=0 populations are genetically identical, and when R=1, populations are fixed for different alleles. The Distance Wagner technique is best when coefficients of variation of branch lengths are large (distantly related taxa).

The phenetic UPGMA analysis is useful for comparison with the Distance Wagner "cladogram" and other studies. Nei's D used in the calculation of UPGMA assumes equal evolutionary rates and is nonmetric, violating the triangle of inequality where the

"genetic" distance between two entities A and C must be less than or equal to the sum of distances between each entity and another "taxa" B.

Modified Roger's D and thus the Diswag procedure does not violate this mathematical law nor assume equal evolutionary rates Nei's single locus diversity measure D and Roger's D were determined using Biosys-1 (Swofford and Selander 1981). A phenetic analysis was carried out utilising the unweighted pair group analysis (UPGMA) procedure (Sneath and Sokal 1973) provided in the Biosys-1 programme. The matrix of Nei's D was used to generate UPGMA phenograms for clustering populations. The Distance Wagner dendogram was similarly generated using The Biosys-1 programmme (Swofford and Selander 1981).

The methods of analysing electrophoretic data for systematics has been reviewed by Richardson et. al (1986), Buth (1984) and Sokal and Sneath (1973).

4.2.2. Morphometrics

4.2.2.1 Collection of Material

Flowers were collected from 5-34 individuals in 12 populations covering 6 taxa within the genus during field surveys throughout 1989 and 1990. Leaves were collected from 8-21 individuals in 6 populations of *Tetratheca* hirsuta. Individual plants were selected at random. Representative voucher specimens collected on field surveys were lodged in the W.A. Herbarium (Perth). Flowering material, leaves, fruit and seeds were sampled from as many populations as possible throughout the geographic range and at the extremities.

4.2.2.2 Pollen sampling

Pollen slides were prepared in the field using Wooler's et.al. (1983) gel. A small cube of gel was placed on a slide and *Tetratheca* anthers held above the gel and vibrated sideways to release pollen. A cover slip was placed over the gel and the slide gently heated to fix the cover.

4.2.2.3 Leaf and Flower measurements

One fully opened flower and three leaves from the top 25 cm of growth were selected at random. Each flower and three leaves were carefully removed from the stem. Several sepals, petals, the peduncle and leaves were placed on pressure sensitive tape and stuck

onto cards. This procedure served the two fold purpose of flattening the material to reduce measurement errors and preserving the specimen for future reference. Stamens were glued to cards for measurement.

Stamen tube length, filament and anther body were measured with a hand held 8 x magnifier with scale. Vernier calipers accurate to 0.1± 0.025 mm were used to measure 3 petal dimensions: sepal length, sepal width and peduncle length; and four leaf characters (Fig. 4.1). Dimensions measured represented a selection of characters considered of taxonomic importance in the genus*Tetratheca* by Thompson (1976). Only leaves of *Tetratheca hirsuta* were considered for analysis. Comparison of the leaves of other species was either not feasible or of much merit, in *Tetratheca* aphylla and *T. paynteri*, leaves occur only in juvenile growth. *Tetratheca deltoidea* and *T. efoliata* leaves are substantially different in shape from leaves of *T. hirsuta*.

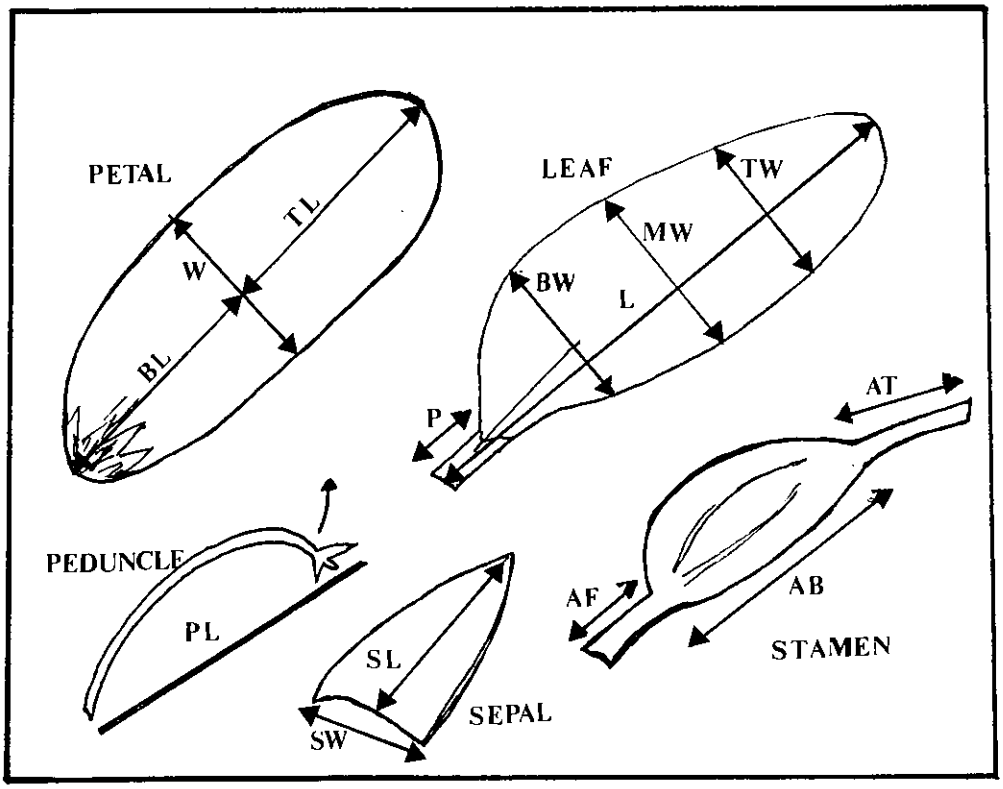

Figure 4.1 Floral and leaf character measurements used in the morphometric multivariate analysis.

4.2.2.4 Multivariate Morphometric Analysis

A multivariate morphometric technique was chosen to analyse the variation in floral and leaf characters between populations and species. Specifically this involved the use of Canonical variate analysis which separates populations or individuals by selecting a canonical vector (axes of variation) which maximizes the ratio of between population sums of sqares to the within population sum of squares for each set of variables. For total samples of 150-200 specimens canonical roots less than 0.75 are rarely associated with separation of any biological importance.

$$f\ (\text{Canonical root}) = C^t B_c / C^t W_c$$

B= between sum of squares. W= within sum of squares.

Ct=first canonical vector, chosen to maximise ratio.

The programme used was written by N.A. Campbell and was run on systems at Commonwealth Scientific Investigation Organisation, Mathematics and Statistics division laboratories in Floreat Park. The interpretation and theory of canonical variate analysis is discussed in Reyment et. al. (1984). Botanical systematic applications include analysis of variation in kangaroo paws (Hopper and Campbell, 1977) and *Clarkia* (Bloom, 1976).

4.3 RESULTS

4.3.1. Allozyme electrophoresis

Allele frequencies for the 28 population representing all seven *Tetratheca* species are presented in Appendix 2. Several unique alleles were detected in some species which provide useful genetic markers. *T. affinis* possessed a common (f=0.439) 'fast' allele at Pgm-1. *T. paynteri* "b" allele at Pgm-1 was distinctive by having monomorphic fixed alleles at Pgm-1, Lap-1, Mdh-1 and a unique 'b' allele at the Pgm-1 locus. *T. harperi* can be identified from *T. aphylla* and *T. harperi* by the presence of the "d" allele at Pgi-2. Within the *T. hirsuta* "complex" populations HIR (6,8,9,10,11) all possessed allele "e" Mdh-1 (which was also found in HAR, APH, and DEL) and HIR7 is monomorphic for LAP-1 "c" and MDH-1 "c:". Considerable variation in the type and frequency of alleles occurs througout the *T. hirsuta* complex.

The averaged mean values of Nei's (1978) genetic similarity between all *Tetratheca* species was I=0.313. The average value for a range of congeneric species reviewed by Gottlieb (1981) was I=0.67.

Table 4.1. Values for Nei's genetic identity (I) between species in *Tetratheca* and putative *Tetratheca* hirsuta 'forms'.

	(I)	
	Mean	Range
aphylla x deltoidea	0.277	0.130-0.427
aphylla x efoliata	0.222	0.062-0.394
aphylla x harperi	0.678	0.346-0.994
aphylla x paynteri	0.266	0.095-0.501
aphylla x affinis	0.508	0.301-0.632
aphylla x hirsuta	0.468	0.112
paynteri x deltoidea	0.184	-
paynteri x efoliata	0.000	-
paynteri x harperi	0.134	0.000-0.264
paynteri x hirsuta	0.210	0.055-0.362
deltoidea x efoliata	0.129	-
deltoidea x harperi	0.289	0.191-0.342
deltoidea x affinis	0.249	0.227-0.272
deltoidea x hirsuta	0.429	0.245-0.676
harperi x affinis	0.717	0.588-0.874
harperi x hirsuta	0.388	0.123-0.725
hirsuta x affinis	0.263	0.023-0.520
affinis x efoliata	0.363	0.303,0.423

Phenetic Analysis

The UPGMA phenogram (Figure 4.2) derived from Nei's (1978) distance between populations. clustered all *T. hirsuta* populations and at least 3 sub-clusters within the complex. *T. deltoidea* was clustered with HIR6 and the"granite" form *T. hirsuta* populations. *T. affinis* was clustered within populations of *T. aphylla* and *T. harperi* ; *T. efoliata* and *T. paynteri* branched well away from all other populations and species.

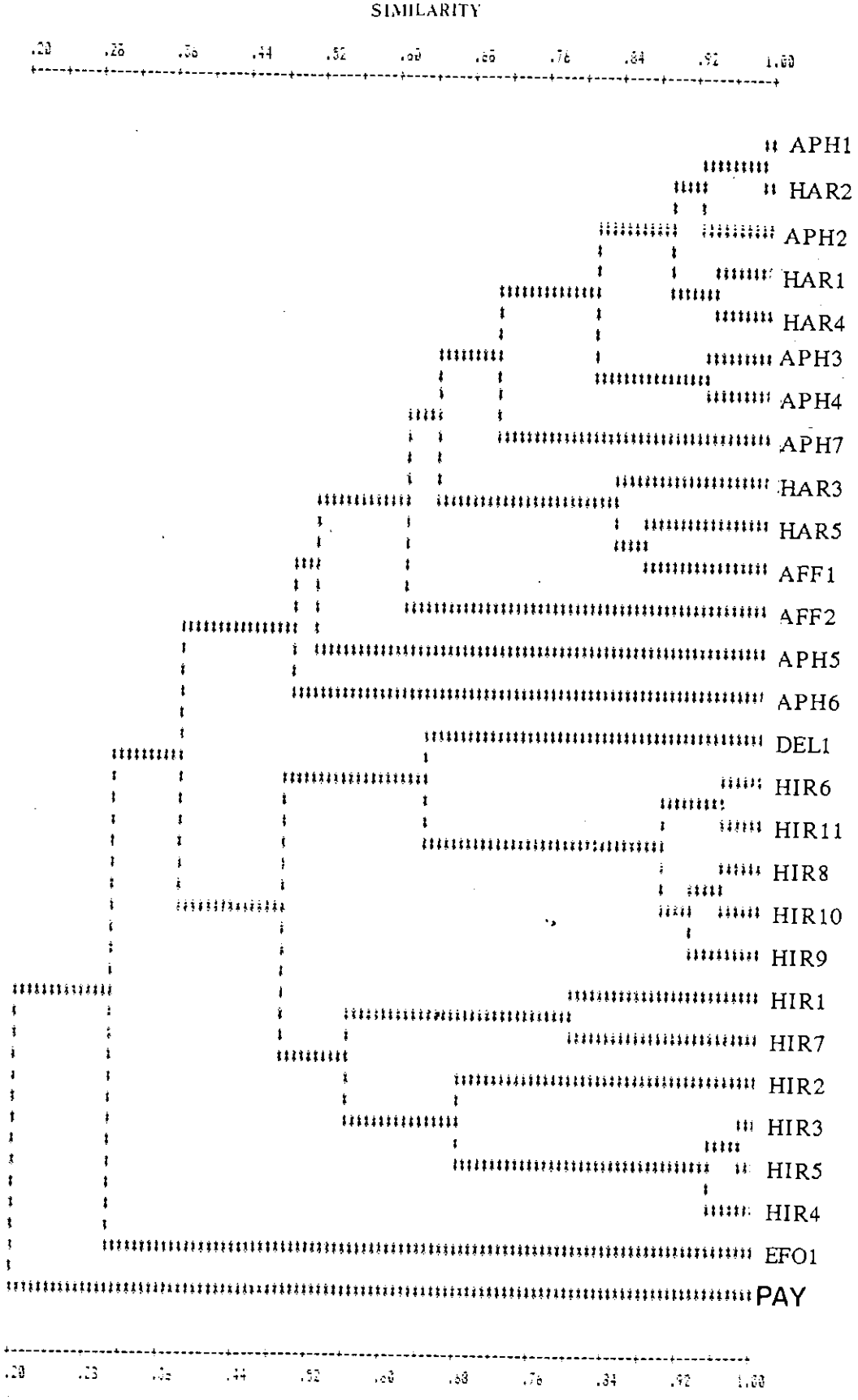

Figure 4.2. Hierachical cluster produced using the unweighted pair-group algorithm (UPGMA) with Nei's (1978) unbaised genetic distance.

Cladistics.

The Distance Wagner dendogram (Fig. 4.3.) clustered *Tetratheca* populations into 2 distinct groups. One tightly clustered group was composed of the leafless species *T. aphylla, affinis, harperi* and *T. paynteri* , and the other was the 'leafy' group *T. hirsuta, T. efoliata* and *T. deltoidea*

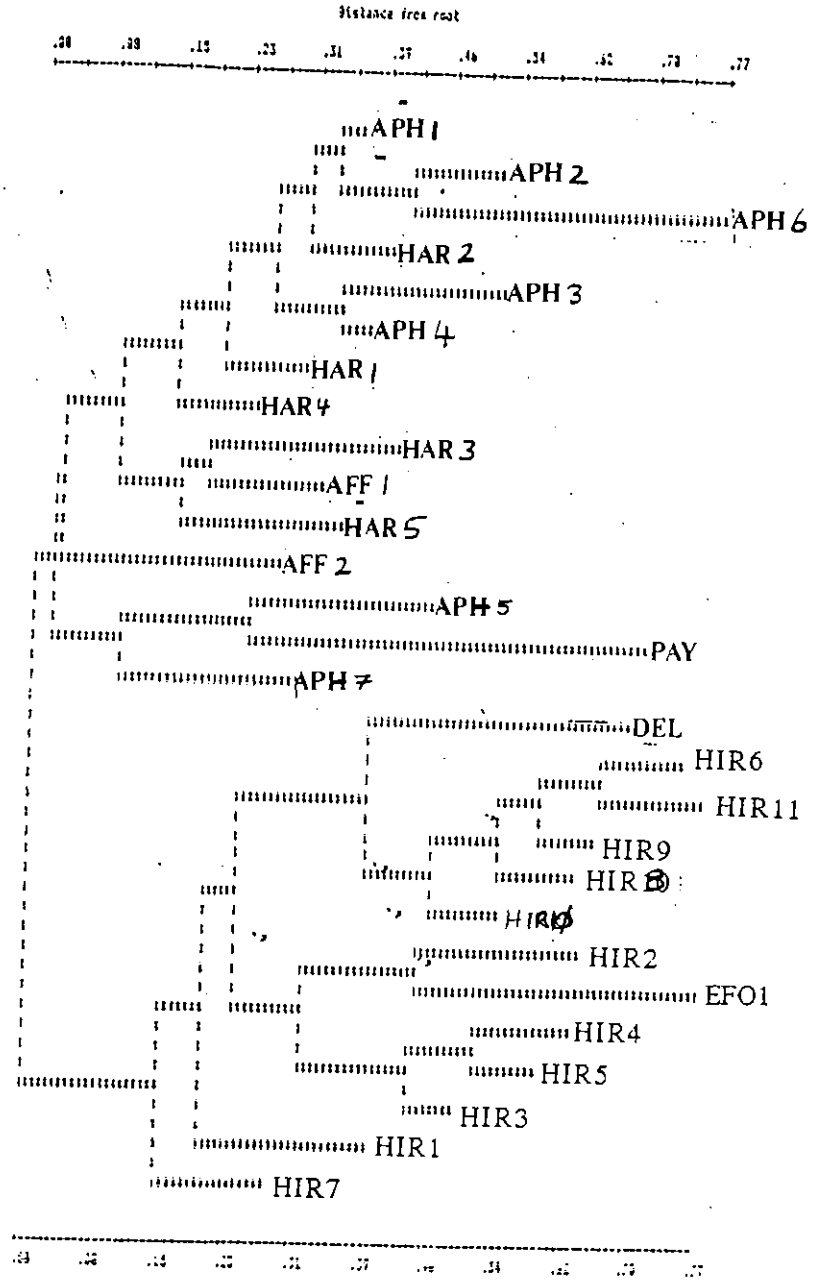

Figure 4.3. Phylogenetic tree produced using the Wagner procedure with modified Roger's distances (Wright, 1978).

4.3.2 Morphometrics

4.3.2.1.Pollen

Pollen was not found to be a useful taxonomic character at 1000x magnification. SEM investigation may prove to be more useful. Size and subtle shape variation was detected in pollen. Percentage of viable/ non viable pollen (collapsed deformed) appeared to vary between species.

4.3.2.2 Multivariate analysis

Canonical variate analysis of floral characters

Table 4.2.Mean morphometric floral character values for *Tetratheca* species. Raw data is presented in Appendix 5.

Character	(n)	W	LB	LT	SL	SW	PL	AB	AF	AT
Taxon										
T.paynteri	29	5.48	6.07	3.60	4.10	1.37	6.79	2.93	0.49	0.95
T.aphylla	9	4.46	4.80	3.30	2.67	1.17	2.71	2.18	0.60	1.03
T.harperi	34	5.96	4.57	7.47	2.58	1.52	5.64	2.30	0.38	1.68
T.deltoidea	15	6.51	1.85	6.62	2.12	1.31	17.8	1.41	0.33	0.44
T.hirsuta										
HIR1	11	8.04	7.36	5.84	3.24	1.49	20.9	1.96	0.36	2.48
HIR3	7	6.00	6.43	3.20	2.38	1.19	21.9	1.64	0.41	1.57
HIR5	5	6.88	6.02	4.42	2.62	1.34	25.9	1.92	0.46	1.70
HIR6	15	7.49	6.62	5.60	3.53	1.37	16.0	2.12	0.43	2.18
HIR7	13	6.82	6.99	6.34	6.33	1.75	16.5	2.10	0.62	2.30
HIR10	11	7.23	6.80	5.73	3.93	1.56	20.1	2.11	0.37	2.28
HIR11	8	6.74	7.10	5.79	4.10	1.46	19.8	2.08	0.53	2.19
T.efoliata	7	5.14	7.74	4.14	3.81	1.87	6.91	2.69	0.91	2.10

W=width of petal at widest point. LB=length from base of petal to W
LT=length from W to top of petal SL=sepal length
SW=sepal width PL=peduncle length
AB=anther body length AF=stamen, filament length
AT=stamen, tube length

The canonical variate analysis of petals, sepals, stamens and peduncle characters of 12 populations representing 7 species of *Tetratheca* resulted in clear discrimination of all recognized taxonomic groups (Fig.4.4).

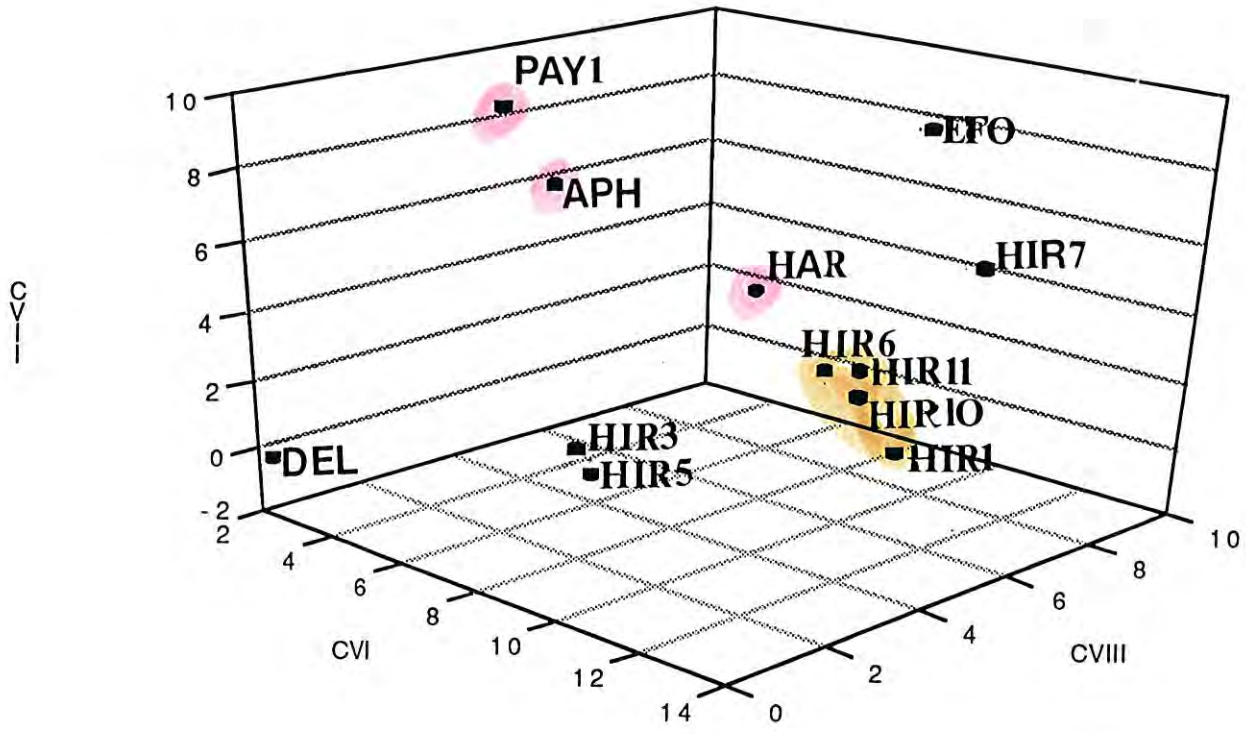

Figure 4.4. Canonical variate analysis of *Tetratheca* floral morphology measurements

The newly described *Tetratheca paynteri* is clearly distinct from its nearest neighbours *T. aphylla*, *T. harperi* and *T. efoliata*.. In the putative *Tetratheca hirsuta* complex proposed in Chapter 2 two populations HIR3 and HIR5 showed close affinities. The HIR7 population is clearly distinct from other *T. hirsuta*.The sepals of this group are much longer than any other population see Table 4.2. and the peduncle is shorter. The HIR10,11 granite form of *T. hirsuta* and HIR6 all clustered together. Individuals in this population are strongly scented and have two forms of leaves and leaf arrangement. The HIR1 population clustered away from the granite form and HIR6. Flowers of five of the six taxa used in the canonical variate analysis are illustrated in Figs.4.5 and 4.6.

Grouping according to analysis:

1. HIR7 different at 2 canonical variates
2. HIR6,10,11 and possibly HIR1
3. HIR3 and HIR5
4. Discrimination of the taxa *T. harperi, T. efoliata, T. paynteri, T. deltoidea, T. aphylla*

A

B

C

Figure 4.5 Flowers of A) *T. paynteri* B) *T. harperi* and C) *T. deltoidea*

A

B

Figure 4.6 A) *T. efoliata* and B) *T. hirsuta* (HIR7)

Mean morphometric values for 5 leaf characters are given in Table 4.3. The measurements taken are illustrated in Fig.4.1

Table 4.3. Mean morphometric values for leaf characters in *Tetratheca* hirsuta populations.

See Figure 4.1 for illustration of measurements.

Character	# Obs.	Petiole length	Total leaf length	Lower width	Middle width	Top width
Population						
HIR1	33	0.97	11.03	3.39	3.34	2.54
HIR3	24	1.39	8.88	4.64	4.78	3.41
HIR5	22	1.42	8.28	4.82	5.46	3.96
HIR6	33	0.63	8.89	2.26	2.44	1.92
HIR7	61	0.62	10.62	1.99	1,87	1.56
HIR11	30	0.86	12.54	2.38	2.41	1.94

Canonical variate analysis of leaf characters

The canonical variate analysis of 203 leaves from 67 individuals in 6 populations of *T. hirsuta* representing groups segregated using floral characters and a range of habitats recognised in Chapter 2, failed to elucidate taxonomic groupings at a level more accurately than could be assessed by normal description (Fig.4.7). The strongest discrimination was between HIR3 and HIR5 with ovate leaves and all other *T. hirsuta* with linear to lanceolate leaves.

The leaf analysis was severely affected by the amount of variation in leaf size within individuals and populations. The magnitude of the range of values may occupy one of the canonical variates corresponding to the largest, and most significant canonical root overemphasising differences in size rather than shape. The conversion of a range of leaf characters into a ratio may be more appropriate and lessen the effects of magnification. The smallest absolute value of the standardized canonical vectors, the vectors for characters standardised to unit standard deviation within populations (canonical vectors x pooled within population standard deviation) generally contribute little to the discrimation.

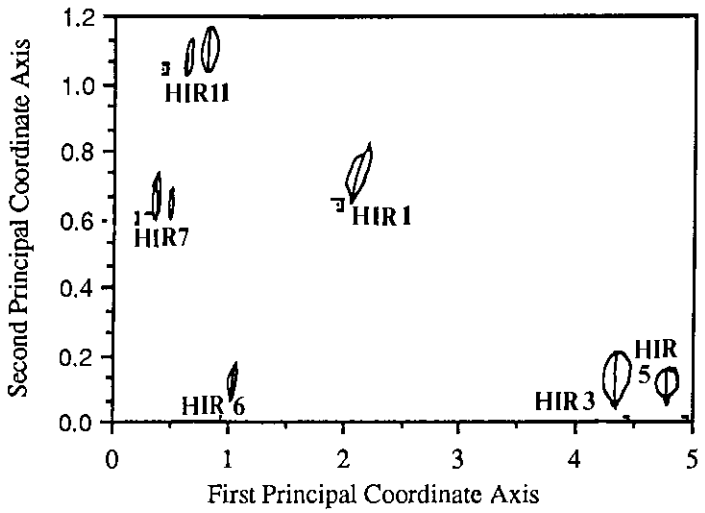

Figure
4.7. Canonical variate analysis of *Tetratheca* hirsuta leaf morphology measurements

4.4.DISCUSSION

Genetic similarity between *Tetratheca* species varied but generally concorded with the current taxonomic status. The levels of genetic similarity between congeneric species were on the whole lower than that (I=0.67) found by Gottlieb (1977). *Tetratheca paynteri* has 4 fixed monomorphic loci and 2 unique alleles which justify its description as a new taxon. *Tetratheca affinis* and *T. efoliata* similarly possess unique alleles which may provide genetic markers for future allozyme studies of *Tetratheca*..

The UPGMA phenetic clustering of *Tetratheca* distinctly grouped all taxa with the exception of *T. aphylla, T. harperi* and *T. affinis*. The dendogram branches clearly separated the species that developed adult leaves (*T. hirsuta, T. efoliata* and *T. deltoidea*) from the leafless species *(T. affinis, T. aphylla, T. paynteri* and *T. harperi).*

T. paynteri had a low average genetic similarity with all other taxa I=0.2033, when compared with the morphologically similar *T. aphylla* (I=0.266). The genetic identity between the (HIR6) and the granite form of *T. hirsuta* averaged a relatively high 0.8343. This is higher than the conspecific comparison even within the granite form populations (I=0.7595). HIR 7 and HIR1 were clustered by UPGMA but separated distinctly by the Distance Wagner procedure.

The canonical variate analysis of *Tetratheca* floral morphology measurements is illustrated in Figure 4.4. Morphometric analysis of a range of floral attributes clearly discriminated between the species *T. paynteri (Fig. 4.5a), T. efoliata* (Fig. 4.6a.), and *T. deltoidea* (Fig. 4.6c), a species with distinct stamens and floral anatomy. *Tetratheca hirsuta* was clustered into 4 sub-groups: the granite forms HIR8,9,10 and 11 were clustered with HIR6, and the laterite forms sub-grouped into HIR7 with longer internodes, glabrous stems and strongly scented flowers, HIR1 hairy, dwarf shrubs and HIR5 and HIR3. The granite forms of *T. hirsuta.* are distinct from all other *T. hirsuta* by their robust habit, 2 forms of leaves and leaf arrangement. Alternate, linear leaves are found in the new growth and three whorled broader leaves occur in older, woodier growth. Granite forms of *T. hirsuta* differ from HIR6 by their associated habitat (see Chapter 2) of massive granite on banks of watercourses compared the massive dry lateritic habitat of HIR6.

The use of leaf dimensions in morphometric analysis proved only to reflect obvious shape differences and did not delineate species boundaries amongst those species with apparently clinal variation in leaf shape. Canonical variate analysis of *Tetratheca hirsuta* leaf measurements is illustrated in Fig. 4.5.

The combined methods of floral morphometric analysis and allozyme electrophoresis clearly discriminated between taxa and separated *T. hirsuta* into 4 sub groups. This suggests that variation in floral characters is reflected by the allozymes found in each species. The floral structures may be more highly conserved and the expression of phenotypic plasticity may be less pronounced (Stace, 1989). One of the major benefits of using allozymes rather than morphological characters in systematic comparisons is that proteins are rarely the products of convergence. Phenotypic plasticity similarly is not a problem in allozyme analyses. Morphological analyses may not indicate the levels of genetic relatedness between species or their evolutionary relationships.

Systematic and Evolutionary relationships

The geology and landscape of Western Australia is ancient. The banded ironstone ranges inhabited by *T. aphylla, T. harperi* and *T. paynteri* are estimated to be Archaean in origin (Williams, 1975). Sands of the Swan Coastal Plain, supporting some forms of *T. hirsuta* are relatively geologically recent, deposited in the Mesozoic (Wyrwoll, 1990). It has been proposed that fluctuating climatic and geological conditions in South-Western Australia has been instrumental in the high levels of diversity in the flora (Hopper et.al., 1990).

The genus *Tetratheca* is represented by a total of 41 species on both sides of the Australian continent (Thompson, 1976). Marine intrusions in the Miocene epoch followed by calcification of soils on the Nullarbor Plain (Williams, 1975).would have prevented migration of plant species as long as 26 million years B.P. This implies that the remarkably similar morphology of Eastern and Western Australian *Tetratheca* species either evolved in parallel or has been maintained for millions of years. Massive extensions of the arid zone started in the Pleistocene, alternating with temperate climatic conditions until the last arid extension 18 000 years B.P. (Wyrwoll, 1990).

The distributions of *Tetratheca* species today are primarily allopatric. Some species such as *T. hirsuta* and *T. confertifolia* do co-occur but do not appear to hybridize. The spatial distribution of *Tetratheca* populations and species indicates that the fluctuating arid conditions experienced over a lengthy time period, especially in the drier inland regions would have forced a gradual contraction of what may have once been a widespread ancestral species. At some point in time conspecific populations became physically and genetically isolated. Geographic speciation is likely to have occcured in *Tetratheca* especially if seed and pollen dispersal was as limited as it is today. Depending on the size of the population, bottlenecks or founder effects may have led to loss of 'rare' alleles due to random genetic drift. Low genetic similarities between extant species (I=0.313) indicates that populations and species have been isolated for a long time.

Tetratheca aphylla, T. paynteri and *T. harperi* are highly adapted for survival in arid conditions. All are virtually leafless and have a thick waxy cortex (Alford, unpub. data), *T. harperi* possess spine like setae. The seeds have a very hard testa and are possibly transported short distances by ants (less than 5 m) to safe-microsites which may be more conducive to germination and survival (Anderson, 1988). The genetic similarity and similar morphology of *T. harperi* and *T. aphylla* suggests that divergence of these taxa was quite recent (Soltis, 1981).Carlquist (1977) examined the wood morphology of several members of Tremandraceae including *Tetratheca retrorsa,* it was concluded that Tremandraceae were not relics of a more mesic past but secondary entrants into wet forests.

It may be that *Tetratheca* species evolved more xeric structures in response to a long interval of arid conditions, and then capitalised by dispersing throughout the drier sites of the wet forests when less well adapted species were foundering. This does not preclude the possibility that the ironstone species *T. harperi, T. aphylla* and *T. paynteri* are relics. While some *Tetratheca* may have spread throughout the lateritic soils of the South-West ,

others in the Transitional rainfall zone could have been forced to retreat to refugial habitats.

Populations of *T. harperi* have very high genetic similarity (I=.805) either reflecting continuous gene flow throughout the populations or the maintainence of diversity within them. *Tetratheca paynteri* is morphologically and ecologically very similar to *T. harperi* and *T. aphylla* yet it is genetically dissimilar (I=0.226,*T. aphylla* ; I=0.1344,*T. harperi) T. paynteri* possesses a limited extract of alleles of the other ironstone species and thus may be derivative of *T. harperi, T. aphylla*.or a now extinct progenitor. A similar situation has been observed in *Cirsium* (Loveless and Hamrick, 1984) and in *Coreopsis* (Gottlieb, 1977).

In *Tetratheca paynteri* the present allozyme complement may reflect the size and isolation of populations at the time of disjuction. Of the 5 loci, 4 were fixed and only two allelic variants were present at Mr-1. *T. paynteri* and *T. harperi* both develop juvenile leaves in new growth and produce few flowers whereas*T. aphylla* possesses decidous deltoid scale-like leaves and flowers profusely. The stamens of *T. paynteri* are most like those of *T. aphylla* (Appendix 4). In the multivariate analysis similar floral characteristics between these species cluster them together. The morphological and ecological similarities between the ironstone species and the maintenance of unique alleles in all three species suggests that they diverged from a common ancestor after the progenitor populations had contracted and effected allopatry. This is supported by the observation that the allelic richness and population genetic diversity in *T. aphylla* is greater than that in *T. harperi* and *T. paynteri*. Thus the genetic diversity found in these ironstone species may be a function of the population size sustainable in the available habitat at the time of genetic isolation and subsequently stochatic events such as random drift, eventuating in low genetic similarity.

T. efoliata is geographically closest to the ironstone species yet has a very low genetic similarity especially when compared to*T. paynteri* (I=0.00). If all the *Tetratheca* species in this region are derived from a single ancestor then *T. efoliata* populations maintained a slightly different subset of two unique alleles not found in any other than the ironstone*Tetratheca.*

Tetratheca deltoidea apppears to be most closely related to *T. hirsuta* .based on the Diswag dendogram and Nei's genetic similarity values. Lamont (1989) has proposed that many plant species persisted despite the extremely arid climate conditions by retreating to the wetter valleys of the Darling Scarp and to the base of granite outcrops. The

progenitors of *T. aphylla.*, *T. harperi*, and *T. paynteri.* may have retreated to the ironstone refugia at the same time that the progenitor of *T. hirsuta* and *T. deltoidea* retreated to the granite gulleys.

CONCLUSION

The evidence from allozyme electrophoresis and multivariate morphometric analyses clearly supports *Tetratheca paynteri* as a distinct taxa and supports the taxonomic status of all other taxa, except *Tetratheca hirsuta* which was divisible into at least four forms. The taxonomic status of *Tetratheca hirsuta* requires further investigation. However, based on all evidence available the group could be divided into 4 distinct forms. A robust shrub, strongly scented with alternate juvenile leaves and whorled leaves on old stems growing to 1.4 m on banks of watercourses amongst granite rocks (possibly including less robust forms such as HIR6). The second *T. hirsuta* form are low shrubs with unscented flowers and hairy, whorled to alternate leaves.The third form is a glabrous weak shrub growing in sand or massive laterite with strongly scented flowers and extremely long reflexed sepals and the fourth form includes the dwarf to low shrubs with hairy leaf margins of the dry jarrah forest sands and laterite.

Further research to clarify the taxonomic status of *Tetratheca hirsuta* populations may profit from careful investigation of the fruits and stem vestiture at the light microscope and SEM levels.(J. Alford, unpublished observations).

CHAPTER 5

GENERAL DISCUSSION

Plant species richness and endemism in the south-western heathlands and transitional rainfall zone of Western Australia is renowned. *Tetratheca* species represent only 0.6% of at least 1400 taxa threatened with extinction within this region (Briggs and Leigh, 1988; Hopper et al., 1990). The formulation of specific conservation programmes for each endangered species is not feasible. In practice general principles can be derived from a model plant taxon and used to assess the conservation status of other rare species which are known to have similar distributions, habitat, mating system or life form. These life history attributes are reflected by the patterns of genetic diversity in plant populations (Hamrick et al., 1979).

The genus *Tetratheca,* with both widespread and highly restricted species which occupy a diverse range of habitats represented an ideal group in which to investigate patterns of genetic diversity and systematic and evolutionary relationships.

5.1 Biogeography and Biology

Population sizes of *Tetratheca* species ranged from 60 to 1000 individuals and were usually isolated from conspecific populations by at least 2 km. The distributions ranged from less than 500 m for *T. deltoidea* to over 300 km for *T. hirsuta.* Spatial distribution was closely related to lithological, surface soil characteristics and the dominant plant species, notably *Eucalyptus.* Flowering occurs between July to December , ironstone species may flower opportunistically.

Tetratheca have capitalised on disturbed sites such as gravel pits and road verges and often live in unique habitats were competition from other plant taxa is minimal. Mymecochorous seed dispersal was observed for *T. paynteri.* The likelihood of fires in the ironstone hills and granites is low. Although the well developed rootstock of *T. hirsuta* appears to resprout after fires, in locations where regular fires have occurred population sizes seem to have been decimated.

Tetratheca appear to be long lived and fecund, primarily outcrossing judged on their floral morphological attributes and are distributed in late successional habitat.

5.2 Genetic diversity within *Tetratheca* species

The mean measures of genetic diversity were generally high for most *Tetratheca* species when compared to average values for a wide range of plant taxa. The levels were of the order of those found for long lived perennials. No relationship was found between population sizes, isolation and genetic distance and so no estimates were made of minimum population sizes.

In accordance with theoretical predictions, the geographically restricted *Tetratheca aphylla, harperi, T. deltoidea* and *T. paynteri* exhibited lower mean levels of genetic diversity than the widespread *T. hirsuta, T. efoliata* and *T. affinis*. Allelic richness and mean polymorphic loci varied greatly between conspecific populations and often reflected the small Nei's (1978) genetic similarity values, this suggests that populations may be fairly genetically isolated from each other and that divergence has or is taking place.

All populations except the granite forms of *T. hirsuta* exhibited considerable heterozygote deficiencies from those expected panmicted values. This may result from inbreeding or artifact of sampling of several sub-populations (pooling the homozygotes). It has been shown that high levels of heterozygosity are not a necessary condition for survival (Selander et al., 1971).

Levels of genetic diversity may be indicative of the length of time that the population has been fragmented and the size of the original 'founding' population. *Tetratheca* species and populations in the Transitional rainfall zone may have been isolated, and functioning as discrete units for longer periods than the more widespread species. The low levels of heterozygosity may be the result of a succession of extreme aridity.

5.3 Systematic relationships within *Tetratheca* species

A multivariate morphometric analysis of floral characters segregated *Tetratheca* species in the same way as allozyme electrophoresis characters (alleles) indicating that floral attributes may be highly conserved (Stace, 1989). Soule (1979) found a positive correlation between morphological and structural gene (electrophoretic) variability.

Tetratheca hirsuta was discriminated into four distinct forms utilising allozyme electrophoresis and multivariate morphometric data.

The relatively high genetic similarity between *T. harperi* and *T. aphylla* and the low genetic similarities between populations suggests that ironstone species are relics of a more mesic past and have been isolated from each other for long enough to adopt superficially similar, yet unique morphological attributes. *T. paynteri* may be an ancient derivative of either *T. aphylla, T. harperi* or an extinct progenetor of all three species.

Nei's genetic similarity data and the Distance Wagner dendogram purports that the leafless *Tetratheca* species *T. affinis, T. aphylla, T. paynteri* and *T. harperi* diverged from an ancestral entity at about the same time as the leafy species *T. hirsuta, T. efoliata* and *T. deltoidea.* The granite forms of *T. hirsuta* and *T. deltoidea* cluster together, however all branch lengths are long. This may signify that *Tetratheca* species have been distributed allopatrically for some time and populations may be operating as discrete units. Recently diverged species may exhibit high genetic similarities with progenitor species (Crawford, 1983). In the populations of the morphologically quite similar *T. hirsuta* a range of quite low genetic identity values indicate that populations may have been isolated and diverging for some time.

5.4 Conservation implications and recommendations for future research

The findings of this study suggest that it is just as important to consider the genetic viability of widespread species as it is for rare *Tetratheca* species. All *Tetratheca* species except the granite*T. hirsuta* expressed deficits in heterozygotes which may indicate that the effective breeding population size is smaller than is apparent or it may indicate that inbreeding is taking place. Maintenance of genetic diversity within widespread species is often overlooked (James, 1982). Two almost identical populations (electrophoretically and morphologically) of the widespread *T. hirsuta* expressed radically different levels of genetic diversity which could only be attributed to a reduction in population size and possibly a loss of pollinators caused by severe degradation of habitat. The conservation of widespread species such as *T. hirsuta, T. efoliata* and *T. affinis* will require a more detailed survey in local areas to gain an understanding of which forms occur where.

Many rare species inhabit relictual geological landforms or land that is of no economic value. Species or populations which have always been genetically depauperate and isolated may suffer less from disturbance than widespread species.

The findings of this study could be extended to investigate how heterozygosity affects fecundity, by comparing seed set and viability within the most heterozygous *T. hirsuta* granite populations and the genetically depauperate *T. paynteri* population.

Crossing experiments between species that have the highest levels of genetic similarity such as *T. harperi* and *T. affinis* may test the validity of the phylogenetic relationships postulated

The clarification of taxonomic relationships and delimitation of species in the *T. hirsuta* complex could be investigated using the four forms revealed by allozyme and floral morphometric analyses as a guideline.

The newly discovered and described species *Tetratheca paynteri* will be recommended for gazettal as Declared Rare Flora and further surveys for this species and the vulnerable and highly restricted *T. deltoidea* will be conducted.

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APPENDIX 1

POPULATION BIOGEOGRAPHIC DATA AND OBSERVATIONS

TETRATHECA APHYLLA

CODE APH1 WEST SADDLE POPULATION, AURORA RANGES
Lat. 30°21'51" Long. 119°41'52"

Land Status: Vacant Crown Land **Population size:** 75 **Habit:** erect shrubs to 50 cm. **Habitat:** banded ironstone outcrops with easterly aspect. **Assoc.veg.:** sparse *Dryandra arborea* to 5 m, *Trachymene pilosa, Brachycome* to 10 cm, *Chamaexeros macranthera* to 30 cm.

CODE APH2 SADDLE POPULATION ON EASTERLY SCREE SLOPE
Lat. 30°21'57" Long. 119°41'58"

Land status: =APH1 **Population size:** 150 **Habit:** erect shrubs to 50 cm. **Habitat:** massive banded ironstone south east slope. **Assoc.veg.:** occ. *Dryandra arborea, Calcopeplus ephedroides, Chamaexeros macranthum* .

CODE APH3 MAIN BUNGALBIN POPULATION
Lat. 30°23'40" Long.119°37'40"

Land status: =APH1 **Population size:** 200. **Flowering** : April, May Sept./Oct. **Habit:** erect, leafless shrubs to 50 cm.. **Habitat:** pockets of heavy brown loam over massive red and black banded ironstone. Slope 30° **Assoc.veg.:***Eucalyptus ebbanoensis* mallees and*Dryandra arborea* to 5 m, *Allocasuarina acutivalvis, Calycopeplus ephedroides, Melaleuca filifolia, M. leiocarpa, Acacia quadrimarginea* and open low sparse shrubs to 1.5 m: *Eriostemon brucei, Mirbelia, Cryptandra leucophracta, Alyxia buxifolia, Leucopogon sp. Sclerolaena diacantha.* and *Chaemaexeros macranthera* to 35 cm, common.

CODE APH4 NORTHERN DIGGINGS- SMALL HILL NORTH WEST OF MAIN BUNGALBIN PEAK Lat. 30°22'29" Long. 119°37'47"

Land status: =APH1 **Population size:** 100 **Flowering** : (1 plant) April 1990, Sept. **Habit:** erect, leafless shrubs to 45 cm. **Habitat:** lower altitude massive banded red and black ironstone outcrops. **Assoc.veg.:** *Dryandra arborea* and *Chamaexeros macranthum, Plectrachne sp.* and *Dianella revoluta* to 40 cm. **Notes:**several flowers which were bagged as buds had developing fruits.

CODE APH5 CAVE POPULATION 7.5 km NORTH EAST OF BUNGALBIN PEAK
Lat. 30°21'21" Long. 119°42'07"

Land Status: =APH1 **Population size:** 100. **Flowering** : **Habit and Habitat:** =APH2 **Assoc.veg.:** =APH2

CODE APH6 NORTHERN POPULATION
Lat. 30°21'11" Long. 119°42'18"

Land Status: =APH1 .**Population size:** 200 **Flowering** :April & a few fruit developing. **Habit:** leafless shrubs to 40 cm. **Habitat:** massive banded ironstone ridges. **Assoc.veg.:** sparse shrubs of *Alyxia buxifolia, Melaleuca filifolia.*and *Dryandra arborea* to 3 m, occas. emergent *Eucalyptus ebbanoensis..* to 8 m. over open and sparse *Plectrachne sp.* (5% cover), *Petrophile sp* <<5% to 60cm.

CODE APH7 GULLY POPULATION 400 m EAST OF MAIN BUNGALBIN POPULATION Lat.30°23'40" Long.119°37'47"

Land Status: =APH1 **Population size:** 200 **Flowering:**Aug.Sept. **Habit:**=APH3 **Habitat:** massive gully with S.E. aspect, red banded ironstone. **Assoc.veg.:***Eucalyptus eudesmoides,* over *Exocarpos aphylla, Dryandra arborea, Allocasuarina acutivalvis* and abundant *Stipa trichophylla*

TETRATHECA PAYNTERI

CODE PAY1 7.4 km West of Evanston Road, 124 km North of Bullfinch Yilgarn Shire Lat. 30°00'40" Long. 119°09'17"

Land status: part of Diemals station - leasehold **Population size:** 1000 **Flowering :**April, August in full bloom, November still flowering. **Habit:** small erect 20 cm to large weeping shrub of 40cm. **Habitat:** growing directly out of rich, red, loamy soil pockets and cracks in massive, black, ironstone rocks, primarily on Northern aspect. **Assoc.veg.:** Sparsely vegetated near *Tetratheca* otherwise occ. *Calyopeplus euphedroides, Acacia tetragonophylla, Dodonaea viscosa* and *Melaleuca filifolia t* o 3 m, over *Exocarpos aphyllus* and *Alyxia buxifolia* to 2 m and *Chenopodium, Ptilotus obovatus,* and *Olearia stuartii* shrubs. *Isotoma petraea, Threlkeldia, *Sonchus oleraceus, *Erodium cicutarium, Parietaria debilis* and ferns. **Notes:** Small black ants tried to remove seed, a little heavy for them. More sheltered and watered N.N.E. aspect supports some very old plants eg: 1 metre long by 60 cm. On hill just East of eastern end of main population c. 50 *T. paynteri.* with *Chamaexeros macranthum.* Many plants appear to have died completely, but some found with new green shoots emerging through dead stems.

TETRATHECA HARPERI

CODE HAR1 WESTERN MOST POPULATION NEAR PEAK OF MT. JACKSON **Lat. 30°15' Long. 119°16'**

Land status: part of Mt. Jackson pastoral lease. **Population size:** 150 **Flowering:** Sept./Oct. **Habit:** spiny, shrubs to 40 cm. **Habitat:** growing on steep slopes in pockets of loam amongst cracks and occasionally at the base of massive, red, ironstone rock. **Assoc.veg.:** *Dryandra arborea, Eucalyptus leptopoda* to 3 m, *Eucalyptus ebbanoensis, E. ewartiana, Prostanthera magnifica* and *Acacia steedmanii.*

CODE HAR2 AMPITHEATRE POPULATION ON NORTH WEST END OF MUDDARNING HILL **Lat. 30°15'26" Long. 119°17'17"**

Land status: HAR1 **Population size:** 250+ plants **Flowering:** Oct./Nov. **Habit:**=HAR1 **Habitat:** massive red ironstone, 60° slope. **Assoc.veg.:** occ.*Calycopeplus euphedroides, Eucalyptus ebbanoensis.* sparse open shrubs.

CODE HAR3 0.3 km NORTH OF END OF TRACK ON SOUTH SIDE OF MUDDARNING HILL **Lat.30°15'36" Long. 119°16'58"**

Land status:=HAR1 **Population size:** 100. **Flowering:** Aug./Oct./Nov. **Habit and Habitat:**=HAR2 **Assoc.veg.:** *Calycopeplus ephedroides* and *Alyxia buxifolia, Jacksonia sp,* occ. *Dryandra arborea* scrub to 4 m

CODE HAR4 GALLERY SLOPE POPULATION BELOW MAIN MT.JACKSON POPULATION **Lat. 30°15' Long. 119°16'**

Land status:=HAR1 **Population size:** 60 **Habit:**=HAR1 **Habitat:**60° slope, massive ironstone rock faces. **Assoc.veg.:** *Dryandra arborea* to 3 m, occ. *Calycopeplus ephedroides* to 1.2 m, occ. *Melaleuca uncinata* to 1.5 m and *Jacksonia sp.* **Notes:**Mesh was wrapped around two sets of stems to prevent pollinators from accessing flowers, fully open flowers were removed. Young fruit were found developing several months later.

CODE HAR5 THE ROCK TO EAST OF MAIN POPULATION **Lat.30°15' Long. 119°16"**

Land status: =HAR1 **Population size:** 100 **Flowering:** Sept./Oct. **Habit and Habitat:** =HAR4 except many plants growing at base of massive boulder. **Notes:** plants in shade seem to develop many more leaves.

TETRATHECA DELTOIDEA

CODE DEL1 MOUNT CAROLINE, 19 km SOUTH OF KELLERBERRIN Lat. 31°48' Long. 117°38'

Land Status: "A" Class Nature Reserve **Population size:** 160 **Flowering:** Sept.-Dec. **Habit:** leafy, multistemmed, spreading trailing shrubs to 1 m **Habitat:**pockets of rich, humus grey soil, in protected S.S.Eastern "swales" near the top of a massive granite monolith. **Assoc.veg.:** amongst sedges *Lepidosperma* , under *Eucalyptus caesia* to 4 metres tall **Notes:** stamens go from blood red in young bud to grey black, abundance of insects but no pollinations observed.

TETRATHECA HIRSUTA

CODE HIR1 PAXWOLD GIRL GUIDES CAMP; end of Gilchrist Road, LESMURDIE Lat. 30°01'37" Long. 116°03'

Land Status ???? **Population size:** 150 plants amongst shrubs **Habit:**inconspicuous shrubs to 30 cm with leaves in whorls of 3 or 4. **Habitat:** Laterite and loam **Flowering:** Sept.-Nov., no perfume noted. **Assoc.veg.:**Open Jarrah forest over low shrubs to 50 cm.

CODE HIR2 HINES ROAD, NORTH DANDALUP NEAR WATER AUTHORITY SIGN

Lat. 32°30'33" Long. 115°59'23"

Land Status: W.A.W.A. catchment area. **Population size:** 100 **Habit:** dwarf shrubs to 35 cm. **Flowering:**Sept.-Nov.. **Habitat:** Loam and some gravel. **Assoc. veg.:** Almost pure stand of skinny (regrowth) Jarrah to 18 m over *Grevillea, Persoonia, Macrozamia* and *Xanthorrhoea* dwarf shrubs to 40 cm. **Notes:** no perfume noted.

CODE HIR3 200 m up Jarrahdale Follow, JARRAHDALE.

Lat. 32°20' Long. 116°04'

Land Status Northern section of Serpentine National Park **Population Size:** more or less continuous population for 1.8 km (1000s of plants) **Flowering:** full bloom and all stages, very few fruits July -Sept. **Habit:** shrubs 30 to 50 cm with whorled leaves (3). **Habitat:** gently undulating approx 5%, couple of inches of hummus dark, loamy sand with 50% laterite pebbles <1cm. **Veg. Association:** Jarrah forest to 25 metres logged in past and burnt lots of young trees c.15% cover total occ Marri leggy to 20 m open understory to 1 metre of *Persoonia, Xanthorrhoea, Acacia, Macrozamia reidliei, Hovea trisperma, Hypocalymma pink,Dryandra nivea, Baeckea and Daviesia decurrens.* **Notes:** West side ?burnt more often, less Tetratheca on this side. On most plants all growth appears new, multi-stemmed, no branching.

CODE HIR4 1.7 km east of DWELLINGUP on road verge near gravel track. Lat. 32°43' Long. 116°05'51"

Land Status Main Roads verge **Population size:** 400 plants **Flowering:** c. 70% not flowering 27th July early bud, full flower 1st Oct. **Habit:** shrubs to 40cm, not scented **Habitat:** laterite pebbles to 3cm diameter with rich brown loamy sand, quite moist.Very gentle slope east. Rich leaf litter. **Veg. Assoc:** Jarrah forest c. 5% to 30 metres occ Marri to 8 m, *Banksia grandis* common 4 to 8 metres over low heath to 60 cm inc. *Pteridium esculetum, Macrozamia reidlei, Daviesia decurrens, Xanthorrhoea, Acacia pulchella, Mesomelaena* and *Lepidosperma* occ. *Leucopogon verticillatus* to 2 m.

CODE HIR5 TORRENS ROAD, 10 km North of DWELLINGUP. Lat. 32°39' Long. 116°02'

Land Status: C.A.L.M. estate Forest **Population size:** 50 plants **Flowering:** some flowering, most in bud 27th July. **Habit:** shrubs to 30cm **Habitat:** gentle southern slope, soil =HIR4 **Veg. Assoc.:** Old heavily logged forest, very damaged.(scorched tree trunks and depauperate understory) Almost pure stand of ? regrowth Jarrah to 18 m, occ Marri, *Persoonia* common, a few *Banksia grandis* to 4 m. *Pteridium esculetum* and *Macrozamia reidlei* abundant. **Notes:** *Tet.* colonizing road edge. Most plants appear young, ?recover from seed or stock. (root system simple).

CODE HIR6 1.4 km West of LOWER CHITTERING ROAD, on MUCHEA ROAD EAST Lat. 31°32'45" Long. 116°06'10"

Land status: Main Roads Department, verge. **Population size:** several hundred plants seen along a 2 km road verge, primarily on south side of road. **Flowering:** full flower **Habit:** shrubs to 50 cm. **Habitat:** heavy laterite boulders and pebbles on gradual westerly hill slope. **Veg.assoc.:** Old sparse, remnant Jarrah with occ. Marri to 15 m. Dwarf shrubs to 60 cm inc. *Xanthorrhoea, Lechenaultia biloba, Bossia eriocarpa, Hibbertia hypericoides, Grevillea synaphea.*

CODE HIR7 4.5 km east of Brand Hwy. on track parallel with Wannamal West Road, GINGIN. Lat. 31°10'27" Long. 115°50'58":

Population 1: Land Status: Vacant Crown Land **Population size:** >500 plants seen in 200 m **Flowering:** Bud (late Aug), Flower (Sept-Nov/?Dec) **Habit:** weak shrubs to 30cm. **Habitat:** almost flat, deep yellow sand over ?laterite. **Assoc.veg.:** Heavily logged Jarrah, many coppicing, open to 15 m. Occ. Marri with *Banksia grandis* to 5 m. over *Xanthorrhoea , Jacksonia sternbergiana* and *Hakea trifurcata* to 2.5 m. Species rich dwarf shrubland to 60 cm including *Acacia pulchella, Hibbertia hypericoides, Bossiae eriocarpha, Calectasia cynea, Daviesia preissii, Mesomelaena stygia, Petrophile serruriae, Synaphea and Hybanthus.* **Notes:** heavily scented flowers with strongly reflexed sepals, fading almost to white with age. Abundant insects, no pollination evidenced. Dense, rich leaf litter.

Population 2: 1.9 km South of Wannamal West Road on track, 7 km East of Brand Highway, Gingin. Lat. 31K12' Long. 115°52"

Population Size: 60 plants, very inconspicuous. **Habitat:** Laterite ridge with massive rock just emergent and gravel with yellow sand. **Assoc.Veg.:** Jarrah/Marri to 18 metres over occasional *Banksia grandis* to 8 metres and *Dryandra sessilis* occ. in large patches to 8 m. Understory: shrubs to 40cm of *Calytrix, Hibbertia, Lechenaultia biloba, Conostephium pendulum, Grevillea synaphea, Synaphea, Gompholobium, Acacia drummondii, Hakea lissocarpha, Bossia eriocarpha* and *Daviesia preissii* with emergent *Xanthorrhoea* and *Allocasuarina.*

CODE HIR8 East end of Gosnells Road East, Gosnells within the grounds of the "Practical Shooting Club". Lat. 32°02'12" Long. 116°01'51".

Population size: >1000, up to 60% of understory in patches. **Habit:** robust shrubs, many branched or lax to 1.35 m. **Flowering:** heavily scented, May-Nov. **Habitat:** rich brown, clay loam over massive granite with c. 5% bank slope into creek. **Assoc.veg.:** *Eucalyptus rudis* with Marri and Wandoo to 18 m. Over species rich, dense shrubland to 1.5 m incl. *Acacia pulchella, Macrozamia reidlei, Phyllanthus calycinus, Hibbertia hypericoides, Hakea trifurcata, Dryandra and Trymalium.* **Notes:** Abundant insects, no pollination events..

CODE HIR9 half way down the ZIG ZAG Scenic drive, GOOSEBERRY HILL Lat. 32°02'12" Long. 116°01'51"

Population size: >400 **Flowering:** Sept. **Habit:** robust shrubs to 1 m tall. **Habitat:** S.E. slope, rich red, clay loam over massive granite boulders. Steep slope 30°. **Assoc. veg.:** Sparse Wandoo and Marri to 15 m over occ. *Nuytsia floribunda* to 8 m over dense shrubs to 1 m incl. *Xanthorrhoea preissii, Hakea trifurcata, Hakea undulata, Trymalium, Allocasuarina, Pimelea, Wumbea diocea and Stackhousia.*

CODE HIR10 OZONE TERRACE, KALAMUNDA. Lat.32°01'06" Long. 116°02'28"

Land Status: road verge. **Population size:** 300 plants. **Habit:** erect shrubs to 80 cm. **Habitat:** Light brown yellow loam over granite on 25° S.E. slope down to Whistlepipe gully. **Assoc.veg.:** Marri and occ. Wandoo to 15 m over dense shrubs to 60 cm incl. *Acacia pulchella, Hakea undulata, Pimelea, Calothamnus, Hakea trifurcata, Daviesia cordata, Mesomelaena stygia,Xanthorrhoea* and occ. *Macrozamia reidlei* to 1.2 m.

CODE HIR11 BICKLEY BROOK Just east of YOUTH SPORT AND RECREATION CAMP, LESMURDIE. Lat. 32°02'14" Long. 116°03'
Land Status: Department of Youth Sport and Recreation Land **Population size:** >450 individuals **Flowering:** strongly scented, full flower Aug. **Habit:** hairy shrubs to 70 cm, with 2 forms of leaf growth on each stem. **Habitat:** primarily on northern banks of Bickley brook in brown loam over massive granite boulders. **Assoc.Veg.:** Wandoo/Marri/*Eucalyptus rudis* sparse over rich heath to 1 metre of *Melaleuca, Darwinia, Trymalium, Acacia Leucopogon, Pimelea, Xanthorrhoea, Grevillea pulchella, Acacia pulchella*

Tetratheca efoliata
CODE EFO1 28.3 km EAST OF SOUTHERN CROSS OFF GREAT EASTERN HIGHWAY Lat.31°16'55"Long.119°37'01"
Land Status: Main Roads reserve. **Population Size:** 100 **Flowering:** Nov. **Habit:** wiry dwarf shrubs to 40 cm. **Habitat:** yellow sand, flat plain off road bank. **Assoc.veg.:** occ. *Grevillea didymobotrya* to 6 m with sparse *Allocasuarina acutivalvis, Banksia elderana, Leptospermum roei, Acacia coolgardiensis, A. signata.* Low shrubs to 30 cm. **Notes:** *Tetratheca* appears to colonize edge of road readily after grading. Plants under shrubs tend to be more lush and leafy than their exposed counterparts. A tough, woody root system is well developed even in plants only 4 cm tall.

TETRATHECA AFFINIS
CODE AFF1 FOOT OF PORONGORUPS 2.2 km EAST OF TURN OFF TO CASTLE ROCK Lat. 34°40'55" Long. 117°54'47"
Land Status: Main roads reserve. **Population size:** 60 plants. **Habit:** erect, leafless, shrubs 25 to 40 cm. **Habitat:** laterite gravel. **Assoc.veg.:**very open Jarrah to 12 m with dense understory.

CODE AFF2 SHEEPWASH STATE FOREST. SOUTH MITCHELL RIVER ROAD. 19.km North of DENMARK Lat. 34°50'43" Long. 117°25'48"
Land Status: C.A.L.M. State Forest **Population size:** 100 **Habit:** erect to lax, leafless shrubs to 40 cm tall. **Habitat:** Laterite gravel and sand, slightly undulating plain. **Assoc.veg.:** very sparse, burnt and logged Jarrah woodland to 20 m, understory to 3 m incl. *Agonis, Casuarina, Banksia grandis, Hovea trisperma* and *Leucopogon verticillatus.*

APPENDIX 2

ALLOZYME ELECTROPHORESIS RECIPES

Phosphoglucomutase

(PGM, E.C. 2.7.5.1)

Buffer	Substrate	Stain
4 ml 0.1M Tris-HCl	6 mg Glucose-1-phosphatase	2 drops PM
pH 8.0	(Sigma Type g 1259)	4 drops MT
	2-3 mg NADH	
	20-30ul G6pd	
	6 drops $MgCl_2$	
	(0.5M)	

Leucine aminopeptidase

(LAP, **E.C.**.....)

Buffer	Substrate	Stain
4 ml Acetate	3-4 mg Na-L Leucyl-Naphylomide	1mg/ml Fas
		Black
pH 5.0	6-10 Drops $MgCl_2$ (0.5M)	K Salt

Phosphoglucose isomerase

(PGI, E.C. 5.3.1.9.)

Buffer	Substrate	Stain
4 ml 0.1M Tris-HCl	4-6 mg Fructose-6-phosphate	1 Drop PMS
pH 8.5	2-3 mg NADP	4 Drops MT
	20-30 ul G6pd	
	5 Drops $MgCl_2$	

Malate dehydrogenase

(MDH, E.C. 1.1.1.37)

Buffer	Substrate	Stain
4 ml 0.1M Tris-HCl	8-10 mg L-Malic Acid	1 Drop PMS
pH 8.0	2-3 mg NAD	4 Drops MT

Menadione reductase

(MR, E.C.)

Buffer	Substrate	Stain
4 ml 0.1M Tris-HCl	6-8 mg Mendadione (2mg/ml)	6 Drops MT
pH 7.0	2-3 mg NADH (1mg/ml)	

Extraction Buffer:

10 mls Double Distilled H_2O

Add

0.5 g PVP
1.0 g Sucrose
0.017 g EDTA
0.002 g Absorbic acid

Then

0.01 g Bovine serum
0.005 g NAD
0.004 g NADP

Lastly

0.0112 g Dithiothreitol

APPENDIX 3

ALLELE FREQUENCIES FOR 28 POPULATIONS OF 7 TAXA OF *TETRATHECA*

(n=sample size per locus)

Locus	Alleles	APH1	APH2	APH3	APH4	APH5	APH6	APH7
Pgm-1	(n)	15	8	18	18	10	7	11
	c	0.53	0.31	1.00	0.78	1.00	0.07	1.00
	d	0.47	0.69	0.00	0.22	0.00	0.93	0.00
Lap-1	(n)	18	10	18	15	11	6	11
	a	0.00	0.00	0.00	0.00	0.00	0.00	0.18
	b	0.11	0.50	0.06	0.00	0.00	0.00	0.64
	c	0.56	0.50	0.44	0.63	0.05	0.00	0.18
	d	0.33	0.00	0.39	0.37	0.32	0.17	0.00
	e	0.00	0.00	0.11	0.00	0.46	0.83	0.00
	f	0.00	0.00	0.00	0.00	0.18	0.00	0.00
Pgi-2	(n)	16	11	18	19	11	6	11
	e	0.88	0.96	0.56	0.61	0.14	0.75	1.00
	f	0.13	0.05	0.44	0.34	0.86	0.25	0.00
	g	0.00	0.00	0.00	0.05	0.00	0.00	0.00
Mr-1	(n)	17	10	15	17	1	1	10
	b	0.35	0.30	0.77	0.53	0.00	0.00	0.60
	c	0.62	0.70	0.20	0.35	1.00	1.00	0.40
	d	0.03	0.00	0.03	0.06	0.00	0.00	0.00
	e	0.00	0.00	0.00	0.06	0.00	0.00	0.00
Mdh-1	(n)	15	10	18	18	1	5	1
	a	0.10	0.20	0.00	0.00	0.00	0.00	0.00
	b	0.07	0.05	0.00	0.00	0.00	0.00	0.00
	c	0.30	0.35	0.06	0.36	1.00	0.00	1.00
	d	0.53	0.40	0.53	0.58	0.00	1.00	0.00
	e	0.00	0.00	0.42	0.06	0.00	0.00	0.00

Locus	Alleles	PAY1	HAR1	HAR2	HAR3	HAR4	HAR5	DEL1
Pgm-1	(n)	49	31	16	11	19	24	40
	b	1.00	0.00	0.00	0.00	0.00	0.00	0.00
	c	0.00	0.68	0.56	0.73	0.79	1.00	0.38
	d	0.00	0.32	0.44	0.27	0.21	0.00	0.60
	e	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Lap-1	(n)	53	23	12	10	16	16	25
	b	0.00	0.26	0.08	0.00	0.25	0.00	0.00
	c	0.00	0.35	0.50	0.20	0.75	1.00	0.54

	d	1.00	0.26	0.42	0.00	0.00	0.00	0.46
	e	0.00	0.13	0.00	0.65	0.00	0.00	0.00
Pgi-2	(n)	53	31	18	10	21	20	51
	d	0.00	0.08	0.06	0.00	0.12	0.00	0.02
	e	0.00	0.58	0.75	0.75	0.81	1.00	0.00
	f	1.00	0.32	0.17	0.00	0.07	0.00	0.27
	g	0.00	0.02	0.03	0.25	0.00	0.00	0.23
	h	0.00	0.00	0.00	0.00	0.00	0.00	0.49
Mr-1	(n)	47	5	11	11	3	3	3
	a	0.79	0.00	0.00	0.00	0.33	0.00	0.00
	b	0.21	0.20	0.09	0.00	0.00	0.00	0.00
	c	0.00	0.40	0.59	0.41	0.33	0.67	0.00
	d	0.00	0.40	0.18	0.32	0.33	0.33	0.00
	e	0.00	0.00	0.14	0.27	0.00	0.00	0.00
Mdh-1	(n)	15	24	11	4	15	7	21
	a	0.00	0.33	0.00	0.00	0.27	0.00	0.00
	b	0.00	0.13	0.27	1.00	0.07	1.00	0.00
	c	1.00	0.15	0.14	0.00	0.27	0.00	0.00
	d	0.00	0.40	0.55	0.00	0.40	0.00	0.00
	e	0.00	0.00	0.05	0.00	0.00	0.00	1.00
Locus	Alleles	HIR1	HIR2	HIR3	HIR4	HIR5	HIR6	HIR7
Pgm-1	(n)	26	16	9	13	8	28	38
	c	0.08	0.00	0.00	0.00	0.00	0.09	0.18
	d	0.25	0.88	1.00	1.00	1.00	0.91	0.67
	e	0.67	0.13	0.00	0.00	0.00	0.00	0.15
Lap-1	(n)	24	14	9	13	1	22	35
	a	0.00	0.04	0.00	0.15	0.00	0.18	0.00
	b	0.00	0.79	1.00	0.65	0.00	0.25	0.00
	c	0.90	0.14	0.00	0.19	0.00	0.55	1.00
	d	0.08	0.04	0.00	0.00	0.00	0.02	0.00
	e	0.02	0.00	0.00	0.00	0.00	0.00	0.00
Pgi-2	(n)	24	14	9	13	7	27	38
	d	0.00	0.07	0.00	0.00	0.00	0.00	0.13
	e	0.00	0.29	0.06	0.00	0.00	0.43	0.50
	f	0.27	0.46	0.33	0.08	0.43	0.50	0.20
	g	0.19	0.18	0.22	0.54	0.43	0.07	0.12
	h	0.54	0.00	0.39	0.39	0.14	0.00	0.03
	i	0.00	0.00	0.00	0.00	0.00	0.00	0.03
Mr-1	(n)	21	14	9	13	8	15	32

	b	0.00	0.07	0.00	0.00	0.00	0.00	0.00
	c	0.33	0.14	0.39	0.67	0.69	0.97	0.30
	d	0.10	0.39	0.56	0.19	0.25	0.03	0.55
	e	0.41	0.32	0.06	0.15	0.06	0.00	0.13
	f	0.00	0.07	0.00	0.00	0.00	0.00	0.03
Mdh-1	(n)	11	8	9	2	7	17	7
	c	1.00	0.13	1.00	1.00	1.00	0.00	1.00
	d	0.00	0.88	0.00	0.00	0.00	0.00	0.00
	e	0.00	0.00	0.00	0.00	0.00	1.00	0.00
Locus	Alleles	HIR8	HIR9	HIR10	HIR11	EF01	AFF1	AFF2
Pgm-1	(n)	26	15	12	10	12	17	18
	a	0.00	0.00	0.00	0.00	0.00	0.00	0.39
	b	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	c	0.00	0.00	0.00	0.00	0.17	0.91	0.61
	d	1.00	0.80	1.00	1.00	0.42	0.09	0.00
	e	0.00	0.20	0.00	0.00	0.42	0.00	0.00
Lap-1	(n)	27	14	12	4	4	17	18
	a	0.35	0.00	0.17	0.50	0.00	0.00	0.00
	b	0.19	0.14	0.46	0.13	0.88	0.38	0.00
	c	0.41	0.86	0.38	0.38	0.13	0.44	0.19
	d	0.06	0.00	0.00	0.00	0.00	0.18	0.81
Pgi-2	(n)	27	15	12	9	11	19	18
	b	0.00	0.00	0.00	0.00	0.32	0.00	0.00
	c	0.00	0.00	0.00	0.00	0.18	0.00	0.00
	d	0.00	0.03	0.00	0.00	0.23	0.00	0.17
	e	0.00	0.03	0.13	0.00	0.27	1.00	0.83
	f	0.46	0.27	0.21	0.56	0.00	0.00	0.00
	g	0.22	0.43	0.38	0.11	0.00	0.00	0.00
	h	0.13	0.20	0.13	0.28	0.00	0.00	0.00
	i	0.19	0.03	0.17	0.06	0.00	0.00	0.00
Mr-1	(n)	24	5	5	4	17	18	17
	c	0.35	0.40	0.70	1.00	0.00	0.00	0.00
	d	0.44	0.30	0.30	0.00	0.47	0.67	0.29
	e	0.17	0.30	0.00	0.00	0.53	0.28	0.59
	f	0.04	0.00	0.00	0.00	0.00	0.06	0.12
Mdh-1	(n)	6	0	12	4	0	16	17
	a	0.00	0.00	0.00	0.00	0.00	0.16	0.12
	b	0.00	0.00	0.00	0.00	0.00	0.84	0.32
	c	0.00	0.00	0.00	0.00	0.00	0.00	0.56
	d	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	e	1.00	1.00	1.00	1.00	0.00	0.00	0.00

APPENDIX 4

FORMAL DESCRIPTION OF *TETRATHECA PAYNTERI*

This paper has been submitted and accepted for publication in Nuytsia, 1991.

A new species of *Tetratheca* Smith from the Coolgardie district of Western Australia.

J.J. Alford

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Abstract

Alford, J.J. *Tetratheca paynteri*, a new species of Tremandraceae from the Coolgardie district of Western Australia. One new, endemic species of *Tetratheca* is described. This species is apparently endangered, being restricted to a small range of hills north of Bullfinch. *Tetratheca paynteri* has affinities with *T. aphylla* however, several features such as floral and stem morphology serve to distinguish this as a distinct species. Major features which distinguish the new species from *T. aphylla* and *T. harperi* are described.

Introduction

The genus *Tetratheca* (Tremandraceae) consists of 39 species, 21 of which are restricted to the south-west and transitional zone of Western Australia. The conservation and taxonomic status of these taxa is being investigated. (Alford, unpub. Honours thesis).

In the process of collating distribution data for *Tetratheca aphylla*, Rae Paynter, a botanist and naturalist of Toodyay brought the author's attention to specimens collected in 1988. Initially it was considered the specimens represented a range extension of *Tetratheca aphylla* but further investigation supports separation of these plants as a distinct species.

Further collection of flowering and fruiting material was made by the author in 1990 and this description was based on measurements of 50 individual plants.

Taxonomy

Tetratheca paynteri J.J.Alford, sp. nov. (Figure 1)

Typus: Unnamed hills, 11 km south of Pigeon Rocks, 124 km North of Bullfinch. 8th November, 1989. J.J.Alford 1360 (holo:PERTH)

Shrubs 15 to 40 cm high, erect to slightly weeping with divaricate, fairly stout stems branching alternately and often terminating in a brown or silver slender, point. ***Vestiture*** of minute, warty tubercules. ***Stock*** woody with fine masses of offshoot roots. ***Leaves*** generally absent in adult tissue. Juvenile type growth in resprouting plants, alternate and 8 mm. long. ***Flowers*** with distinctive dank musky odour, usually occuring singly (occasionally double) in axils of clustered bracts. ***Bracts*** fleshy, keeled and acuminate 0.5 to 1.5 mm long, reddish and tuberculate. Some short white hairs. ***Peduncles*** often slightly recurved 4.8 to 11.0 mm, glossy green and red coloured with sparse, scattered

warty, tubercules; some minute, white trichomes and occasional glandular hairs; longitudinally striated, thickening gradually as goes into 1.0 to 1.5 mm diameter receptacle. ***Calyx segments*** 5 occ. 6, deciduous 3.3 to 5.5 mm long, narrowly deltoid, acute; green with red near base and on margins, odd red glandular hairs or stiff, short white setae; outer primarily glabrous, inside appressed white short pubescence; prominent ridge extending from base to 2/3 on underside. 5 occ. 6, obovate to elliptical with small yellowish white patch at base. 8 to 12.6 mm long and just over half as wide with the widest part being c. 1/3 from the top, acuminate, deciduous. ***Stamens*** 10 occ. 12 c. 5 mm long, pairs of stamens sharing a common filament, strongly infolded together in bud, 0.5 mm long, body of anther 3.5 mm, anther tube 1.1 mm and slightly curved, a few minute stiff hairs. ***Ovary*** covered in dense, stiff, short hairs tapering finely to stigma, occasional glandular hairs; greenish colour with red on junction of carpels and on margins. ***Ovules*** 4, 2 in each loculus. ***Fruit*** obovate, 5.6 to 8 mm long and 4 to 6 mm broad in lower third, prominent ring of old receptacle persistent; green, glabrous capsule with tiny white hairs on outer margin. ***Seed*** ca. 3.6 mm long, pale to medium brown with hard, shiny, brown testa covered in soft, white hairs; including a well developed, cream-coloured elaiosome ca. 0.9 mm long; pale green, cigar-shaped embryo.

Flowering period April to November, ?rain opportunist.

Distribution Endemic to the Coolgardie botanical district of Western Australia, occurring on a range of hills 11 km south of Pigeon Rocks, Yilgarn Shire.

Habitat. Found growing in crevices of rich, red loam amongst massive banded ironstone rock.

Conservation Status. The unique habitat of this species has been thoroughly investigated and to date less than 1000 individuals have been found at the only known location. The locality is not within a conservation reserve and supporting the recommendation of Declared Rare Flora status.

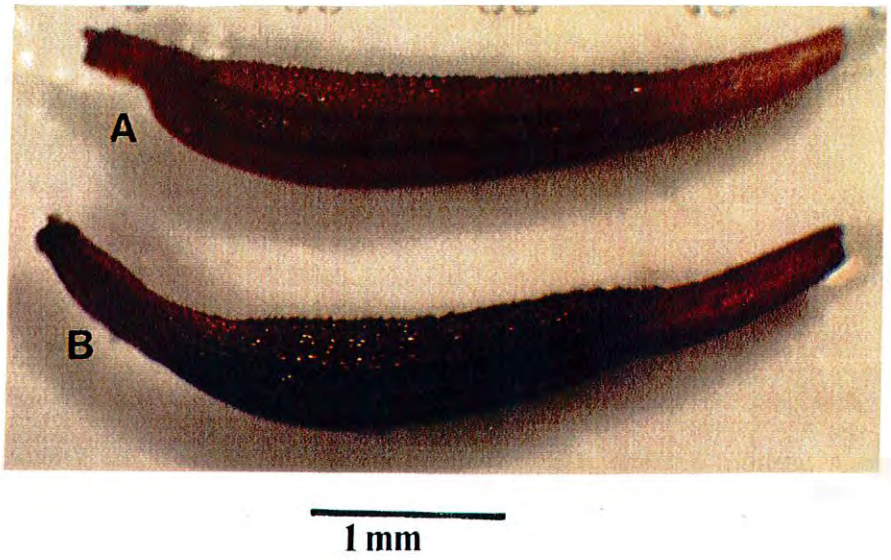
Etymology. The specific epithet honours the efforts of Rae Paynter of Toodyay who has contributed greatly to the conservation of the flora of the State.

Discussion

Tetratheca paynteri superficially resembles *Tetratheca aphylla* and occupies the same habitat type. Distinguishing characters are the warty tubercules of the stems of *Tetratheca paynteri* cf. *Tetratheca aphylla*'s minute, stiff trichomes; longer, more acute sepals, glabrous peduncle and presence of 4 ovules as opposed to *T. aphylla* with only 2 ovules. The stamens of *T. paynteri* differ slightly from those of *T. aphylla* (Fig. 1.). The habit, flowers, fruit and juvenile growth form of *T. paynteri* are illustrated in Fig. 2. The primary distinguishing characters of *T. paynteri*, *T. aphylla* and *T. harperi* are summarised in Table1.

Reference

THOMPSON, J. (1976) A Revision of the genus Tetratheca (Tremandraceae). Telopea 1:139-215.



A B
Figure 1. Stamens of *T. paynteri* and *T. aphylla*.

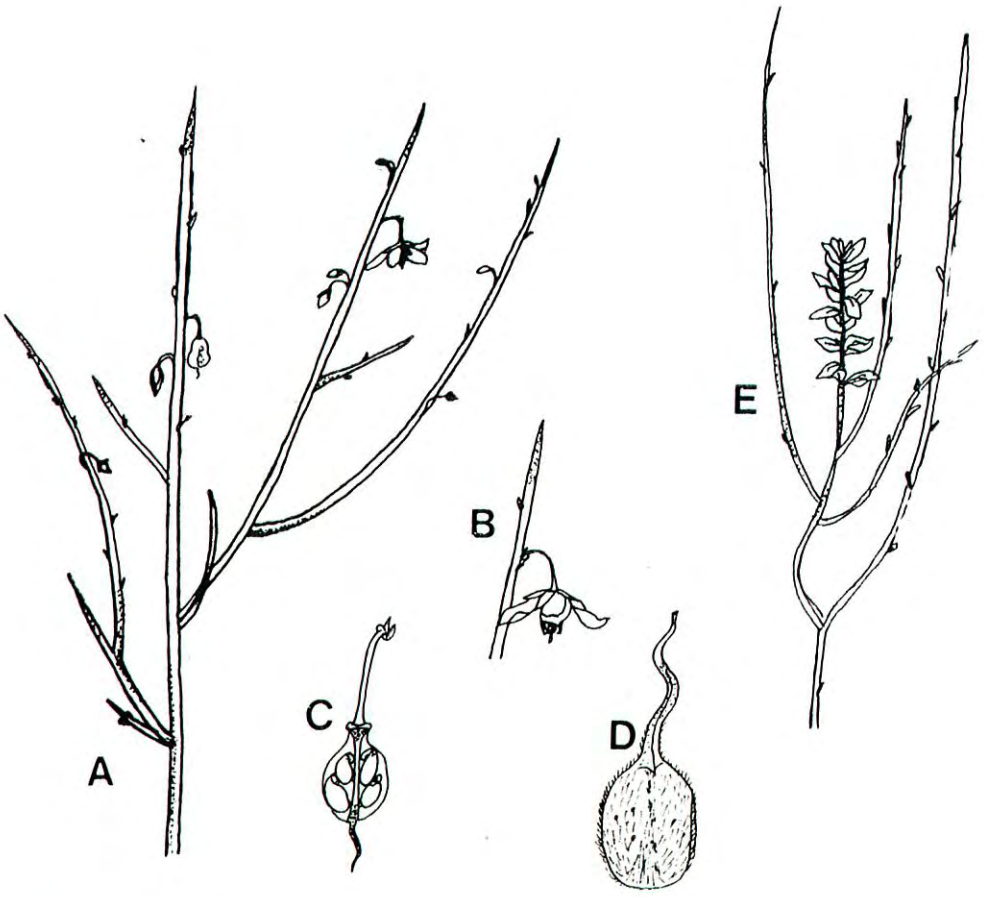


Figure 2. *T. paynteri*. A. Habit. B. Flower. C. Section of fruit showing developing seed. D. Ovary. E. Juvenile growth form.

Table 1. Morphological characters which distinguish *T. paynteri*, T. aphylla and *T. harperi*.

	T. paynteri	*T. aphylla*	*T. harperi*
Ovule number	4 (2/loculus)	2 (1/loculus)	2 (1/loculus)
Peduncle	4.8 - 11 mm glabrous, green & red	4 mm dark dense, minute stiff hairs	5 mm glabrous red &green
Vestiture	warty tubercules	dense, minute tubercules	tubercule based setae, 1- 2 mm
Ovary surface	dense, stiff short hairs taper out on style	dense, stiff short hairs almost to stigma	glabrous
Sepals	5; 3.3 - 5.5 mm narrowly deltoid; scattered glandular hairs	5; 3 mm long, narrowly deltoid; scattered glandular hairs	4 or 5; 2 - 2.5 mm ovate to elliptical obtuse, short point
Petals	5 occ. 6 8 - 12 mm c.1/2 as wide in upper 1/3	5 10 mm long c. 1/2 as wide at midpoint	4 or 5 10 - 12 mm c. 1/2 as wide in upper 1/3
Stamens	10 occ. 12 4.4 mm. long filament 0.5 mm. tube 0.95 mm.	10 4 mm long filament flat 0.6 mm tube 1.0 mm	10 4.4 mm long fil. stout 0.4 mm; tube 1.7 mm

APPENDIX 5

MORPHOMETRIC DATA USED FOR ANALYSES IN CHAPTER 4

Table 1. Floral measurements (mm) used in multivariate morphometric analysis.

W=petal width
BL=bottom length of petal
TL=top length of petal
SL=sepal length
SW=sepal width
PL=peduncle length
AB=anther body length
F=filament length
T=tube length

	W	BL	TL	SL	SW	PL	AB	F	T
Tetratheca aphylla									
APH 01	3.8	3.9	3.3	3.0	1.2	2.8	2.4	0.5	0.6
APH 02	4.4	4.8	3.0	2.9	1.4	3.8	2.3	0.5	1.0
APH 03	4.1	4.5	2.8	2.6	1.2	2.0	2.0	0.7	1.2
APH 04	4.9	5.6	4.1	3.0	1.0	2.8	2.0	0.7	1.4
APH 05	5.1	5.0	3.9	2.6	1.0	2.3	2.2	0.5	0.9
APH 06	4.5	5.1	2.9	2.6	1.3	2.7	1.8	0.7	1.0
APH 07	3.5	3.0	2.5	2.7	1.0	-1	2.1	0.6	1.2
APH 08	4.9	5.8	3.2	2.0	1.0	2.8	2.1	0.7	1.1
APH 09	4.3	4.5	2.6	2.4	1.4	3.5	-1	-1	-1
APH 10	5.5	5.8	3.8	2.8	1.0	3.9	-1	-1	-1
APH 11	4.0	4.6	2.7	2.3	1.0	-1	1.7	0.6	1.0
APH 12	4.6	4.4	3.5	2.7	1.4	2.7	2.4	0.6	1.0
APH 13	5.0	6.1	4.0	3.2	1.0	-1	2.3	0.7	1.0
APH 14	5.9	5.4	4.5	3.3	1.2	-1	2.3	0.5	1.3
APH 15	3.8	4.1	3.0	2.6	1.0	2.5	2.4	0.5	1.1
Tetratheca paynteri									
WIN 01	5.9	5.8	4.0	4.5	1.4	6.4	2.8	0.4	0.9
WIN 02	5.4	6.3	3.1	3.3	1.3	7.1	2.8	0.4	1.1
WIN 03	4.3	4.9	3.9	4.3	1.3	7.6	3.2	0.5	1.1
WIN 04	5.5	6.4	4.1	4.8	1.4	7.5	3.0	0.5	0.9
WIN 05	6.1	7.0	2.4	4.3	1.6	5.8	3.1	0.4	1.0
WIN 06	5.5	7.2	2.5	4.3	1.3	6.0	2.8	0.6	0.9
WIN 07	5.0	4.7	3.3	3.9	1.4	6.5	2.9	0.5	0.8
WIN 08	6.1	7.8	4.8	5.5	1.3	11.0	3.2	0.7	1.2
WIN 09	4.4	5.7	2.8	4.5	1.3	5.2	2.9	0.6	0.8
WIN 10	4.9	6.2	3.6	4.6	1.4	5.7	2.9	0.5	1.0
WIN 11	4.9	6.2	3.8	3.8	1.3	7.5	3.4	0.5	1.0
WIN 12	4.1	4.1	2.8	3.6	1.4	7.3	2.6	0.4	0.8
WIN 13	5.3	4.9	3.9	4.6	1.5	6.4	2.9	0.5	1.0
WIN 14	4.6	5.7	3.0	4.2	1.2	6.5	3.0	0.5	1.1
WIN 15	6.0	5.6	3.6	4.5	1.5	6.8	-1	-1	-1
WIN 16	5.5	5.5	3.0	3.6	1.4	5.1	3.1	0.5	1.2
WIN 17	5.1	4.7	4.1	4.1	1.4	6.9	3.2	0.5	1.1
WIN 18	5.7	5.0	3.2	3.8	1.5	6.2	2.5	0.4	0.8
WIN 19	6.3	6.4	3.6	3.8	1.4	6.3	2.7	0.5	0.9
WIN 20	7.8	6.7	4.8	4.6	1.7	7.3	3.3	0.5	1.2
WIN 21	5.0	4.9	4.5	4.1	1.5	6.5	2.6	0.5	1.1
WIN 22	5.9	6.3	4.1	4.6	1.3	7.5	3.1	0.5	0.9
WIN 23	5.1	5.7	3.8	3.8	1.1	6.3	2.6	0.4	0.9
WIN 24	5.6	9.3	3.5	3.4	1.3	6.5	3.0	0.4	1.0
WIN 25	6.2	7.0	3.4	3.6	1.2	5.8	2.8	0.5	1.0
WIN 26	4.5	5.1	3.1	3.4	1.2	6.1	2.7	0.5	0.7
WIN 27	6.4	7.5	4.3	4.0	1.5	5.7	2.9	0.5	0.8
WIN 28	6.3	6.5	4.3	4.5	1.4	8.3	3.3	0.4	1.0
WIN 29	5.7	6.1	3.4	3.8	1.3	7.3	2.7	0.4	0.7
WIN 30	5.9	6.4	3.4	3.7	1.5	8.5	3.0	0.6	0.8
Tetratheca harperi									
HAR 01	5.6	4.3	5.7	2.6	1.5	3.8	1.9	0.2	1.2
HAR 02	5.3	4.5	8.0	2.9	1.4	6.2	3.0	0.2	2.0
HAR 03	5.9	6.1	5.9	3.0	1.7	6.3	2.1	0.2	1.6
HAR 04	5.9	5.7	7.9	2.4	1.9	7.0	2.6	0.3	2.0
HAR 05	5.9	4.6	7.3	2.2	1.3	5.9	2.1	0.4	1.5
HAR 06	6.5	5.6	8.2	3.1	2.1	6.2	2.6	0.5	1.8
HAR 07	4.8	3.5	6.3	2.5	1.5	4.6	2.6	0.5	1.5
HAR 08	5.0	4.3	7.5	2.6	1.5	4.6	2.4	0.4	1.4
HAR 09	5.6	4.7	7.5	2.7	1.7	7.5	2.5	0.4	1.4
HAR 10	4.1	5.4	5.5	2.8	1.7	5.0	2.1	0.3	1.7
HAR 11	4.5	3.5	5.8	2.5	1.5	6.6	2.2	0.4	1.8
HAR 12	6.0	5.1	6.7	2.0	1.1	6.5	2.3	0.5	1.7
HAR 13	5.3	4.3	6.7	3.0	1.4	5.4	2.0	0.4	1.6
HAR 14	6.9	5.4	6.5	2.6	1.6	5.4	2.5	0.4	1.5
HAR 15	5.0	4.4	6.0	2.3	1.4	2.9	2.0	0.3	1.4
HAR 16	5.9	4.9	7.9	2.5	1.4	7.1	2.4	0.5	2.0
HAR 17	7.6	4.8	9.3	2.5	1.4	5.5	2.4	0.4	2.0
HAR 18	6.0	4.6	7.9	2.6	1.5	3.7	2.2	0.5	1.6
HAR 19	5.8	4.5	7.3	2.3	1.5	4.8	2.5	0.4	1.8
HAR 20	6.4	3.9	8.1	2.6	1.6	8.5	2.4	0.4	1.5
HAR 21	7.5	4.5	8.0	2.7	1.6	4.9	2.3	0.4	1.6
HAR 22	6.0	4.0	8.5	3.0	1.3	7.3	2.6	0.4	1.4
HAR 23	6.3	4.0	8.7	2.5	1.4	6.5	2.3	0.4	1.6
HAR 24	7.0	4.5	8.8	3.1	1.8	8.5	2.1	0.2	2.0
HAR 25	5.4	3.8	8.8	2.8	1.4	5.5	2.3	0.3	1.4
HAR 26	6.5	4.9	7.8	2.6	1.5	6.1	2.0	0.2	1.8
HAR 27	6.9	5.8	6.7	2.5	1.6	4.1	2.4	0.5	2.0
HAR 28	5.8	4.7	7.4	2.5	1.6	6.0	2.3	0.5	1.6
HAR 29	7.9	4.5	6.3	2.0	1.3	3.2	2.0	0.5	1.7
HAR 30	5.6	4.6	9.5	2.6	1.5	5.5	2.5	0.5	2.0
HAR 31	5.6	4.5	7.7	2.5	1.5	4.2	2.2	0.3	1.8
HAR 32	5.8	4.3	6.1	2.2	1.6	4.9	2.0	0.4	1.7
HAR 33	5.6	3.5	8.9	2.7	1.3	5.2	2.3	0.3	1.6
HAR 34	6.6	3.7	8.9	2.3	1.7	6.3	2.2	0.3	1.8
Tetratheca deltoidea									
DET 01	7.2	2.0	7.4	2.0	1.4	19.0	1.3	0.4	0.5
DET 02	6.5	2.8	6.2	2.5	1.3	19.2	1.2	0.3	0.5
DET 03	6.5	1.5	6.5	2.1	1.1	15.1	1.3	0.4	0.5
DET 04	7.1	2.0	7.6	2.4	1.5	18.7	1.7	0.3	0.4
DET 05	5.8	1.6	6.3	2.5	1.3	15.9	1.7	0.3	0.4
DET 06	4.9	1.3	5.3	2.0	1.0	15.6	1.1	0.3	0.3
DET 07	6.9	1.6	5.9	2.0	1.3	18.6	1.4	0.2	0.5
DET 08	7.3	2.5	8.1	2.1	1.3	18.9	1.5	0.4	0.4
DET 09	6.0	2.3	5.8	1.3	1.6	18.8	-1	-1	-1
DET 10	7.1	1.9	7.5	2.3	1.1	20.2	1.6	0.3	0.4

DET	11	6.2	1.7	5.9	1.9	1.5	16.0	1.2	0.3	0.4
DET	12	6.5	2.0	6.9	2.1	1.3	15.5	1.2	0.4	0.5
DET	13	5.5	1.5	5.0	1.8	1.5	24.2	1.6	0.3	0.4
DET	14	8.0	2.1	7.4	2.0	1.6	18.8	1.7	0.3	0.5
DET	15	6.8	1.5	6.8	2.3	1.4	15.7	1.6	0.4	0.5
DET	16	5.4	1.8	6.5	1.8	1.1	15.7	1.1	0.4	0.4

Tetratheca hirsuta

HIR1 GIRL GUIDES CAMP

GIR	01	9.0	8.8	6.4	3.1	1.8	23.2	1.9	0.4	2.5
GIR	02	8.5	7.4	5.5	3.7	1.4	20.1	1.8	0.3	2.2
GIR	03	6.9	6.8	4.3	2.5	1.4	15.2	1.8	0.3	2.1
GIR	04	7.3	7.4	5.6	3.3	1.4	22.5	1.8	0.3	2.5
GIR	05	7.8	6.2	6.2	3.1	1.3	25.2	1.9	0.5	2.5
GIR	06	7.5	7.4	5.0	3.2	1.7	17.8	2.1	0.3	2.7
GIR	07	7.0	6.6	5.1	2.9	1.2	18.1	2.1	0.4	2.5
GIR	08	7.5	6.7	5.5	3.0	1.6	18.8	2.0	0.4	2.5
GIR	09	7.4	7.6	6.9	4.0	1.4	19.9	1.7	0.3	2.8
GIR	10	9.3	7.8	6.9	3.5	1.4	19.2	2.2	0.4	2.5
GIR	11	10.2	8.3	6.8	3.3	1.8	30.6	2.2	0.4	2.5

HIR3 JARRAHDALE

JAR	01	6.9	6.4	3.2	2.9	1.3	19.2	1.8	0.4	1.4
JAR	02	6.5	6.4	3.9	2.4	1.1	25.6	1.7	0.5	1.6
JAR	03	6.1	5.5	3.0	2.5	1.2	19.2	1.7	0.4	1.6
JAR	04	6.9	8.9	2.5	2.0	1.1	27.2	1.7	0.4	1.7
JAR	05	5.6	5.4	3.8	2.5	1.3	27.2	1.5	0.4	1.7
JAR	06	5.9	8.1	2.6	2.5	1.1	18.5	1.5	0.4	1.5
JAR	07	4.1	4.3	3.4	1.8	1.2	16.6	1.6	0.4	1.5

HIR5 TORRENS RD. DWELLINGUP

TOR	01	6.9	7.2	3.5	2.4	1.3	20.4	2.0	0.4	1.8
TOR	02	7.5	6.1	4.2	3.2	1.8	30.8	1.8	0.5	1.7
TOR	03	6.2	5.4	5.3	3.0	1.2	25.7	1.8	0.5	1.8
TOR	04	6.4	6.5	4.3	2.5	1.2	22.6	2.1	0.5	1.5
TOR	05	7.1	5.5	4.6	2.2	1.2	-1	2.0	0.5	1.7
TOR	06	-1	-1	-1	2.5	1.3	20.5	1.8	0.4	2.1
TOR	07	7.4	4.9	4.8	2.0	1.2	30.2	1.9	0.4	1.7
TOR	08	-1	-1	-1	2.5	1.2	25.7	1.6	0.5	1.7

HIR6 CHITTERING

CHI	01	7.0	7.5	4.8	4.3	1.3	11.0	2.5	0.3	2.5
CHI	02	9.5	7.5	6.9	3.5	1.3	15.1	1.6	0.3	2.5
CHI	03	9.1	6.9	7.5	3.6	1.4	15.4	1.8	0.5	2.0
CHI	04	8.5	6.3	5.8	3.1	1.2	13.5	2.2	0.4	2.0
CHI	05	7.4	7.1	5.3	3.0	1.4	13.0	2.2	0.5	2.6
CHI	06	8.2	6.1	6.1	3.4	1.3	13.8	2.4	0.5	2.1
CHI	07	7.2	7.3	3.8	3.5	1.3	13.6	1.9	0.6	2.4
CHI	08	8.1	5.8	5.5	3.7	1.7	15.5	1.8	0.6	2.0
CHI	09	5.9	6.6	4.6	3.7	1.3	15.9	2.2	0.5	1.5
CHI	10	4.9	6.3	4.8	3.3	1.1	19.9	2.0	0.4	2.2
CHI	11	7.8	5.3	5.3	3.7	1.6	12.5	2.4	0.4	2.0
CHI	12	8.3	7.1	6.5	3.4	1.5	17.1	2.1	0.3	2.2
CHI	13	5.3	7.1	6.8	3.8	1.3	21.5	2.2	0.4	2.0
CHI	14	8.4	6.8	5.6	3.5	1.6	24.6	2.3	0.4	2.4
CHI	15	6.7	5.6	4.7	3.4	1.3	17.8	2.2	0.3	2.3

HIR7 BOONANARRING V.C.L., GINGIN

BOO	01	6.1	7.4	5.6	6.5	2.0	14.8	2.2	0.7	2.3
BOO	02	7.2	6.5	7.0	7.0	1.9	16.8	2.0	0.7	2.5
BOO	03	5.5	6.6	5.4	6.2	1.8	11.6	2.0	0.4	2.5
BOO	04	8.6	8.6	5.8	5.9	2.0	19.6	1.8	0.5	2.5
BOO	05	6.8	5.5	5.8	5.6	1.3	20.1	2.2	1.0	2.7
BOO	06	5.9	6.8	6.4	7.1	1.9	11.5	2.5	0.6	2.5
BOO	07	8.0	7.8	6.4	6.4	1.9	19.7	2.0	0.4	2.0
BOO	08	6.6	6.4	7.2	7.2	1.6	18.0	2.0	0.5	2.0
BOO	09	5.5	6.9	6.3	5.0	1.6	18.3	1.8	0.5	1.9
BOO	10	6.8	5.8	8.6	7.1	1.6	17.3	-1	-1	-1
BOO	11	6.9	5.3	7.9	7.1	1.7	15.7	2.2	0.8	2.3
BOO	12	8.5	7.8	7.8	6.9	1.8	16.3	-1	-1	-1
BOO	13	8.5	10.5	6.5	6.6	2.0	19.9	2.1	0.7	2.4
BOO	14	6.5	7.1	6.6	5.0	1.6	15.8	2.2	0.7	2.0
BOO	15	6.6	5.5	5.5	6.7	1.4	12.8	2.3	0.6	2.3

HIR8 GOSNELLS RIFLE RANGE

GOS	01	7.3	7.1	4.9	4.0	1.4	27.8	-1	-1	-1
GOS	02	6.6	5.6	5.6	2.6	1.5	17.0	2.0	0.4	2.2
GOS	03	6.9	5.0	6.6	4.0	1.5	26.4	-1	-1	-1
GOS	04	6.2	4.3	6.0	3.5	1.5	-1	-1	-1	-1
GOS	05	5.2	3.7	5.0	4.0	1.3	-1	-1	-1	-1
GOS	06	7.2	5.3	6.1	2.8	1.3	-1	2.0	0.4	2.0
GOS	07	7.0	4.9	5.1	3.0	1.8	-1	2.4	0.4	2.0

HIR10 OZONE TERRACE, KALAMUNDA

OZO	01	7.5	6.5	6.6	3.9	1.5	20.8	2.1	0.4	2.3
OZO	02	7.8	8.6	5.7	4.0	1.5	24.1	1.9	0.3	2.3
OZO	03	7.0	6.9	4.5	4.3	1.4	17.1	2.0	0.4	2.2
OZO	04	7.3	7.6	5.0	4.2	1.5	17.4	2.2	0.4	2.5
OZO	05	6.4	5.9	3.8	3.9	1.5	16.8	2.3	0.3	2.3
OZO	06	6.1	5.9	6.4	3.8	1.4	18.5	2.0	0.3	2.1
OZO	07	6.8	6.4	5.7	3.5	1.5	19.2	2.1	0.3	2.3
OZO	08	7.6	5.8	5.8	3.5	1.6	19.9	2.0	0.5	2.2
OZO	09	7.9	7.8	6.3	5.1	2.0	24.1	2.3	0.5	2.5
OZO	10	8.4	6.3	7.3	3.6	1.7	24.0	2.2	0.4	2.2
OZO	11	6.7	7.1	5.9	3.4	1.5	18.6	2.1	0.3	2.2

HIR11 YOUTH S. & R.CAMP, BICKLEY

YSR	01	5.1	5.4	4.9	3.8	1.3	14.1	2.2	0.5	2.2
YSR	02	8.7	6.8	6.1	4.0	1.5	19.2	2.0	0.5	2.0
YSR	03	7.1	6.6	6.4	4.4	1.4	23.0	2.1	0.6	2.2
YSR	04	6.7	8.6	5.5	4.0	1.3	16.3	2.1	0.7	2.0
YSR	05	6.6	7.7	5.6	4.5	1.6	17.7	2.1	0.4	2.2
YSR	06	6.9	7.7	6.5	3.9	1.7	23.3	1.8	0.5	2.5
YSR	07	6.3	6.3	5.1	4.3	1.4	21.5	2.1	0.5	2.2
YSR	08	6.5	7.7	6.2	3.9	1.5	23.3	2.2	0.5	2.2

EFO1 SOUTHERN CROSS

EFO	01	7.8	7.7	5.2	3.8	2.1	-1	2.6	1.0	2.0
EFO	02	6.3	7.6	4.5	3.0	2.0	5.2	-1	-1	-1
EFO	03	6.4	8.9	4.7	3.8	1.9	5.4	2.6	0.8	2.0
EFO	04	5.9	4.8	4.0	4.0	2.3	8.0	-1	-1	-1
EFO	05	5.3	9.1	5.6	3.8	1.8	8.5	-1	-	-1
EFO	06	6.1	9.5	4.4	4.4	2.3	5.9	3.0	1.0	2.2
EFO	07	6.6	8.8	4.0	3.5	2.1	10.3	3.0	1.0	2.2
EFO	08	5.0	9.0	5.2	4.2	2.1	9.8	-1	-1	-1
EFO	09	3.6	6.6	3.8	3.2	1.6	5.8	2.5	1.0	2.0
EFO	10	5.6	7.4	3.8	3.5	1.8	8.5	-1	-1	-1
EFO	11	6.0	8.5	5.9	4.4	2.0	4.5	3.2	1.0	2.0
EFO	12	4.8	6.5	3.5	3.0	2.2	8.7	-1	-1	-1

EFO 13 4.1 5.9 3.0 3.8 1.7 9.7 2.0 0.6 2.3
EFO 14 3.2 6.0 3.2 3.6 1.5 6.8 2.5 1.0 2.0
EFO 15 6.8 9.7 5.6 3.5 2.0 -1 2.8 1.3 2.2

TABLE 2. Measurements of leaf dimensions of *Tetratheca hirsuta*, used in analysis in Chapter 4.

P=petiole length
L=Total leaf length
BW=bottom 1/4 width
MW=middle width
TW=top 1/4 width

P L BW MW TW

HIR1 GIRL GUIDES, BICKLEY

GIR 01 1 0.8 8.8 3.2 2.5 2.1
GIR 01 2 0.8 8.9 3.0 3.0 2.5
GIR 01 3 0.8 9.3 2.9 2.8 2.2
GIR 02 1 1.0 11.5 1.9 2.1 1.7
GIR 02 2 0.8 13.4 2.9 2.8 2.2
GIR 02 3 0.8 15.3 3.3 3.0 2.6
FIR 03 1 1.0 8.0 2.9 2.8 2.0
GIR 03 2 1.0 7.8 2.8 3.1 2.5
GIR 03 3 0.7 4.4 1.6 1.5 1.2
GIR 04 1 0.8 10.7 4.0 4.3 3.3
GIR 04 2 0.8 10.7 4.2 4.2 3.2
GIR 04 3 1.0 10.5 3.5 3.5 2.8
GIR 05 1 1.0 15.8 4.9 4.6 3.3
GIR 05 2 1.0 12.9 3.0 2.8 2.3
GIR 05 3 0.5 11.3 2.3 2.5 2.1
GIR 06 1 0.8 8.5 3.8 3.8 2.3
GIR 06 2 1.5 10.8 3.0 3.1 2.4
GIR 06 3 1.0 9.1 3.8 3.5 2.6
GIR 07 1 1.0 14.7 4.9 5.3 3.5
GIR 07 2 1.0 14.0 4.2 4.3 3.1
GIR 07 3 1.0 13.0 4.4 4.2 3.0
GIR 08 1 0.8 9.4 3.8 3.5 2.9
GIR 08 2 1.0 9.8 3.7 3.7 2.4
GIR 08 3 1.0 9.9 3.8 3.5 2.7
GIR 09 1 1.0 10.8 2.4 2.3 1.8
GIR 09 2 1.2 11.1 2.8 2.8 2.6
GIR 09 3 1.2 9.7 1.8 1.8 1.2
GIR 10 1 0.8 9.5 2.8 3.2 2.6
GIR 10 2 1.5 12.6 5.3 5.3 4.3
GIR 10 3 1.5 13.6 5.1 5.3 4.1
GIR 11 1 1.0 11.7 2.8 2.8 1.5
GIR 11 2 1.0 12.8 3.5 3.0 2.4
GIR 11 3 1.0 13.6 3.5 3.3 2.5

HIR3 JARRAHDALE

JAR 01 1 1.8 8.2 4.3 4.7 3.2
JAR 01 2 1.8 8.1 5.0 5.1 3.5
JAR 01 3 1.4 7.2 3.7 3.5 2.1
JAR 02 1 1.5 11.1 4.0 4.1 3.1
JAR 02 2 1.0 10.2 3.6 3.9 3.0
JAR 02 3 1.5 10.0 4.0 4.2 2.8
JAR 03 1 1.3 10.6 4.4 4.3 3.3
JAR 03 2 1.3 10.9 4.5 4.8 3.5
JAR 03 3 1.0 10.8 4.5 4.8 3.4
JAR 04 1 2.3 11.5 7.3 7.0 4.5
JAR 04 2 2.5 12.4 7.8 7.1 3.9
JAR 04 3 2.3 10.4 5.9 5.9 4.2
JAR 05 1 1.0 5.9 4.1 3.1 1.9
JAR 05 2 1.0 5.6 3.3 2.9 1.9
JAR 05 3 1.5 6.0 4.3 3.4 2.2
JAR 06 1 1.0 9.1 4.5 5.0 3.8
JAR 06 2 1.0 7.8 4.1 4.7 3.8
JAR 06 3 1.0 7.8 3.6 4.8 4.1
JAR 07 1 1.0 8.2 4.7 5.1 3.8
JAR 07 2 1.2 8.0 4.9 6.0 4.5
JAR 07 3 1.0 7.5 6.3 7.5 5.9
JAR 08 1 0.7 6.5 3.0 3.0 2.2
JAR 08 2 1.8 9.6 4.3 4.1 3.2
JAR 08 3 1.5 9.6 5.3 5.8 4.0

HIR5 TORRENS RD., DWELLINGUP

TOR 01 1 3.0 12.2 4.8 7.0 5.5
TOR 01 2 1.4 10.7 3.5 3.4 2.7
TOR 01 3 1.5 13.1 4.5 6.3 4.7
TOR 02 1 1.0 7.1 4.9 4.8 3.2
TOR 02 2 1.5 8.6 7.4 8.0 1.1
TOR 02 3 0.5 6.8 4.8 5.3 4.1
TOR 03 1 2.0 7.4 6.0 7.1 5.4
TOR 03 2 1.0 6.6 5.0 5.7 4.5
TOR 04 1 1.5 7.1 4.8 5.0 3.8
TOR 04 2 0.8 5.0 2.6 2.7 1.8
TOR 04 3 -1 5.3 3.9 3.8 2.8
TOR 05 1 0.8 5.9 4.1 4.1 2.6
TOR 05 2 1.5 7.6 4.4 4.8 3.8
TOR 05 3 1.5 7.3 4.8 5.5 4.4
TOR 06 1 1.0 6.1 3.1 3.5 2.5
TOR 06 2 -1 9.5 4.9 4.9 3.8
TOR 06 3 1.5 9.2 5.0 5.0 3.8
TOR 07 1 1.0 8.5 4.4 4.5 3.5
TOR 07 2 1.3 6.8 3.0 3.9 3.0
TOR 07 3 2.0 8.1 5.0 5.3 4.0
TOR 08 1 1.5 8.1 5.2 5.9 4.4
TOR 08 2 2.0 12.6 7.7 9.3 7.9
TOR 08 3 1.5 8.7 5.8 6.9 5.3
TOR 09 1 1.5 8.7 5.3 6.0 5.0

HIR6 CHITTERING

CHI 01 1 0.6 8.1 3.0 3.2 2.7
CHI 01 2 0.7 5.9 2.0 2.1 1.7
CHI 01 3 0.5 5.8 3.0 8.3 2.8
CHI 02 1 1.0 15.8 2.3 2.3 2.1
CHI 02 2 1.0 15.6 2.1 2.2 2.0
CHI 02 3 0.5 8.5 1.5 1.6 1.4
CHI 03 1 0.5 6.5 1.9 2.0 1.7
CHI 03 2 0.5 6.6 2.3 2.4 1.9
CHI 03 3 0.5 4.8 1.9 2.0 1.6
CHI 04 1 0.6 7.0 1.7 1.7 1.3
CHI 04 2 0.6 6.5 1.3 1.3 1.0
CHI 04 3 0.5 6.2 1.2 1.0 1.0
CHI 05 1 0.8 8.1 2.3 2.6 1.8
CHI 05 2 0.7 9.3 2.3 2.3 1.8
CHI 05 3 0.5 6.0 1.7 1.6 1.3

CHI 06 1 0.6 5.0 1.3 1.4 1.4
CHI 06 2 0.5 5.5 1.3 1.6 1.3
CHI 06 3 0.5 5.4 1.5 1.8 1.5
CHI 07 1 1.0 12.3 3.2 3.1 2.8
CHI 07 2 1.0 11.8 3.0 3.2 2.9
CHI 07 3 0.5 7.3 2.4 2.5 2.0
CHI 08 1 0.5 7.0 1.8 1.9 1.6
CHI 08 2 0.6 12.8 2.0 2.1 1.8
CHI 08 3 0.5 7.1 1.8 1.8 1.4
CHI 09 1 0.5 9.1 3.4 3.5 3.0
CHI 09 2 0.5 12.6 3.4 3.5 2.8
CHI 09 3 0.5 12.3 3.5 3.4 3.0
CHI 10 1 0.6 12.8 2.8 2.7 2.4
CHI 10 2 0.5 13.8 3.5 3.3 2.7
CHI 10 3 1.0 12.2 3.1 2.5 2.0
CHI 11 1 0.8 9.1 2.1 2.0 1.7
CHI 11 2 0.6 7.9 2.0 1.9 1.5
CHI 11 3 0.6 8.8 2.0 1.8 1.4

HIR7 BOONANARRING V.C.L., GINGIN

BOO 01 1 0.4 11.8 2.2 1.8 1.4
BOO 01 2 0.4 9.6 2.2 1.9 1.4
BOO 01 3 0.4 15.6 2.0 2.0 1.8
BOO 02 1 1.0 19.2 3.2 3.1 2.1
BOO 02 2 1.3 15.9 2.8 2.2 1.9
BOO 02 3 -1 16.3 2.9 2.4 1.8
BOO 03 1 0.4 8.7 2.0 2.0 1.8
BOO 03 2 0.4 9.4 1.8 1.8 1.8
BOO 03 3 0.4 8.8 2.0 2.0 1.9
BOO 04 1 0.3 7.5 2.5 2.5 2.1
BOO 04 2 0.5 9.0 2.7 2.3 1.8
BOO 04 3 0.4 8.8 2.5 2.5 1.8
BOO 05 1 0.6 12.4 3.1 3.0 2.3
BOO 05 2 0.5 6.8 1.9 1.9 1.4
BOO 05 3 0.8 11.4 2.7 2.5 2.3
BOO 06 1 0.6 8.8 1.5 1.5 1.4
BOO 06 2 0.6 9.0 1.8 1.7 1.5
BOO 06 3 0.5 5.1 1.3 1.2 0.9
BOO 07 1 0.7 11.9 1.5 1.4 1.3
BOO 07 2 0.5 7.8 1.1 1.1 0.9
BOO 07 3 0.5 8.1 1.4 1.2 0.8
BOO 08 1 0.2 8.4 2.2 1.9 1.7
BOO 08 2 0.6 8.9 1.6 1.5 1.3
BOO 08 3 0.7 8.5 2.0 2.0 1.6
BOO 09 1 0.5 5.4 1.5 1.5 1.0
BOO 09 2 0.8 19.9 2.3 2.6 2.0
BOO 09 3 0.8 19.9 2.5 2.4 1.9
BOO 10 1 0.5 8.8 1.3 1.2 1.1
BOO 10 2 0.6 6.7 1.9 1.7 1.6
BOO 10 3 0.6 7.1 1.8 1.8 1.4
BOO 11 1 -1 11.5 1.5 1.5 1.3
BOO 11 2 0.5 11.5 1.3 1.1 0.9
BOO 11 3 0.5 11.2 1.3 1.2 1.0
BOO 12 1 0.6 9.2 2.4 2.3 2.2
BOO 12 2 0.8 13.6 2.0 1.8 1.5
BOO 12 3 0.5 10.1 1.9 2.0 1.8
BOO 13 1 0.7 10.9 2.3 2.4 2.2
BOO 13 2 0.7 8.8 1.6 1.6 1.4
BOO 13 3 0.5 9.7 1.8 1.8 1.5
BOO 14 1 0.5 10.6 1.4 1.4 1.2
BOO 14 2 0.5 12.5 1.6 1.5 1.3
BOO 14 3 0.8 12.4 1.8 1.6 1.4
BOO 15 1 1.0 16.8 2.5 2.8 2.3
BOO 15 2 0.7 13.0 2.0 1.6 1.1
BOO 15 3 0.5 12.3 2.1 1.7 1.3
BOO 16 1 0.5 9.3 2.3 1.6 1.3
BOO 16 2 0.5 6.9 1.7 1.4 1.0
BOO 16 3 0.5 6.9 2.0 1.8 1.4
BOO 17 1 0.5 13.4 1.5 1.5 1.3
BOO 17 2 0.7 13.6 1.2 1.1 1.0
BOO 17 3 0.5 13.6 1.3 1.1 1.0
BOO 18 1 0.5 11.9 2.1 1.9 1.7
BOO 18 2 0.5 10.5 2.8 2.5 2.3
BOO 18 3 0.7 10.6 2.8 2.2 1.8
BOO 19 1 1.0 14.8 2.4 2.4 1.8
BOO 19 2 1.0 13.6 2.3 2.0 1.8
BOO 19 3 1.0 13.1 2.6 2.7 2.0
BOO 20 1 1.0 10.3 1.9 1.8 1.7
BOO 20 2 1.0 9.9 1.8 1.5 1.4
BOO 20 3 1.0 6.0 2.7 2.9 2.1
BOO 21 1 0.5 5.8 1.4 1.4 1.1
BOO 21 2 0.5 8.7 1.7 1.8 1.8
BOO 21 3 0.5 7.3 1.3 1.3 1.3

HIR11 YOUTH SPORT & RECREATION

YSR 01 1 1.7 25.3 5.8 5.8 4.9
YSR 01 2 1.0 18.4 3.5 3.5 3.0
YSR 01 3 1.0 13.5 2.5 2.5 2.0
YSR 02 1 0.5 7.3 1.3 1.3 1.4
YSR 02 2 0.5 8.5 1.4 1.3 1.2
YSR 02 3 0.5 6.0 1.2 1.1 1.1
YSR 03 1 1.0 13.7 1.3 1.3 0.9
YSR 03 2 1.0 13.4 1.8 1.8 1.1
YSR 03 3 1.0 10.3 1.8 1.9 1.4
YSR 04 1 1.0 13.4 3.8 3.3 2.9
YSR 04 2 1.0 13.3 3.3 3.4 2.9
YSR 04 3 1.0 12.4 2.8 2.8 2.4
YSR 05 1 0.5 13.4 2.1 2.2 1.8
YSR 05 2 1.0 14.2 2.0 2.2 1.9
YSR 05 3 0.8 13.2 1.5 1.8 1.3
YSR 06 1 0.5 8.3 1.8 1.8 1.8
YSR 06 2 0.6 8.9 1.6 1.8 1.4
YSR 06 3 0.6 9.1 2.3 2.0 1.6
YSR 07 1 1.0 13.5 1.6 1.6 1.4
YSR 07 2 1.0 16.6 2.0 2.1 1.5
YSR 07 3 1.0 15.9 1.6 1.8 1.3
YSR 08 1 0.8 9.1 2.1 1.8 1.4
YSR 08 2 1.0 10.0 2.2 2.3 1.5
YSR 08 3 1.0 10.7 2.0 2.0 1.6
YSR 09 1 0.5 17.5 3.3 3.4 2.7
YSR 09 2 0.8 11.9 2.3 2.9 2.4
YSR 09 3 0.8 12.0 2.7 2.8 2.3
YSR 10 1 1.0 9.8 3.8 4.0 2.8
YSR 10 2 1.0 11.1 2.9 2.9 2.0
YSR 10 3 0.8 15.4 3.1 2.8 2.2