CONSERVATION GENETICS AND POPULATION ECOLOGY OF FIVE RARE AND THREATENED WESTERN AUSTRALIAN ORCHIDS

ANCA ESP Project No 19

By

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FINAL REPORT TO THE ENDANGERED SPECIES UNIT, AUSTRALIAN NATURE CONSERVATION AGENCY

December 1994

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PO Box104, Como, WA 6152

This study (ANCA ESP Project No. 19) was funded by the Endangered Species Program of the Australian Nature Conservation Agency.

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1. *CALADENIA ELEGANS* (THE ELEGANT SPIDER ORCHID)

The elegant spider orchid, *Caladenia elegans* Hopper & A.P.Brown, was described from material collected near Northampton in the period 1986-1990 (see Hoffman and Brown, 1992). *C. elegans* is morphologically uniform throughout its range, except for plants from near Kalbarri (Population 1), where flowers occur only in cream and white and have slightly shorter (5 mm) petals and sepals.

C. elegans is related to *Caladenia varians*, and closely resembles *C. varians* subsp. *pendans* which is more robust with larger cream or white flowers. Both species are included in the *filamentosa* species complex. The two species are not associated in the field.

1.1 DISTRIBUTION AND CONSERVATION STATUS

1.1.1 Distribution

Although extensively surveyed in the wild, *C. elegans* is known from only a few remnant populations on road verge, and from one population on a shire reserve. All of the populations occur in the CALM Midwest Region. The northern most population is located near Kalbarri and another four populations were distributed over several kms north-west of Northampton (Figure 1.1). It has a total range of 75 km.

Most of the land in this area has been cleared for agriculture, leaving little evidence with which to reconstruct a distribution and density map of *C. elegans* populations. However the large number of remnant populations found near Northampton suggest that although its distribution is localized, *C. elegans* once comprised many populations.

1.1.2 Populations

The *C. elegans* population on a shire reserve near Kalbarri was relatively undisturbed. The remaining populations, however, occurred on degraded road verge. The locations, conservation status and condition of the sites are summarised below. Precise locality details are contained in CALM Departmental Declared Rare Flora files.

<u>Population 1</u> - near Kalbarri. 200 adult plants were scattered along the banks of an ephemeral creek, and distributed over a 2.5 acre area (approx. 70 m x 150 m) on a relatively undisturbed shire reserve.

Conservation status: Although not currently under threat, this small population is extremely localised and is at risk because of low numbers and long term habitat degradation. Special attention is required for its long-term survival.

<u>Population 2</u> - north-west of Northampton. 2000 adult plants were distributed over a 3 acre area (approx. 50 m x 250 m) along a weed infested road verge, and bordered by paddocks.

Conservation status: This population is at risk of extinction within the next two decades if habitat degradation, due largely to invasive weeds, continues. Close monitoring of population numbers is required and weed control, if feasible, and germ plasm collections may need to be considered.

Population 3 - north-west of Northampton. 200 adult plants were distributed over a 1/2 acre area (approx. 25 m x 80 m) along a weed infested road verge adjacent to cleared paddocks.

Conservation status: This population is at risk of extinction within the next two decades if habitat degradation, due largely to invasive weeds, continues. Close monitoring of population numbers is required and weed control, if feasible, and germ plasm collections may need to be considered.

Population 4 - west-north-west of Northampton. 2000 adult plants were distributed over a 3 acre area (approx. 50 m x 250 m) along road verge adjacent to cleared paddocks. A weed infested gravel pit is situated alongside the population.

Conservation status: This population is at risk of extinction within the next two decades if present land use and habitat degradation continues. Close monitoring of population numbers is required and weed control, if feasible, and germ plasm collections may need to be considered.

Population 5 - west-north-west of Northampton. 10000 adult plants were distributed over a 12.5 acre area along a 1 km strip of degraded road verge adjacent to cleared paddocks.

Conservation status: Although large this population is still under threat from habitat degradation primarily due to weed invasion. Close monitoring of population numbers is required, and weed control and germ plasm collections may need to be considered if a continuous decline in numbers is evident.

1.2 HABITAT AND ASSOCIATED SPECIES

C. elegans is found on red clay-loam soils over laterite, along the banks of ephemeral creeks (Population 1), and in the winter wet depressions (Populations 2-5) that occur around and between hillocks 5-15 km from the coast. It grows among large scattered *Acacia* sp. and *Melaleuca uncinata* shrubs, in low dense heath of *Thryptomene saxicola*, *Dampiera* sp. and *Conostylis neocymosa*. Herbs were plentiful in winter. Other orchids found with *C. elegans* included *C. flava*, *C. footiana*, *C.* aff. *huegelii*, *C.* aff. *longicauda*, *Diuris* aff. *laxiflora*, *D.* aff.*longifolia*, *D. emarginata* var. *pauciflora*, *Prasophyllum macrostachyum* var. *ringens*, *Pterostylis cycnocephala*, *Thelymitra antennifora* and *T. nuda*.

The climate of the area is dry-mediterranean. The summers (December-March) are hot and dry, and winters are cool. Most rainfall occurs in late autumn, winter and early spring, and the mean annual rainfall at Northampton is 496 mm (Bureau of Meteorology).

1.3 LIFE HISTORY

Throughout its range *C. elegans* experiences environmental conditions that are cyclical and therefore very predictable. Short, cool and wet winters alternate with long, hot and dry summers. Under such conditions *C. elegans* has developed physiological and morphological traits, such as underground perennating tubers, which are regarded as being of selective advantage in promoting good establishment and carryover of reserves within and between seasons (Pate and Dixon 1982).

Flowering occurs annually and during the growing season. This has advantages because pollinators are in highest frequency, and current photosynthesis is harnessed directly to the production of seeds.

1.3.1 Phenology of growth and reproduction

In late autumn (April-May) underground tubers of *C. elegans* commence growing. In expanding the new season's shoot, roots and leaf, most of the water and carbohydrate reserves of the tuber are consumed. Thereafter, completion of the plant's growth and reproductive cycle depends on the photosynthetic gains made by the new foliage. From May to July the products of photosynthesis are directed toward replacing the parent tuber. In a good season, daughter tubers may also be produced. In mid to late August the inflourescences quickly elongate and the flower bud expands. During mid and late August the leaf shrivels, and capsule and seed maturation relies on the nutrients stored in the scape. In late September the capsule dries and dehisces, releasing huge quantities of seed. The underground perennating tubers are all that remain over the summer months.

Seed germination relies on the presence of a species specific mycorrhizal fungus. This symbiotic relationship is essential for any seedling establishment. Although very large quantities of seed are released from each capsule only a fraction become mature plants.

1.3.2 Recruitment after fire

C. elegans does not require fire to complete its life cycle and should be protected from uncontrolled fires. However, there is some evidence to suggest that it may benefit from fire every ten years or so . In the summer of 1981 the site of Population 5 was burnt. When the site was visited in August 1983 vegetation was recovering well from the effects of the fire and the size of the *C. elegans* population at that time was 200+ plants (A. Brown *pers comm*). By 1990 there was no evidence of fire, and the number of plants in the population had increased to 10 000. The number of adult plants in a quadrat stationed permanently at Population 5 did not fluctuate between 1990 and 1991, suggesting that the size of the population had stabilized. *C. elegans* possibly benefits from fire, as it opens up the heath thereby reducing competition for space and light, and provides a source of nutrients for the growth of mycorrhiza on which *C. elegans* seedlings depend for their establishment and growth.

1.3.3 Pollination biology

In 1990 and 1991 the flowering period of *C. elegans* was restricted to August and peaked in the second week. A solitary flower characterized most inflorescences, although some had as many as five flowers displayed simultaneously. While a small percentage of plants produced up to five inflorescences in a clump, singles and pairs are the norm.

Adult plants are probably long-lived (tens of years), and flower regularly every year. In a 10 meter square quadrat stationed permanently at Population 5, those plants that flowered in 1990 were also observed to flower in 1991. In 1991 there was approximately 15000 flowers in Population 5. Flowering is synchronized within and among populations to produce an expansive bloom, of short duration, with many individuals producing only a few flowers each.

When kept in cool and humid conditions *C. elegans* flowers are long lived, 15-20 days, whereas in the field they display for only 1-5 days. The flowers are strongly scented with the source of the fragrance being the single- and multi-celled trichomes located on the lower two thirds of the petals and sepals. The trichomes form a dense cover thereby increasing the surface area through which the floral fragrance diffuses.

Williams (1982) suggests that a number of insects are able to detect floral fragrances over a distance of at least several meters, and male euglossine bees will fly up to 1 km over water to an odour source (Ackerman 1981). *C. elegans* flowers attract only male thynnid (common name) wasps, and the primary distance attractant is the fragrance produced by the petals and sepals. Once the wasp had been attracted to the flower, visual and tactile cues stimulate further pollination responses.

C. elegans has a zygomorphic, or "lipped" (labellum), flower. Maroon lines on the labellum converge toward two rows of calli at the entrance of the flower. This "guide pattern" acts as a near goal orientation cue for the male thynnid wasp.

The pollen of *C. elegans* is not available as food for wasp visitors. In 1990 and 1991 less than 1% of flowers at Population 5 produced small quantities of nectar which collected between the 2 rows of calli on the dorsal surface of the labellum. Samples of this nectar were analysed and found to contain sucrose (27.17 mg/ml), glucose (3.47 mg/ml), and traces of fructose but no amino acids.

Most thynnid wasps went unrewarded when they were deceived into visiting *C. elegans* flowers. This does not seem to stop the wasps however, which were observed visiting flowers at Population 5 shortly after 10.00 am on August 9 1990. At that time the ambient temperature was 21 c, and only while the weather remained warm and sunny did the thynnid wasps continue pollinating flowers. The latest pollination event observed was on August 21 1991 at 3.30 pm.

Just as the flowering season of *C. elegans* was of short duration, so was the active pollination period of the thynnid wasps. At peak flowering time (August 9) in 1990, 52% of *C. elegans* flowers at Population 5 had been pollinated. By late flowering (August 21) in 1991, 60% of the flowers had been pollinated, a difference of only 8%. This equates to approximately 87% of pollination events occurring in the first 10 days of flowering, and 13% in the second 10 days. An estimated 45 million *C. elegans* seeds were produced at Population 5 in 1991.

Clearly thynnid wasps play an integral part in, and are a necessary requirement for the completion of, the reproductive cycle of *C. elegans*.

1.3.4 Phenology of thynnid wasp pollinators and associated species.

Mating season (August) is the only time, in the their annual life cycle, when male thynnids wasp pollinators of *C. elegans* are active above ground. At Population 5 on August 9 1990 and again in 1991 on August 20-21, single males, and males and females in copula (females are flightless) were observed milking nectar from *Thryptomene saxicola* flowers. This was the only nectar producing species on which thynnids were observed. Nectar is an energy source for patrolling male thynnids (those seeking females), and may be a necessary nutrient requirement for fertilized females in egg production and embryo development.

Thryptomene saxicola is a widely distributed, low (1/2 meter high), spreading, woody, perennial shrub in the Myrtaceae family. It forms dense (100% cover) thickets over several square meters, is insect pollinated, and flowers profusely during August in the Northampton area.

These observations and data indicate a complex interaction between *C. elegans*, thynnid wasp pollinators and *Thryptomene saxicola. C. elegans* depends upon the thynnid wasp as its pollinator but it also appears to rely indirectly on the presence of *Thrypyomene saxicola* as a major, perhaps the only, nectar source for the thynnid wasps.

1.4 GENETIC DIVERSITY AND POPULATION STRUCTURE

A range of factors are known to affect patterns and levels of genetic variation in plant populations. One of the most important considerations is population size because gene (allele) frequencies are more likely to fluctuate in small populations and loss of genetic variation through genetic drift and inbreeding is more likely. In several studies and reviews of the relationships between ecological and life history traits and the genetic structures of plants, the importance of the breeding system has also been recognized for its effect on the structure of populations (Jain 1975; Brown 1978, 1979; Hamrick *et al* 1979; Gottlieb 1981; Hamrick 1983; Loveless and Hamrick 1984; Coates 1988; Hamrick and Godt 1990). In general, ecological and life history traits which maintain high population numbers and promote outbreeding and gene flow are associated with higher levels of intrapopulational genetic diversity, most of which is partitioned within populations, and lower differentiation between them. This is the situation expected for most Western Australian terrestrial orchid species.

C. elegans currently exists in populations of varying sizes some of which have no doubt been substantially reduced in size in the last few years by land clearing and habitat degradation. Population size may therefore be a critical factor in maintaining genetic diversity. However, in order to fully appreciate how *C. elegans* is able to maintain its genetic integrity, full consideration will also need be given to certain ecological and life history traits outlined in previous sections.

1.4.1 Genetic variation and structure within populations

Measures of genetic diversity within populations (Table 1.1) such as *P, A He, Ho* indicate that *C. elegans* is similar to other orchid species (Rossi *et al,* 1992) and typical of a diploid sexually reproducing plant species which is animal pollinated and has a mixed mating system (Hamrick and Godt, 1990).

Of the four *C. elegans* populations examined, Population 1 had the least number of individuals (the smallest, Ne = 200), and the lowest mean gene diversity index (expected heterozygosity, *He* = 0.13) (Table 1.1). This apparent relationship between population size and genetic diversity may not be real however, as the sample size of Population 1 used in this study was small (N = 12). Otherwise the gene diversity index for the species as a whole is relatively high. In this respect *C. elegans* has similar levels of intra-population genetic variation to other spider orchids and European orchids (Table 1.2).

Several studies have documented lower than expected heterozygosity levels in largely outcrossing species (see Brown, 1979 and Coates, 1991). A similar pattern was also observed in *C. elegans*. In three populations (1, 4 and 5) the mean observed heterozygosity (*H*o) was lower than expected (*H*e), and they had slightly positive values for Wright's fixation index (*F*) (Table 1.1). For all of the populations collectively the genotype frequencies of 19% of the polymorphic loci deviated significantly from Hard-Weinberg expectations due to an excess of homozygotes. There are two likely explanations for the excess of homozygotes. Firstly, late in the 1991 flowering season some *C. elegans* flowers in Population 5 were observed to autofertilize. Secondly population structuring associated with limited clonality is highly likely. Population 5 occupied a long (1 km) narrow strip along a road verge, and it is very likely that in this population rarer alleles are non-randomly distributed. Therefore the excess of homozygotes observed in Population 5, and in the others as well, can be attributed to a sampling (Wahland) effect caused by structuring in the population, and to the mating system which involves some self pollination.

1.4.2 Differentiation between populations and gene flow

The partitioning of genetic variation within and between populations based on Nei's diversity measures (Table 1.2) indicates that only 2% (GST = 0.02) of the total genetic variation is distributed between populations and the total genetic diversity (*H*T = 0.24) is somewhat lower than that found in other plant species with comparable breeding systems and life-histories.

Cluster analysis using genetic distance supports the conclusion that there is little divergence between the populations (Fig. 1.2). Populations 2 & 4 were less distant from Population 1 than from Population 5, indicating that geographic distance and genetic distance are not related in this species.

The estimate of gene flow between the four *C. elegan* populations (*N*m = 11.1) suggests a very high rate of gene exchange. However, given the extent to which the land between the populations has been cleared for agriculture, it seems more likely that this estimate is a reflection of historical rather than current patterns of gene exchange. (see Larson *et al* 1984, Samson *et al.* 1989).

Patterns of gene exchange between populations (Table 1.2) appear to conflict with data which suggests that gene flow may be limited within populations. Whereas genetic diversity surveys and estimates of gene flow indicate that the exchange between *C. elegans* populations was high prior to clearing, and an important force retarding the differentiation of populations, structuring may have occurred in some populations limiting gene flow within them. In a review, Loveless and Hamrick (1984) found that this pattern was not unusual in plants. It seems that high gene flow between populations does not eliminate the possibility of within-population gene flow being restricted so that local neighbourhoods form and become genetically different as a result of genetic drift.

1.5 RECOMMENDATIONS FOR CONSERVATION OF GENETIC RESOURCES AND MANAGEMENT

1.5.1 Strategies for conserving genetic resources

From the investigations of *C. elegans* described in the previous sections it was shown that Population 1 was geographically isolated from the other populations, and was morphologically distinguishable from them by its shorter petals and sepals. This is the only evidence of variation between the populations since the allozyme studies indicate that 98% of the genetic diversity occurs within populations. Arguably then, in order to preserve a minimal representative proportion of all of the genetic variation observed in *C. elegans*, Population 1 from near Kalbarri and one other from near Northampton would need to be preserved. As mentioned above, historically *C. elegans* was probably characterised by high levels of gene exchange between populations throughout its range. Today the likelihood of exchange between Population 1 and the others is considerably reduced, and consequently it would be now expected to evolve as an isolated and independent lineage. This emphasises the importance of conserving this population. In addition it is proposed that at least one further population near Northampton be given priority for conservation as insurance against localised extinction of one of the populations due to an uncontrolled catastrophic event.

The previous paragraph was concerned with determining the minimum number of populations required to conserve the genetic resources of *C. elegans*, yet it is also important to determine the minimum viable size (MVP) of a population for that purpose. Ordinarily this is a complex procedure, but in this report MVP's are estimated from a genetic view point to represent the size of populations below which the loss of genetic variation would become a problem. Franklin (1980) proposed a population size of 500 as the minimum requirement for maintaining genetic variation at a level that would allow adaptive evolution, and Frankel and Soule (1981) suggested an effective population size (*Ne*) of 50 as 'the irreducible minimum size consistent with short-term preservation of fitness for a randomly breeding population, based upon lose of heterozygosity'. Research in Western Australia on hermaphrodite plant species (James 1970, James 1982, Burbidge and James 1991, Coates, 1992) have emphasized the importance of genetic mechanisms which elevate the fitness of heterozygous individuals in small populations thereby maintaining high genetic diversity. Peakall (1987) and Carstairs and Coates (upublished data, see Chapters 2 and 4) attributed high levels of heterozygosity in two other Western Australian ground orchids to post-zygotic selection mechanisms which elevate the frequency of heterozygotes in the adult population. There was no evidence to suggest that such systems were operating in *C. elegans* populations. Here genetic diversity is maintained by a classical, unencumbered genetic system characterized by large population sizes, high exchange between populations, long generation time, wasp pollination which ensures the near random mixing of gametes, high recombination and high compatibility between gametes irrespective of their relatedness.

If the size of populations of this rare flora are severely reduced, to say <10 individuals, they may well suffer a reduction of allelic frequency and average heterozygosity per locus (Nei *et al.* 1975; Chakraborty & Nei 1977). Populations 1 & 3 had the smallest number (200) of adult individuals. Population 1 also had slightly less genetic diversity (Table 1) than populations with 1000's (2 & 4) or 10000 (5) individuals. However, the difference was not significant and could also be attributed to the small number of individuals sampled from Population 1. There is little evidence to suggest that Population 1 has suffered any loss of genetic diversity as a consequence of its smallness, nor that it is below the minimum viable size for this species. It is therefore assumed that all of the extant populations of *C. elegans* have numbers in excess of the MVP.

Research has indicated that some *C. elegans* populations may be subdivided into genetically different neighbourhoods with limited gene flow between them. When populations are subdivided in this way, it is important to preserve as much of the entire population as is practicable because the loss of a portion of it may be as critical in a genetic sense as the loss of the entire population (Hamrick 1983, Samson *et al* 1990). Therefore, entire populations of *C. elegans* should be conserved, and from near Northampton the larger ones should take priority (i. e., Populations 2, 4 and 5). Distribution maps of the alleles in the populations, particularly those of the highly variable allozyme loci, namely PGI-2, PGM-1 and SDH-1, would be a helpful management tool.

Each of the populations were found to occupy very small areas of land (usually less than 12.5 acres), and will therefore require some management to prevent their degradation. Whereas the recovery of the original ecosystem may not be possible for all of the populations, the rehabilitation or restoration of certain desirable attributes may be possible for most.

1.5.2 Management actions

1.5.2.1 Liaison with landowners and shires

The Department of Conservation and Land Management (CALM) staff are required to provide landholders and other agencies with advice regarding the conservation and management of populations of Declared Rare Flora on land under their control. At present the survival of all *C. elegans* populations relies on close cooperation with local shires, particularly in relation to their road maintenance operations. Owners of farming land adjacent to road verge populations should be requested to arrange their operations so that the species is not destroyed or damaged in any way. In order of priority, the populations for CALM liaison with shires and landowners are:

(a) Populations 1 and 4
(b) Populations 5 and 2
(c) Population 3

1.5.2.2 Land acquisition

There are no *C. elegans* populations on land which has been reserved for conservation of flora and fauna, and only one population occurs on uncleared land. Therefore, it is strongly recommended that CALM acquire land on which *C. elegans* occurs. Ideally all the populations should be conserved, but if this is not possible then priority should be determined by:

(a) the size and quality of the site and its habitat,
(b) conserving the range of genetic and morphological diversity, and
(c) reducing the risk of sudden extinction.

Research has shown that the preservation of Population 1 and two others from near Northampton would conserve the range of genetic and morphological diversity of this species, and reduce the risk of its sudden extinction. Although 200 plants spread over about 1 ha of natural habitat constitutes what appears to be a viable population, the preservation of viable populations of thynnid wasps and *Thryptomene saxicola* must also be considered. Therefore, to ensure and enhance the survival of *C. elegans* it is essential to provide and maintain areas containing a suitable variety of species and habitats. For this purpose a minimum reserve area of 5 ha is recommended. Population 1 occurs on an uncleared site greater than this size, and Population 4 occurs on a site which approaches this size. These two sites then, have highest priority for conservation. Clearly the other populations will still be useful in ensuring and enhancing the survival of *C. elegans* in the short term, but their value for long term survival is likely to be less.

In order of priority, the following sites should be acquired:

(a) Populations 1 and 4
(b) Populations 5 and 2
(c) Populations 3

1.5.2.3 Protection from grazing

With the onset of summer the country-side dries and the winter wet depressions where *C. elegans* grows become the preferred grazing sites of feral pigs and rabbits. In particular, feral pigs were responsible for considerable damage to the habitat at Population 5 until the Agriculture protection Board (APB) conducted a successful eradication program in 1989. The site remained free of pigs until 1991 when in August there was again evidence of habitat degradation by pigs. It is therefore recommended that either the APB institutes a bi-annual eradication program for all of the sites, or the sites are fenced to exclude all herbivorous mammals.

In order of priority, all herbivorous mammals should be excluded from the following sites:

(a) Populations 1 and 4
(b) Populations 5 and 2
(c) Populations 3

1.5.2.4 Protection from accidental destruction

C. elegans is vulnerable to damage or destruction owing to the small areas occupied by its populations, and should be protected from accidental destruction by bulldozing, rubbish dumping, spraying of potentially damaging herbicides and insecticides and erosion by run-off from farm paddocks.

Mining in areas adjacent to or within sites should be prevented or terminated and the land rehabilitated. The gravel pit adjacent to Population 4 is the highest priority for closure and rehabilitation (see Section 1.6.2.9).

In order of priority, the following sites should be protected from accidental destruction:

(a) Population 4 and 5
(b) Populations 1 and 2
(b) Population 3

1.5.2.5 Protection from fire

C. elegans does not require fire to complete its life cycle although there is evidence to suggest that it benefits from fire as it opens up the heath thereby reducing competition for space and light. Fire also provides a source of nutrients for the growth of mycorrhiza on which *C. elegans* seedlings depend for their establishment and growth. As a general rule it is recommended that frequent fires be prevented in the vicinity of *C. elegans* populations. However, the occasional control burn could be considered on any population which shows a significant and continued decline in numbers of plants, providing weeds can be adequately controlled.

Research has indicated that adult plants are most vulnerable during their vegetative stage (April-July), when replacing their parent tuber(s). If it is decided that a site should be burnt, then it is strongly recommended that this should not take place in April-July. October-November is the recommended time for a control burn.

1.5.2.6 Weed control

The control of weeds in or near sites that are to be preserved for conservation of *C. elegans* is desirable. All of the populations near Northampton were in agricultural land and therefore surrounded by weeds. CALM officers should liaise with other CALM staff with expertise in the area, the APB, Main Roads Department (MRD) and private landowners.

Weeds should be removed by hand with minimum soil disturbance where use of selective herbicides may damage *C. elegans* or other native species. Weed control should be exercised after *C. elegans* has completed flowering, and before the weeds shed their seed. It is recommended that weeds be controlled biannually in the month of October. Department of CALM Information Sheets Nos. 1-87 and 2-88 provide information on the control of weeds in natural and direct seeded regeneration areas.

In order of priority, weeds should be removed from the following sites:

(a) Populations 4 and 5
(b) Populations 1 and 2
(c) Population 3

1.5.2.7 Linear marking

The four populations near Northampton are located on roads and are subject to damage by maintenance operations. Linear marking would provide a minimum protection for these populations. The MRD has developed a field marking system for demarcating environmentally significant areas on road reserves, and local shires have been encouraged to adopt this system (Kelly *et al.* 1990).

In order of priority, *C. elegans* populations should be linear marked at the following sites:

(a) Populations 4, 5 and 2
(b) Population 3

1.5.2.8 Ex situ conservation

The preservation of *C. elegans* in its natural habitats is the highest priority, however, the *ex situ* conservation of this species is also advisable to reduce the possibility of its sudden extinction through catastrophes such as accidental bulldozing or disease. This may be achieved through cultivation and long-term seed storage.

The underground tubers of *C. elegans* would provide the most suitable material for the propagation of this species. Western Australian ground orchids can persist as potted plants in shade houses for many years, and *C. elegans* would be a suitable candidate for this method of *ex situ* conservation.

Samples of 1 or 2 tubers could be collected from plants having 5 or more ramets. Tubers should be collected at the end of the flowering season in such a manner as to minimize the disturbance to the remaining tubers. The tubers should be representative of the range of genetic and morphological diversity, and in practice all of the populations could be sampled. Within populations, sampling should be random.

In order of priority, the following sites should be considered for *ex situ* cultivation:

(a) Populations 1, 4, 5 and 2
(b) Population 3

1.5.2.9 Rehabilitation of existing populations

Rehabilitation of the road verge populations near Northampton is required to fulfil the objective of management. It should be done in co-operation with landowners, Shires and the MRD. Officers are referred to the Department of CALM Information Sheets Nos. 5-87 and 2-88, and Edmiston (1987) for advice on methods of rehabilitation.

The area of the smaller sites to be preserved should be increased to 5 ha, and it is essential that the size of the *Thryptomene saxicola* populations in these sites be elevated to their MVP. The gravel pit adjacent to Population 4 is the highest priority area for rehabilitation.

Hopper and Coates (ibib) recommend that caution be exercized in the rehabilitation of degraded lands, and that local seed stock be used if viable breeding populations are to be maintained.

In order of priority, the following sites should be rehabilitated:

(a) Population 4
(b) Populations 5 and 2

(c) Population 3
(d) Population 1

1.5.2.10 Artificial gene flow

Artificial gene flow between existing populations is not recommended because most genetic variation in *C. elegans* is maintained within populations. Historically gene flow between Population 1 and the populations from near Northampton probably occurred through a series of now extinct populations. In the absence of information about the possible adverse effects (see James 1982) of direct cross pollination between plants from these geographically distant populations, artificial gene flow between them is not advised.

1.5.2.11 Monitoring

A quadrat stationed permanently in Population 5 has already provided useful demographic information, and biannual monitoring of it should continue. Similarly quadrats should be stationed on the other populations and monitored.

In order of priority, permanent quadrats should be established in the following populations, and monitored:
(a) Populations 1 and 4
(b) Population 5 and 2
(c) Population 3

Table 1.1. Summary of allozyme variation based on 17 loci and four populations of *Caladenia elegans*.
*N*e, estimated number of flowering plants; N, mean sample size per locus; *P*, proportion of polymorphic loci; *A*, mean number of alleles per locus; *A*e, mean effective number of alleles per locus; *H*o, mean observed heterozygosity; *H*e, mean expected heterozygosity; and *F*, mean fixation indices (Wright's) for the polymorphic loci.

Species	*N*e	N	*P*	*A*	Ae	*Ho*	*He*	*F*
All Populations	**90**		**0.82**	**2.9**	**1.18**	-	**0.15**	-
Population 1	200	12	0.41	1.7	1.15	0.09	0.13	0.18
Population 2	2000	8	0.47	1.6	1.18	0.15	0.15	-0.09
Population 4	2000	23	0.65	2.4	1.19	0.12	0.16	0.17
Population 5	10000	47	0.65	2.5	1.18	0.11	0.15	0.15
Mean			**0.54**	**2.1**	**1.18**	**0.12**	**0.15**	**0.10**

Table 1.2. Summary of allozyme variation, genetic diversity and distribution of diversity within and between populations of *Caladenia elegans* and other orchid species of widespread and regional distributions, and a number of plant groupings arranged by Hamrick & Godt (1990). Moran and Hopper (1987) define widespread species as having a geographic range of 600 km in at least one direction; regional species range between 150 and 600 km; and localized species are endemic to an area of less than 100 km; P, mean proportion of polymorphic loci; A, mean number of alleles per locus; and *He*, mean expected heterozygosity; *Hs*, mean genetic diversity within populations; *Ht*, total genetic diversity; *Dst*, mean genetic diversity among populations, *Gst*, mean proportion of diversity between populations.

	P	*A*	*He*	H_S	H_T	G_{ST}	Source
WIDESPREAD							
Australian							
Drakaea livida	0.66	2.6	0.27	0.39	0.42	0.06	1
Drakaea glyptodon	0.67	2.3	0.24	0.29	0.31	0.05	1
REGIONAL							
Australian							
Drakaea elastica	0.45	1.7	0.16	0.20	0.25	0.21	1
European							
Orchis morio	0.48	1.7	0.12	0.12	0.12	0.05	2
Orchis longicornu	0.54	1.9	0.16	-	-	0.01	2
LOCALIZED							
Australian							
Caladenia elegans	**0.63**	**2.3**	**0.22**	**0.23**	**0.24**	**0.02**	1
Caladenia caeseria sub sp. *maritima*	0.36	1.8	0.17	0.39	0.41	0.03	1
ANIMAL POLLINATED	0.50	1.8	0.15	-	-	0.20	3
SELFING	0.20	1.3	0.07	-	-	0.51	3
WIND-DISPERSED SEED	0.43	1.7	0.12	-	-	0.14	3

Sources of data: (1) this study, (2) Rossi *et al.*(1992), Hamrick & Godt (1990).

Figure 1.1. Location of *C. elegans* populations in relation to CALM Regions.

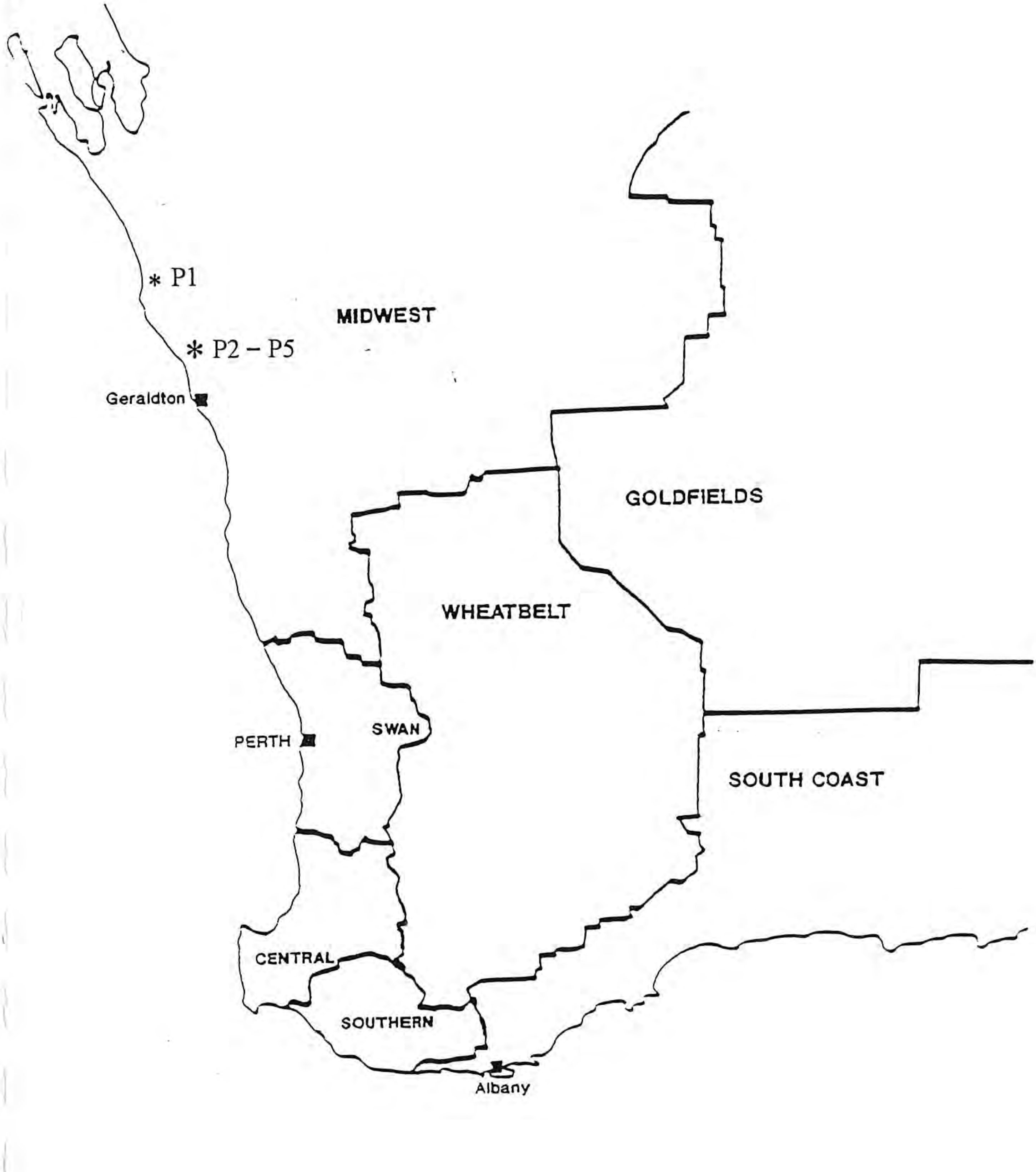


Figure 1.2. Dendogram of UPGMA cluster analysis based on Nei's genetic distance between 4 populations of *C. elegans*.

```
                        Distance

.04       .03       .02       .01       .00
+----+----+----+----+----+----+----+----+

                                     ****  Population 5
                                   ***
                                     * **  Population 1
                                     ***
                                       **  Population 2
                                       **
                                        *  Population 4

+----+----+----+----+----+----+----+----+
.04       .03       .02       .01       .00
```

2. *CALADENIA CAESARIA* SUBSP. *MARITIMA* (CAPE SPIDER ORCHID)

The Cape spider orchid, *Caladenia caesaria* subsp. *maritima* Hopper & A.P.Brown, was first collected from near Cape Naturaliste by Bruce Maslin in 1972. It is morphologically uniform throughout its range and it is distinguished from three other sub species by its earlier flowering time and isolated coastal distribution (see Hoffman and Brown, 1992)

2.1 DISTRIBUTION AND CONSERVATION STATUS

C. caesaria is distributed over a range of 200 km in the south west of Western Australia. *C. caesaria* subsp. *transiens* is the most northerly distributed subspecies occurring between Williams and Wagin. *C. caesaria* subsp. *subdita* is found near Woodanilling and *C. caesaria* subsp. *caesaria* occurs between Tenterden and Bridgetown. *C. caesaria* subsp. *maritima* has the most restricted distribution being found over a few kilometres between Dunsborough and the northern tip of Cape Naturaliste as a linear series of 12 populations which are confined to coastal granite outcrops which have a north-eastern aspect (Fig 2.1).

Most of the land where *C. caesaria* subsp. *maritima* occurs is relatively undisturbed. Only two roads, a few tracks and some recreation and camping facilities currently impact on this orchid. Three populations had been invaded by exotic weeds, but otherwise most of the populations are in near pristine condition. The locations, conservation status and condition of the sites are summarised below. Precise locality details are contained on CALM Departmental rare flora database and files.

<u>Population 1</u> - north-west of Rocky Point. 5 mature plants were distributed over a 0.25 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: Although not currently threatened at this site, this small population is distributed over a small area and could be at risk through depletion over a longer period. Monitoring population numbers is recommended.

<u>Population 2</u> - south-east of Rocky Point and 250 m south-east of Population 1. Fifteen adult plants were distributed over a 0.5 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: Although not currently threatened at this site, this small population is distributed over a small area and could be at risk through depletion over a longer period. Monitoring population numbers is recommended.

<u>Population 3</u> - south-east of Rocky Point and 350 m south-east of Population 2 with 150 mature plants distributed over a 3.25 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 4 - south-east of Rocky Point and 300 m south-east of Population 3 with 200 mature plants distributed over a 5 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 5 - south-east of Eagle Bay and 1.25 km south-east of Population 4 with 150 mature plants distributed over a 3.5 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 6 - south-east of Eagle Bay and 600 m south-east of Population 5 with 250 mature plants distributed over a 5 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 7 - south-east of Eagle Bay and 500 m south-east of Population 6 with 350 mature plants distributed over a 7.5 acre area. A gravel road has been cut into the site, otherwise the population, which is on Shire Recreation Reserve, is relatively undisturbed.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 8 - north-west of Meelup Brook and 1.0 km south-east of Population 7 with 600 adult plants distributed over a 15 acre area adjacent to road reserve. The population is on Shire Recreation Reserve and is largely undisturbed.

Conservation status: This population is not under any immediate threat, however, monitoring of population numbers is recommended.

Population 9 - south-east of Meelup Beach and 350 m south-east of Population 8. Five adult plant were distributed over 0.25 acres of weed infested land on Shire Recreation Reserve.

Conservation status: This population is under serious threat from habitat degradation due to weed invasion. Special attention may be required for its survival over the next decade.

Population 10 - north-west of Castle Rock and 750 m south-east of Population 9 with 125 adult plants distributed over 1.0 acres of weed infested land on Shire Recreation Reserve.

Conservation status: This population is under serious threat from habitat degradation due to weed invasion. Special attention may be required for its survival over the next decade.

Population 11 - near Castle Rock and 250 m south-east of Population 10 with 30 adult plants distributed over a 0.5 acre area on relatively undisturbed Shire Recreation Reserve.

Conservation status: Although not currently threatened at this site, this small population is distributed over a small area and could be at risk through depletion over a longer period. Monitoring population numbers is recommended.

Population 12 - south-east of Castle Rock and 100 m south-east of Population 11 with 10 adult plants distributed over a 0.25 acre area on a relatively undisturbed Shire Recreation Reserve.

Conservation status: Although not currently threatened at this site, this small population is distributed over a small area and could be at risk through depletion over a longer period. Monitoring population numbers is recommended.

2.2 HABITAT DISTRIBUTION AND POPULATION DENSITY

Sites with the geological and temperature requirements for habitation by *C. caesaria* subsp. *maritima* were fragmented and occurred in a linear sequence parallel to the coast. There were several size classes of suitable habitat, and these were not randomly distributed throughout its range. The largest suitable habitat areas occurred in the middle of the range, and habitat areas were smallest at the ends of the range. There was a high positive correlation ($r = 0.99$; $P < 0.01$) between suitable habitat area and population size. *C. caesaria* subsp.*maritima* populations were larger in the centre of the range of the species, and smallest toward the ends.

2.3 HABITAT AND ASSOCIATED SPECIES

C. caesaria subsp.*maritima* grew on the reddish brown sandy-loam soils that collects in the crevices between rocks on coastal granite outcrops and bare rock. The associated vegetation was usually open dwarf shrub over low heath, sometimes with scattered small trees, and was comprised of *Acacia pulchella*, *Banksia grandis*, *Boronia tenuis*, *Calothamnus graniticus* subsp.*graniticus*, *C. sanguineus*, *Cheilanthes tenuissima*, *Dampiera alata*, *Darwinia citriodora*, *Dodonaea ceratocarpa*, *Dryandra nivea*, *Eucalyptus calophylla*, *Gastrolobium spinosum*, *Hakea trifurcata*, *H. lissocarpha*, *Hibbertia hypericoides*, *H. racemosa*, *Hypocalymma robusta*, *Loxocarya flexuosa*, *Macrozamia reidlei*, *Nuytsia floribunda*, *Phyllanthus calycinus*, *Pimelea ferruginea*, *Rulingia* sp., *Trymalium ledifolium*, *Viminaria juncea* and *Xanthorrhoea preissiiia* amongst others.

Other orchids found with *C. caesaria* subsp.*maritima* included *Acianthus reniformis* var. *huegelia*, *Caladenia chapmanii*, *C. longicauda*, *C. gardnerii* subsp. *valida*, *Diuris* aff. *longifolia*, *Prasophyllum parvifolium*, *Pterostylis nana*, *P.* aff. *nana* and *Thelymitra nuda*.

The area has a temperate climate. The summers (December-March) are hot and dry, and winters are cool and wet. Most rainfall occurs in late autumn, winter and early spring, and the mean annual rainfall at Busselton (some 40 Km to the NE) is 821 mm (Bureau of Meteorology).

2.4 LIFE HISTORY

C. caesaria subsp. *maritima* experiences environmental conditions that are cyclical with cool and wet winters and hot and dry summers. Under such conditions *C. caesaria* subsp. *maritima* like *C. elegans* has developed physiological and morphological traits, such as underground perenating tubers, which are regarded as being of selective advantage in promoting good establishment and carryover of reserves within and between seasons (Pate and Dixon 1982).

Flowering occurs annually and during the growing season. This has advantages because pollinators are in highest frequency, and current photosynthesis is harnessed directly to the production of seeds.

2.5.1 Phenology of growth and reproduction

C. caesaria subsp. *maritima* has an extremely similar pattern of growth and reproduction to *C. elegans.* However, it flowers nearly a month later with inflorescences elongating and the flower buds expanding in late August - early September. During mid and late September the leaf shrivels, and capsule and seed maturation relies on the nutrients stored in the scape. In late September - early October the capsule dries and dehisces, releasing large amounts of seed. The underground perenating tubers are all that remain over the summer months.

Seed germination relies on the presence of a species specific mycorrhizal fungus. This symbiotic relationship is essential for any seedling establishment. Although very large quantities of seed are released from each capsule only a fraction become mature plants.

2.4.2 Recruitment after fire

C. caesaria subsp. *maritima* does not require fire to complete its life cycle and should be protected from frequent fires. In chapter 1 we suggested that *Caladenia elegans*, a species related to *C. caesaria* subsp. *maritima*, benefited from the effects of fire, ie. opening up the canopy thereby reducing competition for light and space, and releasing nutrients locked up in other species, and it may be that occasional fire benefits *C. caesaria* subsp. *maritima* in the same way.

2.4.3 Pollination biology

In 1990 and 1991 *C. caesaria* subsp. *maritima* began flowering late in August and continued to flower throughout September. Although a solitary flower characterizes most inflorescences, some have two or three flowers displayed simultaneously. While a small percentage of plants produced up to fifteen inflorescences in a clump, most plants (45%) produced three inflorescences. In 1991 an estimated 1750 flowers were produced in Population 8 alone.

Adult plants are probably long-lived (tens of years or more), and flower every year. Flowering is synchronized within populations but not between them. In the last week of

August (in 1990 and 1991), Populations 1 and 2 were the first to begin flowering. They were also the highest above sea level, had less cover, and may therefore have had the warmest sites at that time of the year. As time progressed, successive populations commenced flowering in a south-easterly direction, such that by the time Population 12 was in full flower in mid to late September, Populations 1 an 2 had finished flowering.

C. caesaria subsp. *maritima* flowers are displayed for only 3-10 days and are strongly scented. Like other *Caladenia* species (See Chp 1) the source of the fragrance is the single- and multi-celled trichomes located on the lower two thirds of the petals and sepals.

C. caesaria subsp. *maritima* flowers attract only male thynnid (common name) wasps, and the primary distance attractant was the fragrance produced by the petals and sepals. Once the wasp had been attracted to the flower, visual and tactile cues stimulated further pollination responses. *C. caesaria* subsp. *maritima* has a zygomorphic, or "lipped" (labellum), flower. Maroon lines on the labellum converge toward two rows of calli at the entrance of the flower. This "guide pattern" acted as a near goal orientation cue for thynnid wasp visitors.

The pollen of *C. caesaria* subsp. *maritima* is not available as food for wasp visitors, and flowers did not produce nectar. Thynnid wasps then, go unrewarded when they are deceived into visiting *C. caesaria* subsp. *maritima* flowers. In *C. elegans* thynnid wasps actively pollinate flowers for only a short period (10-15 days). At any one of the *C. caesaria* subsp. *maritima* populations thynnid wasps could possibly be active for as much time, but because flowering was not synchronized between populations thynnids would be pollinating flowers of this species for a more extended period (25-30 days). In the last week of August 1991 thynnids were most active in Populations 1, 2 and 3, and became less active as distance away from these populations increased.

In *C. elegans* thynnid wasps pollinate 60% of flowers. By August 29 1991 only 10% of *C. caesaria* subsp. *maritima* flowers in Populations 1 and 2 had been pollinated, and in Population 3 only 15%. This four fold difference in pollination rate between these two species may be due to a difference in the activity time of the wasp pollinators. Wasps fly actively when air temperature is above 20 C and the sky is cloudless (Stoutamire 1983, Chp 1). The regular cycles of high and low pressure fronts that sweep through southern Australia during late August and September also control wasp activity (Stoutamire 1983), and would therefore influence the pollination rate in *C. caesaria* subsp. *maritima*. A passing front is accompanied by a drop in temperature followed by cloudy weather which changes over a period of several days to a short period of warm sunny weather before the next front passes. Throughout *C. caesaria* subsp. *maritima's* flowering period then, the actual time spent by wasps pollinating flowers may be brief, a few hours each week, since wasps are active only during these short periods of rising temperatures. Consequently the pollination rate of *C. caesaria* subsp. *maritima* flowers may not greatly exceed, on average, the 15% observed at Population 3, and it is estimated that 1.75 million *C. caesaria* subsp. *maritima* seeds were produced by Population 8 (the largest population) in 1991. *C. elegans* with its northern distribution is affected less by these fronts, and has longer periods of warm sunny weather which allows for more wasp activity and results in a higher pollination rate of flowers.

The staggered flowering times of *C. caesaria* subsp. *maritima's* populations, which results in a protracted flowering time for the species relative to some other orchids, may have long term evolutionary significance for the species. The warm sunny periods required for pollination are brief and infrequent, but when they do occur there is a high probability that somewhere throughout its very restricted range there will be a *C. caesaria* subsp. *maritima* population in full flower ready to take advantage of any available wasp pollinators. As with *C. elegans* thynnid wasps clearly play an integral part in, and are a necessary requirement for the completion of, the reproductive cycle.

2.4.4 Phenology if thynnid wasps and associated food plants

Mating season (late August and September) is the only time, in the whole of their annual life cycle, when male thynnid wasp pollinators of *C. caesaria* subsp. *maritima* are active above ground. At Population 10 on August 29 1991 male wasps were observed milking nectar from *Hakea tricuspis* flowers. This was the only nectar producing species on which these thynnids were observed.

Hakea tricuspis is a widespread woody perennial shrub, is 0.5 to 2.5 meters high, spreading, and belongs in the Proteaceae family. It sometimes forms dense thickets over several square meters, is insect pollinated, and in the Dunsborough area it flowers profusely during late August and September. The apparent reliance of the thynnid wasps on this species as a food source indicates that *Hakea tricuspis* probably plays an integral part in the life cycle of *C. caesaria* subsp. *maritima* and its thynnid wasp pollinators.

2.5 GENETIC DIVERSITY AND POPULATION STRUCTURE

For general references and a brief review see section 1.4.

2.5.1 Genetic variation and structure within populations.

Measures of genetic diversity within populations (Table 2.1) such as *P, A He, Ho* indicate that *C. elegans* is similar to other orchid species and typical of a diploid sexually reproducing plant species which is animal pollinated and has a mixed mating system (Hamrick and Godt, 1989).

Of the nine *C. caesaria* subsp. *maritima* populations examined, Population 1 had the least number of individuals ($N = 5$; Table 2.1), had fewer alleles per locus ($A = 1.6$) than any other population, and the mean expected heterozygosity ($He = 0.16$) and proportion of polymorphic loci ($P = 0.33$) were among the lowest. However the correlation between population size and allele frequency per locus ($r = 0.34$; $P > 0.05$) was not significant.

The mean expected heterozygosity ($He = 0.17$) for the species as a whole was high. In this respect *C. caesaria* subsp. *maritima* is like other spider orchids with which it is closely related, other orchid species and animal pollinated species in general (Table 2.2). The total genetic diversity ($Ht = 0.41$; Table 2.2) was also very high, and this was due to the influenced of a small proportion (28%) of highly variable loci.

The study on *C. elegans* (Chapter 1) revealed lower than expected heterozygosity levels within populations which was attributed to a mating system involving some autofertilization, and to man-made disturbance which had introduced structuring into its populations. In *C. caesaria* subsp. *maritima,* however, the pattern was quite the opposite with higher than expected frequencies of heterozygotes. In all populations examined the mean observed heterozygosity (*Ho*) was greater than that expected under panmixia (*He*) indicating an excess of heterozygotes (Table 2.1), and all but one of the populations (# 10) had slightly negative values for Wright's fixation index (F = -0.08, averaged over all populations; Table 2.1). Moran & Bell (1983) and Ledig (1986) found in some plant species that heterozygosity in isoenzyme alleles increased as cohort age increased. It remains to be proven whether there is selection favouring outcrossed individuals throughout the life cycle of *C. caesaria* subsp. *maritima*, however, the contrast with *C. elegans* indicates it warrants further study.

2.5.2 Differentiation between populations and gene flow

Most of the genetic diversity was partitioned within *C. caesaria* subsp. *maritima* populations (Hs = 0.39), and only 3% of the total genetic variation is distributed between populations (Gst = 0.03) (Table 2.2). Cluster analysis based on Nei's genetic distance (Figure 2.2) identified the two geographically separated population groups, but supports the conclusion that there has been little divergence between any of the populations. Thus when the genetic distances between populations from north-west and south-east of Eagle Bay were compared with genetic distances between populations within these groups, they were not found to be significantly different (t = 0.2745; P > 0.1).

Gene flow estimates based on Wright's method (1951) indicated that there was a high rate of exchange (Nm = 7.3) between each of the nine *C. caesaria* subsp. *maritima* populations. *C. caesaria* subsp. *maritima* can be separated into two population groups, those that occur north-west of eagle Bay and those that occur to the south-west of it. The exchange of migrants between the north-west populations (Nm = 22.3) was three times that for all of the populations, and the rate of exchange between the south-east populations (Nm = 8.08) was also higher than that for all populations.

2.6 RECOMMENDATIONS FOR CONSERVATION OF GENETIC RESOURCES AND MANAGEMENT

2.6.1 Strategies for conserving genetic resources

For general references and a brief review see section 1.5.1

In the previous sections it was established that *C. caesaria* subsp. *maritima* exists in near pristine condition in the wild, and in order to maintain the genetic diversity of this threatened species certain management strategies need to be implemented. Some of the management strategies recommended by Hopper and Coates (1990) for that purpose, include selection of priority populations for conservation, reserve and corridor design, and active management of species through propagation and translocation in the wild.

Ideally all of the *C. caesaria* subsp. *maritima* populations should be preserved, and given that they exist as a linear sequence, all of which are currently on Shire Recreation Reserves, this should not be a costly exercise. However, if there are insufficient funds to preserve all of the populations, then a choice has to be made on the allocation of the available resources. Based on the level of differentiation between populations Hopper and Coates (1990) were able to recommend priority populations for the conservation of genetic diversity in three distinctly different species, *Eucalyptus caesia, E. diversicolor* and *Banksia cuneata*. The distribution of genetic resources of *C. caesaria* subsp. *maritima* documented in previous sections were found to be similar in many respects to those of *E. diversicolor* and *B. cuneata*. From the UPGMA dendrogram based on genetic distance for population divergence (Figure 2.2) it is evident that there are low but clear levels of divergence between the two population groups separated by Eagle Bay. Based on these data the preservation of a minimum of one large population from each of the two groups of populations would probably ensure adequate short term protection of the genetic resources of this taxon.

Information from UPGMA dendrogram (Fig. 2.2) provides a first estimate of the minimum number of populations that would probably protect this species' genetic resources. However our investigations indicate that population demographics and the reproductive strategy of the species need to be considered when setting a minimum viable number of populations. *C. caesaria* subsp. *maritima's* populations are very small and average 157 adult individuals. Four populations (#s 4, 6, 7 and 8) are larger than the average, and occur in the centre of this species' range. Population 4 is in the north-west population group and the remaining three are in the south-west. 7 of the 8 smaller populations occur at the ends of the species range, and these populations are most likely to experience occasional temporary extinctions. It was shown that *C. caesaria* subsp. *maritima's* flowering season was not synchronized between its populations, and it was suggested that the staggered flowering times of its populations maximizes the probability that the seed set will be high under unpredictable environmental conditions. *C. caesaria* subsp. *maritima* minimizes the risk of not setting seed, and under this system is able to maintain a high rate of migration between its populations. This would indicate that the smaller populations at the ends of the species range are just as important for maintaining genetic diversity as any other population. An appropriate conservation strategy for this species then would be to preserve Population 4 and one other (Population 3) in the north-west group, and Population 8, either of Populations 6 or 7, and Population 10 in the south-east group.

As discussed in Section 1.7.1 strategies for conserving the genetic resources of a species may also require one to determine the minimum viable size (MVP) of a population. Ordinarily this is a complex procedure, but in this report MVP's are estimated from a genetic view point to represent the size of populations below which the loss of genetic variation would become a problem. In *C. caesaria* subsp. *maritima* populations heterozygotes occurred in higher than expected frequencies, and there may be a post-zygotic selection mechanism operating in this species to elevate the frequency of heterozygotes in the adult population. This would serve to offset the effects of inbreeding in small populations, i.e. loss of alleles and reduced total genetic diversity, as was observed in Populations 1 ($Ne = 5$) and 2 ($Ne = 15$) which had not suffered any loss as a consequence of their smallness. Assuming, that a high rate of exchange is maintained between *C. caesaria* subsp. *maritima* populations, then a MVP of five seems plausible.

Each of the populations were found to occupy very small areas of land (usually less than 15 acres), and will therefore require some management to minimize their invasion by exotic weeds and subsequent degradation.

2.6.2 Management actions

2.6.2.1 Liaison with landowners and shires

At present the survival of all of the *C. caesaria* subsp. *maritima* populations relies on the good will of the local shire. CALM staff are required to provide landholders and other agencies with advice regarding the conservation and management of populations of threatened flora on land under their control, and owners are requested to arrange their operations so that populations will not be destroyed or damaged in any way. In order of priority, the populations for staff liaison with the shire are:

(a) Populations 8, 10 and 6
(b) Populations 7, 5 and 9
(c) Population 4, 11, 3, 2, 10 and 1

2.6.2.2 Land acquisition

There are no *C. caesaria* subsp. *maritima* populations currently on land reserved for the conservation of flora and fauna. Therefore, it is strongly recommended that the Department of CALM acquires land on which *C. caesaria* subsp. *maritima* occurs. Ideally all populations should be conserved, but if this is not possible then priority should be determined by:

(a) the size and quality of the site and its habitat,
(b) conserving the range of genetic and morphological diversity, and
(c) reducing the risk of sudden extinction.

Conservation of Populations 8, 4, 6, 3 and 10 would conserve the range of genetic and morphological diversity found in this Rare Flora, and reduce the risk of its sudden extinction. Although 125 plants spread over about 1/2 ha (Population 10) of natural habitat constitutes a MVP, the preservation of viable populations of thynnid wasps and *Hakaea tifurcata* must also be considered. Therefore, to ensure and enhance the survival of *C. caesaria* subsp. *maritima* it is essential to provide and maintain areas containing a suitable variety of dependent species and habitats. For this purpose a minimum reserve area of 5 - 15 ha is recommended as this would accommodate an appropriate range in population sizes.

In order of priority, the following sites should be acquired:

(a) Populations 8, 4, 6, 3 and 10
(b) Populations 7, 5, 11 an 2
(c) Populations 12, 9 and 1

2.6.2.3 Protection from grazing

There was no evidence of direct grazing of *C. caesaria* subsp. *maritima* plants by rabbits or any other introduced herbivores. However in view of the potential such introduced species have for degrading habitats, it is strongly recommended that populations be monitored for any possible threat from grazing.

2.6.2.4 Protection from accidental destruction

C. caesaria subsp. *maritima* is vulnerable to damage or destruction owing to the small areas occupied by its populations, and should be protected from accidental destruction by bulldozing (of access roads, firebreaks or for nature walks), rubbish dumping, and spraying of potentially damaging herbicides or insecticides.

In order of priority, the following sites should be protected from accidental destruction:

(a) Populations 8, 10 and 6
(b) Populations 7, 5 and 9
(c) Population 4, 11, 3, 2, 10 and 1

2.6.2.5 Protection from fire

C. caesaria subsp. *maritima* does not require fire to complete its life cycle and where feasible should be protected from frequent uncontrolled fires by the construction of fire breaks or by fuel reduction in surrounding areas. However it may be that *C. caesaria* subsp. *maritima* benefits from fire as it opens up the heath thereby reducing competition for space and light, and provides a source of nutrients for the growth of mycorrhiza on which the seedlings depend for their establishment and growth.

Research has indicated that adult plants are most vulnerable during their vegetative stage (April-August), when replacing their parent tuber(s). If control burns are considered for the area it is strongly recommended that they should not take place in April-August rather late October and November is the recommended time.

In order of priority, the following sites should be protected from uncontrolled burning:

(a) Populations 8, 4, 6, 3 and 10
(b) Populations 7, 5, 11 an 2
(c) Populations 12, 9 and 1

2.6.2.6 Weed control

The control of weeds in or near sites that are to be preserved for conservation of *C. caesaria* subsp. *maritima* is desirable. Some populations are more susceptible to invasion by weeds than others. Populations near recreation facilities, car parks and rest areas are particularly vulnerable as weeds seeds are transported to these sites by vehicles. As to the control of weeds CALM officers should liaise with other CALM staff with expertise in the area, the APB, Main Roads Department (MRD) and private landowners.

Weeds should be removed by hand with minimum soil disturbance where use of selective herbicides may damage *C. caesaria* subsp. *maritima* or other native species. Weed control should be exercised after *C. caesaria* subsp. *maritima* has completed flowering, and before the weeds shed their seed. It is recommended that weeds be controlled biannually in the month of October. Department of CALM Information Sheets Nos. 1-87 and 2-88 provide information on the control of weeds in natural and direct seeded regeneration areas.

In order of priority, weeds should be removed from the following sites:

(a) Populations 8, 10, 7, and 9

(b) Populations 6 and 5
(c) Population 4, 3, 11, 2, 12 and 1

2.6.2.7 Linear marking

Populations 5 through to 8 are located adjacent to Meelup Road or are dissected by it, and are subject to damage by maintenance operations. Linear marking would provide a minimum protection for these populations. The MRD has developed a field marking system for demarcating environmentally significant areas on road reserves, and local shires have been encouraged to adopt this system (Kelly *et al.* 1990).

In order of priority, Populations 8, 7, 5 and 6 should be linear marked.

2.6.2.8 *Ex situ* conservation

Intensive genetic management has yet to be carried out on populations of any endangered native Australian plant, however it is a viable option and needs to be considered in the future if extinction in the wild is to be prevented (Hopper and Coates 1990). Hopper and Coates (ibib.) suggest that plant species s may be managed by cultivation followed by their re-introduction or translocation into areas where they were previously known to exist. The preservation of *C. caesaria* subsp. *maritima* in its natural habitats is the priority, however the *ex situ* conservation of this species is also advised to reduce the possibility of its sudden extinction through catastrophes such as fires or disease. This may be achieved through cultivation and long-term seed storage.

The underground tubers of *C. caesaria* subsp. *maritima* would provide the most suitable material for the propagation of this species. Many West Australian ground orchids persist as potted plants in shade houses for many years, and *C. caesaria* subsp. *maritima* would be a suitable candidate for this method of *ex situ* conservation.

Samples of 1 or 2 tubers could be collected from plants having 5 or more ramets. Tubers should be collected at the end of the flowering season in such a manner as to minimize the disturbance to the remaining tubers. The tubers should be representative of the range of genetic and morphological diversity, and in practice all of the populations could be sampled. Within populations, sampling should be random.

In order of priority, the following sites should be considered for *ex situ* cultivation:
(a) Populations 8, 4, 6, 3 and 10
(b) Populations 7, 5, 11 an 2
(c) Populations 12, 9 and 1

2.6.2.9 Rehabilitation of existing populations

Rehabilitation of the road verge populations (# 7 and 8), and Population 9 which is near to recreation facilities, is required to fulfil the objective of management. It should be done in co-operation with Shires and the MRD. Officers are referred to the Department of CALM Information Sheets Nos. 5-87 and 2-88, and Edmiston (1987) for advice on methods of rehabilitation.

When rehabilitating an area it is essential to ensure that a MVP of *Hakea trifurcata* is also conserved. Hopper and Coates (ibib) recommend that caution be exercized in the rehabilitation of degraded lands, and that local seed stock be used if viable breeding populations are to be maintained. It should be noted that any introduction or re-introduction of this taxon must be carried out under approved Wildlife Management Programs or Interim Management Guidelines as outlined in CALM Policy Statement No. 29.

In order of priority, the following sites should be rehabilitated:

(a) Population 8
(b) Populations 7 and 9

2.7.2.10 Artificial gene flow

Assuming that the number of populations recommended in this report are preserved, then artificial gene flow between them should not be necessary.

2.7.2.11 Monitoring

Quadrats have been stationed permanently in populations of other orchids (see Chpt. 1) and it is recommended that similar quadrats be stationed in each of the *C. caesaria* subsp. *maritima* populations, and that they be monitored at least annually.

In order of priority, permanent quadrats should be established in the following populations, and monitored:

(a) Populations 8, 4, 6, 3 and 10
(b) Populations 7, 5, 11 an 2
(c) Populations 12, 9 and 1

Table 2.1. Summary of allozyme variation based on 18 loci and 9 populations of *Caladenia caesaria* sub sp. *maritima*. Ne, estimated number of flowering plants; N, mean sample size per locus; P, proportion of polymorphic loci; A, mean number of alleles per locus; *Ho*, mean observed heterozygosity; *He*, mean expected heterozygosity; and *F*, mean fixation indices (Wright's).

Species	*Ne*	*N*	*P*	*A*	*Ho*	*He*	*F*
<u>All Populations</u>			**0.44**	**2.1**	**-**	**0.18**	**-**
Population 1	5	4.0	0.33	1.6	0.17	0.16	-0.16
Population 2	15	14.0	0.39	1.7	0.21	0.19	-0.11
Population 3	150	23.1	0.33	1.8	0.19	0.16	-0.06
Population 4	200	26.0	0.44	1.9	0.22	0.20	-0.06
Population 6	250	29.9	0.44	1.9	0.19	0.17	-0.08
Population 7	350	27.6	0.33	1.7	0.20	0.17	-0.11
Population 8	600	32.0	0.39	1.8	0.20	0.17	-0.12
Population 10	125	29.2	0.28	1.7	0.16	0.15	0.02
Population 11	30	23.1	0.39	1.8	0.18	0.17	-0.05
Mean			**0.36**	**1.8**	**0.19**	**0.17**	**-0.08**

Table 2.2. Summary of allozyme variation, genetic diversity and distribution of diversity within and between populations of *Caladenia caesaria* sub sp. *maritima* and other orchid species of widespread and regional distributions, and a number of plant groupings arranged by Hamrick & Godt (1990). Moran and Hopper (1987) define widespread species as having a geographic range of 600 km in at least one direction; regional species range between 150 and 600 km; and localized species are endemic to an area of less than 100 km; *P*, mean proportion of polymorphic loci; *A*, mean number of alleles per locus; and *He*, mean expected heterozygosity; *Hs*, mean genetic diversity within populations; *Ht*, total genetic diversity; *Gst*, mean proportion of diversity between populations.

	P	*A*	*He*	H_S	H_T	G_{ST}	Source
WIDESPREAD							
Australian							
Drakaea livida	0.66	2.6	0.27	0.39	0.42	0.06	1
Drakaea glyptodon	0.67	2.3	0.24	0.29	0.31	0.05	1
REGIONAL							
Australian							
Drakaea elastica	0.45	1.7	0.16	0.20	0.25	0.21	1
European							
Orchis morio	0.48	1.7	0.12	0.12	0.12	0.05	2
Orchis longicornu	0.54	1.9	0.16	-	-	0.01	2
LOCALIZED							
Australian							
Caladenia elegans	0.63	2.3	0.22	0.23	0.24	0.02	1
Caladenia caeseria* sub sp. *maritima	**0.36**	**1.8**	**0.17**	**0.39**	**0.41**	**0.03**	**1**
ANIMAL POLLINATED	0.50	1.8	0.15	-	-	0.20	3
SELFING	0.20	1.3	0.07	-	-	0.51	3
WIND-DISPERSED SEED	0.43	1.7	0.12	-	-	0.14	3

Sources of data: (1) this study, (2) Rossi *et al.*(1992), Hamrick & Godt (1990).

Figure 2.1. Location of *C. caesaria* sub sp. *maritima* in relation to CALM Regions

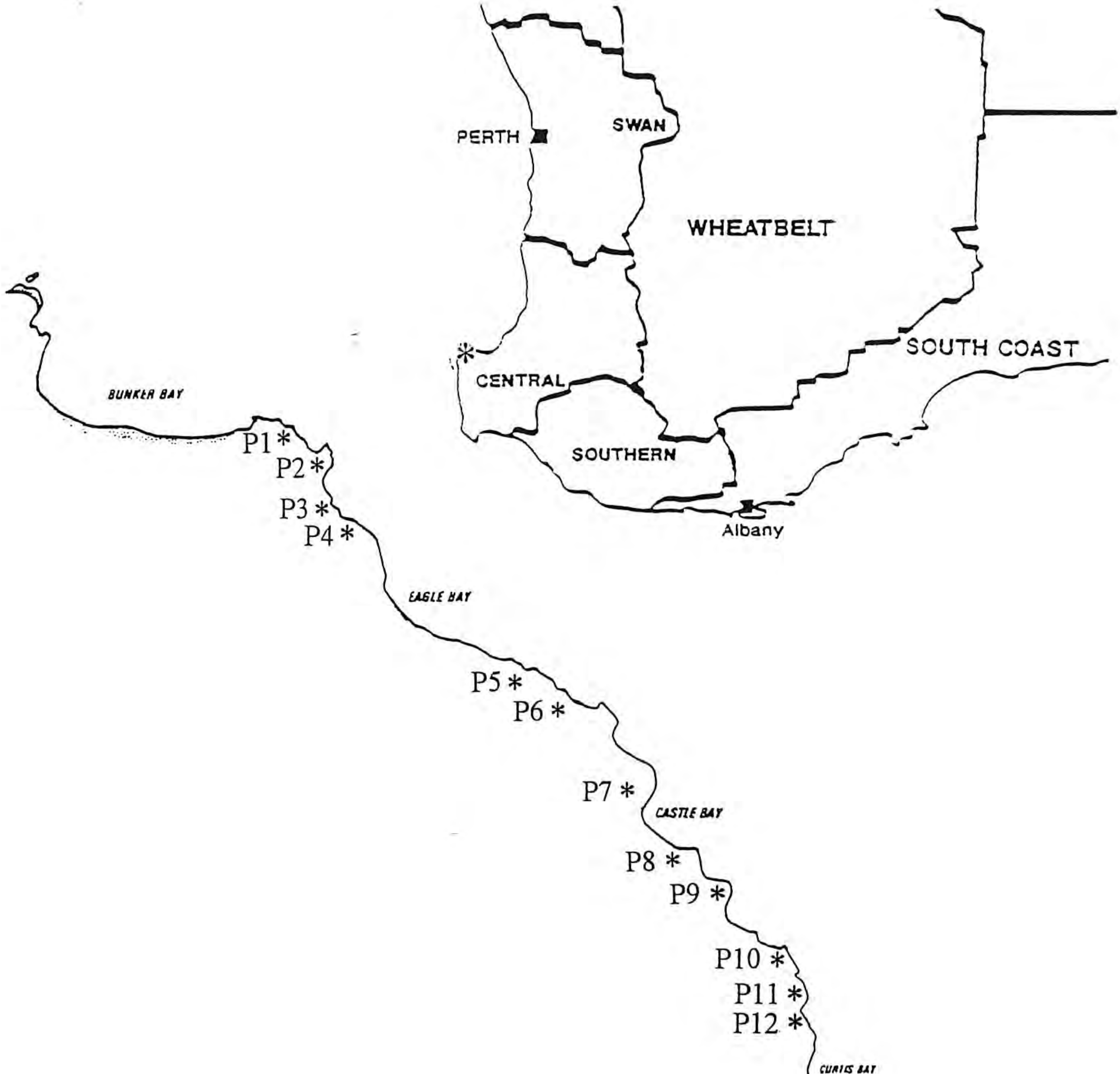


Figure 2.2. Dendogram of UPGMA cluster analysis based on Nei's genetic distance between 9 populations of *C. caesaria* sub sp. *maritima*.

```
                Distance

.03       .02       .01       .00
+----+----+----+----+----+----+

                              *  Population 1
                           ****
                          **  *  Population 2
                          **                      north-west of
                       ********  Population 4       Eagle Bay
                       *  *
                       *  *****  Population 3
                       *
                       *
                    ****
                    *  *      *  Population 7
                    *  *     **
                    *  *   ****  Population 11
                    *  *   * *
                ***** ***** **  Population 10     south-east of
                    *      *                        Eagle Bay
                    *      ****  Population 8
                    *
                    ***********  Population 6


+----+----+----+----+----+----+
.03       .02       .01       .00
```

3. *DIURIS MICRANTHA* (THE DWARF BEE ORCHID)

The dwarf bee orchid, *Diuris micrantha* D. Jones, remained undiscovered until 1974 when Andrew Brown and Warren Stoutemire found it in a single winter wet swamp in the Perth metropolitan area. It was gazetted Declared Rare Flora under the WA Wildlife Conservation ACT in 1989.

D. micrantha is readily distinguished from its closest relative the common bee orchid, *D. laxiflora*, by its smaller pale yellow flowers and shorter labellum midlobe. *D. micrantha, D. laxiflora* and a third, later flowering donkey orchid, *D. carinata*, were found growing together at the *D. micrantha* site. It is reported to form hybrids with *D. carinata* (A. Brown *pers. comm.*), however none were found in either of the 1990 or 1991 flowering seasons and are probably extremely rare.

3.1 DISTRIBUTION AND CONSERVATION STATUS

Although having been extensively searched for in the wild, the original discovery site remained for many years the only known population of *D. micrantha* . However in the spring following a publication by Carstairs and Brown (1992), a second population was reported to have been discovered (A. Brown *pers. comm.*). The original site occurs near Fremantle in the CALM Swan Region, and the new site, which needs to be investigated further, occurs east of Collie in the Central Forest Region (Figure 3.1). *D. micrantha* currently has a distribution range around 150 km.

Much of the land between the two known *D. micrantha* sites has been cleared for agriculture, and the likelihood of finding additional populations is low. Both the original discovery site and the Collie site occur on and adjacent to degraded road verge. The locations, conservation status and condition of the sites are summarised below. Precise locality details are contained on CALM Departmental Rare Flora database and files.

<u>Population 1</u> - near Fremantle. 250 adult plants were distributed over 4 acres of Crown Land along a weed infested road verge, and bordered by a drain and paddocks.

Conservation status: This population is in serious danger of disappearing from this site within one to two decades if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

<u>Population 2</u> - about 30 km east-south-east of Collie. Approximately 1000 adult plants were distributed over a 4 acre area along a weed infested road verge (A. Brown *pers. comm.*).

Conservation status: Although not presently in danger of extinction at this site, this population was distributed over a small area and is therefore at risk through depletion over a longer period. Special attention is required for its long-term survival.

3.2 HABITAT AND ASSOCIATED SPECIES

D. micrantha grew on a dark sandy-loam substrate in winter wet depressions or swamps. The Fremantle site is a southern extension of the Spectacles, a series of temporary lakes that run parallel (north-south) to the coast. The first environmental factor that determines the distribution and abundance of this orchid, then, is hydrological - the distribution of ephemeral winter wet depressions.

The associated vegetation was dominated by the sedge *Baumia acuta*. Low dense heath characterized the northern end of the swamp, and *Melaleuca preissiana* were scattered throughout. *Eucalyptus marginata* occurred around the northern periphery of the swamp, and mixed Banksia woodlands (*B. attenuata*, *B. ilicifolia* and *B. menziesii*) with *Allocasuarina fraseriana*, *E. calophylla*, and *Kunzia ericifolia* thickets occurred to the south. Herbs were plentiful in winter. Other orchids found with *D. micrantha* were *Caladenia heuglii*, *D. carinata*, *D. laxiflora* and *Prasophyllum macrostachyum* var. *ringens*.

The area has a mediterranean climate. The summers (December-March) are hot and dry, and winters are cool and wet. Most rainfall occurs in late autumn, winter and early spring, and the mean annual rainfall at Perth is 870 mm (Bureau of Meteorology).

3.3 LIFE HISTORY

Throughout its range *D. micrantha* experiences environmental conditions that are cyclical and therefore very predictable. Short, cool and wet winters alternate with long, hot and dry summers. Under such conditions *D. micrantha*, like many other south west orchids, has developed physiological and morphological traits, such as underground perenating tubers, which are regarded as being of selective advantage in promoting good establishment and carryover of reserves within and between seasons (Pate and Dixon 1982). Flowering occurs annually and during the growing season. This has advantages because pollinators are in highest frequency, and current photosynthesis is harnessed directly to the production of seeds.

3.3.1 Phenology of growth and reproduction

In late autumn (April-May) underground tubers of *D. micrantha* commence growing. In expanding the new seasons' shoot, roots and leaves, most of the water and carbohydrate reserves of the tubers are consumed. Thereafter, completion of the plant's growth and reproductive cycle depends on the photosynthetic gains made by the new foliage. From May to August the products of photosynthesis are directed toward replacing the parent tuber. In a good season, daughter tubers may also be produced. In late August and September the inflourescences quickly elongate and the flower bud(s) expand. During October the leaf shrivels, and capsule and seed maturation relies on the nutrients stored in the scape. In late October the capsule dries and dehisces, releasing large amounts of seed. The underground perenating tubers are all that remain over the summer months.

Seed germination relies on the presence of a species specific mycorrhizal fungus. This symbiotic relationship is essential for any seedling establishment. Although very large quantities of seed are released from each capsule only a fraction become mature plants.

3.3.2 Pollination biology

Adult *D. micrantha* plants are probably long-lived (tens of years). In four 1/2 meter square quadrats stationed permanently at the Fremantle site, 26% of flowering plants recorded in 1990, flowered again in 1991 and were relocated. Some of the remaining 74% would have perished, but it is likely that most of the underground tubers produced in 1990 either remained dormant in 1991 or produced vegetative plants only, and were not relocated. 54% of the plants that flowered in the quadrats in 1991 had not flowered in 1990. Within populations plants synchronized their flowering times, and although the flowering times of the Fremantle and Collie populations overlapped in 1991, the southern Collie population flowered 2 weeks later than the Fremantle population.

In 1990 and 1991 *D. micrantha* at the Fremantle site began flowering in the third week of August and continued flowering into the first week of October. Some plants produced as many as 10 inflorescences, most produced 1 - 3, and each inflorescence averaged 3.2 flowers. The buds at the base of the inflorescences were the first to open, and as the season progressed the buds opened up in sequence from the bottom to the top of the inflorescence. Inflorescences often had 2 or more flowers displaying simultaneously. An estimated 500 flowers were produced at the Fremantle site in 1990, and in 1991 800 flowers were produced.

To humans, *D. micrantha* flowers do not give off any detectable fragrance, and yet they attracted small flighted beetles (A. Brown *pers. comm.*). The continued survival of beetle population is dependent upon the success of its individuals in finding food and suitable mates. Two of the primary mechanism involved in insect movement are (1) a simple random dispersion resulting from undirected migration, and (2) a directed movement caused by the release of pheromones. Apparently *D. micrantha* does not employ a distance attractant, such as a scent, to attract its pollinators, as do *Drakaea* and some *Caladenia* species (Stoutamire 1974 & 1983, Peakall 1987 and Carstairs & Coates unpub. reps. 1, 2 & 4), and probably relies on primary mechanism (1) above of insect movement, i. e. the random dispersion of insects, for the initial discovery of its flowers by beetles. Once a beetle is close enough to its flowers *D. micrantha* relies on visual cues to stimulated further pollination responses. *D. micrantha* has a zygomorphic flower with two prominent paddle shaped petals positioned at the back of the flower, and a broad labellum at the front. The petals are probably the first visual attractant for beetles. The brown markings on the yellow labellum, which converge toward the entrance of the flower, is probably a guide pattern and acts as a near goal orientation cue for beetle visitors.

A. Brown (*pers. comm.*) observed beetles aggregating on flowers and mating. Once a beetle has settles on a flower it probably releases pheromones, and primary mechanism (2) above, i. e. directed movement toward pheromones, is the likely cause of beetle aggregation on *D. micrantha* flowers. Male beetles with *D. micrantha* pollinia attached were also observed visiting other open flowers on the same inflorescence, and flowers on other inflourescences (A. Brown *pers. comm.*).

The pollen of *D. micrantha* is not available as food for beetle visitors, and flowers did not produce nectar. Therefore, the only reward received by beetle visitors of *D. micrantha* flowers, is a higher probability of finding a mate(s). Stoutamire (1974, 1983), Peakall (1987) and Carstairs and Coates (this report) found that male thynnid wasps, whose flight is also directed toward sex pheromones, fly actively when air temperature is above 20^0C and the sky is cloudless. At the Fremantle site in 1990 and 1991, no beetles were observed when the temperature was below 20^0C or on cloudy days, and it may be that the same environmental conditions apply for beetle activity as for wasp activity.

The regular cycles of high and low pressure fronts that sweep through southern Australia during late August and September would also control beetle activity, and therefore influence the pollination rate of *D. micrantha.* A passing front is accompanied by a drop in temperature followed by cloudy weather which changes over a period of several days to a short period of warm sunny weather before the next front passes. Thus throughout the flowering period of *D. micrantha*, the actual time spent by beetles pollinating flowers may be brief, a few hours each week, since beetles are active only during these short periods of rising temperatures.

When kept *ex situ* in cool and humid conditions, *D. micrantha* flowers were long lived, 15-20 days. At the Fremantle site in September 1991, some flowers were observed to display for 16 days, and it is likely that the cool wet conditions of *D. micrantha's* preferred habitat promotes flower longevity. The longevity of *D. micrantha's* flowers, its protracted flowering time (45 days for some plants at the Fremantle site in 1991), and multiple flowers per inflorescence, may have long term evolutionary significance for the species. The warm sunny periods required for pollination are brief and infrequent, but when they do occur there is a high probability that plants will have flowers ready to take advantage of any available beetle pollinators.

In the first two weeks of the 1991 flowering season at the Fremantle site 34% of the flowers were fertilized, and a further 56% were fertilized during the next two week period. In all 80% of the flowers were fertilized. In 1990 only 61% of flowers were fertilized. There are two likely reasons for the difference between successive years. Firstly, it may be due to the 1990 flowering season having less favourable weather conditions than those in 1991. This was probably a significant factor. Secondly it may be that pollination rate is positively correlated with flower number. In 1991 there were 60% more flowers than in 1990, and the pollination rate was 31% higher. Given that *D. micrantha* relies on beetles locating its flowers by a simple random dispersion mechanism of undirected migration, then pollination rate would be expected to be positively correlated with flower density. There was a high positive correlation ($r = 0.96$; $P < 0.05$) between the number of flowers displayed simultaneously on an inflorescence and the probability that a flower will be pollinated, and there was also a high positive correlation ($F = 15.17$; $P = 0.027$) between the available number of flowers in the Fremantle population and the rate of pollination. This is consistent with the prediction, and suggests that in this species selection would favour (1) plants that produce and display 2 or more flowers simultaneously, and (2) populations with a high density of flowers. From this it may be concluded that there is a minimum threshold number of flowering plants below which the pollination rate would effectively be zero, and that there is a minimum number of plants below which the pollination rate would not be at its optimum. The polynomial equation that describes the relationship between the available number of flowers in the Fremantle population and the rate of pollination is given by :

$$y = -0.36 + 0.003x^2 - 1.699E\text{-}6x$$

From this it was estimated that 40 flowering plants is the minimum threshold number below which the pollination rate in the Fremantle population would effectively be zero, and below 220 flowering plants the pollination rate is below its optimum. In 1990 there was only 150 flowering plants in the Fremantle population , and so in that flowering season the pollination rate would have been below its optimum potential.

Clearly beetles play an integral part in, and are a necessary requirement for the completion of, the reproductive cycle of *D. micrantha.*

3.4 GENETIC DIVERSITY AND POPULATION STRUCTURE

For general references and a brief review see section 1.4.

3.4.1 Genetic variation and structure within populations.

The isozyme banding patterns of *D. micrantha* were typical of those found in diploid, sexually reproducing plants. Ten of the 14 (71%) loci examined were polymorphic, and each locus averaged 2.9 alleles (Table 3.1). Six (43%) of the loci had 3 alleles, and at these loci the frequency of the common allele was about average, 0.79 (average of all the loci), for polymorphic loci. This was due to the high diversity of two loci (IDH-2 and 6PG-1). The data reflect the invariant nature of *D. micrantha,* and indicate that allozyme variation in this species is not distributed evenly across all the loci, but is restricted to a small proportion of them (28%).

The mean number of alleles per locus, proportion of polymorphic loci, and the expected heterozygosity (*He* = 0.24) for *D. micrantha* was very high. In this respect it is like other donkey orchids with which it is closely related, and contrasts with other Western Australian orchids (Chapters 1,2 4, and 5), European orchids and animal pollinated species (Table 3.1), all of which have lower values for these parameters.

Lower than expected frequencies of heterozygous individuals in *C. elegans* (Chp. 1), another outcrossing orchid, was attributed to its mating system, which involved some autofertilization, and to man-made disturbance which had introduced structuring into its populations. In *D. micrantha* the pattern was similar. At the Fremantle site the mean expected heterozygosity (*He* = 0.26; Table 3.1) was slightly greater than that observed, (*Ho* = 0.22), indicating a slight excess of homozygotes. Wright's fixation index for this population, F = 0.10, was positive which was again indicative of an excess of homozygotes. The genotype frequencies of 20% of the polymorphic loci deviated significantly from Hardy-Weinberg expectations due to an excess of homozygotes. There are two likely explanations for the excess of homozygotes. Firstly, as seen from earlier sections there was a high rate of migration of pollinators between flowers within inflorescences, and the mating system of *D. micrantha* undoubtedly involves partial self-fertilization. Secondly, neighbourhood size is probably small and matings are more than likely to occur between related adjacent individuals within the population. A contributing factor here may also be localised clonality.

3.5 RECOMMENDATIONS FOR CONSERVATION OF GENETIC RESOURCES AND MANAGEMENT

3.5.1 Strategies for conserving genetic resources.

For general references and a brief review see section 1.5.1

From the investigations of *D. micrantha* described above it was shown that Populations 1 and 2 are geographically isolated from each other, and vary slightly in their flowering times. Although Population 2 is a recent discovery and requires further investigation, it is recommended that both populations be managed and protected to adequately conserve the genetic resources of this species.

In addition to determining the minimum number of populations required to conserve the genetic resources of *D. micrantha* it is also important to determine the minimum viable size (MVP) of a population for that purpose. In this regard mechanisms which maintain or increase heterozygosity may be critical. High levels of heterozygosity in two Western Australian ground orchids (Chapter 1 and Peakall 1987) can be attributed to post-zygotic selection mechanisms which elevate the frequency of heterozygotes in adult populations. There was no direct evidence to indicate that similar system is operating in the Fremantle population of *D. micrantha*, in spite of the amount of self pollination that occurs in this population and the structuring which can promote crossing between near relatives. However, only 20% of the polymorphic loci were not in Hardy-Weinberg equilibrium due to an excess of homozygotes. This suggests that there may be a system in the population operating to elevate the frequency of heterozygotes in the reproducing individuals.

Nei *et al.* (1975) and Chakraborty & Nei (1977) have shown that if population sizes are severely reduced, to say <10 individuals, they may well suffer a reduction of allelic frequency and average heterozygosity per locus. However, as *D. micrantha* appears to have a system operating in its Fremantle population which selects for heterozygosity, it may be that it could tolerate being reduced to very small numbers without losing a significant amount of genetic diversity due to drift. In previous sections it was shown that there is a minimum threshold number of flowering individuals ($Ne = 40$), below which the reproductive rate is effectively zero. If the Fremantle population were to be reduced to this number for any considerable length of time, it is more likely that the population would go to extinction for stochastic reasons before it suffered any ill effects from the loss of genetic diversity.

Research has indicated that the Fremantle population of *D. micrantha* may be subdivided into genetically different neighbourhoods with limited gene flow between them. When populations are subdivided in this way, it is important to preserve as much of the entire population as possible because the loss of a portion of it may be as critical as the loss of the entire population (Hamrick 1983, Samson *et al* 1990). Distribution maps of the alleles in the populations, particularly those of the highly variable loci, namely PGI-2, IDH-2, PGM-1 and 6PG-1, would provide managers with useful markers for delimiting sub-populations.

Both *D. micrantha* populations were found to occupy very small areas of land (about 4 acres), and will therefore require some management to prevent their degradation.

Whereas the recovery of the original ecosystem may not be possible for the Fremantle population, the rehabilitation or restoration of certain desirable attributes may be possible.

3.5.2 Management actions

3.5.2.1 Liaison with landowners and shires.

At present the survival of the *D. micrantha* populations relies on the good will of local shires and landholders of adjacent private land. CALM staff are required to provide landholders and other agencies with advice regarding the conservation and management of populations of Rare Flora on land under their control. Private landholders with land adjacent to road verge populations should be requested to arrange their operations so that the area of Rare Flora will not be destroyed or damaged in any way.

3.6.2.2 Land acquisition

There are no *D. micrantha* populations on land which has been reserved for the conservation of flora and fauna. Therefore, it is strongly recommended that the department of CALM acquire land on which *D. micrantha* occurs. Ideally both Populations 1 & 2 should be conserved, but if this is not possible then priority should be determined by:

(a) the size and quality of the site and its habitat,
(b) conserving the range of genetic and morphological diversity, and
(c) reducing the risk of sudden extinction.

Although Population 2 has not been researched as much as Population 1 initial reports indicate that it is at present the larger of the two, and is the least degraded site. Population 2 also flowers later. If both populations are preserved then the known range of genetic and morphological diversity of this rare flora would conserved, and the risk of its sudden extinction would be reduced. Although 250 plants spread over about 4 acres (Population 1) of natural habitat constitutes a MVP, the preservation of viable populations of beetle pollinators must also be considered. Therefore, to ensure and enhance the survival of *D. micrantha* it is essential to provide and maintain areas containing a suitable variety of species and habitats. For this purpose a minimum reserve area of 5 ha is recommended. Both Populations 1 & 2 occur on uncleared sites that approach this size.

3.5.2.3 Protecting from grazing

With the onset of summer the winter wet depressions where *D. micrantha* grows become the preferred grazing sites for rabbits. Although rabbits are most prevalent in *D. micrantha* sites when there are no above ground parts of the orchid they are still a serious threat to *D. micrantha's* long term survival. By the beginning of autumn (April) in 1990 & 1991 rabbit droppings were scattered throughout the Fremantle site, and there were several areas of very high density. Large quantities of urea can be released in very small areas, raising the nitrogen and acids levels of the water and soil. This appears to have resulted in the death of associated plant species at the Fremantle site. Further, it is providing ideal conditions for weed establishment and growth. In 1974 this site was virtually weed free (A. Brown *pers. comm.*) whereas now weeds are a serious problem.

Rabbits appear to be one of the primary causes of increased habitat degradation at the Fremantle site. It is therefore recommended that either the APB institutes a biannual eradication program for both sites, to be conducted in the summer months, or the sites are fenced to exclude all herbivorous mammals.

3.5.2.4 Protection from accidental destruction

D. micrantha is extremely vulnerable to accidental damage or destruction because of the small areas occupied by both populations. Protection from accidental destruction by off road vehicles, bulldozing, rubbish dumping, and spraying of potentially damaging herbicides and insecticides is strongly recommended.

In the 12 day period between July 15 - 27, 1990, 6% of the Fremantle site was damaged by off road vehicles. This was equivalent to a 35% increase in off road use of this site by vehicles. Subsequently CALM officers constructed fences around the area to divert traffic away from the *D. micrantha* site.

3.5.2.5 Protection from fire

D. micrantha does not require fire to complete its life cycle and should be protected from uncontrolled fires. Research has indicated that adult plants are most vulnerable during their vegetative stage (April-August), when replacing their parent tuber(s), and particular care must be taken to ensure that they are not destroyed by fire at this stage.

The adjacent area west of the Fremantle site gets burnt most summers. During a 1984 burn the south-west corner of the Fremantle site was also burnt, removing all of the native vegetation (A. Brown *pers. comm.*). Weeds quickly recolonised the area, and in 1991 the area was still weed infested and there was little evidence of regeneration of local vegetation. It is recommended that, where possible, fire breaks be constructed around the populations and that a fuel reduction programme be adopted for adjacent areas.

3.5.2.6 Weed control

The control of weeds in or near both populations is desirable. CALM officers should liaise with other CALM staff with expertise in the area, the APB, Main Roads Department (MRD) and private landowners.

Weeds should be removed by hand with minimum soil disturbance where use of selective herbicides may damage *D. micrantha* or other native species. Weed control should be exercised after *D. micrantha* has completed flowering, and before the weeds shed their seed. It is recommended that weeds be controlled biannually late in the month of October. Department of CALM Information Sheets Nos. 1-87 and 2-88 provide information on the control of weeds in natural and direct seeded regeneration areas.

3.5.2.7 Linear marking

Both *D. micrantha* populations are located on and adjacent roads and are subject to damage by maintenance operations. Linear marking would provide a minimum protection for these populations. The MRD has developed a field marking system for demarcating environmentally significant areas on road reserves, and local shires have been encouraged to adopt this system (Kelly *et al.* 1990).

3.6.2.8 *EX SITU* CONSERVATION

Intensive genetic management has yet to be carried out on populations of any endangered native Australian plant, however it is a viable option and needs to be considered in the future if extinction in the wild is to be prevented (Hopper and Coates 1990). Hopper and Coates (ibib.) suggest that plant species s may be managed by cultivation followed by their re-introduction or translocation into areas where they were previously known to exist. The preservation of *D. micrantha* in its natural habitats is the priority, however the *ex situ* conservation of this species is also advisable to reduce the possibility of its sudden extinction through catastrophes such as fires or disease.

In vitro propagation and cultivation techniques have been developed for *D. micrantha* at Kings Park and Botanic Gardens (Collins, 1993; unpublished report to CALM) which provide an ideal means for the *ex situ* conservation of this species. This material can also be readily used for population enhancement if required.

3.5.2.9 Rehabilitation of existing populations

As both populations of *D. micrantha* occur on road verge their rehabilitation is required to fulfil the objective of management. The area of both sites to be preserved should be increased to 5 ha. It should be done in co-operation with landowners, Shires and the MRD. Officers are referred to the Department of CALM Information Sheets Nos. 5-87 and 2-88, and Edmiston (1987) for advice on methods of rehabilitation.

Hopper and Coates (ibib) recommend that caution be exercized in the rehabilitation of degraded lands, and that local seed stock of associated species be used if viable breeding populations are to be maintained.

3.5.2.10 Monitoring

Ehrlich and Murphy (1987) and Hopkins *et al.* (1987) among others, have outlined the value of monitoring in populations. Quadrats stationed permanently in Population 1 have already provided useful demographic information about this population, and biannual monitoring of it should continue. Similarly quadrats should be stationed in Population 2 and monitored.

Table 3.1. Summary of allozyme variation, based on 14 loci, in 1 population of *Diuris micrantha*. These data are compared with that of other orchid species. Ne, estimated number of flowering plants; N, mean sample size per locus; P, proportion of polymorphic loci; A, mean number of alleles per locus; *Ho*, mean observed heterozygosity; *He*, mean expected heterozygosity; and *F*, mean fixation index (Wright).

Species	Ne	N	*P*	*A*	*Ho*	*He*	*F*	Source
AUSTRALIAN								
Diuris micrantha	**250**	**45.1**	**0.71**	**2.9**	**0.22**	**0.24**	**0.10**	**1**
Diuris laxiflora			0.64	2.9	-	0.26	-	1
Diuris carinata			0.78	2.7	-	0.26	-	1
Caladenia varians spp.*pendens*			0.42	1.8	-	0.16	-	2
Caladenia elegans			0.54	2.1	0.12	0.15	0.10	1
Caladenia maritima			0.36	1.8	0.19	0.17	-0.08	1
Drakaea elastica			0.45	1.7	0.16	0.16	-	1
Rhizanthella gardneri			0.5	1.6	0.04	0.11	0.58	1
EUROPEAN								
Orchis morio			0.48	1.7	-	0.12	-	3
O. longicornu			0.54	1.9	-	0.16	-	3
Animal pollinated			0.50	1.8	-	0.15	-	4
Selfing			0.20	1.3	-	0.07	-	4
Wind-dispersed seed			0.43	1.7	-	0.12	-	4

Sources of data: (1) this study, (2) Carstairs (unpublished data), (3) Rossi *et al.*(1992), (4) Hamrick & Godt (1990).

Figure 3.1. Location of *Diuris micrantha* populations in relation to CALM Regions.

4. *DRAKAEA ELASTICA* (THE VIRGIN PRAYING ORCHID)

Drakaea elastica the praying virgin orchid was first described by Lindley (1840) and was subsequently called *Drakaea jeanensis* by Rogers (1920). In 1989 Clements reinstated it as *D. elastica* (see Hoffman and Brown, 1992). It is morphologically uniform throughout its range, readily distinguished from other hammer orchids, and is often found growing with *D. livida*, and *D. glyptodon*.

4.1 DISTRIBUTION AND CONSERVATION STATUS

D. elastica has been extensively searched for in the wild and occurs in the CALM Northern Forest, Swan and Central Forest Management Regions (Fig. 4.1, Table 4.1). Populations are restricted to the coastal plain between Bindoon and Busselton. Four *D. elastica* populations occur on three nature reserves, and the remaining populations are on private or Crown Land some of which are on degraded road verge. The locations, conservation status and condition of the sites are summarised below. Precise locality details are contained on CALM Departmental Rare Flora database and files.

Population 1 - near Bindoon. September 1985: 2+ adult plants were distributed over a small area. This population is on private property, in near virgin bush surrounded by paddocks. The current land owner is a keen conservationist and wishes to maintain the site in its present condition.

Conservation status: Although not presently in danger of extinction, this population is distributed over a small area and is therefore at risk through depletion over a longer period. Special attention is required for its long-term survival.

Population 2 - Mandogalup, Metropolitan Area south-west of Perth. October 1982: 30+ adult plants were distributed over a 1/2 acre area. The bulk of the population is on the uncleared portion of a residential block, and a few plants occur on the two adjoining residential blocks. The current owners of the block containing the bulk of the population are keen conservationists and wish to maintain the site in its present condition. Notwithstanding this the population has declined in numbers and in 1991 was about one third of its 1982 size (L. Mutter *pers. comm.*).

Conservation status: Although not presently in danger of extinction at this site, this small population was distributed over a small area and is therefore at risk through continued depletion over a longer period. Special attention is required for its long-term survival.

Population 3 - Metropolitan Area south of Perth. October 1986: 7 adult plants were distributed over a small area on private property (A. Brown *pers. comm.*). No plants were located at this site in July 1991 although the habitat was in pristine condition. The site has been zoned for semi-rural use, and is to be developed in the future. The current land owner(s) needs to be informed of the presence of a Declared Rare Flora on this site.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

Population 4 - Armadale, Metropolitan area south of Perth. October 1978: 2+ plants were distributed over a small area. This small road verge population was on Crown Land adjoining a public golf course. Although the habitat was in a healthy condition no plants were located in 1986, and have not been sighted in recent years (A. Brown *pers. comm.*).

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

Population 5 - south of Byford. July 1991: 19 adult plants were distributed over a 10 meter square area on private property. This population occurs on the uncleared portion of a paddock used for grazing sheep.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

Population 6a & 6b - Lowlands. (6a) July 1991: 16 plants were distributed over a small area in pristine bush along the edge of a fire break on private property. (6b) July 1991: 4 plants were located 200 m from 6a, and were distributed over a small area in pristine bush on private property.

Conservation status: Although not presently in danger of extinction at these sites, these small populations are distributed over small areas and are therefore at risk through depletion over a longer period. Special attention is required for their long-term survival.

Population 7a & 7b - West of Pinjarrah. (7a) September 1991: 300 plants were distributed over a 200 square meter area along a disused track in otherwise pristine bush on a nature reserve. (7b) September 1991: 150 plants were located 200 m from 7a, and were distributed over a 200 square meter area along a disused track in otherwise pristine bush on a nature reserve.

Conservation status: Although not presently in danger of extinction at these sites, these populations are distributed over small areas and are therefore at risk through depletion over a longer period. Special attention is required for their long-term survival.

Population 8 - West of Pinjarrah. September 1991: 4 plants were distributed over a small area in pristine bush on a nature reserve.

Conservation status: Although not presently in danger of extinction at this site, this population is distributed over a small area and is therefore at risk through continued depletion over a longer period. Special attention is required for its long-term survival.

Population 9 - West of Waroona. October 1978: 1 adult plant occurred near a road verge on private property. The area of land adjacent to this site has since been cleared, and although the habitat of the *D. elastica* site is still intact no plants were found there in 1985.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

<u>Population 10</u> - Near Yarloop. October 1979: 7+ adult plants were scattered over a 20 m square area near the road verge on a recreation and camping reserve. Current land status and the present condition of this population needs to be assessed.

Conservation status: Although not presently in danger of extinction at this site, this population was distributed over a small area and is therefore at risk through continued depletion over a longer period. Special attention is required for its long-term survival.

<u>Population 11</u> - West-south-west of Yarloop. October 1977: 2+ adult plants were scattered over a small area of pristine bush near a flora and aquatic wildlife conservation reserve (# 12682, 4778). Land status and the present condition of this population needs to be assessed.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

<u>Population 12</u> -North-west of Brunswick Junction. June 1987: 300 adult plants were distributed over a 1/4 acre area on two adjacent private properties.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

<u>Population 13</u> - Near Capel. August 1991. 63 adult plants were distributed over a small area on a Public Utility (# 3249), near a degraded road verge and adjacent to cleared paddocks.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

<u>Population 14</u> - Ruabon. August 1990. 9 adult plants were distributed over a small area on a nature reserve. Adjoining the reserve and near the orchid population is a domestic refuse dumping area.

Conservation status: This population is in serious danger of disappearing from this site within a decade if present land use and other causal factors continue to operate. Special attention is required for its short-term survival.

Most of the land throughout the range of *D. elastica* has been cleared for agricultural or urban use leaving little evidence with which to reconstruct an accurate distribution and density map for this species. Most of the few populations reported by botanists were from easily accessed areas with 21% of reported populations occurring in the Perth Metropolitan area, and another 36% found near the South West Highway. It is likely however, that before land clearing *D. elastica* consisted of many small disjunct populations and that some populations may still remain undiscovered.

4.2 HABITAT AND ASSOCIATED SPECIES

D. elastica grows on white sand over a dark sandy loam on flat damp areas near ephemeral lakes, or on the slopes adjacent to winter wet depressions, swamps and water causes. The vegetation associated with *D. elastica* was Banksia (*B. attenuata, B. ilicifolia* and *B. menzesii*) woodland with scattered Marri (*E. calophylla*) and *Allocasuarina frazeriana*. All of the populations, except for Population 5, occur in *Kunzia ericifolia* thickets.

Other orchids found with *D. elastica* included *Acianthus reniformis* var. *huegelii, Caladenia flava, C. reptans, Drakaea glyptodon, D. livida, Leporella fimbriata, Elythranthera brunonis, Lyperanthus nigricans, Paracaleana nigrita, Prasophyllum parvifolium, Pterostylis nana, P.* aff. *nana, P. recurva, P. vittata* var. *vittata* and *Thelymitra* sp.

The area has a Mediterranean climate. The summers (December-March) are hot and dry, and winters are cool and wet. Most rainfall occurs in late autumn, winter and early spring, and the mean annual rainfall at Perth is 870 mm (Bureau of Meteorology).

4.3 LIFE HISTORY

Throughout its range *D. elastica* experiences environmental conditions that are cyclical and therefore very predictable. Short, cool and wet winters alternate with long, hot and dry summers. Under such conditions *D. elastica* like other terrestrial orchids in this study (Chapters 1,2, 3) has developed physiological and morphological traits, such as underground perenating tubers, which are regarded as being of selective advantage in promoting good establishment and carryover of reserves within and between seasons (Pate and Dixon 1982).

Flowering occurs annually and toward the end of the growing season. This has advantages because pollinators were then in highest frequency, and resources from current photosynthesis could be harnessed directly into flower development and seed production.

4.3.1 Phenology of growth and reproduction.

In late autumn (April-May) underground tubers of *D. elastica* commenced growing (Table 4.2). In expanding the new seasons' shoot, roots and leaf, most of the water and carbohydrate reserves of the tuber are consumed. Thereafter, completion of the plant's growth and reproductive cycle depends on the photosynthetic gains made by the new foliage. From June to September the products of photosynthesis are directed toward producing replacement tubers. The inflorescence begins to elongate in July and develops slowly over winter and early autumn. In September the inflorescence reaches its full height and in late September and early October the single floral bud matures and opens. During October the leaf shrivels, and capsule and seed maturation relies on the nutrient stores in the scape. In late October and early November the capsule dries and dehisces, releasinf very large amounts of seed. The underground perenating tubers are all that remain over the summer months.

Seed germination relies on the presence of a species specific mycorrhizal fungus. This symbiotic relationship is essential for any seedling establishment. Although very large quantities of seed are released from each capsule only a fraction become mature plants.

4.3.2 Population dynamics and factors affecting population size

4.3.2.1 Population size and disturbance

The estimated sizes of the *D. elastica* populations at different sampling times for the recorded sites are presented in Table 4.1. Not only do these data demonstrate the differences in population sizes between sites, but also that at the same site the population size varies between sampling times. The Capel (7931) population, which comprised more than a 100 ramets in 1984, was reduced to 4 plants in 1986, experienced a resurgence in 1990 when there were 21 ramets, and then trebled its size in 1991 when there were 63 ramets. At the Carrabungup Nature Reserve S1 site in 1990 there were 700 ramets, and in the following year the population fell to 250 ramets. In the same period the Carrabungup Nature Reserve S2 population doubled its 1990 size of 50 ramets, when in 1991 100 ramets were recorded.

Population size (number of plants), for the various sampling times at the Ruabon site ($n = 3$) and the sites mentioned in the previous paragraph ($n = 9$), was plotted against autumn (April-May) rainfall and was found to be highly correlated with it ($p = 0.022$). High population sizes were associated with high autumn rains, and low population sizes were associated with low autumn rains. This indicates that autumn rainfall is a good predictor of population size in the forthcoming season at any given site.

This study demonstrates the value of long term (decades) monitoring programmes of many sites for the management of this rare flora, and indicates that caution needs to be taken when assessing the conservation value of populations. Of the *D. elastica* sites listed in Table 4.1 60% had population sizes of less than ten individuals. Of these 75% had been assessed only one or two times. Long term monitoring of sites, particularly after favourable autumn rains, would provide accurate estimates of their population sizes, from which their conservation value may be meaningfully assessed.

In order to understand more fully how environmental conditions affect the survival of plants, and therefore population size, 4 permanent 1/2 meter square quadrats (SQ1, SQ2, SQ3 and SQ4) were established at the Carrabungup Nature Reserve S1 site and monitored during 1990 and 1991 (Tables 4.2 and 4.3). Three quadrats (SQ1, SQ2 and SQ3) were exposed to approximately 4 hours of direct sunlight during the day, while a fourth sheltered quadrat (SQ4) was exposed to an hour of direct sunlight. In 1991 two permanent 1/2 meter square mesh enclosed quadrats (MQ1 and MQ2) were also established and monitored at the S1 site. Also in 1991 two permanent quadrats, one standard type (SQ5) situated in a sheltered area and one mesh enclosed (MQ3), were established and monitored at the Carrabungup Nature Reserve S2 site. The objective of this study was to compare the survival rate of plants in naturally or artificially sheltered conditions with that of plants which were exposed.

At the S1 site in 1990, there was no apparent difference in the survival rates of plants in exposed or sheltered conditions (Table 4.2). However only 27% of the plants in exposed quadrats (SQ1, SQ2 and SQ3) in October 1990 survived and were recorded in May 1991 compared with 87% of the plants in the sheltered quadrat (SQ4). By October 1991 only 7.5% of the plants recorded in the exposed quadrats in May were alive compared with the sheltered quadrat in which all of the plants recorded in May had survived (100% survival rate). Over this period seedlings increased the size of the sheltered quadrat population by 131%. At the S1 site the survival rate of plants in artificially sheltered quadrats was the same as that of plants in naturally sheltered conditions (100%), and their populations sizes were also increased by the addition of seedlings (Table 4.2). At the S2 site the plants in naturally and artificially sheltered conditions also had high survival rates, 93% and 82% respectively (Table 4.2).

The poor survival rate in 1991 of plants exposed to 4 hours of sunlight at the S1 site may be attributed to abiotic (environmental) and biotic factors. The average daily temperature in April 1991, the month when the underground tubers sprout, was hotter than the average by 1.8 degrees Celsius. It is possible that these unusually hot conditions caused some tubers in exposed areas to remain dormant. In spite of above average April rains some tubers in exposed areas may have sprouted but died from desiccation before reaching the earths surface, while it was noted that some young plants had dried out and died at the earths surface. These unseasonably warm conditions continued into May and June 1991 when the average daily temperatures were approximately 0.6 degrees Celsius above the average. During these months it was noted that the plants in the warmer exposed areas were being heavily grazed by insects. The warm conditions, higher than average rainfall and fresh insect injuries resulted in a high incident of fungal infection and death in plants in exposed quadrats. In contrast with this none of the plants in the naturally sheltered quadrat or the artificially sheltered quadrats appeared to suffered from heat stress or lack of water. In the cooler sheltered areas and in the artificially sheltered quadrats there was no evidence of insect attack and no fungal infection (Table 4.3). Consequently the survival rate of plants in cool sheltered areas was 100% compared to 7.5% in warmer exposed areas.

At the end of the 1991 season (October) the average number of plants in the 4 standard quadrats at the S1 site was 65% lower than the October 1990 average. This value reflects the estimated change in the overall size of the S1 population between these years, as in 1991 the population had declined to be only 36% of its 1990 size, see Table 4.1. This suggests that the observed changes in the plants frequencies in the quadrats used in this study reflect the changes in the whole of the S1 population for the same period.

4.3.2.2 Population size and disturbance

The size of *D. elastica* populations was also highly correlated with disturbance to the habitat. The mean population size at sites which had a recent (< 7 yrs.) history of disturbance was 144 plants (n = 6), whereas undisturbed sites had a mean of 6.5 plants per population (n = 11). The difference between the size of populations at disturbed and undisturbed sites was highly significant (Chi square. = 127.61; P < 0.05). *D. elastica* is therefore considered to be a 'disturbance opportunist', and its preference for disturbed sites may be attributed to the gross morphology of its vegetative plants. They consist of a small (usually <2.0 cm) heart shaped leaf, which is adpressed to the ground, and most frequently occur in small clearings where there are few herbaceous competitors. The types of disturbance with which high population size was correlated included fire (Population 2), disused off road vehicle tracks (Populations 7a and 7b), kangaroo runs (Population 7a), fire breaks (Populations 12 and 13) and farm stock (population 5).

4.4 GENETIC DIVERSITY AND POPULATION STRUCTURE

For general references and a brief review see section 1.4.

4.4.1 Genetic variation and structure within populations

The isozyme banding patterns of *D. elastica* were typical of those found in diploid, sexually reproducing plants. Thirteen of the sixteen (81%) loci examined were polymorphic, and the average number of alleles per locus was 2.75 (Table 4.4). There was a negative correlation between the number of alleles and the frequency of the common allele ($r = -0.98$; $P < 0.05$). These data reflect the invariant nature of *D. elastica*, and showed that most of the variation in this species was distributed amongst 25% of the loci.

Of the seven *D. elastica* populations examined, populations 7902 and 7904b had the least number of individuals (G = 3; Table 4.4), and had fewer alleles per locus (A = 1.2 and 1.3 respectively) than the other populations. There was a significant correlation between population size and frequency of alleles per locus ($r = 0.86$; $P < 0.05$), indicating that as the size of the populations increased the amount of genetic variation contained within them also increased.

The mean expected heterozygosity ($He = 0.16$) for *D. elastica,* like other insect pollinated plant species, was high (Table 4.5), but lower than that found in widespread *Drakaea* species. The total genetic diversity ($Ht = 0.25$; Table 4.5) of *D. elastica* was most like that of *Caladenia elegans*, which is also a regionally distributed species.

Peakall (1984) estimated the self pollination rate to be high in *Drakaea glyptodon*, but found that the observed frequency of heterozygotes in its populations were not lower than the expected frequency of heterozygotes as predicted by its mating system. A similar result to this was also found in *D. elastica* where the observed frequency of heterozygotes was the same as the expected frequency (Table 4.5). This was also reflected in the values of the fixation index (F) for loci within populations (Table 4.6). In all populations or sub-populations with the exception of sub-population S1b F was either zero or less than 0. Moran & Bell (1983) and Ledig (1986) found in some plant species that heterozygosity increased as cohort age increased. It may be that a similar mechanism is operating in populations of *D. glyptodon* investigated by Peakall (1987) and populations of *D. elastica*.

4.4.2 Differentiation between populations and gene flow

Most of the genetic diversity was partitioned within *D. elastica* populations ($Hs = 0.2$) and the coefficient of differentiation between them was high ($Gst = 0.21$; Table 4.7). In this respect *D. elastica* was like other insect pollinated plants, but contrasted with other orchid species (Table 4.6).

Cluster analysis using genetic distance (Figure 4.2) supports the conclusion that there has been considerable divergence between the populations analysed in this study.

Gene flow was calculated using Wright's method (1951):

$$Nm = \frac{1 - Gst}{4Gst}$$

indicating a low rate of gene exchange (0.95 migrants per generation, Table 4.8) between all of the *D. elastica* populations sampled..

4.5 RECOMMENDATIONS FOR CONSERVATION OF GENETIC RESOURCES AND MANAGEMENT

4.5.1 Strategies for conserving genetic resources

For general references and a brief review see section 1.5.1

In the previous sections it was established that few *D. elastica* populations occur in undisturbed vegetation systems. It was also established that the population sizes at many sites may be grossly underestimated as may be the estimates of genetic diversity in presumed small populations. In order to maintain the genetic diversity of this rare flora, certain management strategies need to be implemented. Some of the management strategies recommended by Hopper and Coates (1990) for that purpose, include selection of priority populations for conservation, reserve and corridor design, and active management of species through propagation and translocation in the wild.

Based on the level of differentiation between populations Hopper and Coates (1990) were able to recommend priority populations for the conservation of genetic diversity in three distinctly different species, *Eucalyptus caesia, E. diversicolor* and *Banksia cuneata*. The distribution of genetic resources in *D. elastica* is similar in many respects to *Eucalyptus caesia,* that is, each population is genetically distinct. From the genetic distance dendrogram for population divergence (Figure 4.2), it was concluded that there is significant interpopulation differentiation in *D. elastica.* Based on these data the preservation of several populations, representing its distributional range, is recommended to ensure adequate short term protection of the genetic resources of this species.

A second consideration is to estimate the minimum viable size (MVP) of a population for the purpose of preserving the species' genetic resources. In *D. elastica* populations heterozygotes occur more often than expected given that it has a high self pollination rate and no self incompatibility (Table 4.4) . This suggests there may be post-zygotic selection mechanisms operating in this species to elevate the frequency of heterozygotes in the adult population. This would serve to offset the effects of inbreeding in small populations, i.e. loss of alleles and reduced total genetic diversity. Nei *et al.* (1975) and Chakraborty & Nei (1977) have shown that if population sizes are severely reduced, to say <10 individuals, they may well suffer a reduction in allelic diversity and average heterozygosity per locus. It is therefore recommended that populations in excess of 10 individuals may serve the purpose of preserving the genetic resources of this species.

4.5.2 Management actions

4.5.2.1 Liaison with land owners and shires

At present the survival of 75% of *D. elastica* populations relies on the good will of local shires and private land owners. CALM staff are required to provide landholders and other agencies with advice regarding the conservation and management of populations of Rare Flora on land under their control. Owners with land adjacent to road verge populations should be requested to arrange their operations so that populations will not be destroyed or damaged in any way. In order of priority, the populations for staff liaison with landowners are:

(a) Populations 12, 3 and 5
(b) Populations 2, 6, 1, and 13
(c) Populations 9, 11, 4 and 10

4.5.2.2 Protection from accidental destruction

D. elastica is vulnerable to damage or destruction owing to the small areas occupied by its populations, and should be protected from accidental destruction by bulldozing, rubbish dumping, and spraying of potentially damaging herbicides and insecticides.

In order of priority, the following sites should be protected from accidental destruction:

(a) Populations 7, 14 and 8
(b) Populations 5, 13 and 10
(b) Population 6, 1 and 3

4.5.2.3 Protection from fire

D. elastica does not require fire to complete its life cycle. However, research suggests that *D. elastica* is a disturbance opportunist which benefits from fires which open up the heath thereby reducing competition for space and light, and provide a source of nutrients for the growth of mycorrhiza on which *D. elastica* seedlings depend for their establishment and growth.

The number of adult plants in quadrats stationed permanently in Populations 7a and 7b fluctuated considerably between 1990 and 1991, demonstrating the vulnerability of *D. elastica* populations to environmental perturbations. It is therefore recommended that similar quadrats be stationed in other populations, and that they be monitored biannually. If the number of plants in any of the quadrats shows a significant and continued decline then the whole site should be considered for a controlled burn.

Since adult plants are most vulnerable to fire during their vegetative growth stage (May-October), when replacing their parent tuber(s), it is strongly recommended that this species be excluded from fire during this period. The months of April or November are the recommended times for controlled burns.

In order of priority, the following sites should be protected from uncontrolled burning:
(a) Populations 7, 14 and 8
(b) Populations 13 and 10
(c) Populations 6, 1 and 5

4.5.2.4 Weed control

The control of weeds in sites preserved for the conservation of *D. elastica* is desirable. *D. elastica* inhabits the coastal plain between Bindoon and Dunsborough, most of which has been cleared for agriculture making all populations vulnerable to weed invasion. CALM officers should liaise with other CALM staff with expertise in the area of weed control, the APB, Main Roads Department (MRD) and private landowners.

Weeds should be removed by hand with minimum soil disturbance where use of selective herbicides may damage *D. elastica* or other native species. Weed control should be exercised before *D. elastica* begins flowering, and before the weeds shed their seed. It is recommended that weeds be controlled biannually in the months of June and July. Department of CALM Information Sheets Nos. 1-87 and 2-88 provide information on the control of weeds in natural and direct seeded regeneration areas.

In order of priority, weeds should be removed from the following sites:
(a) Populations 7, 14 and 8
(b) Populations 13 and 10
(c) Populations 6, 1 and 5

4.5.3.5 Linear marking

Three populations (12, 11 and 9) are located on road verge and are subject to damage by maintenance operations. Linear marking would provide a minimum protection for these populations. The MRD has developed a field marking system for demarcating environmentally significant areas on road reserves, and local shires have been encouraged to adopt this system (Kelly *et al.* 1990).

4.5.3.6 *Ex situ* conservation

The preservation of *D. elastica* in its natural habitats is the priority, however the *ex situ* conservation of this species is also advisable to reduce the possibility of its sudden extinction through catastrophes such as fires or disease. This may be achieved through cultivation and long-term seed storage.

The underground tubers of *D. elastica* would provide the most suitable material for germ plasm storage and propagation of this species. Many West Australian ground orchids persist as potted plants in shade houses for many years, and *D. elastica* would be a suitable candidate for this method of *ex situ* conservation.

Samples of 1 or 2 tubers could be collected from plants having 5 or more ramets. Tubers should be collected at the end of the flowering season in such a manner as to minimize the disturbance to the remaining tubers. The tubers should be representative of the range of genetic and morphological diversity, and in practice all of the populations could be sampled. Within populations, sampling should be random.

In order of priority, the following sites should be considered for *ex situ* collections:
(a) Populations 12, 3, 4, 9 and 11
(b) Populations 1, 2, 5, and 6
(c) Populations 10 and 13
(d) Populations 7, 14 and 8

4.5.3.7 Rehabilitation and maintenance of existing populations

Rehabilitation of road verge and shire utility and recreational reserve populations is required to fulfil the objective of management. It should be done in co-operation with landowners, Shires and the MRD. Officers are referred to the Department of CALM Information Sheets Nos. 5-87 and 2-88, and Edmiston (1987) for advice on methods of rehabilitation.

The shire rubbish dump situated alongside Population 14 should be relocated and the area rehabilitated. A large portion of the *K. ericifolia* population at the site of Population 14 has been removed by horticulturalists for bean and tomato stakes. This site needs rehabilitating, and measures (eg. signs, publicity) should be taken at this and all of the *D. elastica* sites to prevent further habitat destruction of this kind.

Research has shown that to conserve the range of genetic diversity found in *D. elastica* many populations would need to be preserved, and that a few plants spread over a small area of natural habitat constitutes a MVP. Although this suggests that a suitable management strategy for this species might be the preservation of many small reserves, this would not necessarily fulfil management goals if viable populations of thynnid wasp pollinators were not also conserved with the orchid. Therefore, to ensure and enhance the survival of *D. elastica* it is essential to provide and maintain areas containing a suitable variety of species and habitats. For this purpose it is recommended that minimum reserve areas should exceed 5 ha.

In order of priority, the following sites should be rehabilitated:
(a) Populations 14, 13 and 10
(b) Populations 5
(c) Populations 11, 9 and 4

4.5.3.8 <u>Artificial gene flow</u>

Artificial gene flow between existing populations is not recommended. There is significant genetic divergence between the *D. elastica* populations and in the absence of information about the possible adverse effects (see James 1982) of direct cross pollination between plants from geographically distant populations, artificial gene flow between them is not advised.

4.5.3.9 Monitoring

Ehrlich and Murphy (1987) and Hopkins *et al.* (1987), among others, have outlined the value of monitoring in populations. A quadrats stationed permanently in Population 7a and 7b have already provided useful demographic autecological information about these populations, and biannual monitoring of them should continue. Similarly quadrats should be stationed in the other populations and monitored.

In order of priority, permanent quadrats should be established in the following populations, and monitored:

(a) Populations 7a and 7b
(b) Populations 6 and 13

Table 4.1. Locations and estimated sizes of *D. elastica* populations

Population No	Name	Popn #	Estimated # of ramets per year (1977-1991)										
			77	78	79	82	84	85	86	87	89	90	91
4	Armadale			2					0				
1	Bindoon NW							2	0				
12	Brunswick Jn									>500			
13	Capel	7931				<100	>100		4			21	63
7a	Carrabungup	S1									>300	700	250
7b	Carraburyup	S2										50	100
6a	Lowlands	7904a*										7	12
6b	Lowlands	7904b*											4
2a	Mandogalup A					7			0				0
2b	Mandogalup B					25			0				
2c	Mandogalup C								3				
8	Mealup N.R.	7911*											4
5	Pioneer	7902*											18
14	Ruabon							6	15			9	
9	Waroona			1					0				
3	Wharton Rd								5				0
11	Yarloop		2			0							
10	Yarloop N				7				2				

* New populations discovered during the course of the project

Table 4.2. ***Drakaea elastica*** **population dynamics at the Carabungup Nature Reserve S1 site for the 1990 and 1991 seasons and at the S2 site for the 1991 season from observations of ramets protected by rabbit exclosures.** SQ and postscript number, designates permanent 1/2 meter square rabbit exclosures; R, the number of ramets per quadrat for the given date; i, the number of inflourescences per quadrat; fl, the number of flowers; fr, the number of fruits; Ps, the number of ramets which were "seedlings".

Year		1990				1991						
Month		Ju	A	O	N	May	Ju	Jy	A	S	O	N
Day		14	23	12	15	26	28	13	25	26	10	11
S1 exposed quadrats receiving 4 hours full sunlight.												
SQ1	R	15	18	19	0	0	0	0	0	0	0	0
	i	7	4	1	0	0	0	0	0	0	0	0
	fl	-	-	1	0	0	0	0	0	0	0	0
	fr	-	-	-	0	0	0	0	0	0	0	0
SQ2	R	32	37	38	0	18	2	1	2	3	2	0
	i	15	7	3	2	-	1	0	0	0	0	0
	fl	-	-	3	0	-	-	-	-	-	0	0
	fr	-	-	-	0	-	-	-	-	-	-	0
SQ3	R	25	23	46	0	10	2	1	1	0	0	0
	i	12	11	4	2	-	2	0	0	0	0	0
	fl	-	-	4	0	-	-	-	-	-	0	0
	fr	-	-	-	2	-	-	-	-	-	-	0
Mean	R	24.0	26.0	34.3	0	9.3	1.3	0.7	1.0	1.0	0.7	0
Mean	i	11.3	7.3	2.7	1.3		1.0	0.0	0.0	0.0	0.0	0
Mean	fl			2.7							0.0	
Mean	fr				0.7							0
Mean	Ps	3.7	5.7	14.0		0.0	0.0	0.0	0.3	0.7	0.3	
S1 sheltered quadrat receiving 1 hour full sunlight.												
SQ4	R	6	6	15	0	13	13	13	18	30	30	0
	i	4	4	4	4	-	7	7	7	7	6	6
	fl	-	-	4	0	-	-	-	-	-	6	1
	fr	-	-	-	4	-	-	-	-	-	-	5
	Ps	0	0	9		0	0	0	5	17	17	
S2												
SQ5	R					11	14	13	13	13	7	0
	i					-	2	1	0	0	0	0
	fl					-	-	-	-	-	-	-
	fr					-	-	-	-	-	-	-
	Ps					0	3	2	2	2		

Table 4.3. ***Drakaea elastica*** **population dynamics at the Carabungup Nature Reserve S1 and S2 sites for the 1991 seasons from observations of plants protected by insect exclosures.** MQ and postscript number, designates permanent 1/2 meter square insect exclosures; R, the number of ramets per quadrat for the given date; i, the number of inflourescences per quadrat; fl, the number of flowers; fr, the number of fruits; Ps, the number of ramets which were "seedlings".

		1991						
		May 26	Ju 28	Jy 13	A 25	S 26	O 10	N 11
S1								
MQ1	R	11	10	10	13	19	21	0
	i	-	4	4	4	4	4	4
	fl	-	-	-	-	-	3	0
	fr	-	-	-	-	-	-	3
MQ2	R	29	32	37	44	50	53	0
	i	-	4	4	4	4	4	4
	fl	-	-	-	-	-	4	0
	fr	-	-	-	-	-	-	4
Mean	R	15.0	21.0	23.5	28.5	34.5	37.0	0
Mean	i	4.0	4.0	4.0	4.0	4.0	4.0	
Mean	fl						3.5	
Mean	fr							3.5
Mean	Ps	0.00	0.07	0.17	0.32	0.43	0.47	
S2								
MQ3	R	9	11	9	10	10	10	0
	i	-	4	4	4	3	3	3
	fl	-	-	-	-	3	3	0
	fr	-	-	-	-	-	-	3
Mean	Ps	0.00	0.22	0.11	0.20	0.20	0.20	

Table 4.4. Summary of allozyme variation based on 16 loci for five populations of *Drakaea elastica*. G, estimated number of genets; N, the mean sample size per locus; P, proportion of polymorphic loci; A, mean number of alleles per locus; Ae, mean effective number of alleles per locus; *Ho*, mean observed heterozygosity; and *He*, mean expected heterozygosity.

	G	N	P	A	Ae	*Ho*	*He*
Drakaea elastica							
All Populations		145.2	0.81	2.8	1.23	-	0.19
S1a 1990	150	31.0	0.50	1.9	1.20	0.18	0.17
S1b 1991	75	37.1	0.56	2.0	1.16	0.14	0.14
S2	50	43.1	0.62	1.9	1.22	0.18	0.18
7902	3	3.0	0.19	1.2	1.11	0.10	0.10
7931	40	21.0	0.62	2.1	1.28	0.21	0.22
7904a	10	8.0	0.44	1.6	1.19	0.15	0.16
7904b	3	2.0	0.25	1.3	1.16	0.16	0.14
Mean			0.45	1.7	1.19	0.16	0.16

Table 4.5. Summary of allozyme variation, genetic diversity and distribution of diversity within and between populations of *Drakaea elastica* and other orchid species of widespread and regional distributions, and a number of plant groupings arranged by Hamrick & Godt (1990).

Moran and Hopper (1987) define widespread species as having a geographic range of 600 km in at least one direction; regional species range between 150 and 600 km; and localized species are endemic to an area of less than 100 km; P, mean proportion of polymorphic loci; A, mean number of alleles per locus; and *He*, mean expected heterozygosity; *Hs*, mean genetic diversity within populations; *Ht*, total genetic diversity; *Dst*, mean genetic diversity among populations, *Gst*, mean proportion of diversity between populations.

	P	*A*	*He*	H_S	H_T	G_{ST}	Source
WIDESPREAD							
Australian							
Drakaea livida	0.66	2.6	0.27	0.39	0.42	0.06	1
Drakaea glyptodon	0.67	2.3	0.24	0.29	0.31	0.05	1
REGIONAL							
Australian							
Drakaea elastica	0.45	1.7	0.16	0.20	0.25	0.21	1
European							
Orchis morio	0.48	1.7	0.12	0.12	0.12	0.05	2
Orchis longicornu	0.54	1.9	0.16	-	-	0.01	2
LOCALIZED							
Australian							
Caladenia elegans	**0.63**	**2.3**	**0.22**	**0.23**	**0.24**	**0.02**	1
Caladenia caeseria sub sp. *maritima*	0.36	1.8	0.17	0.39	0.41	0.03	1
ANIMAL POLLINATED	0.50	1.8	0.15	-	-	0.20	3
SELFING	0.20	1.3	0.07	-	-	0.51	3
WIND-DISPERSED SEED	0.43	1.7	0.12	-	-	0.14	3

Sources of data: (1) this study, (2) Rossi *et al.*(1992), Hamrick & Godt (1990).

Table 4.6. Fixation indicies (*F*) for the polymorphic loci in 5 populations of *Drakaea elastica*. Highlighted are heterozygote proportions significantly greater than Hardy-Weinberg expectations based on a Chi Square test (P<0.05).

Locus	Population							
	S1a	S1b	S2	7902	7904a	7904b	7931	Mean
PGI2	0.10	0.29	-0.25	-	0.33	-	-0.04	0.09
IDH1	-0.02	-0.01	-0.09	-	-	-	-	-0.04
PGM1	-	-	0.08	-	-	-	-0.14	-0.03
PGM2	-	-	-	-	-0.07	-	-	-0.07
SDH1	-0.12	-0.17	0.24	-1.00	-	-1.00	**0.31**	-0.19
ADH1	**0.20**	**0.38**	0.26	0.33	0.19	-0.33	0.16	0.17
MDH1	-0.05	-0.01	-0.01	-	-	-	-0.05	-0.03
MDH2	-	-	-	-	-	-	-0.05	-0.05
MDH3	-	0.23	-0.11	-0.20	-	-	-0.05	-0.04
MRR3	-0.29	-0.13	-0.04	-	0.02	-0.33	0.10	-0.11
GDH1	**-0.45**	-0.05	-0.28	-	-0.23	-0.33	-0.14	-0.21
EST1	-0.09	-0.15	-0.08	-	-0.07	-	-0.08	-0.09
LAP1	-	-	-	-	-0.14	-	-	-0.14
Mean	-0.10	0.04	-0.03	-0.29	0.00	-0.50	0.00	

Table 4.7. Total genetic diversity and the distribution of diversity within and between 5 populations of *Drakaea elastica*. Hs, mean genetic diversity within populations; Ht, total genetic diversity; Dst, mean genetic diversity among populations, Gst, mean proportion of diversity between populations.

	Hs	Ht	Dst	Gst
PGI2	0.304	0.459	0.155	0.338
IDH1	0.035	0.034	0.000	0.000
PGM1	0.132	0.170	0.038	0.226
PGM2	0.017	0.017	0.000	0.000
SDH1	0.397	0.590	0.193	0.327
ADH1	0.533	0.713	0.180	0.252
MDH1	0.036	0.034	0.000	0.000
MDH2	0.015	0.014	0.000	0.000
MDH3	0.109	0.109	0.000	0.000
MRR3	0.347	0.380	0.033	0.086
GDH1	0.367	0.418	0.051	0.123
EST1	0.223	0.245	0.022	0.090
LAP1	0.033	0.034	0.001	0.034
Mean	0.196	0.247	0.051	0.208

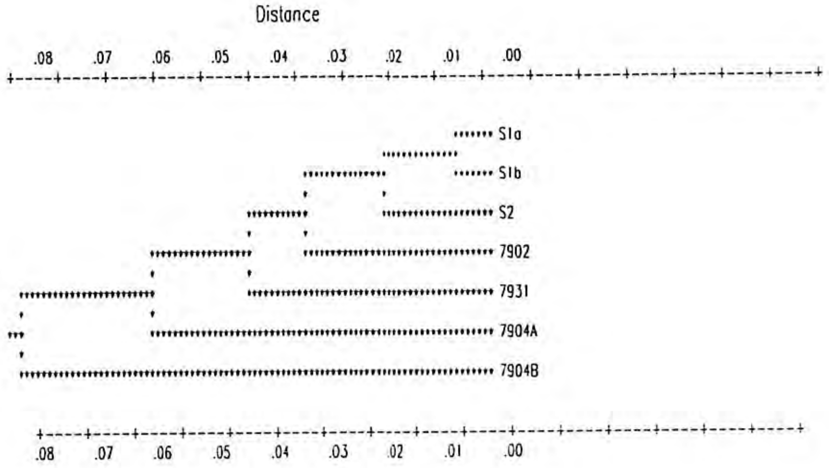
Table 4.8. Estimates of the mean number of migrants (Nm) exchanged between *Drakaea* populations. Nm, was obtained using Wright's(1951) method which uses Gst:

$$Nm = \frac{1 - Gst}{4Gst}$$

Populations	Gst	Nm
D. elastica		
All populations	0.208	0.95
S1a and S1b	0.047	5.07
S1 and S2	0.079	2.91
D. livida	0.061	3.85
D. glyptodon	0.049	4.85
Caladenia elegans	0.022	11.11
Eucalyptus crusis	0.240	0.79

Figure 4.1. Location of *D. elastica* populations in relation to CALM Regions.

Figure 4.2. Phenogram of UPGMA cluster analysis based on Nei's genetic distance between 6 samples from 5 populations of *D. elastica*.



5. *RHIZANTHELLA GARDNERI* (THE UNDERGROUND ORCHID)

For a full account the reader is referred to Rogers (1928), George (1980), George and Cooke (1981) and Dixon (1985). *Rhizanthella gardneri* R. Rogers is a saprophitic orchid which spends its entire life below ground. Adult plants are comprised of succulent 1 - 2+ cm long rhizomes, 6 - 12 cm below ground level, bearing small, adpressed bracts and scattered fine hairs. Lateral branches are produced, by which the species reproduces asexually forming small clones. At anthesis the apices of its inflorescence bracts protrude through the soil and litter forming an access pore to the capitulum chamber.

5.1 DISTRIBUTION AND CONSERVATION STATUS

R. gardneri has been extensively surveyed (Dixon and Pate 1984). It occurs in the CALM Wheatbelt and South Coast Regions (Fig. 5.1), and may be described as widespread (Moran & Hopper 1987). It is currently gazetted as Declared Rare Flora under the WA Wildlife Conservation Act.

R. gardneri populations cluster in two disjunct locations. In the central wheatbelt it occurs NE of Goomalling in the north, east to Babakin and south to Corrigin while the south coast populations cluster around Munglinup.

Although most of the land between known *R. gardneri* sites has been developed for agriculture, undiscovered populations may still exist. Two of the three eastern wheatbelt populations occur on nature reserves, and the third occurs on a town site reserve. Of the three south coast populations one occurs on a reserve, a second on crown land and the third is the 1979 rediscovery site located on private property. Precise locality details are contained on CALM departmental rare flora database and files.

Population 1 - Townsite Reserve. May-June 1982: 110+ plants were distributed over a wide area (Dixon and Pate, 1984). The site was originally set aside for water catchment via an earth catchment, and to provide facilities for town refuse, timber for domestic heating and gravel for road building.

The site is bordered by farm paddocks. These are likely to be a constant source of weed seeds, and in 1990-91 infestations by *Briza* spp., *Arctotheca calendula* (capeweed), *Rhaphanus raphanistrum* (wild turnip) and *Trifolium* spp. were evident in the reserve. Dixon and Pate (1984) attribute the spread of weeds in the reserve to the movements of rabbits which feed on adjacent farmlands before taking refuge in the reserve during the daytime.

Conservation status: At present *R. gardneri* is not in danger of extinction at this site. This population is distributed over a large area and therefore has considerable conservation potential. Special attention is therefore recommended to ensure its long-term survival.

Population 2 - West of Babakin. May 1982 : 4+ plants were found distributed over a small area on a 108 ha reserve (Dixon and Pate 1984). In 1990 there was no evidence of recent burning, and Dixon and Pate (1984) estimated that the reserve had not been burnt within the last 30-40 years. The reserve is a daytime refuge for rabbits which feed in the farm paddocks which border the reserve on all sides.

Conservation status: Although not presently in danger of extinction at this site, this population is distributed over a small area and is therefore at risk through depletion over a longer period. Special attention is required for its long-term survival.

Population 3 - Townsite Reserve. May-June 1988: 36+ plants were distributed over a wide area. In May 1990 20 capitula were discovered, 17 of which were located within a area of only ten meters square. The reserve was originally set aside as a refuse site for nearby farms, and for timber. In May 1990 and again in June 1991 there was evidence of recent dumping of refuse on the reserve. The reserve is also a daytime refuge for rabbits which feed in the farm paddocks which border the reserve on all sides. The reserve showed no evidence of recent burning in 1990, and appeared not to have been burnt within the last 30-40 years.

Conservation status: Although not presently in danger of extinction at this site, this population has the majority of its individuals distributed over a small area and is therefore at risk through localized disturbance followed by depletion over a longer period. Special attention is required for its long-term survival.

Population 4 - The Munglinup rediscovery site. June 1979: 15 plants were distributed over a small area in an uncleared paddock on a farm Near Munglinup east of Ravensthorpe and some 500 km from the Babakin site. In September of 1979 the paddock was mistakenly scrub-rolled (George 1980). While the orchids themselves were untouched, much of the above ground portions of the associated vegetation were damaged. George (1980) estimated that the site had been burnt ten years prior to his 1979 survey.

Conservation status: As this population occurs on private property its short-term survival is at risk from agricultural accidents of the type described. Special attention is therefore required, and liaison between Wildlife officers and the land owner (and future owners) should be frequent enough to ensure that the population is put at minimum risk.

Population 5 - Nature Reserve. July-October 1981: 6 plants were distributed over 17 ha. The reserve itself covers an area of more than 6800 ha. Vacant crown land with pristine bushland extends north west from the reserve, but otherwise all other margins of the reserve are bordered by agricultural lands. Pate and Dixon (1982) found no evidence of fire within the previous 30 years on the north west perimeter, whereas on the southern and eastern perimeter fires had escaped into the reserve from clearing operations on adjoining farmland. The orchid population and sites with highest underground orchids potential, occur within 2 km of a southern boundary road (Dixon and Pate, 1984). The orchid therefore occurs in an area of the reserve most at risk of being disturbed by farming practices.

Conservation status: Although not presently in danger of extinction at this site, this small population is at risk through localized disturbance followed by depletion over a longer period. Special attention is required for its long-term survival.

Population 6 - The Oldfield River Location. August 1981: 5 plants were distributed over a three hectare area. This population occurs on an unvested and relatively unspoilt tract of crown land. Dixon and Pate (1984) found little weed activity but observed that rabbit infestations along road verges and adjacent to the Oldfield River may exacerbate the situation. They also found no evidence of the orchid site being burnt in the 30 years preceding their survey.

Conservation status: Dixon and Pate (1984) believe the Oldfield population is the best situated for the long-term conservation of the species and suggested that a nearby proposed Nature Reserve be extended to include this population and adjacent sites having high underground orchid potential.

Other collections (from George 1980)
- Private property 16 km SE of Corrigin. May 1928: 43 plants in recently ploughed virgin land .
- Shackleton. June 1928: 1(+?) plant.
- Goomalling. June 1928: 1(+?) plant.
- Moonigin NE of Goomalling. June 1938: 1(+?) plant.
- Corrigin. August 1938: 1 plant.
- Babakin. May 1959: 1 plant.

5.2 HABITAT AND ASSOCIATED SPECIES

Dixon and Pate (1984) found the features of the soil in which the underground orchid grows to be similar at populations 1,2 ,3,5 and the Oldfield River site except that the sand layer at the Oldfield site is deeper (50 cm) than at the others (<30 cm). Their description is similar to that given for the Munglinup rediscovery site by George (1980) and for the soil types found at population 3. Dixon and Pate (1984) describe the soils as conforming to categories Cd2 and Bb4 of Northcote (1971), where subsoils are highly sodic and of low permeability resulting in seasonally perched water tables, with attendant saturation of upper soil profiles. General fertility is low, and organic matter averages less than 5% of wet soil weight.

Dixon and Pate (1984) observed that the 1979 rediscovery site near Munglinup, and all discoveries prior to it, occurred between the 300 mm and 400 mm isohyets. Their subsequent discoveries, and population 3, also occur between these isohyets (Fig. 5.2). In the original description of the underground orchid Rogers (1928) mentioned the "saprophitic" nature of the species, describing it as living close to the decaying stumps of the broom honeymyrtle, *Melalleuca uncinata*. Trott (sited in George 1980) noted that the 39 specimens of *R. gardneri* he found in 1928 were closely associated with the shrub *M. uncinata*, and George (1980) also observed that the 1959 Babakin specimen was located close to a *M. uncinata* plant. Dixon and Pate (1984) searched for the orchid in 25 sites, 15 of which were within 1 km of confirmed habitats of the orchid, embracing vegetation types which did not contain *M. uncinata* but were unable to find it. In fact the orchid has not been reported as being found outside stands of *M. uncinata* or stands of mixed vegetation including clumps of *M. uncinata*. The results of their study showed that all capitula were found within 30 cm of a *M. uncinata* stump, and they questioned George's (1980) report of finding *Rhizanthella* 100 cm from its nearest *M. uncinata*. During our May 1990 survey of population 3 A Brown uncovered a capitulum isolated 125 cm from the nearest *M. uncinata* stump. Not only does this allow therefore, for the subterranean migration of the orchid between broom myrtle stumps, but also it suggests that estimations of the number of orchids in any given area may be biased since searches have usually been confined to the base of *M. uncinata* plants.

R. gardneri, it seems, has very specific soil, rainfall and vegetation type requirements. Not surprising given its highly specialized saprophitic habit.

5.3 HABITAT DISTRIBUTION

After a comprehensive survey of the area of overlap between the 300 mm and 400 mm isohyets and the distribution of *M. uncinata*, Dixon and Pate (1984) demonstrated that there were two nodes of occurrence of *R. gardneri* populations - one in the central wheatbelt, the other near the south coast. Whether these represent what was always two disjunct population groups, or are relics of what was once a larger continuous distribution, remains according to Dixon and Pate (1984) a matter of conjecture. The disjunct distribution of *R. gardneri* is not a unique phenomenon among Western Australian terrestrial species, there being other examples of it in other genera (Hopper *pers. comm.*).

Pitman (1929) determined the fungus present in the outermost cells of *R. gardneri* to be a species of *Rhizoctonia*. According to Dixon (1985) the fungus *Thanatephorus cucumeris* is the underground orchid's symbiotic mycorrhizal associate, and is required for geminating *R. gardneri* seeds (K Dixon *pers. comm.*). Obviously a site's potential for having the underground orchid may be gauged by the presence or absence of *R. gardneri's* fungal symbiont. Testing soil samples for *T. cucumeris* may be a cost effective method for surveying for *Rhizanthella* in the future. B Shearer (*pers. comm.*) has observed disjunct distributions in some parasitic fungi although their host plants have continuous distributions. The underground orchid's disjunct distribution may be better understood when *T. cucumeris'* distribution is documented.

5.4 LIFE HISTORY AND PHENOLOGY OF GROWTH AND REPRODUCTION

For a full account the reader is referred to Pate and Dixon (1982). J Trott (cited in Serventy, 1979) considered heavy summer rain to be essential to stimulate the growth and flowering of *R. gardneri*, whereas George (1980) thought this unlikely. Dixon and Pate (1984) provide some, but inconclusive, evidence in support of Trott's earlier observation.

Since 1985 Hopper and Brown (*pers. comm.*) have been recording the annual frequency of flowering capitula in a 10 meter square quadrat permanently stationed at population 1. These data, along with those collected from a similar quadrat stationed at population 3 in 1990, are plotted in Figure 5.2. They were used to determine if there was a relationship between rainfall in the months preceding flowering and the number of capitula found in the quadrats. The F-test indicated that the correlation between rainfall in the months preceding flowering and number of capitula was significant ($p = .0026$).This relationship indicates that seasons following high summer rains may be most productive for surveying and studying the underground orchid.

5.5 POLLINATION BIOLOGY AND DISPERSION

Flowers are self compatible but do not appear to autofertilize. Insect vectors are required to transport the pollen to the stigmatic surface, but how this is achieved remains inconclusively proved. While there is virtually no visual attraction, the flowers apparently produce a sweet but faint scent (George 1980, J S Pate *pers. comm.*). George and Cooke (1981) observed a *Megaselia* fly carrying a "pollinia" on its thorax emerging from a capitulum. A pollen sample from the fly corresponded to that of a herbarium specimen of *Ryzanthella* suggesting that the fly might be possible pollinator. Dixon (1985) describes termite pollinators with "pollinia" attached visiting flowers of *Rhizanthella*. The Penguin Dictionary of Biology (1966) describes a pollinium (pollinia pl.) as a "Coherent mass of pollen grains, as in orchids." Our light microscope observations of *R. gardneri* pollen showed it to be granular rather than a coherent mass. The presumed pollinia described by George and Cooke (1981) and Dixon (1985) were not pollinia, but were probably the microsporangial cases, or thecae which may contain residual pollen, left after anthesis. Most of the granular pollen is released prior to the thecae being dislodged. Pollination events probably involve the transport of a few, tens or even 100's of pollen grains to the stigma, but any one event is unlikely to involve thousands of grains.

George (1980) in excavating a capitulum at the Munglinup rediscovery site disturbed several of the dimorphic ant *Camponotus* sp. nesting in it. The same species has been reported on the flowers of other plants (J Majer cited in George, 1980). George and Cooke (1981) observe several ants and beetles entering and leaving capitula. While excavating an inflorescence at population 3 in mid afternoon, May 23 1990, SC disturbed ants were nesting in it, and did the same at population 1 in mid morning, June 12 1990. In both these cases the capitulum chamber was littered haphazardly with pollen and dislodged thecae. Of the nineteen inflorescences uncovered at population 3 in May 23 1990 seven had some pollen and thecae scattered about their capitula in a manner similar to those in which ants had been observed. Ants are reportedly the pollinators of *Leporella fimbriata* (Peakall *et al*, 1987) and *Microtis* (Peakall and Beattie, 1989), and appear to be good candidates as pollinators for *Rhizanthella*. At population 3 in 1990 the visitation rate by pollinators to capitula was 37%.

George (1980) observed that few *Rhizanthella* flowers develop into fruits, some capitular having none at all. Dixon (*pers. comm.*) observed that the flower to fruit ratio in this species might be only 5%. This is low compared to *Caladenia* species in which as many as 60+% of flowers are pollinated and form fruit (Chapters 1 and 2), but is in keeping with the low pollinator visitation rate observed for *Rhizanthella*, and if pollination dispersion within capitular are random or haphazard. George (1980) observed that only a fraction of the ovules in developing fruits were fertilized. The fruits he examined contained 20 - 50 seeds representing only about 2 - 5% of the ovules. The low fertilization rates and low seed set observed in *Rhizanthella* are expected, however, given that - the pollen is granular; and that pollinators such as ants are likely to deposit only a few to tens of grains on the stigmas at any one time.

From the description of the pollination biology above, one might expect that most of the seed set in natural populations are the products of self pollination events. If the self pollination rate is high, it follows that - the coefficient of inbreeding in *Rhizanthella* populations, Wright's F, will be high; and heterozygote frequencies will be significantly lower than those expected from random mating and Hardy-Weinberg equilibrium of allele frequencies. The predictions made here, from our interpretation of the pollination biology of *R. gardneri*, are tested in the section following.

Because mature *Rhizanthella* fruits are indehiscent, unlike most other orchids which are, and because capitula containing mature fruits remain underground, George and Cooke (1981) argued that wind was not involved in seed-dispersal. The seeds are also unusual for orchids in that they are large and actually have a testa. While this was unknown by George (1980) at the time, he proposed that the capitula are dug up and eaten by animals, and the seeds passed through in their faeces. Dixon (*pers. comm.*) was able to get a small Western Australian marsupial to eat *Rhizanthella* seeds in the laboratory and found, as predicted by George, that they passed unscathed through the faeces. Mainland populations of many small Western Australian marsupial species have been totally eradicated by feral fox and cat. As a consequence there are no stools of these animals, in *Rhizanthella* populations, to examine for seeds, and so the mode of seed-dispersion is still a matter of conjecture.

Rhizanthella appears not to have an effective method of dispersing its pollen or its seed. Notwithstanding this we have shown that underground rhizomes are able to migrate at least 1.25 m. An effective method of underground dispersion may be all that is required to compensate for less effective above ground methods.

5.6 GENETIC DIVERSITY AND POPULATION STRUCTURE

For general references and a brief review see section 1.5.

The isozyme banding patterns of *Rhizanthella* were typical of those found in diploid, sexually reproducing plants. Ten of the 15 (67%) loci examined were polymorphic, and each locus averaged 1.6 alleles (Table 5.1). The frequency of the common allele at the polymorphic loci was high in all cases, >0.78, except for the *Adh* locus in which its frequency was 0.62. These data reflect the invariant nature of *Rhizanthell*, and indicates that the low levels of variation we detected was not distributed evenly within loci.

The mean number of alleles per locus, proportion of polymorphic loci, and the expected heterozygosity measure (He = 0.11) was low for *Rhizanthella*. In these respects it is similar to *Orchis morio*, a European orchid, and to animal pollinated species (Table 5.1).

Lower than expected frequencies of heterozygotes have been documented in *C. elegans* and *D. micrantha* (Chps 1 and 3), which are outcrossing insect pollinated orchids. This was attributed to their mating systems which involved partial self fertilization and mating between near relatives. In *Rhizanthella* the pattern is similar with the mean expected heterozygosity (He = 0.11; Table 5.1) almost three times greater than the observed, (Ho = 0.04). Wright's fixation index for this population, F = 0.58, was highly positive which again indicatives an excess of homozygotes. This result was predicted in the preceding section on pollination biology. Firstly, we proposed that there is a high rate of migration of pollinators between flowers within *Rhizanthella* capitula, and that the mating system undoubtedly involves substantial self-fertilization. Secondly, this result is also expected considering that neighbourhood sizes are probably small, and matings are more than likely to occur between related adjacent individuals within populations.

5.7 RECOMMENDATIONS FOR CONSERVATION OF GENETIC RESOURCES AND MANAGEMENT

5.7.1 Strategies for conserving genetic resources

For general references and a brief review see section 1.5.1

An appropriate genetic resource conservation strategy for *R. gardneri* is to set aside several large reserves containing entire populations, and covering the range of its distribution. This system of reserves would need to conserve the range of morphological and genetic diversity, maintain acceptable levels of gene flow and cohesion in the species' gene pool, and maintain habitats to conserve the flora and faunal diversity that contribute to community stability. Data from this study indicate that the remaining six *Rhizanthella* sites, if maintained largely intact, would provide sufficient genetic resources for the ongoing survival of this species. This would be a minimum requirement and indicates the need to conserve populations from both disjunct population groups.

Nei *et al.* (1975) and Chakraborty & Nei (1977) have shown that if population sizes are severely reduced, to say <10 individuals, they may well suffer a reduction of allelic frequency and average heterozygosity per locus. Since, inbreeding, low allelic frequency and average heterozygosity per locus characterize natural populations of *R gardneri*, reduced genetic diversity due to small population size is unlikely to be a major factor in short or medium term population survival.

5.7.2 Management actions

5.7.2.1 Liaison with private landowners and shires

At present the survival of 50 % of recorded *R. gardneri* populations rely on the good will of local shires and private land owners. CALM staff are required to provide landholders and other agencies with advice regarding the conservation and management of populations of Rare Flora on land under their control. In order of priority, the populations for staff liaison with landowners are:

(a) Populations 6 and 3
(b) Populations 4

5.7.2.2 Land acquisition

There are three *R. gardneri* populations, two of which are in the eastern wheatbelt and the third is in the south coast, on land which has been reserved for the conservation of flora and fauna. It is therefore not recommended to acquire land specifically for the conservation of this species at present. However, if the current conservation status of this species changes and land needs to be acquired for its protection then priority should be determined by:

(a) the size and quality of the site and its habitat,
(b) conserving the range of genetic and morphological diversity
(c) reducing the risk of sudden extinction.

Research has shown that to conserve the range of genetic diversity found in *Rhizanthella*, only few populations would need to be preserved, and that a few plants spread over a small area of natural habitat constitutes a MVP. Although this suggests that a suitable management strategy for this species might be the preservation of a few small reserves, this would not necessarily fulfil management goals if viable populations of pollinators were not also conserved with the orchid. Therefore, to ensure and enhance the survival of *Rhizanthella* it is essential to provide and maintain areas containing a suitable variety of species and habitats. For this purpose it is recommended that minimum reserve areas should exceed 100 ha.

If, for whatever reason(s), land needs to be acquired for the preservation of *Rhizanthella*, then the following sites should be considered for acquisition:

(a) Populations 6 and 3
(b) Populations 4

5.7.2.3 Protection from accidental destruction

Rhizanthella is vulnerable to damage or destruction owing to the structure of its populations, which are fragmented over a large area, and should be protected from accidental destruction by scrub rolling (Population 4), bulldozing, rubbish dumping (Population 3), and spraying of potentially damaging herbicides and insecticides.

In order of priority, the following sites should be protected from accidental destruction:
(a) Populations 4 and 3
(b) Populations 5, 1 and 2

5.7.2.4 Protection from fire

Rhizanthella does not require fire to complete its life cycle and should be protected from uncontrolled fires by the construction of fire breaks or by fuel reduction in surrounding areas.
In order of priority, the following sites should be protected from uncontrolled burning:
(a) Populations 5 and 1
(b) Populations 6, 3 and 2
(c) Population 4

5.7.2.5 Weed control

The control of weeds in sites preserved for the conservation of *Rhizanthella* is desirable. As has been mentioned in earlier sections, weeds are already a problem in the eastern wheatbelt sites, and as much of the land adjoining *Rhizanthella* sites has been cleared for agriculture, the threat of weeds escaping into these sites is ongoing. CALM officers should liaise with other CALM staff with expertise in the area of weed control, the APB, Main Roads Department (MRD) and private landowners.

Weeds should be removed by hand with minimum soil disturbance where use of selective herbicides may damage *Rhizanthella* or other native species. Weed control should be exercised before *Rhizanthella* begins flowering, and before the weeds shed their seed. It is recommended that weeds be controlled biannually in the months of October and

November. Department of CALM Information Sheets Nos. 1-87 and 2-88 provide information on the control of weeds in natural and direct seeded regeneration areas.

In order of priority, weeds should be removed from the following sites:

(a) Populations 1, 3, 2 and 5

5.7.2.6 Linear marking

Four populations are located on road verge and are subject to damage by maintenance operations. Linear marking would provide a minimum protection for these populations. The MRD has developed a field marking system for demarcating environmentally significant areas on road reserves, and local shires have been encouraged to adopt this system (Kelly *et al.* 1990).

In order of priority, *D. elastica* populations should be linear marked at the following sites:

(a) Populations 1, 3, 2 and 5

5.7.2.7 *Ex situ* conservation

The preservation of *Rhizanthella* in its natural habitats is the priority, however the *ex situ* conservation of this species is also advisable to reduce the possibility of its sudden extinction through catastrophes such as fires or disease. This may be achieved through cultivation and long-term seed storage.

In order of priority, the following sites should be considered for *ex situ* cultivation:

(a) Populations all

5.7.2.8 Artificial gene flow

Artificial gene flow between existing populations is not recommended although research has indicated that the genetic difference between eastern wheatbelt populations is minimal. In the absence of information about the possible adverse effects (see James 1982) of direct cross pollination between plants from geographically distant populations, artificial gene flow between the eastern wheatbelt and southcoast populations is definitely not advised.

5.7.2.9 Monitoring

Ehrlich and Murphy (1987) and Hopkins *et al.* (1987), among others, have outlined the value of monitoring in populations. Quadrats stationed permanently in Population 1 and 3 have already provided useful demographic and autecological information about this species, and annual monitoring of them should continue. Similarly quadrats should be stationed in a south coast population.

In order of priority, permanent quadrats should be established in the following populations, and monitored:

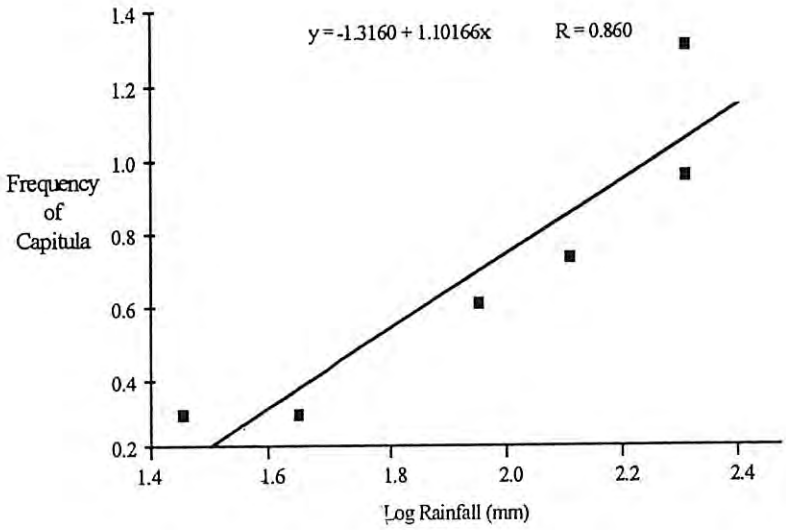
(a) Populations 5 and 6

Table 5.1. Summary of allozyme variation based on 18 loci and 9 populations of *Rhizanthella gardneri*. N, mean sample size per locus; P, proportion of polymorphic loci; A, mean number of alleles per locus; *Ho*, mean observed heterozygosity; *He*, mean expected heterozygosity; and *F*, mean fixation indices (Wright's).

Population	N	P	A	*Ho*	*He*	*F*
All Populations	**41.0**	**0.67**	**2.0**	**0.04**	**0.12**	**-**
3	17.7	0.33	1.4	0.02	0.06	0.53
1	23.3	0.67	1.9	0.06	0.16	0.63
Mean		**0.5**	**1.6**	**.04**	**0.11**	**0.58**
Caladenia caesaria spp *maritima*		0.36	1.8	0.19	0.17	-0.08
Diuris micrantha		0.71	2.9	0.22	0.24	0.10
Orchis morio		0.48	1.7		0.12	
O. longicornu		0.54	1.9		0.16	
C. elegans		0.54	2.1		0.15	
Animal pollinated		0.50	1.8		0.15	

Figure 5.1. Location of *Rhizanthella gardneri* populations in relation to CALM Regions.

Figure 5.2. Relationship between number of flowering capitula and rainfall at populations 1 and 2.



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