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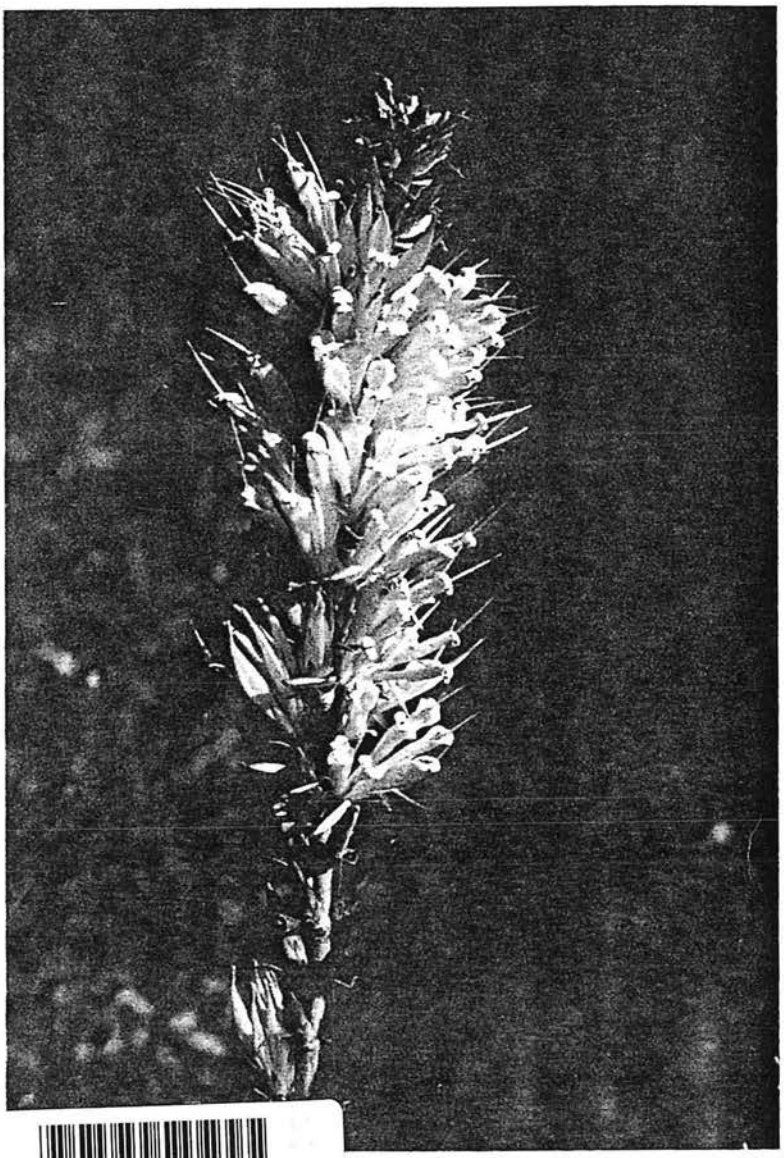
Conservation biology and management of endangered *Lambertia* species.



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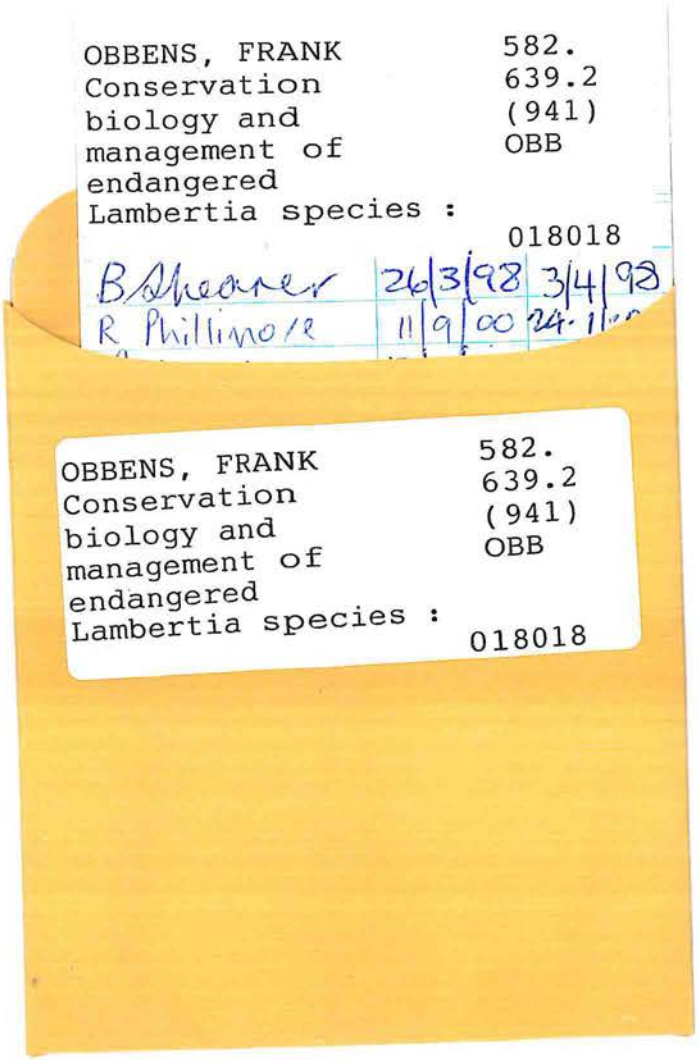

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Conservation biology and management of endangered *Lambertia* species

Project No. 443

Written and compiled by

Frank Obbens

David Coates

FINAL REPORT SUBMITTED TO THE
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1.0 *LAMBERTIA ORBIFOLIA* C.A. Gardner

Round Leaf Honeysuckle

1.1 Introduction

Lambertia orbifolia is a large woody shrub restricted to the lower south west coastal region of Western Australia. It is Declared Rare (Threatened) Flora under the Western Australian Wildlife Conservation Act and is currently considered to be endangered by the Western Australian Threatened Species Scientific Committee, and endangered according to ANZECC categories

1.2 Distribution, habitat and conservation status

Lambertia orbifolia has a disjunct distribution with populations occurring in two discrete areas some 200 km apart (Fig. 1.1). Five populations are located in the Scott River Plains area east of Augusta while the remaining two are found near the small hamlet of Narrikup, ~30 km north west of the regional centre, Albany.

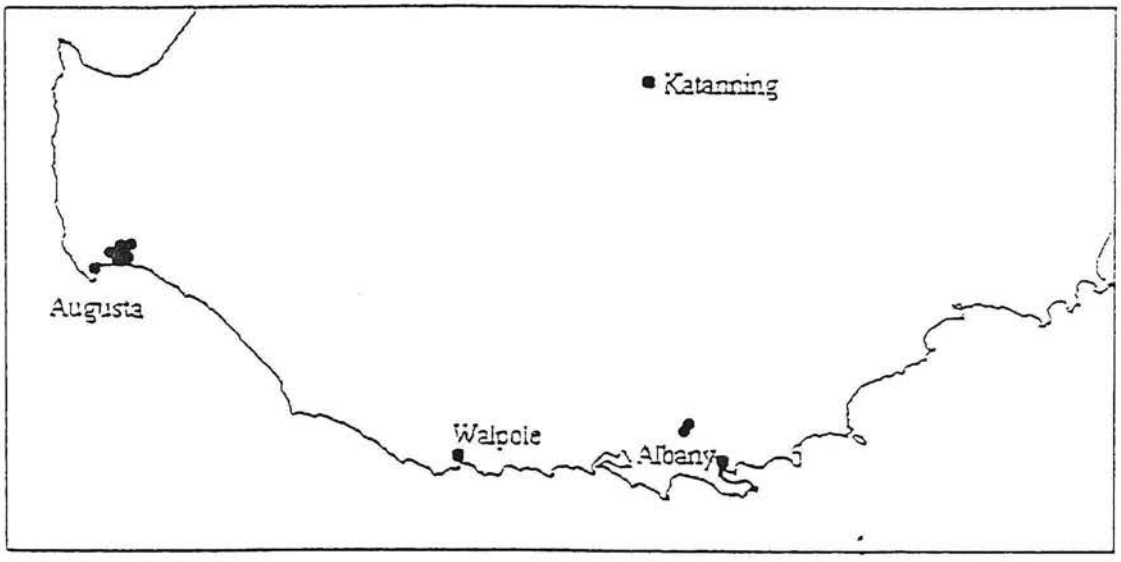

Figure 1.1 - Distribution of *Lambertia orbifolia*

The Scott River Plains populations are found on sandy ironstone soils or on grey sands over ironstone (i.e. mid to lower parts of this low topography), and on shallow sands associated with ironstone around winter wet areas nearer the coast (Hopper *et al.*, 1990; Sage and Lamont, 1994). *L. orbifolia* is found alongside communities of *Agonis flexuosa* and *Eucalyptus marginata* low woodland nearer the coast (two populations behind the secondary dunes). Here it forms a dominant component (thickets) of the community with associated species including *Banksia littoralis, B. ilicifolia B. grandis, Hakea prostrata, Xanthorrhoea preissii, Kunzea recurva, Pimelea rosea, Isopogon formosus, Anthocercis littorea, Lysinema ciliatum, Melaleuca thymoides, Patersonia sp., Loxocarya sp., Lepidosperma sp.* Population details for *L. orbifolia* are in Table 1.1. Although in relatively good condition, the two coastal populations exist on partially cleared private property. Some grazing (subsequent weed invasion) and agro-forestry activities occur adjacent to these populations.

The three more inland populations are located on ironstone uplands (i.e. shallow soils over ironpan) in dense shrub/heath. In these locations *L. orbifolia* occurs as clumps of several plants or as dispersed individuals. These areas can be seasonally inundated and have some similar associated species, including most of the above *Banksia* species. *Kunzea recurva, Hakea tuberulata, Calothamnus aff. crassus* and sedges are also common. Populations 1C and 1D are in relatively pristine condition being larger, undisturbed remnants, although cleared farmlands abut them. Population 1E is similarly large, but has confirmed infections of *Phytophthora cinnamomi* (dieback) and there are sand mining operations nearby. The Brennans Ford population is in moderate condition, but has some plant deaths (of unknown cause). The remaining shire road population (Dennis 3) is also in moderate condition with some weeds encroaching and damage due to firebreak maintenance activity.

The *L. orbifolia* populations, at Narrikup, occur in gently undulating country on brown/grey sandy loams over laterite. The community differs to Scott River Plains being open low woodland of *Eucalyptus marginata* and *E. calophylla*. Associated species include *Banksia grandis, Agonis parviceps, A. hypericifolia, Xanthorrhoea preissii, Hakea ferruginea* and some smaller understorey shrubs. These populations

are in relatively poor condition being affected by aerial canker and *P. cinnamomi* infections, and some weed invasion. Both populations are found on narrow Shire road verges and are under ongoing threats from road maintenance activities.

L. orbifolia is killed by fire and regenerates from seed (a 'seeder'). Its response to soil disturbance and weed invasion is essentially unknown, although field observations suggest weed invasion at the Narrikup site is likely to affect recruitment.

Table 1.1 - Population data for *Lambertia orbifolia*

LOCATION	POPULATION NUMBER-CALM	NUMBER OF LIVE PLANTS	POPULATION TENURE & CONDITION
Scott River Plains			
Dennis Rd 1	1C	173	Res. 42377 - good
Dennis Rd 2	1D	195	Priv. prop. - good
Scott River Rd	1E	100+	Mining lease - moderate
Brennans Ford	4	60	NR42942 - moderate
Dennis Rd 3	5	68	Shire Rd Res - moderate
Adelaide Spring	6	6810	Priv. prop. - moderate
Adelaide Spring	6*	6343	Priv. prop. - moderate
Snake Spring	7	10,000+	Priv. prop. - moderate
Snake Spring	7*	210+	Priv. prop. - moderate
Narrikup			
Spencer Rd	2	139	Shire Rd Res. - poor
Sleeman Rd	3	30	Shire Rd Res. - poor

6* & 7* denote sub-populations used by Sage (1994).

1.3 Taxonomic description

Lambertia orbifolia was first named by Charles Gardner, with the specific name *orbis* referring to the circular-shaped leaves (Gardner, 1964). It is an erect and spreading shrub to 3 m tall which apparently lacks a lignotuber (Hnatiuk, 1995). Hopper *et al.* (1990) have noted that *L. orbifolia* can be as tall as 5 m at some sites. Younger branches are brown and variously hairy (villous to pilose), while the leaves are opposite (rarely whorled), broadly round (orbicular), 20-50 mm long, 20-40 mm wide, and glabrous (Hnatiuk, 1995; Blackall and Grieve, 1988).

Four to six orange-red flowers (florets of the inflorescence) form in the upper leaf axils subtended by a involucre of small bracts (Blackall and Grieve, 1988). The perianth is 40-50 mm long, with a slightly curved tubular-shape and is hirsute externally. At maturity, the four perianth segments roll back tightly and the abaxial suture becomes much deeper than the others (Marchant *et al.*, 1987; Hnatiuk, 1995; Sage, *pers. comm.*). Fertile stamens are also borne on the ends of these perianth segments. There are four nectar (hypogynous) glands occurring at the flower's base and also the small, densely pilose ovary which contains two pendulous ovules (Marchant *et al.*, 1987; Wrigley and Fagg, 1989). Prominently extruded beyond the perianth is a glabrous pollen presenter (Hnatiuk, 1995; Sage, *pers. comm.*). This flower arrangement is typical of many Proteaceous species, however, Keighery (1982) describes this species as having brush flowers with 'gullet' florets and potentially a self-fertile breeding system. Flowering occurs throughout the year, but peaks happen during Jan-Feb and May-July (Hopper *et al.*, 1990; Wrigley and Fagg, 1989; Sage, *pers. comm.*). The follicles (fruit) are asymmetric, around 10 mm in diameter, flattened and smooth, and brown/grey in colour (Hopper and Rye, 1981; Hnatiuk, 1995). Follicles also possess a short oblique beak that represents the remains of the style and each follicle may contain up to two seeds. Seeds are asymmetric, cuneate, ~10 x 6 mm with a narrow wing along one edge (Blackall and Grieve, 1988; Hnatiuk, 1995).

L. ericifolia and *L. inermis* appear to be most closely related to *L. orbifolia*. Hnatiuk (1995) has stated that, "These three, unlike other species, have flowers that face

outward. The bracts do not constrain the flowers which spread widely so that they can be readily probed by birds such as honeyeaters which perch on the stems below the flowers".

1.4 Disease susceptibility and control

Dieback caused by *Phytophthora cinnamomi* and other *Phytophthora* species is considered to be a critical management issue in relation to the conservation of all *Lambertia* species. Field observations associated with isolation of *Phytophthora cinnamomi* from soils near dead plants of *L. orbifolia* suggest that *Lambertia orbifolia* is very susceptible to *Phytophthora cinnamomi* (CALM Vegetation Health Service)

Although dieback due to *P. cinnamomi* has been confirmed from a number of sites in the Scott River Plains area only one population (1E) of *L. orbifolia* is currently considered to be under threat from this disease (Table 1.2). This population occurs in low scrub on very shallow, often inundated soils over ironstone, a habitat considered to be extremely favourable to the spread of the pathogen. The site is covered by a mining lease and there is a commitment from the mining company to manage the area for control of the disease. It is recommended that consideration be given to the application of phosphonate over the area to control any further spread of the disease and protect the population of *L. orbifolia.*

Several plant deaths have been recorded from the Brennans Ford population. However, it is not clear whether these are due to disease or senescence of old plants. Both the Brennans Ford (pop 4) and Dennis Rd (pop 5) populations appear to be the most vulnerable to introduced disease and should be closely monitored.

Serious canker and *P. cinnamomi* infections have been confirmed at both Narrikup sites. These populations have been sprayed with phosphonate in the autumn/winter periods of 1994 and 1995 (Table 1.2). Although fire could be used to eradicate canker, *P. cinnamomi* would still remain in the soil and *L. orbifolia* seedlings are likely

to be even more susceptible to *P. cinnamomi* than the adult plants. Apart from killing the adult *L. orbifolia* plants, fire would also encourage weed invasion within these small remnants. Fire is not currently recommended as a management action for the control of canker at these sites.

Table 1.2 - Disease threat due to *P. cinnamomi* (dieback) and canker infections, and control information for *Lambertia orbifolia*

POPULATION NUMBER-CALM	POPULATION DISEASE STATUS
1C	Res. 42377 - No apparent *P. cinnamomi* (dieback) infection noted
1D	Priv. prop. - No apparent *P. cinnamomi* (dieback) infection noted
1E	Mining lease - Tested positive for *P. cinnamomi*.
4	NR42942 - Several plants dead, cause unknown (vulnerable)
5	Shire Rd Res - No apparent *P. cinnamomi* (dieback) infection noted but considered vulnerable
6	Priv. prop. - No apparent *P. cinnamomi* (dieback) infection noted
7	Priv. prop. - No apparent *P. cinnamomi* (dieback) infection noted
2	Shire Rd Res - *P. cinnamomi* & canker(confirmed). Phosphonate treated
3	Shire Rd Res - *P. cinnamomi* & canker(confirmed). Phosphonate treated

1.5 Seed biology - germination data and storage

The CALM Threatened Flora Seed Centre has made collections of seed from seven of the nine populations of *L. orbifolia*. Seed lots from seven populations are now stored at -18^{0}C (two smaller seed lots are held at 4^{0}C).

Initial subsamples (sample size is variable due to the amount of seed collected) were tested for germination performance (viability) from each lot. Subsamples of the same size will then be tested for germination one year later and thereafter at five year and

then at 10 year intervals depending upon the amount of seed available. Seed germination data and seed storage data are shown in Table 1.3.

Table 1.3 - Seed germination and storage data for *L. orbifolia.*

Colln date	Location	Storage date	Total seed stored	Initial % germ.	Test date	1 year % germ.	Retest date
18/01/96	Adelaide Spring	19/02/96	1120	96.0	1/02/96	-	-
16/05/93	Adelaide Spring	05/04/94	39	96.0	10/06/93	-	-
19/01/96	Scott River Rd (mine)	15/02/96	953	77.0	1/02/96	-	-
15/05/93	Brennans Ford	03/09/93	111	96.0	10/06/93	96.5	15/09/94
13/12/95	Brennans Ford	09/04/96	176	90.0	15/02/96	-	-
15/05/93	Dennis Rd 3	03/09/93	322	85.0	10/06/93	93.0	15/09/94
09/11/93	Dennis Rd 3	25/02/94	362	84.0	22/11/93	73.5	01/03/95
13/12/95	Dennis Rd 3	09/04/96	377	90.0	15/02/96	-	-
16/05/93	Snake Spring	05/04/94	88	100.0	10/06/93	-	-
18/01/96	Snake Spring	05/03/96	1147	92.0	1/02/96	-	-
13/12/92	Spencer Rd	13/09/93	477	100.0	09/06/93	53.0	15/09/94
15/06/95	Spencer Rd	31/07/95	310	100.0	20/06/95	100.0	1/08/96
23/02/96	Spencer Rd	09/04/96	800	95.0	6/03/96	-	-
15/06/95	Sleeman Rd	31/07/95	156	97.5	20/06/95	95.0	1/08/96
23/02/96	Sleeman Rd	09/04/96	390	96.6	5/03/96	-	-

The initial percentage germination rates are high (> 90%) with only three seed lots showing lower germinibility levels (i.e. 19/01/96-Scott River Rd, 15/05/93-Dennis Rd 3 and 09/11/93-Dennis Rd 3). Of the six lots that have been retested all appeared to retain their viability, except one result (i.e. 13/12/92-Spencer Rd). These results indicate that long term storage of *L. orbifolia* seed is likely to be successful.

1.6 Pollination biology

Introduction and methods

There have been numerous studies on pollination vectors of Australian (Collins and Rebelo, 1987; Paton and Turner, 1985; Pyke, 1982) and in particular Western Australian Proteaceae (Collins *et al.*, 1994; Collins and Spice, 1986; Hopper, 1980; Keighery, 1982; Lamont and Collins, 1988; Ramsey, 1988).

Much of the following information on the pollination biology of *L. orbifolia* is based on a one year research program conducted at two populations in the Scott River Plains area (Whitaker and Collins, 1997). Population 1E (Beenup mine site area - Scott River Rd) and population 6 (Adelaide Spring). Whitaker and Collins (1997) identified all foraging bird species feeding on individual plants of *L. orbifolia* at both sites. To detect any possible insect pollinators Whitaker and Collins (1997) randomly checked open flowers during four hour periods in the afternoons by observing groups of inflorescences for two minutes at a time. Five Elliot traps were used adjacent to *L. orbifolia* stems over a two night period to detect the presence of any mammal pollinators.

Potential bird pollinators were assessed for their pollen loads by collecting separate smears from captured birds' crowns and chins using glycerine gel cubes containing basic fuchsin dye (Wooller *et al.*, 1983). These smears were assessed under a light microscope (5 random fields of view) for the quantities of various pollen grains. The percentage abundance of *L. orbifolia* pollen in each smear was categorised as either: very light, 0-4%; light, 5-32%; moderate, 33-65%; heavy, 66-94% and very heavy, 95-100%.

Narrowing down potential pollen vectors

The results indicated that insects were unlikely to be significant pollen vectors of *L. orbifolia* because most of the low numbers of ants, flies and bees observed did not come into contact with flower stigmas (Whitaker and Collins, 1997). Honey Possums (*Tarsipes rostratus*), another potential pollen vector, were not trapped, although they are known to be present at the Beenup site (Kelly *et al.*, 1990).

Of the several different bird species observed foraging on *L. orbifolia* inflorescences only the New Holland Honeyeater (*Phylidonyris novaehollandiae*) was a frequent visitor. Past research has found that New Holland Honeyeaters and White-Cheeked Honeyeaters (*Phylidonyris nigra*) are major pollen vectors for *Lambertia formosa* (Pyke and O'Connor, 1993), *L. inermis* (Burbidge *et al.*, 1979; Collins *et al.*, 1990) and *L. uniflora* (Hopper, 1980; Collins *et al.*, 1994).

Whitaker and Collins (1997) concluded the New Holland Honeyeater was the most likely major vector for *L. orbifolia* pollen. This bird's foraging patterns were observed, including movements (distance moved) between and within plants and the number of inflorescences visited. It was found that New Holland Honeyeaters usually fed at more than one inflorescence per plant before moving to the next plant (93.1%; N=66), although they sometimes fed on perianths whose stigmas had not yet released. Foraging birds moved up to 20 m between plants, but most moved less than 5 m (67.9%; N=53) between neighbouring plants (Whitaker and Collins, 1997). This suggests that potential pollen transfer is likely to be more favourable between flowers of the same or adjacent plants, however, New Holland Honeyeaters also appeared territorially unrestricted moving into and out of the study sites (i.e. some longer distance outcrossing may also be potentially possible). These data are supported by the mating system estimates of outcrossing rate which indicate relatively high levels of selfing and crossing between related neighbouring plants (see section 1.9).

Pollen loads

There was a wide percentage range of pollen loads of *L. orbifolia* removed from New Holland Honeyeaters (Table 1.4) (after Whitaker and Collins, 1997). Most pollen was found on the crown (68.8%; N=32), rather than the chin of these birds (t=7.9; P<0.0001) probably because flower lengths are usually longer than the bird's bill length. Similar results found by Paton and Collins (1989) and Burbidge *et al.* (1979) suggest that most pollen is transferred to honeyeaters in this way.

Table 1.4 - Incidence of New Holland Honeyeaters with *Lambertia orbifolia* pollen loads.

	Density of New Holland Honeyeater Pollen Loads					
Site/date	V. light 0-4%	Light 5-32%	Moderate 33-65%	Heavy 66-94%	V. heavy 95-100%	Total
AS 17/12/96	4	-	-	1	3	8
AS 18/12/96	-	3	-	1	8	12
AS 27/01/97	-	-	2	2	21	25
AS 28/01/97	-	-	-	4	15	19
B 19/12/96	6	1	1	1	-	9
B 20/12/96	7	2	3	4	-	16
B 29/01/97	1	-	3	5	1	10
B 30/01/97	1	7	5	8	1	22

AS = Adelaide Spring B = Beenup

Whitaker and Collins (1997) recorded a significantly greater percentage of *L. orbifolia* pollen loads on New Holland Honeyeaters at Adelaide Spring than at Beenup (F=77.5; P<0.0001) (Table 1.4 and Fig. 1.2) even though honeyeaters at Beenup visited plants

more frequently than at Adelaide Spring (Fig. 1.3). The Beenup population suffers more from summer drought (i.e. shallower soils over ironpan) than Adelaide Spring and it is also affected by dieback. These stresses may result in decreased flower and pollen production. Figure 1.2 also reveals that the overall pollen loads of *L. orbifolia* were greater in January than in December ($F=22.6$; $P<0.0001$) which corresponds to the rising peak in flower production over this period. Sage and Lamont's (1994) research on the reproductive biology of *L. orbifolia* also found this flowering peak occurred during summer (section 1.7).

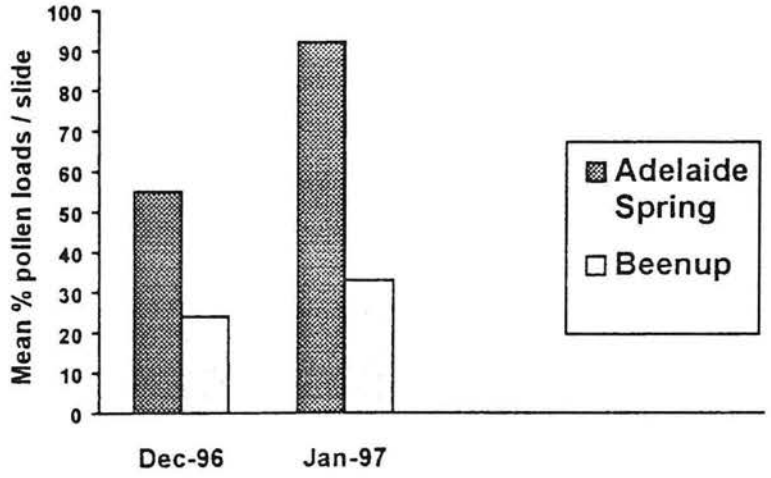

Figure 1.2 - Mean percentages of total pollen loads detected on New Holland Honeyeaters due to *Lambertia orbifolia*.

Honeyeater foraging frequency

Whitaker and Collins (1997) determined the frequency with which honeyeaters foraged at *L. orbifolia* flowers, at each site, by observing them for 5 minutes each at ten randomly selected plants. Observations started within half an hour after dawn and continued at four-hourly intervals (i.e. 4 censuses/plant/day) (McNee 1995). Flower counts were conducted for all ten plants at each site. There was a significant increase in the frequency of honeyeater visits from December 1996 (1.0 honeyeaters / plant / 5 minutes) to January 1997 (1.8 honeyeaters / plant / 5 minutes) ($F=13.7$; $P=0.0002$; Fig. 1.3).

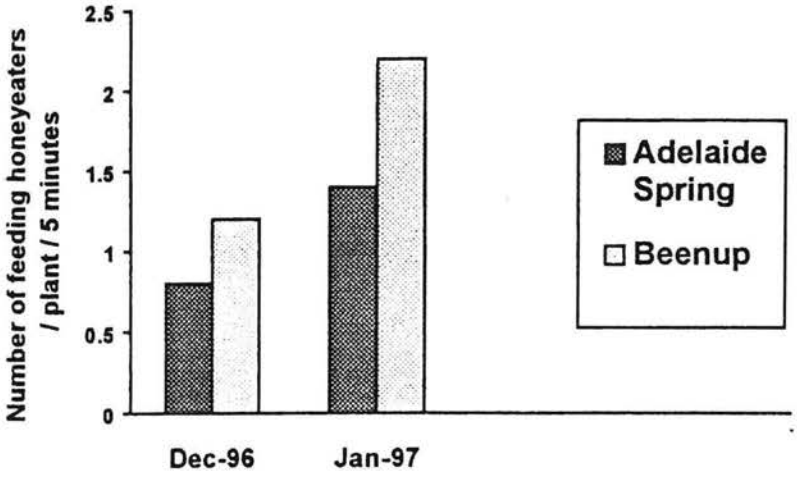

Figure 1.3 - Frequency with which New Holland Honeyeaters visited *Lambertia orbifolia* at Adelaide Spring and Beenup.

The increased foraging was directly related to the significant increase in flower production from December to January at both sites ($F=7.3$; $P=0.01$). Increased flower production allows honeyeaters to obtain all their food requirements from *L. orbifolia* plants rather than from a variety of other widely dispersed plant species. Thus foraging movements become more concentrated with a probable decrease in outcrossing during January compared to December (Whitaker and Collins, 1997).

The frequency of flower visitation by feeding honeyeaters can vary significantly throughout the day. For example, honeyeaters feeding on *Banksia prionotes* are most active the three hours after dawn (Collins and Spice, 1986), whereas, foraging on *Banksia grandis* occurs mainly during the three hours before dusk (Hopper and Burbidge, 1982). This study found that New Holland Honeyeaters foraged actively for the first few hours of each morning in December (low flowering period), although foraging in January (high flowering period) was consistently high throughout the day (Fig. 1.4).

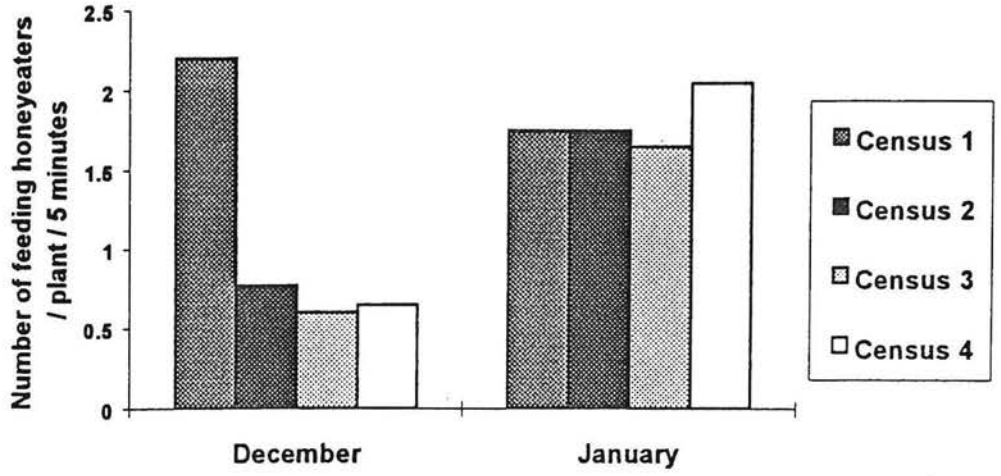

Figure 1.4 - Frequency with which New Holland Honeyeaters visited *Lambertia orbifolia* at Adelaide Spring and Beenup (combined data). Census 1 commenced within the first half hour after dawn, with the following censuses conducted at four-hourly intervals.

As indicated previously the transfer of pollen by New Holland Honeyeaters becomes more pronounced with the increase in *L. orbifolia* flowering (over mid to late summer) and will occur predominantly between the same or neighbouring plants (Fig. 1.4). Longer distance crossing may occur more frequently at other times of the year within a population and occasional crossing probably occurs between nearby populations.

1.7 Reproductive biology

Much of the following information on the reproductive biology of this species is based on an Honours research program (Sage, 1994). Data was gathered from the two coastal sites in the Scott River Plains area and at Narrikup. Major objectives of this research included:

i) assess plant size and population age structure;
ii) assess flowering and factors affecting seed production, viability and longevity;
iii) assess population recruitment patterns with particular reference to the impact of fire and plant disease; and
iv) assess population health in relation to fungal pathogens and insect attack.

Plant age, flowering and follicle production

L. orbifolia flowers throughout the year, although Sage and Lamont (1994) observed peaks in Jan-Feb (Scott River Plains populations) and May-July (Narrikup populations). Pollination and follicle (fruit) production are highest following these peak flowering periods. However, information on fruit set and hence seed production were not recorded. Sage (1994) counted the numbers of recent inflorescences on 15 plants at each population and then inspected 50 inflorescences from each of these plants to determine initial fruit production at each sampling period. The same plants were then measured and their age estimated by counting the node to node growth pattern (Lamont, 1985). These data are presented in Tables 1.4 and 1.5.

Table 1.4 - Dimensions and ages of *Lambertia orbifolia* for three populations. Values are means ± standard deviation for 15 plants per population. *P* refers to probability of significance for 1 way ANOVA. Different letters indicate results significantly different at $P < 0.05$ for Tukey-Kramer multiple range test.

	AS	SN	N	*P*
Height (m)	3.83 ± 0.94 a	5.07 ± 1.34 b	3.33 ± 0.99 a	0.00024
Crown area (m2)	7.9 ± 6.6 a	17.1 ± 12.4 b	7.4 ± 6.5 a	0.0054
Volume (m3)	18.2 ± 20.1 a	51.9 ± 48.0 b	14.2 ± 14.8 a	0.0023
Age (yrs)	24.1 ± 5.8 a	21.3 ± 4.6 a	14.8 ± 3.0 b	0.000003

AS = Adelaide Spring, SN = Snake Spring, N = Narrikup

The largest plants in terms of height, canopy area and volume were from the Snake Spring population (Table 1.4), although these plants were marginally younger than the Adelaide Spring population. This difference may be due to more favourable growing conditions at Snake Spring. The larger Snake Spring plants also had the highest mean numbers of recent inflorescences per plant while the smaller (younger) Narrikup plants

had the least. Several studies of Proteaceae species in Western Australia have obtained similar results (Obbens *et al.*, 1991; Witkowski *et al.*, 1991; Collins *et al.*, 1994). It is also possible that site conditions may be affecting mean numbers of recent inflorescences per plant at Narrikup. This site is more exposed and the population is threatened by canker, dieback and weeds.

Table 1.5 - Number of recent inflorescences per plant and initial fruit production on an inflorescence basis on *L. orbifolia*. Results are means and percentage means ± standard deviation for 15 plants and 50 inflorescences per plant for Feb/May. *P* refers to probability of significance for 1 way ANOVA. Different letters indicate results significantly different at $P < 0.05$ for Tukey - Kramer multiple range test.

	AS	SN	N	*P*
No. of recent infl./plant (Feb/Mar plus May counts)	128 ± 77 b	367 ± 425 a	76 ± 69 b	0.0048
Recent inflorescences fertile (%)	32 ± 13 a	32 ± 13 a	31 ± 16 a	0.9920
No. initial fruits/fertile inflorescence	2.6 ± 0.5 a	2.5 ± 0.6 a	1.6 ± 0.3 b	0.0001

AS = Adelaide Spring, SN = Snake Spring, N = Narrikup

While there appear to be numerous inflorescences being produced continually, it is striking that the percentage of fertile inflorescences (containing young follicles) is virtually identical for all populations (Table 1.5). However, the initial follicle (fruit) set is significantly less for the Narrikup population. This suggests that *L. orbifolia* populations have a similar level of pollination or fertilisation, but fruit set (probably seed set too) is controlled by other factors (Sage and Lamont, 1994). Pyke (1982) concluded that fruit set in *L. formosa* was limited by available resources and not by other factors such as the level of pollinators, available ovules, fruit growing space or herbivory on flowers or zygotes. Limited (or more competition for) resources may

account for the lower initial fruit set at the Narrikup population. Alternatively, disease due to *P. cinnamomi* (dieback) and aerial canker could also be a major contributing factor.

The number and fate of immature and mature follicles

The follicles of *L. orbifolia* undergo several stages of development to maturity prior to any seed release from them. This follicle development phase appears to take approximately 9 - 12 months and is ongoing for the life of the plant, once flowering has been reached (Sage *pers. comm.*). It starts with small green immature follicles (i.e. < 5 mm long - closed and not ripe) which grow some months to become green mature follicles (i.e. > 10 mm long - closed and probably ripened). These follicles become grey and mature (i.e. > 10 mm long - closed and ripe) within 8 - 9 months and eventually open to dehisce their seeds. It should be noted that sometimes green mature follicles do not attain > 10 mm long, although these do sometimes proceed to maturity (i.e. open to reveal supposedly intact seed) and sometimes not (i.e. appear dead). However, the peak in green mature follicle production is still reflected some 3-4 months after the peak in flowering times.

The peak in follicle production occurred around July for Adelaide Spring and Snake Spring (with peak means of 136.5±81.3 and 227.9±169.8 follicles per plant respectively), while at Narrikup it occurred during September (peak mean of 74.4±68.8 follicles per plant) (Sage and Lamont, 1994). The lowest mean number of follicles per plant occurred in Feb/Mar for Adelaide Spring (52.1±45.6) and Snake Spring (122.9±89.1), while at Narrikup (49.9±48.6) this happened around May. Thus *L. orbifolia* has an annual cycle of follicle production with a peak occurring in winter/spring when resources are more abundant, but steadily decreasing till summer/autumn when resources are least available.

To determine the fate of immature follicles Sage (1994) tagged 20 immature green (< 5 mm long) follicles for each of the 15 test plants and followed their fates at two periods during the study. These data are shown in Table 1.6.

Table 1.6 - Fate of immature follicles (<5 mm long) left for two periods (1 : Feb/May to July/Sept; 2 : July/ Sept to Oct). Results are mean percentage ± standard deviation (arcsin transformed) for 6 categories based on 20 follicles per plant on 15 plants at each of 3 populations. *P* refers to probability of significance for 1 way ANOVA. Different letters indicate results significantly different at $P < 0.05$ for Tukey - Kramer multiple range test.

Follicle Condition	Period	AS	SS	N	*P*
Green /closed	1	6.0 ± 11.8	13.1 ± 11.4	28.4 ± 14.3	-
	2	7.9 ± 6.9 b	15.5 ± 14.4 ab	25.7 ± 12.3 a	0.0164
Grey /closed	1	15.2 ± 25.4	8.0 ± 13.9	22.1 ± 16.2	-
	2	16.1 ± 18.3 a	8.5 ± 13.9 a	14.4 ± 12.7 a	0.3822
Grey/open/has seed	1	0.3 ± 0.0	2.2 ± 8.3	2.5 ± 7.9	-
	2	0.0 ± 6.48 a	0.0 ± 5.6 a	2.0 ± 8.6 a	0.6107
Grey/open/no seeds	1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	2	0.0 ± 0.0 a	0.0 ± 0.0 a	0.0 ± 0.0 a	0.9999
Grey/damaged	1	0.0 ± 0.0	2.2 ± 8.2	4.4 ± 10.8	-
	2	0.6 ± 4.2 b	3.2 ± 10.3 ab	6.7 ± 11.9 a	0.0359
Missing	1	82.0 ± 23.3	72.8 ± 11.9	43.0 ± 15.4	-
	2	82.0 ± 16.9 a	74.3 ± 17.3 a	51.0 ± 13.2 a	0.9024

AS = Adelaide Spring, SN = Snake Spring, N = Narrikup

There was a marginal increase in green/closed follicles at Adelaide and Snake Springs and a marginal decrease at Narrikup, between periods 1 and 2, yet the former sites

have significantly fewer green/closed follicles than the Narrikup site. A similar pattern occurs with grey/closed follicles. Sage and Lamont (1994) attributed the significant difference in means between the two areas to be caused by bird granivores which were abundant at Adelaide and Snake Springs, but not at Narrikup. They also suggested that the decreases only seen at Narrikup between periods 1 and 2, are the results of the detrimental advancement effect of canker on the follicles as plants are killed, branch by branch. This argument is supported by data which show that the mean percentage of open follicles with seeds (aborted, immature or intact) was again marginally higher at Narrikup (i.e. advanced maturity). This population also sustained higher levels of damaged follicles which may indicate large numbers of invertebrate granivores and/or higher abortion levels. The percentage missing follicle category was relatively substantial for all the populations, although Adelaide and Snake Springs sites appear much higher than Narrikup. The missing follicles may reflect the activity of parrots (granivores) at the two former sites as these birds seemed to be absent at Narrikup, over the study period (Sage and Lamont, 1994). The data indicate that approximately 17% of initial follicles reach full maturity (dehisce seed) at Adelaide and Snake Springs, while at Narrikup approximately 43% may reach maturity (Table 1.6).

The fate of seeds recovered from mature green and grey follicles

Twenty green (closed) and twenty grey (closed) and/or open follicles were collected from the Adelaide Spring site at each sampling period (Sage, 1994), to determine whether the seeds inside were fully developed, aborted or eaten. Examination of green advanced follicles collected from live plants revealed that 85% of seeds were either aborted or immature, with the remaining 15% intact seed (and viable presumably). Of the grey follicles collected from dead plants, 59% contained either aborted or immature seed, 5% intact seed and the remainder (36% of unknown condition) had already dehisced. Thus between 17-43% of follicles reach full maturity and of these only 5-15% appear to have intact seed. This suggests that even during the peak period of follicle production there may be only relatively small amounts of viable seed stored within each *L. orbifolia* canopy. *L. orbifolia* is considered to be an obligate reseeder

(nonsprouter), it also appears to be nonserotinous (i.e. seed is continually being dehisced into the leaf litter or soil).

Intact and aborted seeds recovered from litter and soil samples

Sage (1994) collected three litter (500 x 500 mm areas) and three soil (500 x 500 x 30 mm) samples at each site which were sorted for any *L. orbifolia* seeds. Only the Narrikup site had a small quantity of intact seed in the litter (9.7±2.5) and even less in the soil (0.7±1.2). Very small quantities of aborted seeds were recorded in the litter at all sites. The small amount of seed found suggests that significant quantities of seed are not stored within the litter or soil or that they are subsequently eaten by granivores not long after being dehisced. Alternatively, more intensive litter and soil sampling may be needed at these sites.

Seed dispersal experiments

To investigate possible seed dispersal by ants Sage (1994) left seeds of *L. orbifolia* and some other species near ant trails at the Narrikup site. No seeds where taken within a 24 hr period. In addition, no *L. orbifolia* seeds were found during sieving of a large ant nest at this same site. Majer and Lamont (1985) noted that ants taking seeds of Proteaceous species in Australia is rarely recorded.

Population recruitment

A hot fire in autumn 1991 through half of the Adelaide Spring population resulted in a significant recruitment event. Sage (1994) recorded 5860 three year old seedlings (86.9% of population), 399 dead adult plants from the fire (5.9%) and 483 live adults not burnt (7.2%) at this site. He also noted that there appeared to be little interfire seedling recruitment, although, low to moderate levels of interfire recruitment have

been observed at Narrikup (D. Coates *pers. comm.*). Fire appears to be the stimulus for major recruitment events from seed for this species. The current data suggests, however, that there is little viable seed (either on the plant or at any ground level) to cater for such a major event. It is not unusual to have high annual loss rates on dispersed seeds caused by a variety of granivores and physical damage (Enright and Lamont, 1989). Additionally, a litter stored seed would need some fire protection to ensure viable propagation rates, although none of this seed was located within soil out of fire's reach. This suggests that the original 399 adult plants must have produced an average of 14.7 germinants each which assumes that a far greater number of viable seeds were produced per plant than normally expected for Western Australian reseeders.

The data gathered so far gives an inconclusive picture of how this recruitment occurred. There is a suggestion that enough mature green and/or grey closed follicles are maintained on the plants at any given time and that these dehisce seed post fire. However, the current seed production data even at the peak production period would only just suffice if seed germination and survival rates were extremely high. More research on the seedbank of *L. orbifolia* is required to resolve this dilemma.

1.8 Population genetic structure

Population genetic structure and patterns of differentiation among populations were investigated using isozyme markers. Ten enzyme systems were assayed: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH), esterase (EST), glucosephosphate isomerase (GPI, E.C. 5.3.1.9), glutamate dehydrogenase (GDH, E.C. 1.4.1.3), leucine aminopeptidase (LAP, E.C. 3.4.17.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), menadione reductase (MDR, 1.6.99.22), phosphoglucomutase (PGM, E.C. 2.7.5.1) and shikimate dehydrogenase (SKUD). A total of 20 isozyme loci were scored. Only three, loci *Gpi*-1, *Gpi*-2 and *Mdh*-1 were not polymorphic. Genetic variability was studied at 17 isozyme loci based on ten enzyme systems.

Single locus genetic diversity measures *A* (mean number of alleles per locus), *P* (mean percentage polymorphic loci), *He* (expected panmictic heterozygosity), and *Ho* (the average observed heterozygosity) are presented in Table 1.7. These data indicate that *L. orbifolia* has relatively high levels of genetic diversity for an endemic species although there are marked differences between populations (see Hamrick and Godt, 1989). These differences are particularly evident when comparing the Scott River Plains populations with the Narrikup populations.

Table 1.7 Single locus genetic diversity measures: *A* (mean number of alleles per locus), *P* (mean percentage polymorphic loci), *He* (expected panmictic heterozygosity), and *Ho* (the average observed heterozygosity) for each population of *L. orbifolia*.

Population (See Table 1.1)		N_e	*A*	*P*	H_e	H_o
Scott River Plains	1E	≈100	1.2	15.0	0.066(0.038)	0.031(0.022)
	4	60	1.4	30.0	0.080(0.038)	0.038(0.016)
	5	68	1.4	35.0	0.119(0.046)	0.060(0.023)
	6	6810	1.4	35.0	0.081(0.036)	0.052(0.028)
	7	≈10,000	1.4	35.0	0.095(0.038)	0.048(0.023)
Mean			1.36	30.0	0.088	0.046
Narrikup	2	139	1.5	45.0	0.149(0.047)	0.076(0.025)
	3	30	1.6	65.0	0.116(0.034)	0.063(0.027)
Mean			1.55	0.55	0.133	0.067
Mean			1.41	37.1	0.129	0.053

Both Narrikup populations have significantly higher genetic diversity levels, based on all single locus diversity. These results are unexpected given the small fragmented nature of the Narrikup populations compared with the three larger populations in the Scott River Plains area. They suggest that quite different evolutionary mechanisms may be operating in the two population systems. These results also indicate that there is a suitable broad range of genetic diversity in the Narrikup populations to support successful translocation and population enhancement programs.

The large discrepancy between the observed heterozygosity (*Ho*) and the expected panmictic heterozygosity (*He*) also suggest significant levels of inbreeding within all populations. This is confirmed by the mating system studies discussed in detail in the following section. The apparent level of inbreeding was unexpected when compared to other bird pollinated Proteaceae (Sampson *et al.*, 1996), although heterozygote deficits are not unusual in plants with mixed mating systems and significant population structuring (Brown, 1979).

A UPGMA analysis of genetic differentiation between populations reveals striking genetic differences between the Narrikup and Scott River Plains areas. The level of genetic divergence between these two population groups (> 0.20) is indicative of species differences in some plant groups (Gottleib, 1981). This result clearly supports the conclusion that these two population groups are distinct evolutionary units or evolutionary significant units (ESUs) as discussed by Moritz (1995), and should be managed as separate conservation units. That is, any translocation programs should not consider mixing of stocks from these two population groups.

There is also a detectable pattern of genetic divergence among the Scott River Plains populations. Although not significantly different, in terms of allele frequency, the coastal populations cluster separately from the inland population. These two population groups are characterised by different vegetation systems and may correspond to ecotypes although further data based on ecological studies are needed to clarify these differences. Reciprocal transplant experiments would provide the necessary data to resolve the issue of ecotypes.

It is recommended, until further ecological data is available, that the these two population groups be treated as separate management units and that any translocation programs avoid mixing germplasm from these areas.

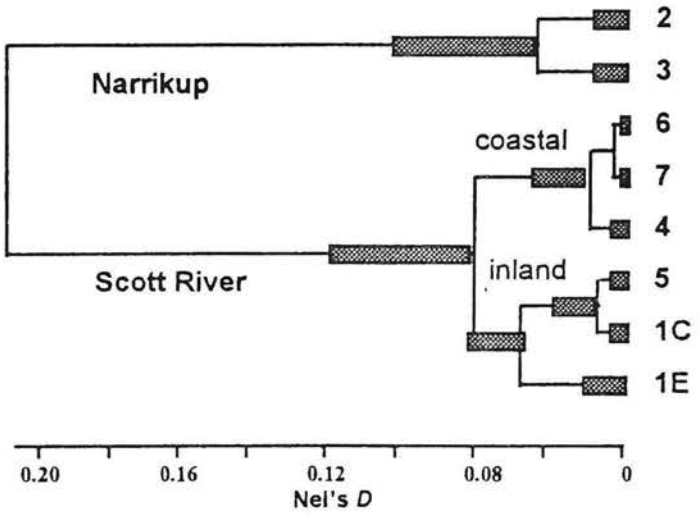

Figure 1.5. UPGMA clustering (based on Nei's genetic distance, *D*) of *Lambertia orbifolia* populations. A cluster in the UPGMA phenogram is significant if the shaded standard error bar is half the branch length.

1.9 Mating Systems

The mating system, level of outcrossing versus selfing, is a critical process in shaping genetic structure and evolutionary potential within plant populations. When combined with the mode of pollination it is considered to be the best predictor of the distribution of genetic variation within and between populations. Changes in mating system parameters between populations or within populations over time can be valuable indicators of population survival following reduction in population size or change in pollinator syndrome (Sampson *et al.*, 1996).

Three allozyme loci, Est-1, Est-2 and Sdh-1 were used to estimate mating system parameters in three Scott River Plains populations of *Lambertia orbifolia*. Based on the mixed mating model (Brown and Allard, 1970), outcrossing rates(t) and their standard errors were calculated for single locus and as multilocus estimates using the joint maximum likelihood methods of Ritland and Jain (1981).

Table 1.8. Single locus and multi locus estimates of outcrossing (t) based on the mixed mating model and calculated from progeny genotype arrays for three populations.

	Locus				
Population	***Est*-1**	***Est*-2**	***Sdh***	**Mean**	**Multilocus**
1. Beenup (1)	0.61(0.20)	0.81(0.24)	0.33(0.11)	0.58(0.15)	0.70(0.11)
2. Adelaide Sp. (6)	0.71(0.10)	0.29(0.07)	0.38(0.09)	0.46(0.05)	0.53(0.06)
3. Snake Sp. (7)	0.53(0.09)	0.79(0.13)	0.34(0.08)	0.55(0.06)	0.72(0.07)

Single locus and multilocus estimates of outcrossing rates for the Beenup, Adelaide Springs and Snake Springs populations are given in Table 1.8. All estimates in all populations differed significantly from random mating (t=1). They indicate significant levels of selfing and/or mating between related plants within populations. Higher multilocus estimates of outcrossing compared with single locus estimates, in all three populations, suggest that both selfing and mating between related plants are contributing to the reduced estimates of outcrossing. Although the difference between the multilocus estimate and mean single locus estimate is not significant except for the Snake Springs population.

The levels of outcrossing within *L. orbifolia* populations are some of the lowest recorded for the Proteaceae and compared with *Banksia* species appear to be at least partly due to mating between relatives. These findings support the pollinator

observations that honeyeaters tend to spend much of their nectar gathering activities within a relatively small area probably covering a few neighbouring closely related plants.

Although *L. orbifolia* populations appear to tolerate significant levels of inbreeding it is not clear how high these levels can go before population viability is likely to be affected. It is recommended that current levels of outcrossing should be maintained within populations by ensuring adequate numbers of bird pollinators. This can only be achieved through the maintenance of suitable areas of vegetation adjacent to the *L. orbifolia* populations which are large enough to maintain permanent bird pollinator populations.

Recommendations for conservation and management

1. Conservation status of populations

The genetically distinct Narrikup populations should be managed as a separate conservation unit. Any management strategies for these populations should be developed independently of those for the Scott River Plains populations.

The Narrikup form is critically endangered and requires a number of immediate management actions such as ongoing disease control and the establishment of a new population in an appropriate disease free and weed free area. Population markers along the roads should be upgraded to ensure that road maintenance does not impact on either population.

2. Disease assessment and control

- The Beenup population which is known to be infected with *P. cinnamomi* requires the following management actions:
    - Access to the area by foot should be restricted and strict hygiene conditions followed
    - There should be no access to vehicles or stock, at any time of the year.
    - phosphonate treatment should be carried out on the site through aerial application.

- The Narrikup populations require the ongoing application of phosphonate

3. Translocation - population mixing options

- There should be no mixing or exchange of germplasm material between the Scott River Plains populations and the Narrikup populations.

- A new population should be established in the Narrikup area with Narrikup material only. Establishment must be in an appropriate disease and weed free area.

4. Research

Further research is needed on:

- Seed bank dynamics and recruitment patterns.

- Pollination biology, mating systems and fruit set in the Narrikup populations. Data in this report indicate that fruit set is significantly less for the Narrikup form and that there are significantly higher levels of damaged follicles, presumably due to invertebrate granivores.

5. Recovery Plan

Although the species as a whole is not currently critically endangered the genetically and evolutionary distinct Narrikup form is, and warrants immediate recovery action. We recommend that a recovery plan be prepared for the species with particular emphasis on the Narrikup populations or a recovery plan be prepared solely for the Narrikup populations.

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2.0 *LAMBERTIA ECHINATA* R.Br. SUBSP. *ECHINATA* and *LAMBERTIA ECHINATA* SUBSP. *OCCIDENTALIS* Keighery

2.1 Introduction

The *Lambertia echinata* complex consists of three subspecies, *L. echinata* subsp. *echinata* is currently considered to be critically endangered by the Western Australian Threatened Species Scientific Committee and it is recommended that *L. echinata* subsp. *occidentalis* also be listed as critically endangered. Subspecies *occidentalis* was discovered recently as a result of ongoing work for this project (see sections 2.3 and 2.6). The other subspecies *L. echinata* subsp. *citrina*, although not considered threatened, is restricted in distribution and geographically disjunct from the two critically endangered subspecies.

All subspecies flower between September and January. They lack a lignotuber, are killed by fire and regenerate from seed.

2.2 Distribution, habitat and conservation status

Lambertia echinata subsp. *echinata* is Declared Rare (Threatened) Flora under the Western Australian Wildlife Conservation Act 1950 and is recommended to be listed as endangered under the ANZECC categories. It is only known from one location in the Lucky Bay area (within Cape Le Grand National Park) near Esperance (Fig. 2.1). This population grows in lateritic gravels and sandy clay soils on exposed coastal slopes dominated by large granite outcrops and underlayed with granite sheeting. These windswept areas are also dominated by kwongan vegetation of high species richness including low mallees and heaths. Only two subpopulations were known. Population 1A currently has 3 live plants located on a remnant vegetation 'island' within a gravel

pit. Population 1B had seven recorded plants, and was 500 metres upslope of the gravel pit near the base of a large granite outcrop. An inspection in August 1992 found that all these had died, apparently due to *Phytophthora cinnamomi* (dieback) infection, and no plants have since been recorded at this site.

To date, no other populations of *L. echinata* subsp. *echinata* have been found elsewhere within the National Park or in adjacent areas. An extensive survey program was carried out over 18 months, as part of this project, to try and locate further populations within the Cape Le Grand area. Despite this survey effort no further populations were located. This taxon is clearly critically endangered particularly given the ongoing threat from *Phytophthora cinnamomi* which is widespread in the adjacent vegetation.

Lambertia echinata subsp. *occidentalis* is Declared Rare (Threatened) Flora under the Western Australian Wildlife Conservation Act 1950 and is recommended to be listed as endangered under the ANZECC categories. It is also strongly recommended that this taxon be considered for listing as Critically Endangered by the WA Threatened Species Scientific Committee.

L. echinata subsp. *occidentalis* occurs in rich scrub heath on shallow soils over sheet ironstone in the Whicher Range area, near Busselton (Fig. 2.1). Only seven adults and three seedlings are known from a single population. Again the area has been well surveyed (Keighery pers. comm.; Gibson, pers. comm.) and no further populations have been located.

In contrast of *L. echinata* subsp. *citrina* although geographically restricted from Albany to Cheyne Beach, is found in a number of relatively large populations and is not considered to be currently threatened. However, it does occur in areas heavily infected with *Phytophthora cinnamomi* dieback and may be threatened by that pathogen in the future.

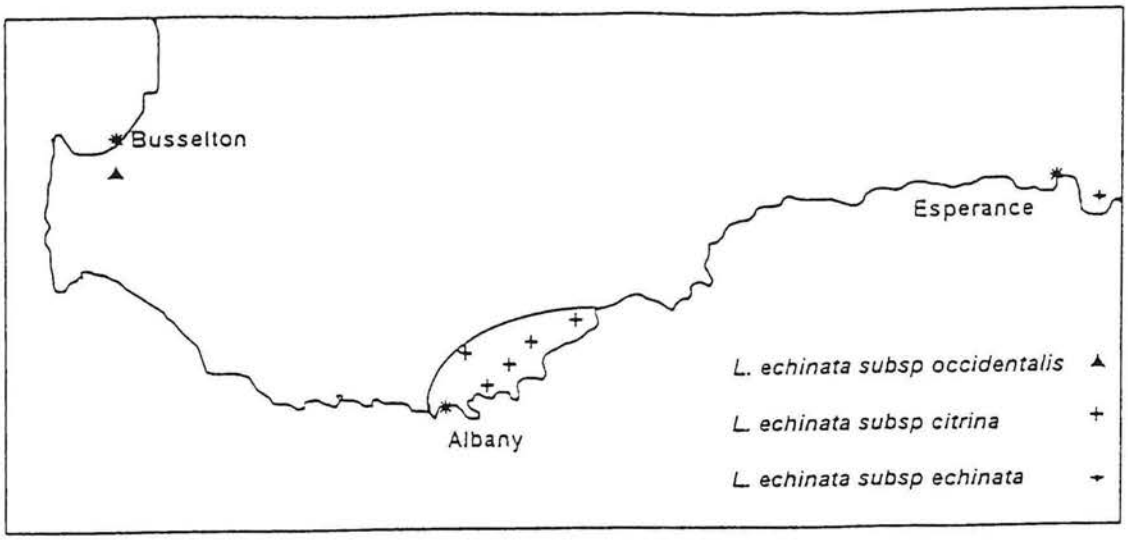

Figure 2.1 - Distribution of all *L. echinata* subspecies

2.3 Taxonomic description

Lambertia echinata was first described in 1810 by noted botanist, Robert Brown, from specimens he collected while in the Esperance area. In 1830, Brown described *L. propinqua* from material collected by W. Baxter from the Albany area. This name was subsequently treated as a synonym of *L. echinata* subsp. *citrina* by Hnatiuk (1995). Hence Brown's *L. echinata* becomes *L. echinata* subsp. *echinata*. Recently, G. Keighery has described another subspecies (Keighery, 1997), *L. echinata* subsp. *occidentalis*, from the Whicher Range, Busselton district. When this subspecies was first discovered it appeared to be subspecies *citrina* although there were some minor differences. Population genetic studies on the *L. echinata* complex (see section 2.6) revealed significant genetic differences between the Whicher Range population and other populations of *L. echinata* subsp. *citrina*. These data lead to a re-assessment of the taxonomic status of this population and it was subsequently described as a new subspecies.

The population genetic studies also revealed an extremely high genetic distance between *L. echinata* subsp. *echinata* and the two other subspecies (Fig. 2.2). These data suggest that this taxon may warrant species status.

L. echinata subsp. *echinata* is a shrub to 1 m tall with erect, spreading branches and no lignotuber known to exist (i.e. a seeder) (Hnatiuk, 1995). The shrub is much branched with younger branches densely villous. Leaf arrangement is opposite or whorled. Leaves have a short petiole (0-2 mm), are up to 30-40 mm long, tapering towards the stems (narrowly cuneate), glabrous and with raised prominent veins on the underside. Usually all floral and vegetative leaves are 3-5 undulate lobed with each lobe having a long pungent point (Erickson *et al.*, 1979; Blackall and Grieve, 1988; Hnatiuk, 1995). Inflorescences are 7-flowered subtended by numerous loosely enclosing bracts, the inner bracts, being two thirds the length of the perianth. The flower is typical of the *Lambertia* genus, approximately 40 mm long, orange-red to pink in colour and at maturity has the upper perianth lobes recurved and a deep adaxial suture (Blackall and Grieve, 1988; Hnatiuk, 1995). Inflorescences are also held on short branchlets in the main body of the plant (Keighery, 1997). There are four nectar glands at the base of the flower near the ovary. The pollen presenter (style) is slender and exerted, and sparsely villous on the lower half. Fruits are approximately 5-8 mm in diameter (beak included), a slightly flattened ovoid shape and often shiny grey in colour and distinctively covered with spines, (Erickson *et al.*, 1979; Hnatiuk, 1995). Each fruit may contain two circular seeds with a narrow annular wing (Hnatiuk, 1995).

L. echinata subsp. *citrina* differs from the above by being a shrub to 2.5 m tall with yellow or yellowish-green flowers (Blackall and Grieve, 1988). The leaves are smaller than subspecies *echinata* at 15-35 mm long, thicker and often lacking the raised veins below (Hnatiuk, 1995). Keighery (1997) described all floral and vegetative leaves of subspecies *citrina* as having 3-5 lobes with rigid points at their apex. He also stated that both subspecies *citrina* and *occidentalis* have inflorescences on short branchlets borne on long erect flowering branches some 3 m tall above the main plant canopy. This growth pattern characteristic is quite different to subspecies *echinata*.

L. echinata subsp. *occidentalis* is a shrub taller again, at 3 m, much branched at the base, but with only several long erect floral branches (as mentioned above). Floral leaves (immediately below inflorescences) have up to 5 pointed lobes, while slightly lower leaves are either trifid (50-80%) or entire (20-50%). The remaining vegetative

leaves are all entire and this is quite different to both subspecies *echinata* and *citrina*. Vegetative leaves are 17-45 mm long, linear-lanceolate with a pungent apex. Flowers are yellow like subspecies *citrina* (7 to an inflorescence), 23-26 mm long, with the floral bracts being longer (15-19 mm) than subspecies *citrina* (12-30 mm), but both being shorter than subspecies *echinata* (Keighery, 1997).

2.4 Disease susceptibility and control

Dieback caused by *Phytophthora cinnamomi* and other *Phytophthora* species is considered to be a critical management issue in relation to the conservation of all three subspecies in the *Lambertia echinata* complex. Field observations associated with isolation of *Phytophthora cinnamomi* from soils near dead plants of *L. orbifolia* and *L. inermis*, indicate that *Lambertia* species are highly susceptible to this pathogen (CALM Vegetation Health Service) and the three subspecies in the *Lambertia echinata* complex occur in or adjacent to vegetation where large scale infections of *P. cinnamomi* are currently known.

The pathogen has been positively identified throughout many areas of the Cape Le Grand National Park. The scrub heath vegetation on shallow sandy soils over laterite, typical of the vegetation in which *L. echinata* subsp. *echinata* is found, is considered to be particularly susceptible to *P. cinnamomi*. The warm, wet conditions experienced throughout most of the year at Cape Le Grand National Park and the shallow sandy soils provide ideal conditions for the rapid spread of this pathogen.

The CALM Esperance district has not undertaken testing for *P. cinnamomi* at or around the vicinity of the gravel pit where *L. echinata* subsp. *echinata* still exists, but field observations indicate it is present in the surrounding area (Table 2.1). Most of the gravel pit has been deep ripped, covered with stockpiled topsoil and left to regenerate. During this time (2 years ago), several *L. echinata* subsp. *echinata* seedlings were also planted out and two are still surviving. At present, natural

regrowth in the pit area appears healthy and vigorous, but the area has not been sprayed with phosphonate to control *P. cinnamomi*.

Table 2.1 - Disease (*Phytophthora cinnamomi* and canker) presence and control information for all *Lambertia echinata* subspecies

POPULATION NUMBER-CALM	POPULATION DISEASE STATUS
subsp. *echinata*	
1A (3 adults)	*Phytophthora cinnamomi* (dieback) confirmed in area adjacent to gravel pit
1B	All plants dead in 1992 and *P. cinnamomi* is strongly suspected
subsp. *citrina*	
Many populations	The location of this subspecies is in coastal heath/woodlands from Albany to Cheyne Bay. *P. cinnamomi* and canker are common throughout this area
subsp. *occidentalis*	
1 (10 plants)	*P. cinnamomi* positively tested ~300 m to the south near the access road

L. echinata subsp. *citrina* is located in an area of coastal heath and woodlands in which a number *P. cinnamomi* and canker infections have been positively identified (CALM Vegetation Health Service). A number of populations of this subspecies are likely to be infected or adjacent to infected sites. Further, *Phytophthora citricola* has been isolated from tissues of a dead *L. echinata* subsp. *citrina* plant (CALM, Vegetation Health Service).

The *L. echinata* subsp. *occidentalis* population is within a few metres of what appears to be a significant *P. cinnamomi* infection. This requires immediate confirmation. *P. cinnamomi* has also been positively identified ~300 m away along the main track into the area and serious *P. cinnamomi* infections are located some distance upslope in State Forest. As yet, no phosphonate treatment or other disease management has occurred at this site.

2.5 Seed biology - germination data and storage

The CALM Threatened Flora Seed Centre, has collected seed from the population of *L. echinata* subsp. *echinata* (3 seed lots) and *L. echinata* subsp. *occidentalis* (2 seed lots). These 5 seed lots are now stored at -18^{0}C. Seed has also been collected from two populations of *L. echinata* subsp. *citrina* and both these seed lots are stored at 4^{0}C. Seed germination information and seed storage data are shown in Table 2.2.

Table 2.2 - Seed germination and storage data for all subspecies collections of *L. echinata*.

Colln date	Location	Storage date	Total seed stored	Initial % germ.	Test date	1 year % germ.	Retest date
subsp. echinata							
29/01/93	Lucky Bay	01/06/93	170	93.0	24/03/93	87.5	02/06/94
14/01/94	Lucky Bay	30/01/94	691	98.0	18/01/94	93.5	31/01/95
20/10/95	Lucky Bay	02/07/96	192	100.0	01/11/95	-	-
subsp. citrina							
15/06/95	Millbrook Rd	30/06/95	47	57.5	20/06/95	-	-
11/01/95	Two Peoples Bay	17/01/95	264	98.0	17/01/95	-	-
subsp. occidentalis							
12/12/95	Whicher Range	01/07/96	62	-	-	-	-
26/02/97	Whicher Range		204	86.8			

The initial percentage germination rates for *L. echinata* subsp. *echinata* are high (> 90%) with a slight reduction in viability 1 year later. Percentage germination for *L. echinata* subsp. *citrina* are variable with one very good result (i.e. 11/01/95-Two Peoples Bay) and one poorer result (i.e. 15/06/95-Millbrook Rd). The initial collection of *L. echinata* subsp. *occidentalis* was not germinated for viability testing because of the small quantity of seed and the need to store as much seed as possible. However, the more recent collection was tested and gave an encouraging initial percentage germination of 86.8%.

2.6 Population genetic structure

Population genetic structure and patterns of differentiation among populations were investigated using isozyme markers. Ten enzyme systems were assayed: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH), esterase (EST), glucosephosphate isomerase (GPI, E.C. 5.3.1.9), glucose 6 phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.17.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), menadione reductase (MDR, 1.6.99.22) and phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphocluconate dehydrogenase (PGD, E.C. 1.1.1.44) and shikimate dehydrogenase (SKUD). A total of 22 isozyme loci were scored. Eight loci *Aat*-1, *Aat*-2, *Adh*-1, *G6pdh*-1, *Gpi*-2, *Mdh*-2, *Mdr*-2, *Pgd*-2, were not polymorphic. Genetic variability was studied at 14 isozyme loci based on ten enzyme systems.

Single locus genetic diversity measures mean number of alleles per locus *A*, mean percentage polymorphic loci *P*, expected panmictic heterozygosity *He*, and the average observed heterozygosity *Ho* are presented in Table 1.3. With the exception of *L. echinata* subsp. *occidentalis* all values are less than those found in *L. orbifolia* populations and are comparable to levels of genetic diversity found in other endemic species (see Hamrick and Godt, 1989). The *L. echinata* subsp. *occidentalis* population has significantly higher genetic diversity levels, based on all single locus

diversity. These results are unexpected given the very small size of this population compared with the larger populations found in *L. echinata* subsp. *citrina*. They suggest that quite different evolutionary mechanisms may be operating in the two subspecies. These mechanisms may relate to fluctuations in population size over time or differences in the mating system.

Table 2.3 Single locus genetic diversity measures: *A* (mean number of alleles per locus), *P* (mean percentage polymorphic loci), *He* (expected panmictic heterozygosity), and *Ho* (the average observed heterozygosity), for populations of *L. echinata* and *L. fairallii*

Taxon	Population		N_e	*A*	*P*	H_e	H_o
L. echinata subsp. *citrina*	Two Peoples Bay	1	≈50	1.2	18.2	.067 (.032)	.029 (.014)
	Cheyne Beach	2	≈100	1.3	22.7	.054 (.031)	.017 (.008)
	Boulder Hill	3	≈40	1.3	18.2	.066 (.035)	.028 (.015)
	Cape Riche	4	≈100	1.3	27.3	.070 (.037)	.026 (.015)
	Millbrook Road	5	≈20	1.1	13.6	.047 (.029)	.015 (.015)
L. echinata subsp. *occidentalis*		1	10	1.5	36.4	.132 (.043)	.080 (.027)
L. echinata subsp. *echinata*		1	3	1.1	13.6	.038 (.025)	.015 (.012)
L. fairallii		1	20	1.0	.0	.000 (.000)	.000 (.000)
		4	≈5,000	1.1	13.6	.031 (.019)	.015 (.011)

The relatively high genetic levels of allelic diversity indicate that there may be a suitable broad range of genetic diversity in this population of *L. echinata* subsp.

occidentalis to support a successful translocation program. Population genetic structure was also studied in two populations of *L. fairallii* and these data will be discussed in section 3.1.

As in *L. orbifolia*, there is a large discrepancy between the observed heterozygosity (*Ho*) and the expected panmictic heterozygosity (*He)* in populations of all subspecies. These data suggest that there are significant levels of inbreeding within populations. We believe that, as in *L. orbifolia*, the high levels of inbreeding are due to significant levels of selfing and mating between related plants within populations. Field observations indicate that all subspecies of *L. echinata* rely largely on bird pollination and although no pollination biology studies were carried out on theses subspecies it is likely that pollinator behaviour will be similar to that observed in *L. orbifolia*. That is, honeyeaters tend to spend much of their nectar gathering activities within a relatively small area, probably covering a few neighbouring closely related plants.

A UPGMA analysis of genetic differentiation between populations of the three subspecies and the closely related species *L. fairallii* is presented in Figure 2.2. It shows a very high level of genetic divergence between *L. echinata* subsp. *echinata* and the other two subspecies (> 0.50) which is indicative of species or even generic differences in some plant groups (Gottleib, 1981). This supports the earlier taxonomic treatments of this species complex which indicated that *L. echinata* subsp. *echinata* was a separate species.

L. echinata subsp. *occidentalis* is also genetically distinct from *L. echinata* subsp. *citrina* although clearly not as divergent as *L. echinata* subsp. *echinata.* As indicated in section 2.3 these data led to a re-assessment of the taxonomic status of this population and it was subsequently described as a new subspecies.

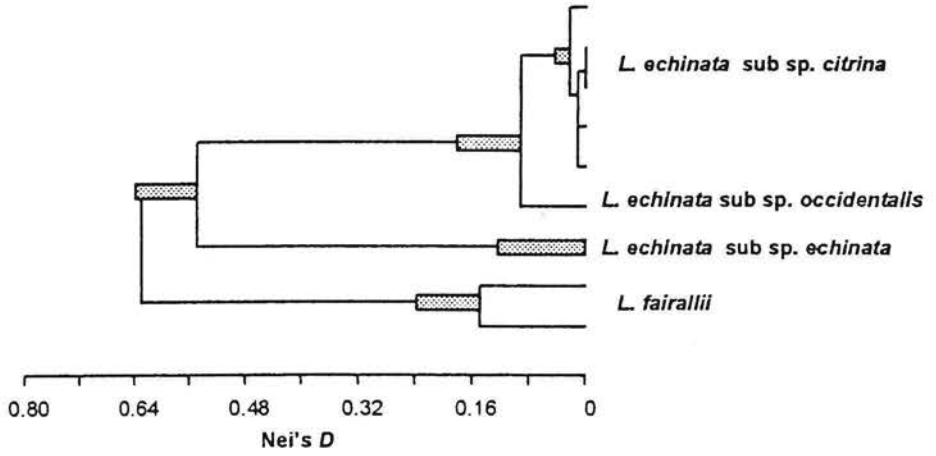

Figure 2.2. UPGMA clustering (based on Nei's genetic distance, *D*) of *Lambertia echinata* and *Lambertia fairallii* populations. A cluster in the UPGMA phenogram is significant if the shaded standard error bar is half the branch length.

Recommendations for conservation and management

1. Disease assessment and control

- *L. echinata* subsp. *echinata* requires the following management actions:
  - The remaining three plants and adjacent vegetation should be treated with phosphonate as soon as possible.
  - Access to the gravel pit should be restricted and strict hygiene conditions followed.

- *L. echinata* subsp. *occidentalis* requires the following management actions:
  - phosphonate treatment should be carried out on the site through aerial application as soon as possible.

- Access to the area by foot should be restricted and strict hygiene conditions followed.
- There should be no access to vehicles at any time of the year.

2. Population augmentation and translocation

- *L. echinata* subsp. *echinata*:
  - Sufficient seed is available, from the three remaining plants, to substantially enhance this population. Seedlings should be established in the gravel pit and in adjacent vegetation, aimed at increasing population size to at least 100 mature plants. This should be carried out in conjunction with ongoing control of *P. cinnamomi* using phosphonate.

- *L. echinata* subsp. *occidentalis*:
  - Although only ten plants are currently recorded, enhancement is not currently recommended for this population. Recent surveys have revealed natural recruitment (three new plants) and close monitoring is recommended over the next two years. If no further recruitment is evident over the next two years then population enhancement is recommended. This must be carried out under strict disease hygiene conditions and in conjunction with phosphonate application.

3. Research

The very low number of plants found in the two critically endangered species makes any population ecology studies extremely difficult to carry out. Further research should focus on the propagation and trial establishment of new populations in association with the appropriate control of *Phytophthora*.

4. Recovery Plan

Both *L. echinata* subsp. *echinata* and *L. echinata* subsp. *occidentalis* are critically endangered and it is recommended that recovery plans be prepared as soon as possible.

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3.0 *LAMBERTIA FAIRALLII* Keighery

3.1 Introduction

Lambertia fairallii is a small woody shrub restricted to the Stirling Range National Park. It is Declared Rare (Threatened) Flora under the Western Australian Wildlife Conservation Act and is currently considered to be endangered by the Western Australian Threatened Species Scientific Committee, and endangered according to ANZECC categories.

3.2 Distribution, habitat and conservation status

L. fairallii was first discovered in 1968 at one small location near Ellen Peak in the eastern end of the Stirling Range National Park. Since then, four further populations have been found, two more around Ellen Peak area, one much further west (Mt Gog) and the last at Mt Success which is an intermediate location to the others (Fig. 3.1).

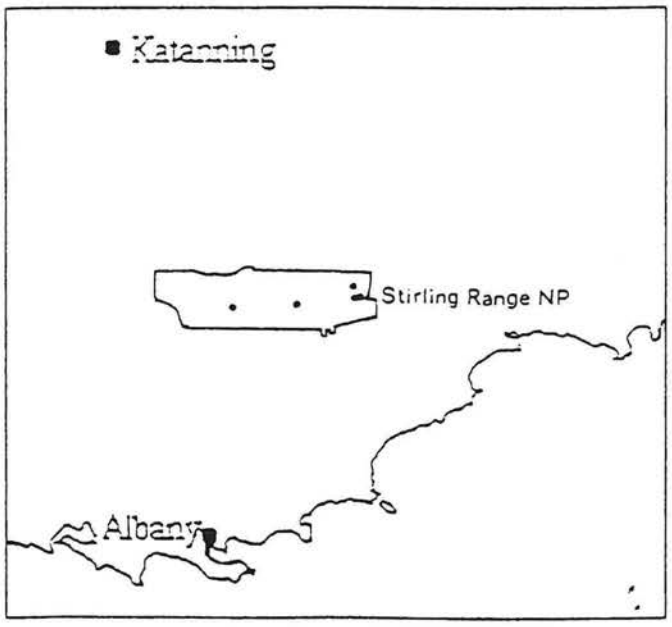

Figure 3.1 - Distribution of *L. fairallii* within the Stirling Range National Park

Population data for *Lambertia fairallii* is shown in Table 3.1, but some explanatory statements are still required. A 1991 wildfire burnt all plants in population 2 and no seedling regeneration has been subsequently recorded. In the same fire population 1 faired better with 25% of the adults unburnt and some seedling regeneration observed (i.e. 10 adults and 10 seedlings). Population 3 (with an unspecified number of plants) was found many years ago and only an approximate location was recorded. In recent years attempts to find it have failed, but it was also affected by the hot 1991 wildfire and any regenerating seedlings may still be too small to be readily recognised.

Recently, a wildfire burnt the largest population (Mt Gog) but it is too early to assess levels of regeneration in the area. The most recently found population, at Mt Success, has ~400+ seedlings which probably regenerated from the 1991 fire. Unfortunately, 10% of these are dead, probably due to *P. cinnamomi* infection.

Currently, nearly all plants in all populations are seedlings or in the case of the Mt Gog population will be very young seedlings in the next 6 months. This may be cause for concern because of the increased susceptibility of seedlings to *P. cinnamomi*. These concerns will be addressed in more detail in section 3.4

Table 3.1 - Population data for *Lambertia fairallii*

LOCATION	POPULATION NUMBER-CALM	NUMBER OF LIVE PLANTS	POPULATION CONDITION
SE of Ellen Peak	1	20	Moderate
SE of Ellen Peak	2	0?	Burnt 1991
N of Ellen Peak	3	?	Unknown
S of Mt Gog	4	~5000+ (before fire)	Good (prior to 1996 fire)
S of Mt Success	5	~400+ seedlings	Moderate

All *L. fairallii* populations appear to grow on the exposed rocky midslopes of these ranges (~350 -500 m ht.) where soils are residual. Hnatiuk (1995) states that the soils are shallow, peaty sand over quartzite or sandy loam with its vegetation being a kwongan type. This infers a species rich community, but at populations 1 & 2 (Ellen Peak) the habitat is unusual in that there appeared to be few associated plants in the community (Keighery pers. comm.). These included *Eucalyptus* sp., *Dryandra fraseri, Beaufortia decussata, Melaleuca* sp., *Hakea ambigua, Andersonia echinocephala* and some others. At kwongan sites the associated species include *Eucalyptus marginata* (mallee form), *Dryandra serecifolia, D. formosa, D. armata, Isopogon latifolius, I. baxteri, Banksia solandri, B. sphaerocarpa, Lambertia ericifolia, Conospermum* sp., *Daviesia trigonophylla, Andersonia echinocephala, Xanthorrhoea platyphylla, Agonis pariceps, Allocasuarina humilis, Kingia australis* and numerous others. *L. fairallii* grows as one or two individuals interdispersed among other shrubs (e.g. population 4) or may also occur as small clusters (e.g. population 1).

This species has been known to flower from January, May and September (Hnatiuk, 1995) and like the other two *Lambertia* species is killed by fire and regenerates from seed.

3.3 Taxonomic description

Lambertia fairallii appears to be quite closely related to the *L. echinata* complex.
It is an erect spreading shrub to 1.5 m tall, lacking a lignotuber (i.e. a seeder), with dense branches and younger branches pilose (Keighery, 1983; Hnatiuk, 1995). Leaves are crowded around short branchlets and are either shortly petiolate to sessile (Blackall and Grieve, 1988). Most mature leaves are very narrowly elliptic to cuneate, revolute, have entire margins and have a short acute point (Blackall and Grieve, 1988; Hnatiuk, 1995). However, there are some leaf variants that are irregularly trifid lobed (Keighery, 1983; Hnatiuk, 1995). Mature leaves are 20-40 mm long, 2-5 mm wide, glabrous above and pilose underneath.

Inflorescence is 5-7 flowered, subtended by involcure of stiff bracts with a tuft of hairs at their apex. The inner most bracts may reach the adaxial suture which is not as deeply split as in many other Lambertias. Perianth is 30-40 mm long, bright yellow, and mostly glabrous, although the perianth lobes are surmounted by a tuft of hairs (Keighery, 1983; Hnatiuk, 1995). Nectar glands are small, basifixed and located near the ovary. The style (pollen presenter) is linear, extruded above the perianth lip and covered by fine sparse hairs (sometimes pilose) (Keighery, 1983). Fruit is 6-7 mm long, mainly has smooth sides, is beaked at stylar end and distinctively 2 horned at the opposite end (Hnatiuk, 1995). There may be 2 seeds/fruit (open upon death of branch) and these are circular (~5 mm dia), flat on one side and domed on the other with a tuberculate pattern and a narrow annular wing (Keighery, 1983; Hnatiuk, 1995).

3.4 Disease susceptibility and control

Dieback due to *Phytophthora cinnamomi* is a major management concern within the Stirling Range National Park with several endangered and numerous priority flora directly threatened by this disease.

P. cinnamomi has been confirmed at or near two sites (populations 1 & 2), but no other populations have been tested. Populations 1 and 2 are on nearby adjoining ridge spurs, south east of Ellen Peak and appear to be in the path of rapidly approaching dieback fronts. Although no deaths have yet been recorded, Population 1 and the surrounding vegetation were treated with phosphonate during mid 1994 and 1995.

Population 3 has yet to be relocated but lies somewhere near the main walk trail to Ellen Peak and it is likely that it will eventually be threatened by dieback.

The recently burnt population 4 (Mt Gog) appeared healthy prior to the fire and there is no indication that *P. cinnamomi* is in the vicinity. However, seedling regeneration should be closely monitored for signs of any disease. The newly found population 5 (Mt Success) has ~10% of it's plants dead from what appears to be dieback. The

presence of *P. cinnamomi* in this population needs to be confirmed as soon as possible. If positive, phosphonate application should be considered immediately. Control of *P. cinnamomi* is of particular concern in this case because of the high proportion of seedlings which are generally considered to be more susceptible than adult plants.

Table 3.2 - Dieback (due to *Phytophthora cinnamomi*) presence and control information for *Lambertia fairallii*

POPULATION NUMBER-CALM	POPULATION DISEASE STATUS
1	Dieback in area. Phosphonate treated mid 1994 & 1995
2	No plants found since 1991. Dieback suspected in the area
3	Pop. not located for sometime. Dieback status unknown
4	Pop. recently burnt. Dieback status unknown, suspect ok
5	Dieback suspected in area. Phosphonate treatment soon

3.5 Seed biology - germination data and storage

The Threatened Flora Seed Centre, Dept of CALM has taken 4 collections of seed from two populations of *L. fairallii* (populations 1 & 4). All seed lots are now stored at -18°C at this facility. Initial percentage germination was determined for all collections including 1 year percentage germinations for three seed lots. Seed germination and storage data are shown in Table 3.3.

The initial percentage germination rates for *L. fairallii* were relatively high (> 90%) for the population 4 (Mt Gog) seed lots with only one slightly lower result (i.e. 11/11/93 collection at 80%). Percentage germination 1 year later for this population was variable with one seed lot retaining viability and another displaying a marked decrease. Initial percentage germination for the population 1 (Ellen Peak) seed lot was very

poor, although there was a significant improvement in viability one year later. This suggests that the initial germination test may be incorrect.

Table 3.3 - Seed germination and storage data for collections of *L. fairallii*.

Colln date	Location	Storage date	Total seed stored	Initial % germ.	Test date	1 year % germ.	Retest date
21/09/93	Mt Gog	05/05/94	80	100.0	27/09/93	-	-
11/11/93	Mt Gog	30/01/94	229	80.0	21/12/93	50.0	31/01/95
15/02/95	Mt Gog	06/02/95	1075	96.5	18/01/95	98.0	07/02/96
12/05/94	Ellen Peak	18/07/94	135	33.0	16/05/94	66.5	21/07/95

3.6 Population genetic structure

Population genetic structure and patterns of differentiation among populations were investigated using isozyme markers. Ten enzyme systems were assayed: aspartate aminotransferase (AAT, E.C. 2.6.1.1), alcohol dehydrogenase (ADH), esterase (EST), glucosephosphate isomerase (GPI, E.C. 5.3.1.9), glucose 6 phosphate dehydrogenase (G6PDH, E.C. 1.1.1.49), isocitrate dehydrogenase (IDH, E.C. 1.1.1.42), leucine aminopeptidase (LAP, E.C. 3.4.17.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), menadione reductase (MDR, 1.6.99.22) and phosphoglucomutase (PGM, E.C. 2.7.5.1), phosphocluconate dehydrogenase (PGD, E.C. 1.1.1.44) and shikimate dehydrogenase (SKUD). A total of 22 isozyme loci were scored. Eight loci *Aat*-1, *Aat*-2, *Adh*-1, *G6pdh*-1, *Gpi*-2, *Mdh*-2, *Mdr*-2, *Pgd*-2, were not polymorphic. Genetic variability was studied at 14 isozyme loci based on ten enzyme systems.

Single locus genetic diversity measures mean number of alleles per locus *A*, mean percentage polymorphic loci *P*, expected panmictic heterozygosity *He*, and the average

observed heterozygosity *Ho* are presented in Table 2.3. All values are less than those found in *L. orbifolia* populations and *L. echinata* subsp. *occidentalis* but are comparable to levels of genetic diversity found in *L. echinata* subsp. *citrina*, *L. echinata* subsp. *echinata*. and other endemic species (see Hamrick and Godt, 1989).

There is no obvious explanation for the lack of genetic diversity found in Population 1. It may simply be due to sampling error because seed could only be collected from a few mature plants or it could indicate clonality. However, the latter seems unlikely given that this species is a seeder and there is no evidence of suckering from lateral root systems.

A UPGMA analysis of genetic differentiation between the two populations of *L. fairallii* and the three subspecies in the *L. echinata* complex is presented in Figure 2.2. It shows significant genetic divergence between the populations at Mt Gog and Ellen Peak, (Nei's $D \approx 0.15$) slightly higher than the divergence found between *L. echinata* subsp. *citrina* and *L. echinata* subsp. *occidentalis*. This genetic differentiation may be the result of prolonged isolation of these populations on different peaks in the Stirling Ranges and warrants further investigation. If confirmed, the level of genetic divergence suggests that populations on different peaks should be treated as separate conservation units.

3.7 Recommendations for conservation and management

1. Disease assessment and control

- All populations, particularly the Mt Gog site, should be assessed or re-assessed for the presence of *P. cinnamomi* and the potential threat this pathogen poses. Spread from adjacent areas, particularly those upslope, is a particular concern.

- Current phosphonate application programs should continue and population 5 should be treated as soon as possible

2. Recovery Plan

This species does not currently warrant a Recovery Plan although it should be given careful consideration in any management actions targeting areas of the Stirling Ranges where it occurs.

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