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Genecological Differentiation in *Leptospermum flavescens* Sm. in the Sydney Region

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Abstract

Evidence is presented for the existence of two genecologically differentiated groups in *Leptospermum flavescens* in the Sydney region. A morphological study of 18 attributes of herbarium specimens indicates three groups of plants: one small-leaved, one large-leaved and one intermediate. Comparative cultivation under standard conditions and seed germination experiments indicate only two discrete groups, because the intermediate group behaves similarly to the large-leaved group. Correlation of population distribution with soil nutrient status suggests that organ size in the large-leaved group is phenotypically plastic in relation to low soil nutrients. This is confirmed by comparative cultivation under high and low nutrient regimes. Reciprocal transplanting of cuttings on a small scale suggests that the two groups are intolerant of each other's habitats.

Introduction

Many plant species are genecologically differentiated (see Heslop-Harrison 1964; Langlet 1971). Phenotypic variation between populations in a plant species may have one of three origins (Heslop-Harrison 1964): (1) the direct plastic modification of individuals; (2) genetic divergence as a result of selection, for which Harberd (1957) coined the term genecological differentiation; and (3) fortuitous genetic divergence (e.g. drift in small populations, or the establishment of deviating colonies from small numbers of founders). The variation arising from the first two origins may show correlation with habitat factors; the third will be random with respect to habitat. To distinguish and study variation arising from (2) is the essential problem of genecology. This can be seen as having: the discrimination of adaptive and random interpopulation variation, and the separation of this adaptive variation into genetic and non-genetic elements.

The answers can be sought non-genetically in two ways (Heslop-Harrison 1964). The direct approach examines experimentally whether populations in different parts of the species range vary in their response to diverse environmental variables, an essentially physiological approach. The indirect approach seeks correlations between 'plant type' and 'habitat type', which may indicate adaptive divergence due to differential selection between the various habitats, or phenotypic conditioning. To date, this indirect approach has been *ad hoc*, but it can be considered systematically in five sequential steps:

(1) classification of morphological variation of the species;
(2) comparative cultivation of classified samples under standardized conditions to differentiate between phenotypic and genotypic variation;
(3) correlation of the genotypic variation with one or more environmental variables;
(4) comparative cultivation of classified samples under varied experimental conditions to test the effect of the hypothesized environmental correlate(s); and
(5) reciprocal field transplants of classified samples.

This last point is particularly important, as this type of study can only be usefully extrapolated to the natural situation when experimental conditions are related to those in nature (Grime 1965).

This study investigated genecological differentiation in *Leptospermum flavescens* Sm. using this indirect approach. Genecological experiments on *Leptospermum* in Borneo, using an *ad hoc* approach, have been successful (Lee and Lowry 1980).

L. flavescens s.l. is an extremely variable group. An adequate solution of the taxonomic problems involved would require a generic revision (cf. Lee and Lowry 1980). The work in this paper concentrates on the variation apparent in four floristic districts of New South Wales: the central and south coasts and the central and southern tablelands. These districts make up an area in the Sydney region and south of it, wherein this species var. *flavescens* and var. *grandiflorum* of Beadle *et al.* (1963) occur, both of which have divergent (rather than parallel) anther cells (J. Thompson, personal communication). The validity of this status became apparent during the study.

Morphological Classification

Methods

As the data available were inadequate to resolve the morphological variation in *L. flavescens*, a more detailed morphological study was undertaken. A sample of 203 dried herbarium specimens from both the National Herbarium of New South Wales and the John Ray Herbarium of the University of Sydney (as listed in Morrison 1979) were examined under a binocular dissecting microscope. Several less complete specimens were rejected. The leaves were examined without further treatment, but the flowers were soaked in a weak detergent solution for approx. 10 min, then dissected. Twenty mature leaves per specimen were examined, but due to the paucity of flowers on many of the specimens, only three flowers could be dissected per specimen.

The 18 attributes eventually chosen for analysis are listed in Table 1, with further details of the multistate attributes in Table 2.

None of the available numerical classificatory methods could be used for determining phenetic relationships between the specimens (i.e. considering each specimen as an operational taxonomic unit) (see Sneath and Sokal 1973), as approx. 60% of the specimens did not have flowers and so could not be coded for over half of the attributes. Substitution for a large number of 'missing values' would have distorted the classificatory procedure, and anyway 18 attributes are not enough for standard numerical taxonomic methods to separate taxa from each other (about 50 attributes are required) (Sneath and Sokal 1973). The specimens were thus classified intuitively. Ordination procedures were also considered but were rejected since, as discussed below, much of the morphological variation observed could be ascribed to phenotypic plasticity so that such procedures would probably have added little if anything to the intuitive analysis (R. Carolin, personal communication).

Results and Discussion

The subjective morphological classification divided the specimens into two major groups, one small-leaved (with 125 specimens), the other large-leaved (55 specimens) (Table 1). Both these groups are homogeneous for number of abaxial leaf veins (in the small-leaved group) and adaxial leaf veins (in the large-leaved group), and the number

Table 1. Attributes and attribute states for the three groups of herbarium specimens from the morphological analysis

Average data include av. measurements for all specimens included in each group; range data include all measurements for each specimen in each group.* See Table 2 for the states of the multistate attributes. Details of each attribute state for each specimen studied are given by Morrison (1979)

Attribute	Group		
	Small-leaved	Large-leaved	Intermediate
Leaf length (mm)	av. <20·0 range 8–22	av. >22·0 range 19–50	av. 14·0–22·0 range 12–30
Leaf breadth (mm)	av. <3·5 range <4·0	av. >3·5 range 3–9	av. 2·0–5·0 range 2–6
Leaf pubescence*	0	0, 1	0 (rarely 1)
Leaf tip*	0, 1	1, 2	1, 2
Abaxial leaf veins*	0 (rarely 1 in largest leaves)	1, 2	0, 1
Adaxial leaf veins*	usually 0, 1 occasionally 2	2	0, 1, 2
Petal length (mm)	av. <5·0 range 2·5–5·0	av. >5·0 range 5·5–9·5	av. >5·5 range 5·5–8·0
Petal breadth (mm)	av. <4·5 range 2·0–5·0	av. >4·5 range 4·0–9·0	av. >4·5 range 4·0–7·0
Petal shape (claw length/ petal length ratio)	usually >0·20 occasionally to 0·10	0·05–0·20	0·05–0·20
Sepal length (mm)	av. <3·0 range 1·0–3·5	av. >2·5 range 2·5–4·0	av. >2·5 range 2·0–3·5
Sepal breadth (mm)	av. <3·0 range 1·0–3·5	av. >2·5 range 2·0–4·0	av. >2·5 range 2·0–3·5
No. of sepaline stamens	2, 3, 4	5, 6	5, 6
No. of petaline stamens	1, 2, 3 (rarely 4)	3	3
Filament length (mm)	av. 2·0–4·5	av. 3·0–5·0	av. 3·0–5·0
Anther gland length*	0, 1, 2	1, 2, 3	2, 3
Style length (mm)	av. 1·0–4·5	av. 2·0–5·0	av. 3·0–4·5
Receptacle diameter (mm)	av. 3·5–6·5	av. 5·0–7·5	av. 5·0–7·0
Capsule diameter (mm)	av. 5·5–9·0	av. 6·5–8·5	av. 6·5–8·0

of sepaline stamens. The other attributes show a gradation from the small-leaved to the large-leaved group, usually with considerable overlap (except in the case of petal length).

A group of 23 specimens did not fit neatly into either of the above groups, but was internally consistent. The floral attributes all have states entirely consistent with those of the large-leaved group of specimens, while the leaf attributes have a mixture of states from both of the other groupings. The two extreme groups relate very closely to those of Bentham (1867) and Beadle *et al.* (1963) in their subdivisions of the species.

The division of the specimens is consistent with what is known of the variation of the other characteristics of *L. flavescens*: both the small- and intermediate-leaved plants have compact bark only, while the large-leaved plants may have rough bark; all small-leaved and intermediate-leaved plants are shrubs less than 3 m high, while the large-leaved plants are sometimes small trees to 5 m; small-leaved plants are recorded in any wet habitat, while the other two groups are known only from creek beds; small-leaved plants flower from late September to early January, large-leaved plants from mid December to early March and intermediate-leaved from mid December to mid January (all based on herbarium specimens). In addition, the leaf chemistry of the small- and large-leaved plants shows differences (see Penfold 1920, 1921).

Table 2. Atrribute states for the multistate attributes from the morphological analysis of the herbarium specimens

Attribute		Attribute states
Leaf pubescence	0	Pubescent only on growing apices, or absent
	1	Pubescence retained by some older leaves
Leaf tip	0	Obtuse
	1	Intermediate
	2	Acute
Abaxial leaf veins	0	One vein only visible
	1	Three veins visible for the whole leaf length on only some leaves, lateral veins usually visible for only part of the leaf length
	2	Three veins visible for the whole leaf length on all leaves
Adaxial leaf veins	0	Most leaves nerveless, occasionally one-veined on largest leaves
	1	One vein only visible for the whole leaf length
	2	Three veins visible for the whole or part of the leaf length on some or all leaves
Anther gland length	0	Gland $\frac{1}{3}$ of cell length
	1	Gland $\frac{1}{3}$–$\frac{1}{2}$ of cell length
	2	Gland $\frac{1}{2}$ of cell length
	3	Gland $>\frac{1}{2}$ of cell length

The geographic distributions of these three groups are shown in Fig. 1. The boundary areas have been drawn bearing in mind the known point distribution of collections, presumed geographic barriers and extensive field observations of most of the boundary areas.

Cultivation under Standardized Conditions

Methods

The six sites chosen, two for each of the three leaf types, appeared to be representative of the full environmental variation under which the three recognized leaf types occur as recorded on the herbarium specimens and as observed in the field. The locations of the sites are shown in Fig. 1.

Twenty mature, woody capsules were collected from each of 10 individual plants randomly sampled in each of the six sites. Three small branches were also randomly gathered from each plant sampled, and the length of a random sample of 10 leaves from each branch was determined for comparison with the offspring raised in the experiment.

Squat plastic pots, 15 cm in diam., were filled with washed river sand and placed in a heated glasshouse in three random blocks along the glasshouse bench. Ten seeds from each plant sampled were sown 1 mm below the surface of the sand in each of the three replicate pots; enough seeds were thus sown to yield at least one seedling per pot. The pots were top-watered twice daily with tap water until every pot contained at least one seedling. A nutrient solution based on that of Hoagland and Snyder (1933) was then added fortnightly, progressively increasing the concentration from a one-tenth

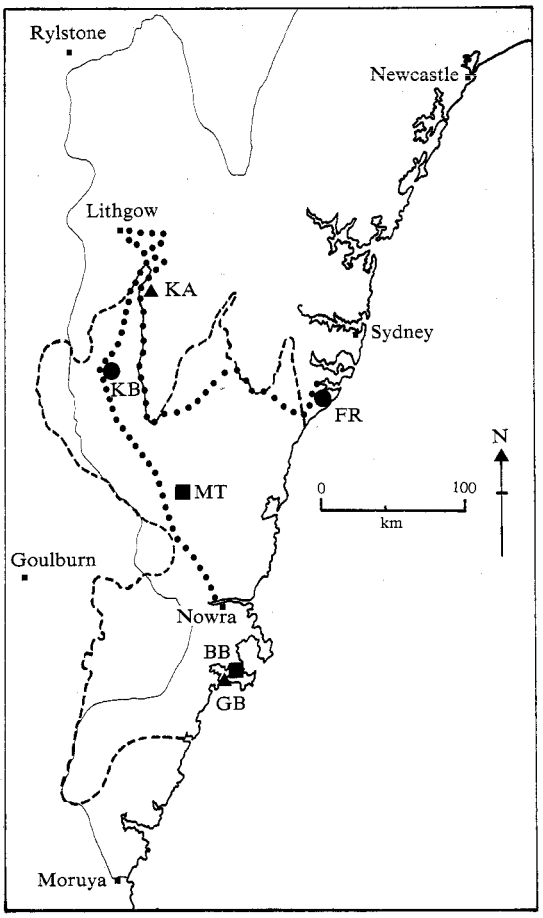

Fig. 1. Distribution of the three classes from the morphological analysis of herbarium specimens. Boundary areas of the small-leaved (solid line), intermediate-leaved (dotted line) and large-leaved (dashed line) specimens are shown. Locations of the study sites for the short-leaved (▲), intermediate-leaved (●) and large-leaved (■) specimens are also given. KA, Katoomba; GB, St George's Basin; FR, Flat Rock Crossing; KB, Kanangra Brook; MT, Mittagong; BB, Bream Beach.

dilution of the nutrient solution until the full concentration was reached 10 weeks later. The sand was kept constantly moist by bottom-watering between nutrient applications. The plants were culled to one per pot 6 weeks after sowing.

The plants were harvested 21 weeks after sowing. The shoot was cut off 2 mm above the uppermost root, and pressed overnight in a paper bag under a heavy weight. The height of each seedling was then measured, along with the length of a random sample of 10 leaves from each. The weight of the shoots after being dried at 90 °C for 48 h was then determined.

Results and Discussion

The offspring of the three parental leaf types were found to differ significantly in dry weight but not in height (Table 3). The small-leaved plants had significantly less dry

weight per shoot than those of either the intermediate- or large-leaved types (Table 4).

In the parent and offspring leaf length data, the four-factor mixed analysis of variance indicated a significant interaction between leaf type and the relationship of parent leaf length to offspring leaf length (Table 3). Parent and offspring leaf lengths were significantly different from each other for all three leaf types, and the leaf length of the intermediate-leaved plants was significantly greater in the offspring as compared with the parent, while no difference was found for either of the other leaf types (Fig. 2).

Table 3. Results of analyses of variance of data for offspring height and shoot dry weight and parent and offspring leaf length from the comparative cultivation experiment

n.s., Not significant at $P<0\cdot05$. n.r., Significant interaction means null hypothesis here is irrelevant. *Significant at $0\cdot025<P<0\cdot05$. ****Significant at $P<0\cdot005$

Source		d.f.	F	Significance
Height				
Leaf type		2	0·10	n.s.
Site		3	9·20	****
Individual		54	1·67	*
Blocks		2	0·14	n.s.
Residual		118		
Dry weight				
Leaf type		2	12·07	*
Site		3	1·70	n.s.
Individual		54	1·57	*
Blocks		2	0·11	n.s.
Residual		118		
Leaf length				
Leaf type	A	2	42·72	n.r.
Site	B(A)	3	14·05	n.r.
Individual	C(AB)	54	6·70	n.r.
Parent/offspring relationship	D	1	18·87	n.r.
A × D interaction		2	13·08	*
B(A) × D interaction		3	4·88	****
C(AB) × D interaction		54	1·79	****
Residual		240		

A clear hypothesis can be made from the leaf length and dry weight data: i.e. given the appropriate environmental conditions, the intermediate-leaved populations can resemble the large-leaved populations. Leaves of their offspring have all the characteristics of the large-leaved group (Table 1), and if these characteristics were found in the field such populations would be interpreted as large-leaved, even though in this experiment average leaf lengths were slightly less than in the two large-leaved populations.

These results are also supported by two seed germination experiments carried out during the growth experiment; in the first seeds of the three morphological leaf types were covered with sand and in the second they were subjected to different nutrient levels (see Fig. 3). In both cases, the intermediate- and large-leaved populations behaved in a statistically identical manner, which is significantly different from the behaviour of the small-leaved populations.

Environmental Correlation

Methods

Fig. 1 shows that the distributions of the intermediate- and large-leaved groups do not extend very far into the Sydney Basin, which is dominated by Hawkesbury and

Table 4. Results of Student-Newman-Keuls testing of data for offspring shoot height and dry weight from the comparative cultivation experiment
The same letters indicate means not significantly different at $P = 0{\cdot}01$

Leaf type	Mean shoot measurement per individual (±s.e.)	Student-Newman-Keuls test
Height (cm)		
Small-leaved	27·8 (5·4)	
Intermediate	28·6 (5·9)	
Large-leaved	27·5 (6·0)	
Dry weight (g)		
Small-leaved	0·567 (0·024)	a
Intermediate	0·843 (0·035)	b
Large-leaved	0·914 (0·044)	b

Narrabeen Sandstones. This suggested a correlation between parent material and leaf types, which was quantified by assigning a scale of 0–5 indicating decreasing amounts of sandstone present in the parent material, to each of the collecting sites of each of the herbarium specimens studied earlier. These data were derived from either the

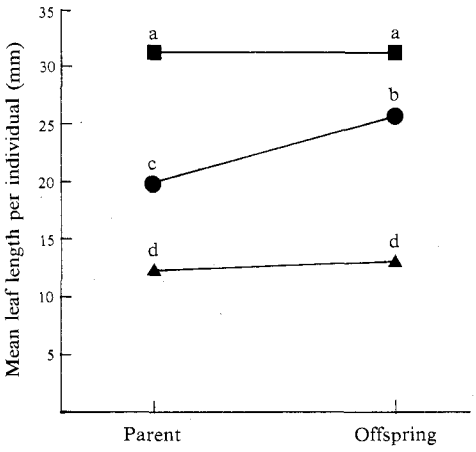

Fig. 2. Comparison of parent and offspring leaf-length data of the three morphological leaf types (small-leaved ▲, intermediate-leaved ●, large-leaved ■) for the comparative cultivation experiment. Points with the same letters are not significantly different at $P = 0{\cdot}01$, as determined by Student-Newman-Keuls tests.

herbarium labels or New South Wales Department of Mines 1:250000 Geological Series Maps. A non-parametric Spearman rank correlation was then carried out on the parent material scale and the average leaf length for each of the specimens. From these data, $r_s = +0{\cdot}2815$, and using a t-statistic as the most appropriate to test the significance of r_s for $n > 100$ (Zar 1972), $t = 4{\cdot}139$ ($n = 201$), which is significant at $P < 0{\cdot}0005$. Thus the larger-leaved specimens tend to have come from sites with less sandstone in the parent material than do the smaller-leaved specimens.

Soil analyses were used to ascertain whether soil factors correlated with leaf form. The observations were carried out in two stages. Firstly, the characteristics of the soils were investigated over the geographical range of plant forms, and then these characteristics were examined over a smaller area.

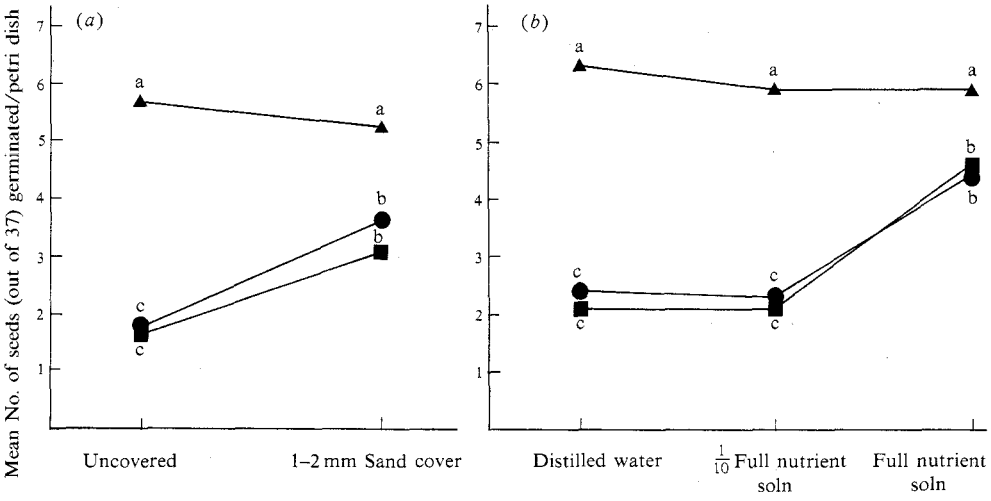

Fig. 3. Comparison of seed germination data of the three morphological leaf types (small-leaved ▲, intermediate-leaved ●, large-leaved ■) for (*a*) covering and (*b*) nutrient experiments. Seeds were germinated on wet filter paper in closed petri dishes, with one seed in each of 37 1-cm squares per dish. The populations sampled were as shown in Fig. 1, with seed from two random individuals and four replicates in each population sampled for each treatment in the covering experiment, and seed from one random individual and six replicates per treatment for the nutrient experiment. The dishes were placed on a laboratory bench adjacent to a window, and the filter paper kept constantly moist. Germination numbers were checked daily, germination being determined by emergence of the radicle. Data analysed were by analysis of variance and Student-Newman-Keuls tests. Points with the same letters are not significantly different at $P = 0{\cdot}05$.

For the general analysis, soils were collected from the six sites shown in Fig. 1. For the small-scale analysis, three sites within 200 m of each other at Echo Head, Kanangra-Boyd National Park, were used.

At each site two random samples were collected with an auger, each from the top 17 cm of the profile (excluding the A_0 horizon). Where it was not possible to sample to 17 cm, the soil was sampled down to bedrock. In most cases, three replicate analyses were carried out on each soil sample for each soil characteristic.

Since differences in parent material may influence several soil characteristics, both physical and chemical properties of the samples were studied. Most of the laboratory analyses performed follow the procedures of Allen *et al.* (1974), with minor modifications (see Morrison 1979).

Data from each soil analysis were subjected to the appropriate nested analysis of variance, and where the analysis indicated a significant difference between the soils of the three leaf types this was investigated using a Student-Newman-Keuls test.

Results and Discussion

Summaries of the results of all the soil analyses are given in Tables 5 and 6.

Those soil analyses which show significant differences between the soils of the three morphological leaf types indicate that soil nutrient status is lower under small- and

intermediate-leaved populations than under large-leaved populations, at both a general level and over a smaller spatial scale. In the small-scale investigation the increased fine clay content associated with the intermediate-leaved population appears unrelated to the other soil characteristics, and so can presumably be considered spurious; except to suggest that the general low nutrient status of the soils is unrelated to the amount of clay present and is thus an inherent characteristic of the soils (perhaps because of low concentrations of nutrients in the parent rocks, leaching of nutrients, erosion etc.).

Table 5. Summary of results of analyses of variance and Student-Newman-Keuls tests of data for soils from populations of the three morphological leaf types

D.f. in all cases are two. n.s., Not significant at $P<0{\cdot}05$. * Significant at $0{\cdot}025<P<0{\cdot}05$. ** Significant at $0{\cdot}01<P<0{\cdot}025$. *** Significant at $0{\cdot}005<P<0{\cdot}01$. **** Significant at $P<0{\cdot}005$. $\overline{A}_1$, $\overline{A}_2$ and $\overline{A}_3$, means for soil of small-, intermediate- and large-leaved types respectively. Underlined values are not significantly different at $P<0{\cdot}01$

Soil analysis	General soil analysis			Small-scale soil analysis		
	F	Significance	Student-Newman-Keuls test	*F*	Significance	Student-Newman-Keuls test
Stone and gravel (particles >2·0 mm diam.)	1·00	n.s.		3·77	n.s.	
Sand (particles 0·02–2·0 mm diam.)	0·87	n.s.		1288·27	****	$\underline{\overline{A}_2\ \overline{A}_1}\ \overline{A}_3$
Silt (particles 0·005–0·02 mm diam.)	0·37	n.s.		0·93	n.s.	
Coarse clay (particles 0·002–0·005 mm diam.)	0·55	n.s.		5·51	n.s.	
Fine clay (particles <0·002 m diam.)	1·17	n.s.		23·09	**	$\underline{\overline{A}_1\ \overline{A}_3}\ \overline{A}_2$
Air-dry moisture	9·34	n.s.		1·06	n.s.	
Oven-dry moisture	0·32	n.s.		15·23	*	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
pH	2·10	n.s.		0·20	n.s.	
Loss on ignition	70·06	****	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	16·50	**	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
Extractable H	34·56	***	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	12·59	*	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
Total extractable metal cations	17·15	**	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	9·83	*	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
Extractable K	16·55	**	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	10·16	*	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
Extractable Mg	69·70	****	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	10·02	*	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$
Extractable Na	11·40	*	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	Heterogeneous data		
Total P	14·30	*	$\underline{\overline{A}_1\ \overline{A}_2\ \overline{A}_3}$	248·30	****	$\underline{\overline{A}_1\ \overline{A}_2}\ \overline{A}_3$

This suggests that the apparent phenotypic plasticity of the intermediate-leaved morphological leaf type may be related to low nutrient levels in the soil.

Cultivation under Varied Conditions

Methods

To test the hypothesis generated by the environmental correlation, seedlings were raised from seed taken from populations of the three leaf types and grown over a range of nutrient levels.

Seed and samples of parental plants were collected from the same sites as described above, with five random plants sampled per population. Three replicate pots of each of two nutrient treatments were set up as described earlier, one treatment being full concentration of the nutrient solution and the other a one-tenth dilution. The sand used in the pots was cleaned by washing with concentrated acid, then alkali and finally distilled water.

Table 6. Data for soils from populations of the three morphological leaf types
All data are means for each leaf type (±s.e.)

Soil analysis	General soil analysis			Small-scale soil analysis		
	Small-leaved	Inter-mediate	Large-leaved	Small-leaved	Inter-mediate	Large-leaved
Stone and gravel (particles >2·0 mm diam.) (%)	8·6 (1·5)	30·8 (2·4)	16·2 (1·1)	9·7 (1·8)	49·0 (2·3)	31·9 (3·3)
Sand (particles 0·02–2·0 mm diam.) (%)	91·6 (0·6)	74·0 (1·2)	84·4 (0·7)	82·7 (0·6)	58·2 (1·1)	84·7 (0·4)
Silt (particles 0·005–0·02 mm diam.) (%)	1·6 (0·3)	4·2 (0·5)	4·0 (0·5)	5·5 (0·6)	7·5 (0·4)	5·1 (0·4)
Coarse clay (particles 0·002–0·005 mm diam.) (%)	0·1 (0·2)	2·4 (0·5)	0·9 (0·3)	1·9 (0·4)	4·7 (0·5)	1·3 (0·3)
Fine clay (particles <0·002 mm diam.) (%)	6·7 (0·4)	19·3 (1·0)	10·7 (0·3)	9·9 (0·4)	29·6 (0·9)	8·9 (0·3)
Air-dry moisture (%)	15·3 (0·4)	15·3 (2·1)	33·9 (1·0)	22·0 (1·2)	23·0 (1·7)	28·7 (0·5)
Oven-dry moisture (%)	0·6 (0·2)	1·1 (0·3)	1·0 (0·1)	1·1 (0·3)	1·8 (0·3)	3·5 (0·2)
pH	3·98 (0·17)	4·28 (0·12)	4·29 (0·16)	3·98 (0·17)	4·13 (0·15)	4·07 (0·21)
Loss on ignition (%)	3·6 (0·3)	9·6 (0·4)	16·4 (0·2)	7·7 (0·6)	10·5 (0·5)	18·1 (0·3)
Extractable H (m-equiv./100 g)	0·17 (0·09)	0·44 (0·14)	1·21 (0·12)	0·51 (0·13)	0·64 (0·16)	1·70 (0·24)
Total extractable metal cations (m-equiv./100 g)	0·20 (0·11)	0·49 (0·14)	0·97 (0·10)	0·51 (0·06)	0·59 (0·14)	0·94 (0·13)
Extractable K (mg/100 g)	8·01 (0·42)	6·86 (0·44)	16·48 (0·59)	7·29 (0·74)	8·19 (0·51)	24·58 (0·91)
Extractable Mg (mg/100 g)	1·43 (0·38)	1·89 (0·35)	11·54 (0·26)	0·90 (0·29)	2·63 (0·55)	15·07 (0·84)
Extractable Na (mg/100 g)	2·74 (0·36)	2·32 (0·33)	5·58 (0·26)	2·14 (0·54)	4·20 (0·96)	5·88 (0·25)
Total P (mg/100 g)	0·96 (0·25)	1·27 (0·29)	3·99 (0·44)	1·64 (0·43)	1·22 (0·24)	8·20 (0·71)

The plants were grown for 24 weeks, and harvested as in the earlier experiment, with similar data collection. Data for offspring height and dry weight and for parent and offspring leaf length were analysed using four-factor mixed analyses of variance.

Results and Discussion

In offspring height or shoot dry weight no significant interactions were found between leaf type and nutrient level owing to variability among the sites and individuals (Tables 7 and 8). The parent and offspring leaf length data do show a

significant interaction of leaf type and the relationship of parent leaf length to the two offspring leaf lengths (Table 8), and this was analysed using a Student-Newman-Keuls test (Fig. 4).

The leaf length data clearly support the hypothesis generated earlier. The height and dry-weight data also show differential growth rates for the two groups of genotypes under different nutrient levels.

Table 7. Results of analyses of variance of data for offspring height and shoot dry weight and parent and offspring leaf length following cultivation of the offspring under two nutrient conditions

n.s., Not significant at $P<0{\cdot}05$. n.r., Significant interaction means null hypothesis here is irrelevant. * Significant at $0{\cdot}025<P<0{\cdot}05$. *** Significant at $0{\cdot}005<P<0{\cdot}01$. **** Significant at $P<0{\cdot}005$

Source		d.f.	*F*	Significance
Height				
Leaf type	A	2	0·40	n.s.
Site	B(A)	3	3·56	n.r.
Individual	C(AB)	24	5·42	n.r.
Nutrient level	D	1	149·65	n.r.
A × D interaction		2	0·22	n.s.
B(A) × D interaction		3	3·35	*
C(AB) × D interaction		24	2·15	***
Blocks		2	5·16	***
Residual		118		
Dry weight				
Leaf type	A	2	2·54	n.s.
Site	B(A)	3	2·84	n.s.
Individual	C(AB)	24	5·21	n.r.
Nutrient level	D	1	169·76	n.r.
A × D interaction		2	2·19	n.s.
B(A) × D interaction		3	1·63	n.s.
C(AB) × D interaction		24	4·27	****
Blocks		2	0·96	n.s.
Residual		118		
Leaf length				
Leaf type	A	2	36·11	n.r.
Site	B(A)	3	12·39	n.r.
Individual	C(AB)	24	3·26	****
Parent/offspring relationship	D	2	59·94	n.r.
A × D interaction		4	18·57	****
B(A) × D interaction		6	7·52	****
C(AB) × D interaction		48	1·08	n.s.
Residual		180		

However, the selective agent and the specific function upon which it impinges have not been positively identified. It is tempting to assume that the environmental factor most obviously correlated with the observed variation between populations must also be the one which controls their relative distributions, and that the facets of variation between the populations studied are those critical under selection. The possibility remains that the 'real' target of selection is an associated or secondary response, or a

function for which the one studied acts as a governor or 'time-keeper' (see Heslop-Harrison 1964).

Table 8. Data for offspring height and shoot dry weight following cultivation of the offspring under two nutrient conditions

Leaf type	Nutrient treatment	Mean shoot measurement per individual (±s.e.)
Height (cm)		
Small-leaved	Full	35·5 (17·8)
	$\frac{1}{10}$ diln	13·9 (4·9)
Intermediate	Full	35·4 (19·9)
	$\frac{1}{10}$ diln	14·0 (7·5)
Large-leaved	Full	29·2 (13·2)
	$\frac{1}{10}$ diln	13·2 (3·9)
Dry weight (g)		
Small-leaved	Full	1·096 (0·114)
	$\frac{1}{10}$ diln	0·144 (0·017)
Intermediate	Full	1·978 (0·171)
	$\frac{1}{10}$ diln	0·252 (0·037)
Large-leaved	Full	1·837 (0·163)
	$\frac{1}{10}$ diln	0·214 (0·021)

Reciprocal Transplants

Methods

Only a very small-scale experiment could be carried out owing to difficulty in obtaining appropriate cuttings (see Bailey 1947). This experiment involved the small- and large-leaved populations used in the small-scale soil analysis.

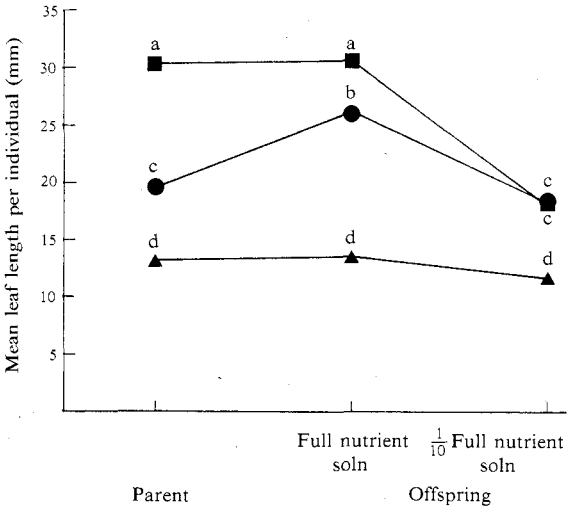

Fig. 4. Comparison of parent and offspring leaf-length data of the three morphological leaf types (short-leaved ▲, intermediate-leaved ●, large-leaved ■) following cultivation of the offspring under two nutrient conditions. Points with the same letters are not significantly different at $P = 0{\cdot}01$ as determined by Student-Newman-Keuls tests.

A series of cuttings was collected from each of 10 individuals at each site: approx. 12–15 cuttings, each about 7–10 cm long with some new growth backed by mature wood, from each individual. The cuttings were then immediately trimmed to a uniform

size, treated with hormones to promote root growth and potted out in a mixture of sand, peat and perlite. The pots were transferred to a mist propagator. As they produced roots, the cuttings were transferred to 5 cm pots, removed from the propagator and placed on a glasshouse bench in a tray of tap water to keep the plants in moist soil.

Ten weeks after collection, the 40 successfully propagated cuttings (two from each individual of each population) were transferred outside the glasshouse to allow acclimation. Two weeks later they were transplanted into the field, one replicate of each individual into the site of the cuttings' origin and one into the alternative site. It was not possible to analyse variability between individual cuttings from each plant (e.g. by transplanting two cuttings from each plant into each site) due to the high mortality rate of cuttings during propagation. Small quadrats, 10 cm^2, were chosen randomly from a grid pattern for each transplant, and were cleared. The transplants were then planted, watered and tagged. Mortality, height and largest leaf length were recorded for each transplant at irregular intervals over 15 weeks. Data for mortality observed after 15 weeks were analysed using an orthogonal analysis of variance.

When the cuttings were taken, 10 capsules were also collected from each individual, and the seeds were pooled for each population. At the time of transplanting the cuttings, six quadrats, 1 m^2, were chosen randomly from a grid pattern at each of the two sites and examined for *L. flavescens* seedlings. None were found in any of the quadrats. Two of the six quadrats were then left as controls. Into each of two of the remaining four quadrats 200 seeds from the small-leaved population were deposited; a further 200 seeds from the large-leaved population were placed into each of the remaining two quadrats. The quadrats were then monitored for seedling emergence at the times of checking of the cutting transplants.

Table 9. Results of analysis of variance of data for cuttings reciprocal transplant mortality

n.s., Not significant at $P<0{\cdot}05$. **** Significant at $P<0{\cdot}005$

Source	d.f.	*F*	Significance
Original population	1	0	n.s.
Transplant site	1	0	n.s.
Interaction	1	12·0	****

Results and Discussion

The mortality rate observed after 15 weeks in the cuttings experiment showed there was a significant interaction between the population of origin of the surviving transplants and the site into which they were transplanted (Table 9). Analysis of this interaction by a Student-Newman-Keuls test indicates that for both the small- and large-leaved populations significantly more transplants survived in the site of the cuttings' origin than in the alternative site (Table 10). Data for height and leaf length showed no obvious differences and so were not analysed. No seedlings were observed to emerge and become established in any of the quadrats.

The higher mortality rate of the cuttings transplanted to the habitat of the other leaf type suggests that some environmental factor may be selecting the different leaf types, which could account for the existence of two such presumably outbreeding populations in close promixity as at Echo Head.

The lack of observed seed germination may be associated with the influence of the covering vegetation. *L. flavescens* seeds are normally only released after a fire, when covering vegetation would be at a minimum. However, in other areas affected by fire very few surviving seedlings were observed, most regeneration being from root stocks.

Table 10. Data for proportional mortality after 15 weeks from the cuttings reciprocal transplant experiment

Leaf type (pop. of cuttings origin)	Transplant site	
	Small-leaved	Large-leaved
Small-leaved	0·6	1·0
Large-leaved	1·0	0·6

Conclusion

Thus, in terms of the five criteria set out initially, there is strong evidence that two distinct groups of genotypes exist in *Leptospermum flavescens* in the Sydney region, one large-leaved and one small-leaved, with the large-leaved type capable of large phenotypic plasticity in relation to substrate nutrient level.

The two genecologically differentiated groups overlap geographically and occur in adjoining habitats. A similar situation is well known in several other species as, for instance, Briggs (1962) showed in species of *Ranunculus* on Kosciusko Plateau, in which she concluded that the identity of the different groups is maintained by their ecological specialization.

Most of the known examples of this situation are for herbaceous plants rather than woody shrubs such as *L. flavescens*. However, Corne and Hiesey (1973) demonstrated genetic control of reduction in leaf and plant size (correlated with altitude) in the *Metrosideros polymorpha* complex in Hawaii. Also, Lee and Lowry (1980) separated *L. flavescens* (=*L. javanicum* Bl.; J. Thompson, personal communication) and *L. recurvum* Hook. f. based on genetically controlled differences in leaf size and chemistry.

The work presented here suggests that, south of Sydney, subdivisions of the species, such as that of Beadle *et al.* (1963), are justified, although morphologically the two forms are difficult to distinguish.

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