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Patterns of morphological diversity in relation to geographical origins of wild *Lupinus angustifolius* from the Aegean region

J. C. Clements[1] & W. A. Cowling[2]
[1]*Botany Department, University of Western Australia, Nedlands, Western Australia 6009;*
[2]*Crop Industries Branch, Department of Agriculture, Baron-Hay Court, South Perth, Western Australia 6151*

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Summary

Patterns of morphological diversity were examined in relation to geographical origins of 157 accessions of wild *Lupinus angustifolius* from the Aegean region using multivariate techniques. Genetic diversity was extremely large for most morphological traits, with significant variation detected among localities in Greece and within and between collection sites for some traits. Canonical variates and correlation analysis showed that early flowering, tall and large-seeded accessions were associated with warm winters and drier climates in southern Greece. Thirteen groups of accessions were identified by hierarchical cluster analysis of 19 morphological traits, accounting for 81% of genotype and 41% of genotype × trait sums of squares. The distinguishing features of these groups were clarified by principal coordinates analysis. Two groups, with very desirable agronomic characteristics, originated from the Dhodhekanisos Islands (Kos, Leros, Patmos) in the south-eastern Aegean: these had rapid and tall growth, prolific podding on the main stem, pods high off the ground, many upper lateral branches, large leaves, pods and seeds, and high seed yield. Accessions from the Kikladhes Islands of the central Aegean were extremely variable and those from Naxos Island were represented in 9 of the 13 groups. Accessions from northern Greece grouped together as later flowering, shorter, and smaller-seeded types, but some accessions from the southern Greek Islands were grouped with the northern mainland types. This study identified regions in Greece, such as the south-eastern islands, where further collection may be warranted for traits of obvious agronomic value for domesticated *L. angustifolius*. Extreme morphological variability occurs within and between collection sites, and between localities in Greece.

Introduction

Lupinus angustifolius L. (narrow-leafed lupin) has the broadest natural distribution of the Mediterranean and North African lupin species, and occurs in most countries surrounding the Mediterranean Sea (Gladstones, 1974). Large genetic variation in wild *L. angustifolius* has been reported from the western Mediterranean (Gladstones & Crosbie, 1979), but few collecting missions have been reported from the eastern Mediterranean for this species (Clements & Cowling, 1990a).

The collection and preservation of wild and semi-domesticated forms is vital for future breeding and improvement of *L. angustifolius*. Collections of wild *L. angustifolius* from the Mediterranean region have already contributed to the development of disease resistant cultivars (Gladstones, 1989). The Australian Lupin Collection contains a significant proportion of the world's genetic resource for this species (Clements & Cowling, 1990b, 1991; IBPGR, 1991), and will provide a broad genetic base for future improvements in crop breeding with this species. However, efficient use of this genetic resource for lupin improvement will require adequate evaluation and recording of the genetic variation in the collection.

Multivariate techniques are useful for evaluation of plant genetic resources when a large number of accessions are to be assessed for several characters of both agronomic and physiological importance (Peeters & Martinelli, 1989). Within the grain legumes, numerical techniques have provided a clearer understanding of the variation among large collections of soybean (Broich & Palmer, 1980) and lentil (Erskine et al., 1989). Principal component analysis and multidimensional scaling were used to investigate patterns of variation in *L. albus* L. by Simpson (1986a, 1986b) in collections from Spain, Portugal, Turkey, the Nile Valley, Greece and Yugoslavia. In this paper we describe the use of multivariate techniques to characterize accessions of *L. angustifolius* from the Aegean region, and to identify localities where desirable traits could be found in future collecting missions.

The Aegean region, with its vast numbers of small islands varying in climate, geology and land-use patterns, may be expected to contain high levels of genetic variation within and among small isolated populations of *L. angustifolius*. A collecting mission to the Aegean region in 1984 discovered wild *L. angustifolius* in most localities visited, and in a wide range of habitats (Cowling, 1986). Preliminary field evaluation of the Aegean collection demonstrated large variation within and between populations and several unique morphological strains were selected from some collection sites (Clements & Cowling, 1991). This study was conducted to investigate the relative genetic variation within and among collection sites and within and among geographical localities in order to improve collecting strategies for wild *L. angustifolius* in the Aegean region, and to identify potentially useful agronomic traits in wild *L. angustifolius* for crossing into cultivated varieties.

Materials and methods

Choice of accessions

Accessions of *L. angustifolius* from the Aegean region were chosen to represent the widest possible geographic distribution, including as many islands as possible. The majority of accessions were collected by Cowling (1986) or Simpson & McGibbon (1982). Accessions from mainland Greece, Crete and Cyprus were also included. Morphological variation within collection sites was identified and verified in field tests of the introductions in South Perth, Western Australia, in 1985 and 1986. Several morphological strains were selected from some collection sites, and were added to the Australian Lupin Collection as new accessions. Evaluation data were recorded for each accession (Clements & Cowling, 1991), as well as collection site data for each introduction where available (Clements & Cowling, 1990b). Soil pH at the collection site was measured with a small colour reaction kit on a sample from 1–5 cm depth. Soil texture was described subjectively on a scale of 1 to 7, where 1 is coarse sand, 2 is fine sand, 3 is loamy sand, 4 is sandy loam, 5 is loam, 6 is clay loam, and 7 is clay (Clements & Cowling, 1990b).

Preliminary evaluation study—1988

A preliminary field evaluation study was established in 1988 to determine which morphological traits were to be measured in the major experiment in 1989. A sample of 11 accessions of *L. angustifolius* from Greece and Cyprus was grown over winter (sown 20 June 1988) in an aphid-proof screenhouse at South Perth in a randomised complete block experiment with two replications. Seed was hand scarified, dusted with peat inoculum of *Bradyrhizobium lupini* group G, and sown in 1 m rows with 5 cm spacing between plants in rows, and 1 m between rows. Plants were irrigated when necessary to avoid drought before maturity. Twenty three traits were measured on five plants per replicate during the growing season. Several traits which did not vary significantly among accessions, or were functionally correlated ($r \geq 0.9$) (Bisby, 1970), were excluded from the major experiment in 1989.

Height to first leaf (from ground level) was measured at 9 weeks after sowing. Leaflet width and length and number of leaflets were measured on the first leaf below the main stem inflorescence as soon as the leaf was fully developed. Height to the top of the main stem inflorescence, inflorescence length and main stem thickness (measured half way up the main stem) were measured when flowering had ceased on the main stem and the inflorescence was fully extended. Height to first pod, length and number of branches, pod length, and number of main stem pods (with at least one fully developed seed) were measured at maturity before pod shattering.

Evaluation of 157 accessions from the Aegean region—1989

The major experiment, sown on 25 May 1989, contained 157 accessions of *L. angustifolius* chosen from 77 collection sites in the Aegean region. *L. angustifolius* was collected in 9 prefectures (local government regions) in mainland Greece, 18 Aegean islands and Cyprus (Table 1). Each of the islands and prefectures was regarded as localities for the purpose of analysis of variance and canonical variates analysis. *L. angustifolius* 'Gungurru' (an early flowering and high yielding commercial cultivar) was included as a control for comparison with the wild lines.

The experimental design and techniques for the major experiment in 1989 were similar to the preliminary evaluation in 1988. However, space limitations in the screenhouse prevented replication of all accessions. Six accessions (including 'Gungurru') were replicated six times among the other accessions in order to estimate experimental error. Twenty two morphological traits were measured on the middle five plants in each row during the growing season.

Analyses of variance were carried out to determine the relative variance among localities, among

Table 1. Summary of collection sites and localities of *L. angustifolius* accessions from the Aegean Region

Region and Prefecture	Locality[1]	Abbreviation	No. Collection Sites	No Accessions
Cyprus	Cyprus	Cypr	5	5
Central Greece—Attiki Pr.	Aegina Is.	Aegi	2	5
Central Greece—Attiki Pr.	Athens	Athe	1	1
Aegean Is.—Kikladhes	Andros Is.	Andr	3	11
Aegean Is.—Kikladhes	Dilos Is.	Dilo	1	2
Aegean Is.—Kikladhes	Ios Is.	Iosi	2	3
Aegean Is.—Kikladhes	Kea Is.	Keai	1	4
Aegean Is.—Kikladhes	Mikonos Is.	Miko	6	8
Aegean Is.—Kikladhes	Naxos Is.	Naxo	9	20
Aegean Is.—Kikladhes	Paros Is.	Paro	3	4
Aegean Is.—Kikladhes	Santorini Is.	Sant	3	8
Aegean Is.—Kikladhes	Tinos Is.	Tino	4	11
Crete Is.	Rethimni Pr.	Cret	1	3
Central Greece	Evia Is.	Evia	3	3
Macedonia	Dhrama Pr.	Dhra	1	2
Macedonia	Kavala Pr.	Kava	2	2
Macedonia	Khalkidiki Pr.	Khal	6	6
Macedonia	Serrai Pr.	Serr	2	2
Macedonia	Thessaloniki Pr.	Thes	2	2
Macedonia	Kilkis Pr.	Kilk	4	6
Aegean Is.—Dhodhekanisos	Kos Is.	Kosi	2	9
Aegean Is.—Dhodhekanisos	Leros Is.	Lero	3	7
Aegean Is.—Dhodhekanisos	Patmos Is.	Patm	3	5
Eastern Aegean Is.	Samos Is.	Samo	1	5
Eastern Aegean Is.	Lesvos Is.	Lesv	3	10
Thessaly	Magnisia Pr.	Magn	1	3
Peloponnese	Lakonia Pr.	Pelo	2	9
Thrace	Samothraki Is.	Smth	1	1
Total	28		77	157

[1]Locality is defined as a distinct geographical area for the purposes of analysis of variance. In this study, a locality may be an island or prefecture.

collection sites within localities, and among accessions within collection sites for each trait. Traits which were considered to be functionally correlated (r ⩾ 0.9) were removed from further analysis.

Canonical variates analysis (Seal, 1964) was carried out using locality means as a grouping factor. With this technique, maximum discrimination was obtained between locality means when tested against the minimised covariance within localities. Principal coordinates analysis and agglomerative hierarchical clustering was carried out on range-standardized data from 19 traits. The measure of dissimilarity was squared Euclidean distance and the clustering strategy was based on incremental sums of squares. A two way hierarchical analysis of variance was carried out in order to choose an appropriate level of truncation. Correlations were calculated between pairs of traits within each genotype group. The list of accessions in each group are available upon request from the authors.

Results

Preliminary evaluation study—1988

There was insignificant variation among the 11 accessions for number of florets per main stem inflorescence, main stem thickness and leaf diameter. These traits were therefore excluded from the 1989 study. There was a high correlation (r = 0.98***) between days to flowering on the main stem and days to flowering on first order lateral branches. The latter trait was also not measured in 1989 due to functional correlation.

Table 2. Analysis of variance for 22 morphological traits in 157 *L. angustifolius* accessions tested in 1989

Character	Abbreviation	MS (Among Localities) df = 28[1]	MS (Among Collection Sites W/in Localities) df = 50[2]	MS (Among Accessions W/in Collection Sites) df = 79[3]	Residual MS df = 26
Plant height at 5 weeks	ph5	25.59***	3.44**	2.34*	1.18
Plant height at 9 weeks	ph9	110.72***	14.36**	9.95*	5.04
Plant height at 13 weeks	ph13	565.25***	91.93***	55.56**	18.76
Plant height at 17 weeks	pH17	800.08***	147.64*	67.78 ns	62.33
Plant height at maturity	phm	338.55***	114.77**	66.82 ns	45.89
Height to top of inflorescence	htm	516.44***	142.53**	75.73 ns	54.67
Number of leaflets per leaf	nol	1.005***	0.556***	0.319***	0.082
Leaflet width	lw	1.153***	0.448**	0.249 ns	0.167
Days to flowering	ft	517.71***	36.20***	22.16***	4.04
Inflorescence length	il	7735***	3490**	1955 ns	1173
Height to first pod	htp	273.59***	67.91**	38.19 ns	22.53
Number of main stem pods	nop	29.37**	28.33**	10.65 ns	9.77
Length longest upper branch	lnub	88.67***	44.49*	27.89 ns	21.90
Length longest lower branch	lnbb	433.31***	81.85 ns	60.14 ns	52.20
Number of upper branches	nobt	12.44***	3.11***	2.02*	0.90
Number of lower branches	nobl	71.48***	10.22 ns	8.65 ns	7.42
Length largest main stem pod	pl	30.79***	17.00***	6.80**	2.66
No. seeds per pod	sdp	0.489***	0.155*	0.116 ns	0.085
Main stem yield	yldm	11.07**	5.33 ns	2.55 ns	3.66
Lateral branch yield	yldl	460.5***	161.2 ns	152.5 ns	129.5
100 seed weight	sdwt	19.95***	3.10 ns	2.10 ns	2.39
Height to first main stem leaf	htfl	6.339***	1.081***	0.914***	0.217

[1]Locality defined as in Table 1. Significance of MS values determined from F = MS(localities)/MS(among collection sites within localities).

[2]F = MS(among collection sites)/MS(accessions within collection sites).

[3]Residual MS estimate is based on replication of control ('Gungurru') and 5 wild accessions in 6 blocks throughout study. Maximum d.f. of 157 (numerator, and 26 (denominator). F = MS(accessions within collection sites)/MS(residual).

Table 3. Analysis of variance for collection site environments, indicating variance among localities for particular collection site characteristics

Collection Site Characteristic	Range among 77 collection sites			Variation among 28 localities[1]	
	Minimum	Mean	Maximum	MS(among localities) /MS(residual)	df(among localities) /df(residual)
Latitude	34.48	37.6	41.16	17.45/0.077***	27/150
Altitude (m)	3	184	1000	18165/6609***	27/149
Annual rainfall (mm)	350	556	900	158000/2176***	23/131
Average January temp. °C	1.0	9.6	12.0	73.47/0.153***	22/115
Average July temp. °C	22.0	24.6	27.0	9.289/0.406***	22/115
Soil texture (1–7)	1.0	3.7	7.0	17.22/1.196***	26/143
Soil pH	4.2	6.7	8.5	3.121/0.491***	26/147

[1]Locality = islands or prefectures (mainland).

Evaluation of 157 accessions from the Aegean region—1989

(i) Analysis of variance for morphological traits

There was significant variation among localities for all 22 morphological traits measured in 1989 (Table 2). There was significant variation among collection sites within localities for all traits except the length and number of lower branches, main stem yield, lateral branch yield and 100 seed weight. Accessions within collection sites contained significant residual variation for height to first main stem leaf, height at 5, 9 and 13 weeks, number of leaflets per leaf, days to flowering, number of upper branches, and length of the longest main stem pod (Table 2). Flower colours of wild accessions ranged from light blue, blue to violet blue.

(ii) Analysis of variance for collection site characters

The 77 collection sites varied widely in latitude, ranging from 34.48°N (CY28 in Cyprus) to 41.16°N (GRC5069A in Dhrama), altitude,

Table 4. Means, minimum, maximum and standard deviation (SD) for 22 morphological traits measured on 157 *L. angustifolius* accessions and 'Gungurru'

Character	Minimum	Mean	Maximum	SD	Control: 'Gungurru'
Plant height at 5 weeks (cm)	2.0	8.1	16.2	2.3	11.8
Plant height at 9 weeks (cm)	3.0	14.3	34.0	4.6	26.4
Plant height at 13 weeks (cm)	7.4	34.6	57.2	11.0	54.9
Plant height at 17 weeks (cm)	18.8	64.9	91.4	13.3	72.1
Plant height at maturity (cm)	42.0	80.4	105.6	10.9	79.1
Height to top main inflorescence (cm)	27.8	65.3	100.2	12.7	53.8
Number of leaflets per leaf	6.0	7.7	9.0	0.7	8.3
Leaflet width (mm)	3.0	5.2	7.2	0.7	6.13
Days to flowering	80.2	106.4	133.0	8.9	80.2
Inflorescence length (mm)	103	252	420	57.5	196.5
Height to first pod (cm)	16.8	40.7	61.2	8.9	33.9
Number of main stem pods	0.1	13.0	24.2	4.1	12.7
Length longest upper branch (cm)	30.2	45.5	58.8	6.4	44.4
Length longest lower branch (cm)	5.0	64.2	87.4	11.0	45.3
Number of upper branches	3.8	8.4	14.6	1.9	7.1
Number of lower branches	4.8	13.0	26.3	3.8	8.4
Length larget main stem pod (mm)	39.4	49.3	60.4	3.6	50.2
No. seeds per largest main stem pod	4.0	5.2	6.3	0.4	4.7
Main stem yield (g)	0.2	4.7	8.8	2.0	7.4
Lateral branch yield (g)	1.0	25.3	86.2	13.5	45.2
100 seed weight (g)	5.6	11.1	18.4	2.1	16.7
Height to first main stem leaf (cm)	0.4	3.4	7.3	1.3	3.8

Table 5 (a). Correlations using plot mean data between 22 morphological traits and collection site data for 157 *L. angustifolius* accessions and 'Gungurru'

Character	ph5	ph9	ph13	ph17	phm	htm	nol	lw	ft	il	htp	nop	lnub
Plant height at 9 weeks	0.94***												
Plant height at 13 weeks	0.85***	0.91***											
Plant height at 17 weeks	0.69***	0.73***	0.77***										
Plant height at maturity	0.40***	0.48***	0.50***	0.80***									
Height to top of inflorescence	0.25**	0.32***	0.31***	0.70***	0.83***								
Number of leaflets per leaf	0.16*	0.13	0.14	0.10	0.03	0.04							
Leaflet width	0.15*	0.17*	0.10	0.35***	0.45***	0.52***	0.07						
Days to flowering	−0.65***	−0.66***	−0.72***	−0.39***	−0.00	0.29***	−0.10	0.19**					
Inflorescence length	0.06	0.11	0.13	0.50***	0.55***	0.80***	0.05	0.45***	0.21**				
Height to first pod	0.33***	0.40***	0.37***	0.70***	0.84***	0.93***	0.01	0.46***	0.27***	0.53***			
Number of main stem pods	0.19**	0.23*	0.22**	0.22**	0.11	0.17*	0.04	0.14*	−0.17*	0.27***	0.07		
Length longest upper branch	0.31***	0.33***	0.40***	0.51***	0.53***	0.28***	0.10	0.40***	−0.31***	0.31***	0.22**	0.14*	
Length longest lower branch	−0.20***	−0.16*	−0.12	0.30***	0.62***	0.62***	−0.06	0.43***	0.48***	0.44***	0.61***	−0.14*	0.36
Number of upper branches	0.57***	0.61***	0.50***	0.67***	0.63***	0.69***	0.08	0.31***	0.00	0.36***	0.76***	0.20**	0.13*
Number of lower branches	−0.12	−0.10	−0.17*	0.05	0.14*	0.30***	−0.02	0.20**	0.42***	0.25**	0.28***	0.13	0.04
Length largest main stem pod	0.41***	0.42***	0.38***	0.52***	0.39***	0.34***	0.06	0.31***	−0.26**	0.27***	0.32***	0.02	0.31***
No. seeds per pod	−0.08	−0.06	−0.03	0.11	0.16*	0.19**	0.12	0.07	0.17*	0.14*	0.17*	0.15*	0.11
Main stem yield	0.40***	0.42***	0.41***	0.50***	0.30***	0.22**	0.11	0.19*	−0.35***	0.22**	0.19*	0.57***	0.33***
Lateral branch yield	−0.04	−0.01	−0.03	0.11	0.30***	0.25**	0.12	0.25**	0.21**	0.16*	0.26**	0.02	0.16*
100 seed weight	0.64***	0.62***	0.56***	0.58***	0.43***	0.25**	0.18*	0.30***	−0.46***	0.12*	0.27***	0.04	0.37***
Height to first main stem leaf	0.93***	0.85***	0.76***	0.63***	0.38***	0.28***	0.13*	0.19**	−0.54***	0.09	0.34***	0.21**	0.28***
Latitude	−0.42***	−0.41***	−0.46***	−0.46***	−0.18*	−0.06	−0.02	−0.09	0.47***	−0.08	−0.05	−0.16*	−0.26***
Altitude (m)	−0.28***	−0.27***	−0.26***	−0.28***	−0.08	−0.01	−0.15*	0.09	0.24**	0.11	−0.08	−0.14*	−0.01
Annual rainfall (mm)	−0.12	−0.07***	−0.10	0.16*	0.36***	0.50***	0.10	0.25**	0.43***	0.45***	0.44***	−0.10	−0.03
Average January temp. (C)	0.60***	0.57***	0.62***	0.56***	0.15*	−0.01	0.13	0.00	−0.66***	−0.01	−0.01	0.21*	0.30***
Average July temp. (C)	0.32***	0.30***	0.33***	0.22*	0.14	0.02	0.17*	−0.20*	−0.31***	−0.13	0.11	−0.10	−0.03
Soil texture (1–7)	−0.03	0.00	−0.01	0.07	0.26**	0.26**	0.14*	0.17*	0.23**	0.21**	0.26**	−0.12	0.04
Soil pH	0.20**	0.24**	0.27***	0.12	0.06	−0.07	0.02	−0.19*	−0.24**	−0.18*	0.02	0.13	0.01
Character	ph5	ph9	ph13	ph17	phm	htp	nol	lw	ft	il	htp	nop	lnub

Table 5 (b). Correlations using plot mean data between 22 morphological traits and collection site data for 157 *L. angustifolius* accessions and 'Gungurru'

Character	lnbb	nobt	nobl	pl	sdp	yldm	yldl	sdwt	htfl	lat	alt	rain	jat	jut	text
Number of upper branches	0.25**														
Number of lower branches	0.28***	0.27***													
Length largest main stem pod	0.17*	0.34***	0.12												
No. seeds per pod	0.17*	0.08	0.18*	0.37***											
Main stem yield	0.08	0.24**	0.13	0.38***	0.26**										
Lateral branch yield	0.37***	0.13	0.28***	0.20**	0.24**	0.03									
100 seed weight	0.05	0.33***	0.01	0.52***	−0.07	0.45***	0.16*								
Height to first main stem leaf	−0.18*	0.56***	−0.08	0.42***	−0.02	0.36***	−0.06	0.58***							
Latitude	0.11	−0.21**	0.11	−0.21**	−0.12	−0.25**	0.08	−0.32***	−0.39***						
Altitude (m)	0.13	−0.25**	0.05	−0.02	0.05	−0.30***	0.19*	−0.20**	−0.27***	−0.02					
Annual rainfall (mm)	0.45***	0.31	0.27**	0.17*	0.12	−0.13	0.31***	−0.05	−0.05	0.27**	0.20				
Average January temp. (C)	−0.26**	0.29**	−0.17*	0.16*	−0.00	0.38***	−0.22**	0.42***	0.55***	−0.94***	−0.55***	−0.27**			
Average July temp. (C)	−0.08	0.20*	−0.33***	0.13	−0.16*	0.02	−0.09	0.26**	0.32***	−0.05	−0.50***	0.03	0.17*		
Soil texture (1–7)	0.34***	0.23**	0.26**	0.19*	0.14*	−0.08	0.28***	0.10	−0.00	0.13	0.15	0.58***	−0.17*	0.09	
Soil pH	−0.21**	0.14*	−0.18*	−0.13	0.09	0.17*	−0.27***	−0.00	0.18*	−0.25**	−0.38***	−0.28**	0.42***	0.14	−0.14*
Character	lnbb	nobt	nobl	pl	sdp	yldm	yldl	sdwt	htfl	lat	alt	rain	jat	jut	text

rainfall, January and July temperature, soil pH and soil texture (Table 3). There was also significant variation among the 28 geographical localities for these characters.

(iii) Patterns of morphological variation

In comparison to wild accessions, 'Gungurru' was among the tallest at 9 and 13 weeks but its height to first main stem leaf and height at maturity were close to average (Table 4). Two accessions exceeded the plant height of 'Gungurru' at the 13 week stage, these being GRC5032A.1 from Andros Island and GRC5039A.3 from Leros Island, which flowered 20 and 26 days after 'Gungurru' respectively. The extremely wide range in growth habits is reflected in the range of heights to first main stem leaf (rosetted types at 0.4 cm compared with tall types at 7.3 cm). All wild accessions flowered later than 'Gungurru' (80.2 days), with the next earliest accession flowering in 83.0 days (MJS373.1 from the Peloponnese) and several others flowering less than one week later. Large variation occurred for number of pods on the main stem, with a maximum of 24.2 pods (CY05 from Cyprus). Few lines exceeded the main stem yield (7.4 g) or 100 seed weight (16.7 g) of 'Gungurru'. Number and length of branches on the upper and lower levels of the plant also varied widely between accessions, reflecting the wide range of growth habits.

Functional correlation ($r \geq 0.9$) existed between plant height at 5 weeks and height to first main stem leaf, plant height at 5 weeks and plant height at 9 weeks, plant height at 9 weeks and plant height at 13 weeks, and height to top of main stem inflorescence and height to first main stem pod (Table 5). Consequently, plant height at 5 weeks, plant height at 9 weeks and height to top of main stem inflorescence were eliminated from further analyses.

Early flowering and taller types tended to come from lower elevation and drier climates in lower latitudes with warmer winters (Table 5). Of these environmental parameters, warm winters were most strongly associated with high main stem yield and cold, wet climates with high lateral stem yield. Overall, there was no association between main stem yield and lateral branch yield, although within cluster groups significant correlations were sometimes observed (see below). Early flowering types were taller in the earlier stages of growth but not necessarily at maturity. Later flowering accessions had the longest basal branches. Tall and early flowering accessions tended to have large pods and seeds and high main stem yield. In general, largeness in one character was associated with largeness in other characters.

The first two variates in canonical variates analysis accounted for 50% and the first three variates for 60% of the variation. Localities were separated on the first axis (Fig. 1) based on plant height at 17 weeks, height to first main stem pod, and to a lesser extent flowering time and seed weight. On the second axis, localities were separated mainly by variation in number of upper lateral branches. Accessions from Aegina Island in southern Greece and 'Gungurru' were relatively tall at 17 weeks (but with first pods close to the ground), were early flowering and had large seed, whereas accessions from Dhrama, Serrai, Thessaloniki and Kilkis in northern Greece were shorter at 17 weeks (but with first pods high above the ground), later flowering and had small seed. On axis 2, accessions from localities such as Aegina, Mikonos, Santorini and Evia islands, and Kilkis and Serrai prefectures, had many upper primary lateral branches, while accessions from Kavala, Ios, Kos and Leros islands had fewer upper primary lateral branches.

Thirteen groups of accessions were identified by hierarchical cluster analysis accounting for 81% of the genotype sums of squares and 41% of genotype × trait sums of squares. The first division of the 158 accessions (including 'Gungurru') at the top of the hierarchical dendrogram (Fig. 2, Table 6) formed one group with 28 accessions (group 312) and the second group with 130 accessions (group 314). The smaller group (312) consisted mainly of northern and some southern accessions which were shorter, lower yielding, later flowering and had smaller seeds than the bulk of the accessions. The larger group 314 was subdivided into groups 311 and 313. Group 311 included 11 accessions from the Dhodhekanisos Islands (groups 272 and 288) with agronomically desirable characters such as vigorous growth, many upper lateral branches, large leaves, seeds and pods, many main stem pods held high above the ground and high seed yield. Group 313 contained 5 subgroups which had relatively rapid growth and early flowering. One of these subgroups included 'Gungurru'—this group contained the earliest flowering accessions with the fewest and shortest basal branches and large seed.

Principal coordinates analysis was carried out to determine which traits separated the 158 accessions in multidimensional space. Positive values on principal coordinate (pc) 1 indicate (in order of importance) taller plant height at 17 weeks, taller height at maturity, taller height at 13 weeks and more upper lateral branches (Fig. 3). Increasing values on pc2 indicate later flowering times and longer basal branches.

Within-group correlations between yield on the main stem and yield on the lateral branches varied greatly between groups. Group 298, which contained short, low yielding accessions, had a high negative correlation (r = −0.82*) which indicates large differences in relative seed production on the main stem and lateral branches in different accessions. A higher yielding group (group 293) had a positive correlation (r = 0.49*), and group 272

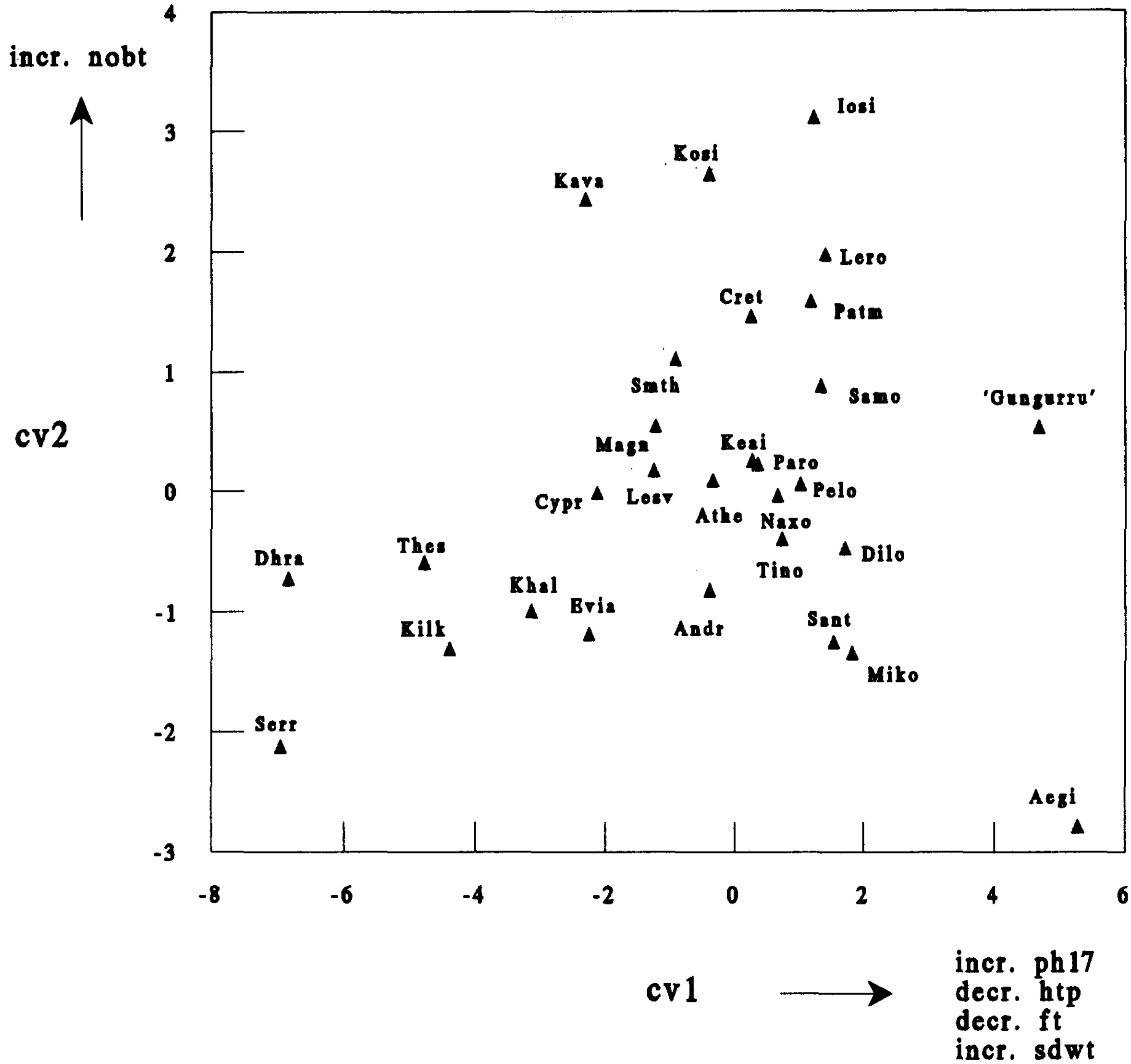

Fig. 1. Scatter diagram of the first two canonical variate mean values for the 28 localities of origin for *L. angustifolius* accessions. Locality abbreviations from Table 1 are used.

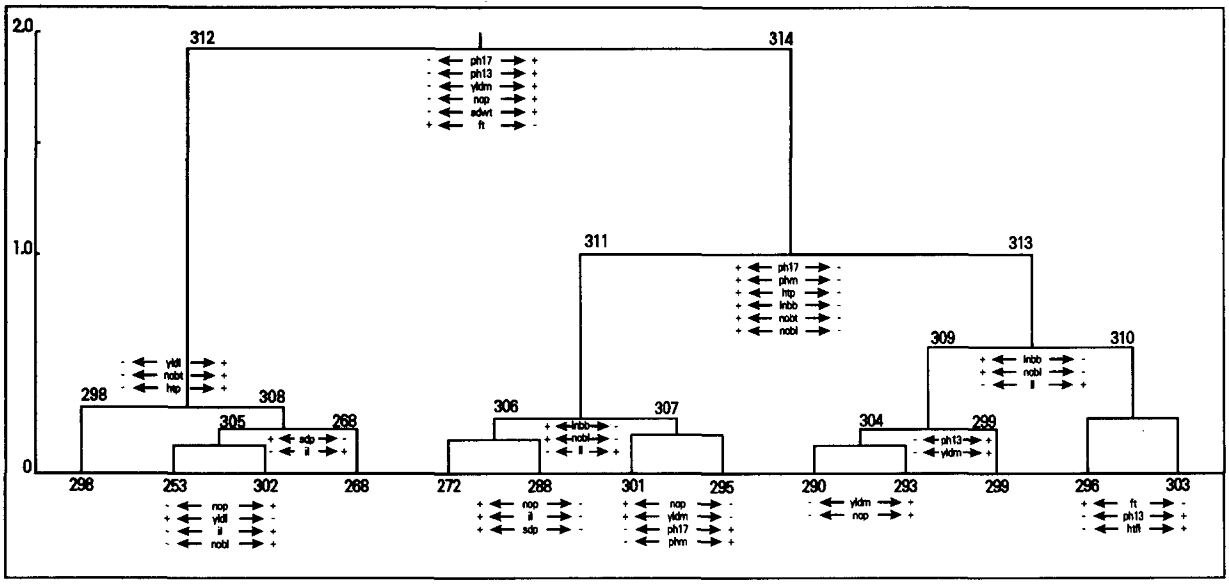

Fig. 2. Dendrogram from hierarchical cluster analysis of 158 *L. angustifolius* accessions using 19 morphological traits.

(with the highest average main stem yield and high lateral yields) showed no correlation between the two traits.

Discussion

Collections of wild *L. angustifolius* were from a diverse range of climates and soil types in the Aegean region. There was significant variation among localities for latitude, altitude, rainfall, temperature, soil texture and pH. There was considerable variation for morphological traits among the 157 accessions, and a strong influence of geographical locality on morphology. Variance among collection sites (assessed relative to variance among accessions within sites) was significant for most traits. Significant variation also occurred for several morphological traits within collection sites. In order to sample this variation, it is necessary to collect in as many geographical localities as possible and to adequately sample the variable populations at each collection site. The data support the contention that the unique geographical and climatic features of the Aegean region, with its many isolated islands and variable microclimates, have assisted the generation of an unusually high degree of genetic variation in wild *L. angustifolius*.

Wild accessions were later flowering than ‘Gungurru’, although some accessions flowered within one week of ‘Gungurru’. Flowering time was influenced by locality with earlier flowering and taller accessions originating from lower elevation and warmer climates in southern Greece. Earlier flowering accessions were usually taller at 13 weeks but mature plant height was not correlated with flowering time. Later flowering was associated with longer and more numerous lower branches and shorter upper branches. Tall and early flowering accessions generally had larger seeds and pods and high main stem yield. Rapid early growth (height to first leaf at 9 weeks) was observed in some accessions. Tall and rapidly growing seedlings may avoid brown spot disease, which is spread by rain splash of fungal spores and may kill young seedlings (Sweetingham, 1986).

Yield on the main stem was positively correlated, to varying degrees, with number of main stem pods and the number and length of upper lateral branches. Excision of upper lateral branches improved main stem pod number in lupin cultivars (Porter, 1982; Downes & Gladstones, 1984). Shading of the main stem inflorescence (Greenwood et al., 1975) and vigour of lateral branch growth (Porter, 1982) also influenced pod set on the main stem. Some accessions in this study supported vigorous upper lateral branch growth and also set *ble 6.* Hierarchical cluster analysis group means for morphological traits of *L. angustifolius*. (Minimums are italicized and maximums are in bold type)

oup	members	ph13	ph17	phm	nol	lw	ft	il	htp	nop	lnub	lnbb	nobt	nobl	pl	sdp	yldm	yldl	sdwt	htfl	Comments
3	5	18.9	*36.7*	*57.2*	7.5	4.2	111.1	172	*23.3*	5.9	36.0	51.6	*5.0*	9.4	*42.2*	5.1	2.0	*8.7*	*7.1*	*1.1*	Shortest pH17, phm, htp, lnbb, htfl, pl and smallest nobt, yldl, sdwt. Low yldm.
3	2	27.9	57.9	88.5	7.3	*4.1*	118.1	*148*	49.3	*4.0*	41.1	**78.0**	8.8	11.5	49.8	5.8	2.2	**80.9**	9.3	2.2	Longest lnbb, highest yldl, narrowest leaflets, shortest il, late ft.
2	16	17.8	45.5	67.8	7.6	5.1	117.6	233	33.7	11.3	39.3	63.1	6.8	**16.4**	48.8	5.4	2.7	27.6	9.2	1.9	Highest nobl, short ph13, late ft.
3	5	*17.4*	49.5	80.3	7.1	5.4	**124.4**	285	49.4	12.6	*35.4*	74.0	9.0	13.4	44.5	*4.4*	*1.7*	23.4	8.7	1.7	Shortest ph13, latest ft, shortest lnub, fewest sdp, lowest yldm. Northern mainland Greece.
2	5	41.7	**82.5**	**96.7**	**8.4**	**6.3**	111.6	**387**	**55.3**	**18.2**	47.1	75.4	11.6	14.9	52.5	**5.9**	**6.1**	34.6	10.9	4.2	Highest nop, longest il, highest sdp, highest yldm, tallest ph17, phm, maximum nol, lw, htp. From Kos Is.
3	6	**49.5**	82.2	91.1	8.2	5.4	103.6	268	53.3	10.3	42.8	65.1	**13.0**	14.3	**53.5**	5.2	5.6	23.7	**14.9**	**5.7**	Largest sdwt, pl, htfl, ph13, most nobt. From Leros, Patmos—SE Greece.
1	24	37.6	70.2	84.8	8.3	5.3	106.3	260	43.7	14.3	49.4	67.3	8.8	15.1	50.2	5.4	5.8	28.1	11.7	3.9	High yldm—SE Greece.
5	17	41.6	79.2	94.3	7.6	5.7	105.6	301	49.1	11.1	**53.1**	74.2	9.3	13.3	52.2	5.3	4.6	30.8	12.5	3.9	Longest lnub, tall ph13, ph17, phm, quite large seed. Includes all Crete accessions.
0	8	30.0	60.0	77.0	*7.0*	5.5	106.6	233	36.6	10.8	46.8	66.2	7.6	11.8	45.6	4.7	2.8	29.5	11.1	3.2	Fewest nol, small pods, above average nop, average for other characters.
3	17	29.9	64.5	79.7	7.2	5.1	106.0	285	37.6	14.2	47.5	66.8	7.2	12.2	50.5	5.4	4.9	24.9	10.7	2.9	Average for all characters.
9	23	39.0	70.9	84.2	7.2	5.4	106.0	249	44.9	15.7	44.0	63.7	9.3	13.0	48.2	5.2	5.9	21.7	11.5	3.8	Quite tall ph13, ph17, phm, average for other characters.
5	17	32.0	57.9	71.3	8.1	5.0	102.1	207	32.2	13.9	43.7	55.8	7.1	10.9	48.0	5.2	4.7	19.4	10.3	3.2	Short lnbb, few nobl, average for other characters.
3	13	48.8	66.3	75.1	7.9	4.8	*89.8*	214	34.0	13.0	46.4	*47.9*	7.7	*8.6*	50.1	4.9	4.8	22.2	12.9	4.8	Earliest ft, fewest nobl, shortest lnbb, large seed. Includes cv. Gungurru control.
ungurru'		54.9	72.1	79.1	8.3	6.1	80.2	196	33.9	12.7	44.4	45.3	7.1	8.4	50.1	4.7	7.4	45.2	16.7	3.8	Control cultivar

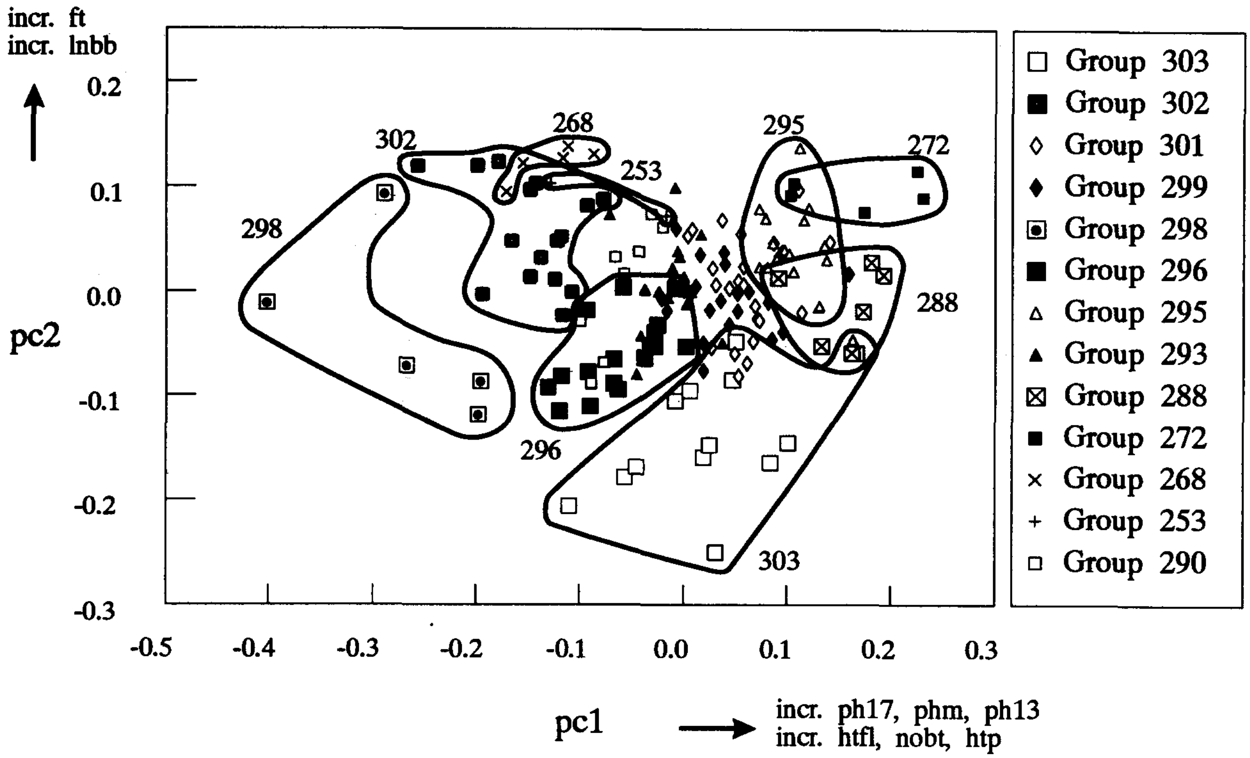

Fig. 3. Principal coordinates analysis scatter diagram of the first two coordinates for 157 wild *L. angustifolius* accessions and control cultivar.

a large number of main stem pods (such as accessions in group 272 from Kos island), and these would be favoured for crossing with domesticated lupins to improve pod set in vigorously growing crops.

Canonical variates analysis using geographical localities as the grouping factor resulted in only a moderate amount of variance explained in the first two variates. This indicates that a lot of morphological variation occurs within localities, which is expected due to the broad range of climatic conditions, geology and land use occurring within localities in Greece. Nevertheless, canonical variates analysis was useful in showing that early flowering and tall, rapidly growing accessions with many upper lateral branches were found in southern Greece where winters are warm and the rainfall lower than in northern Greece.

Principal coordinates analysis was useful for clarifying the grouping of lines as determined by hierarchical analysis. By these methods, it was possible to identify two distinct groups, 272 and 288, with accessions only from the Dhodhekanisos islands Kos, Leros and Patmos in the south-eastern Aegean. These accessions were the most vigorous growing and tallest at maturity, had the greatest number of upper lateral branches, the largest leaves, seeds and pods, the greatest height of pods above the ground, the greatest number of pods on the main stem, and the highest seed yield. These lines have many useful agronomic traits for crossing into domesticated forms.

Accessions from the Kikladhes Islands of the central Aegean were extremely variable and those from Naxos Island were represented in 9 of the 13 groups. 'Gungurru' clustered in group 303 with 12 wild accessions from the Kikladhes, Aegina and the Peloponnese. These accessions were the earliest flowering and had rapid early growth, the fewest and shortest basal branches, and large seed. Group 268 with the lowest main stem yield contained the latest flowering accessions from four collection sites in the northern mainland of Greece. This group also had the shortest plant height at 13 weeks, fewest seeds per pod and small seeds.

The grouping of accessions by multivariate methods in this paper is of practical value to breeders of *L. angustifolius*. Representative accessions may be chosen from particular groups for crossing with domesticated lines. Several potentially important agronomic types have been identified in this study and these accessions will be crossed with domesticated varieties in an attempt to transfer favourable characteristics. Studies such as this will also facilitate the assembly of a core collection of accessions from the larger genetic resource collection (Frankel, 1984; Beuselinck & Steiner, 1992).

The study also helps to define areas for further collection in the Aegean region. Early flowering accessions tended to be found in southern, drier climates, but this trait was not necessarily associated with other important agronomic traits (such as vigorous and tall growth, and profuse podding on the main stem). The Dhodhekanisos islands appear to warrant further collection as many favourable agronomic attributes were found there, but the same may be said for an island such as Naxos, where representatives of several cluster groups were found, indicating extreme genetic diversity. However, morphological variation is not the only concern in assembling plant genetic resource collections. In this region, where wild lupins are relatively rare, it is important to locate as many populations as possible, with adequate sampling of each population to ensure collection of genetic variants. Recently, it was shown that wild *L. angustifolius* from higher pH soils in the Mediterranean region were not necessarily more tolerant of a high pH soil in Western Australia (Cowling & Clements, 1993). It is probably also true that disease resistance and other important traits are difficult to predict from collection site and morphological data, and adequate sampling of the target environment is necessary to ensure that genetic variants are available for future testing.

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