ECOGEOGRAPHY OF THE OLD WORLD LUPINS: CHARACTERISING THE HABITAT RANGE

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ABSTRACT

Lupinus albus **excepted, the smooth-seeded old world lupins have an extremely short history as domesticated crops, and suffer from a narrow genetic background as a result. This is manifested in the absence of specific adaptation, which makes it difficult for breeders to maximise regional potential, and for physiologists to identify key adaptive traits. Wild germplasm can address these problems, because unlike the cultigen, it has undergone natural selection throughout its habitat range for millennia. The 1st step in investigating specific adaptation in wild germplasm is to characterise collection site habitats to develop an understanding of the key selection pressures likely to be imposed by each environment.**

In this study the collection site coordinates for *L. angustifolius, L. luteus, L. albus* germplasm held in the Australian Lupin Collection were ground-truthed using Google Earth and used to extract a wide range of climatic and geographic habitat descriptors for each site. Typical growing season limits for each site were defined using published information and local breeder feedback, and used to calculate site-specific bioclimatic variables (eg. rainfall in the reproductive phase etc.) in order to cluster collection site habitats and identify important selection pressures.

Multivariate analysis indicated that collection sites tended to cluster along stress gradients within all 3 species, to varying extents. The *L. luteus* collection sampled the narrowest habitat range, and could be classified into 3 clusters: 1) short-season, long daylength central European sites with no terminal drought stress; 2) medium altitude northern Iberian sites with cool, frosty vegetative phases and very low terminal drought; 3) central-southern Mediterranean sites with intermediate terminal drought. *L. angustifolius* and *L. albus* habitat types were more complex, grouping into 5 and 6 clusters, respectively. Both species collections contain germplasm from the non-stressful European Cluster 1; a range of Mediterranean habitats with decreasing vegetative phase temperatures and increasing terminal drought stress (*L. angustifolius*, Clusters 2-3; *L. albus,* Clusters 3-5); and high terminal drought stress habitats in northern Africa. In addition, *L. albus* has also been collected from non-stressful habitats in the Ethiopian highlands, characterised by warm, wet vegetative phases and very low terminal drought.

This information is underpinning ecophysiological research currently underway at CSIRO describing how plant traits change in response to environmental selection pressures. By developing an understanding of specific adaptation we hope to be able to guide breeding programs by identifying important adaptive strategies in all 3 species.

KEYWORDS

eco-geography, adaptation, habitat characterisation, *Lupinus angustifolius*, *L. luteus*, *L. albus*

INTRODUCTION

With the exception of *Lupinus albus* L., which was known to Aegean farmers as early as 400 BC (Gladstones, 1998), the old world lupins have a comparatively short history as domesticated crops. Whereas other Mediterranean grain legumes such as chickpea, pea and lentil were domesticated more than 10,000 years BP (Zohary and Hopf, 2000), *L. luteus* L. was introduced to central European agriculture in the 1840s, but not entirely domesticated until the advent of mutation breeding in 1920-30s alongside *L. angustifolius* L. (Hondelmann, 1984). In Australia, the history of the old world lupins is particularly brief. Germplasm evaluation commenced in the 1950s, a breeding program established in the 1960s, and Uniwhite, the first Australian-bred *L. angustifolius* cultivar, was released in 1967 (Gladstones, 1994).

An important consequence of lupins' brief development as domesticated crops is their limited genetic diversity. In *L. angustifolius* this is demonstrated by the absence of specific adaptation among Australian commercial cultivars (Berger *et al.* 2008b). This is a problem for breeders and physiologists alike. Breeders cannot maximise regional yield potential by breeding specifically adapted cultivars, while physiologists cannot identify important traits responsible for specific adaptation because the material under evaluation is too similar. Wild germplasm has the potential to unlock this impasse

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because, unlike the cultigen, it has been subject to natural selection within its distribution range for at least 12,000 years, coinciding with the end of the last glaciation (Gladstones, 1998), and this has led to the formation of distinct ecotypes (Berger *et al.* 2008a). The key to unlocking this potential is to develop an understanding of how adaptive traits change in response to varying selection pressures imposed across the habitat range. The first step in the process is to characterise plant habitats in order to identify significant selection pressures likely to result in ecotype formation. Our capacity to take this step has been greatly improved by the advent of high resolution elevational climate models (Berger 2007; Berger *et al.* 2008a). Altitude, and long term monthly average rainfall, minimum and maximum temperatures are available for the world's terrestrial surface in 30 second grid intervals (ca. 1 km spatial resolution) from the WORLDCLIM model (Hijmans *et al.* 2005). More detailed data such as relative humidity, the number of rain or frost days per month, coefficients of variation for monthly precipitation, sunshine percentage, and wind speed are available at slightly lower resolution (10 minute grid intervals-ca. 9-18 km spatial resolution) (New *et al.* 2002).

In the present study we geo-referenced the Australian Lupin Collection (ALC) by matching latitude/longitude coordinates against site descriptions in order to eliminate errors arising in collections made before the introduction of GPS systems. For each collection site, climate data was extracted and typical growing season phenology defined in order to calculate site-specific bioclimatic variables, and characterise lupin habitats using multivariate techniques. This paper describes the range of habitat types sampled in the Australian Lupin Collection of *L. angustifolius, L. luteus,* and *L. albus.*

MATERIALS AND METHODS

GEO-REFERENCING LUPIN COLLECTION SITES

Much of the ALC comprises material collected before the advent of GPS systems when accurate site coordinate determination was difficult. Because of the high resolution of modern elevational climate models it is important to resolve collection site coordinate errors before extracting climate data. This was done by sorting passport files by collector and collection sites names (typically alphanumeric codes) to regenerate the collection sequence. Google Earth (http://earth.google.com/) was used to virtually recreate the collection trip using the site descriptions, which identified towns, road names and numbers, and km travelled from town A to town B, using the path ruler. At the putative collection site, elevation and site coordinates compared to the original passport data. Where there was ambiguity, the Google Earth maps were discussed with the original collectors to correctly locate the original collection site. Localities were located using the search tool in Google Earth and individual country gazetteers (http://www.diva-gis.org/Data.htm).

CHARACTERISING GERMPLASM HABITATS

Germplasm collection sites (and agricultural research stations used for breeding in the case of cultivars) were mapped with DIVA-GIS (Hijmans *et al.* 2001) and long term monthly climate records extracted. For each site typical growing season phenology (dates of emergence, flowering and maturity) was defined using published information and feedback from local breeders (see Berger *et al.* (2008a)). Seasons were divided into vegetative and reproductive phases, and bioclimatic variables generated for each phase. Rainfall, and the number of frosty and rainy days/month were summed. Temperature, daylength, relative humidity, sunshine percentage, wind speed, and precipitation coefficients of variation were averaged. Minimum and maximum temperatures recorded in each phase were extracted directly. Habitats were characterised by performing principal components analysis and then defined by hierarchical clustering (Ward's method, SPSS Version 10) to facilitate the evaluation of contrasting ecotypes within each species.

RESULTS AND DISCUSSION

GEO-REFERENCING LUPIN COLLECTION SITES

All collection sites in the ALC were checked with Google Earth (n = 1763). In 938 sites the listed coordinates matched the results of Google Earth. 605 site coordinates were incorrect (34%) based on the collection site information and therefore were modified using the Google Earth data. 220 localities could not be found using gazetteers, Google Earth or collector feedback. In this case the listed coordinates were not modified.

The ALC was particularly well suited for accurate geo-referencing because the collection was relatively small and recent, the collection site descriptions very thorough and most of the key collectors available locally to help unscramble ambiguous sites. Moreover, the Google Earth coverage for most of the collection area was detailed enough to include road names or numbers, and significant landmarks.

CHARACTERISING GERMPLASM HABITATS

Multivariate analysis of all species collection sites in a single analysis was unsatisfactory because the classification was dominated by outlying environments such as the Nile Valley (where rainfall is extremely low), and therefore both *L. luteus* and *L. angustifolius* were grouped into <3 clusters (data not presented). In order to increase habitat resolution, and maximise the likelihood of subsequent evaluation identifying distinct ecotypes, collection site analysis was performed within species. Principal components analysis (PCA) was able to explain a large proportion of variance in the 1st two PCs (54-77%), and collection sites tended to cluster along stress gradients within all species (Fig. 1).

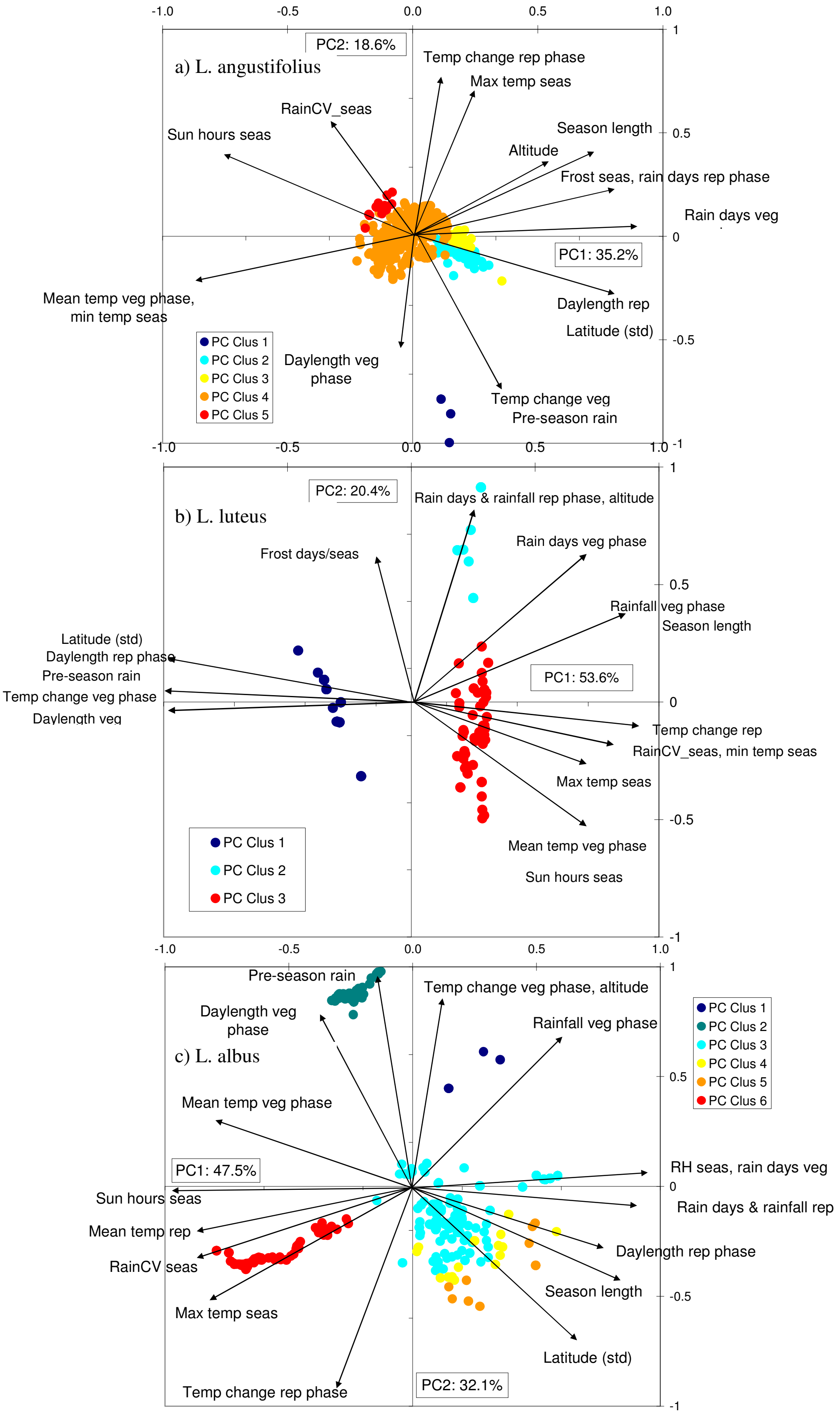

Fig. 1. Principal components ordination of: a) *L. angustifolius,* b) *L. luteus,* and c) *L. albus* collection site habitats sampled in the Australian Lupin Collection. Variable factor loadings for PC1 and PC2 are site specific bioclimatic variables and presented as vectors. Abbreviations are as follows: seas, season; veg, vegetative phase; rep, reproductive phase

L. angustifolius habitats were the most extensively collected, and perhaps the most complex of all 3 species, as shown by the relatively low variance explained in the 1st two PCs, and by the large cluster number required to classify collection sites (Fig. 1a). PC1 was dominated by altitude, rainfall, temperature and their attendant effects on season length, latitude and daylength. Thus, relatively non-stressful cool, high rainfall, long season sites in the northern Mediterranean (Clusters 2, 3) and central Europe (Cluster 1) are located on the right of PC1 (Figs 1a, 2a). The largest number of *L. angustifolius* collection sites came from the central Mediterranean region with intermediate terminal drought stress (Cluster 4), and are clustered around the origin in Fig. 1a. Low latitude northern African sites (Cluster 5, Fig. 2a) represent the strongest terminal drought stress in the collection, as a consequence of high sunshine hours, high, rapidly rising temperatures during the reproductive phase and variable rainfall (Table 1), and map to the upper left quadrant of Fig.1a. The most dissimilar, least terminally drought stressed habitats are the few spring-sown central European sites (Cluster 1) located on the negative of PC2 (Fig. 1a). These are differentiated from the other Mediterranean winter-annual habitats by longer daylength, higher pre-season rainfall, lower mininum and maximum temperatures, less sunshine, and much shorter growing seasons with considerably lower, but less variable rainfall (Table 1). In contrast to Mediterranean environments, the central European growing season has a soft finish, with temperatures falling, rather than rising, and rainfall increasing during the reproductive phase.

The *L. luteus* collection is relatively small and samples 3 distinct habitat types differing in rainfall and its distribution, temperature and daylength (Table 1). Unlike *L. angustifolius* and *L. albus,* advanced breeding lines of *L. luteus* have been extensively collected from central Europe (Cluster 1, Fig. 2b), reflecting the relatively early domestication of the crop in northern Germany in the 1840s (Hondelmann, 1984). As before, these spring-sown environments are very distinct from the Mediterranean habitats of *L. luteus* wild progenitors (Fig. 1b), which can be classified into separate clusters on the basis of geography and terminal drought stress. Cluster 2 comprises long growing season, low temperature/high elevation sites in Spain and Portugal (Figs 1b, 2b) with a high frost incidence, and high, relatively frequent rainfall, experiencing low terminal drought stress (Table 1). Cluster 3 sites in the central-southern Mediterranean are warmer, receiving more sun hours per day than Cluster 2. Although not very stressful compared to some of the habitats from which *L. angustifolius* or *L. albus* are found, the combination of rising reproductive phase temperatures, lowest annual rainfall with the highest variability (Table 1), makes Cluster 3 the strongest terminal drought stress environment in the *L. luteus* collection.

The *L. albus* collection comprises the widest habitat range in the ALC, sampling areas in the Ethiopian highlands, the Nile Valley, Mediterranean Basin and continental Europe (Fig. 2c). PCA was very effective at characterising these diverse habitats: only 2 PCs were required to explain 76.5% variance, and the 6 clusters were clearly separated in the ordination (Fig. 1c). PC1 corresponds to a drought stress gradient, with temperature, sunshine and rainfall variability increasing from right to left (-PC1 loadings), and rainfall, relative humidity, day and season length increasing from left to right (+PC1 loadings). Thus, the highest terminal drought stress is experienced in the Nile Valley (Cluster 6), located in the lower left quadrant of Fig, 1c, combining the highest, most rapidly rising reproductive phase temperatures of any lupin collection habitat with negligible rainfall (Table 1). Clearly these crops are irrigated, but it is likely that high temperatures will impose considerable drought stress. The remaining Mediterranean habitats vary along a vegetative phase temperature/terminal drought gradient moving diagonally down the lower right quadrant of Fig. 1c. Thus, coastal areas with warm vegetative phases and low terminal drought (Cluster 3) plot closest to the origin in Fig. 1c, followed by medium elevation, cool, drier areas (Cluster 4), and finally high altitude inland sites (Cluster 5) with frequent frost, and rapidly warming reproductive phases with intermediate rainfall (Table 1). PC2 contrasts high pre-season rainfall, long daylength and rapidly rising temperatures in the vegetative phase (+PC2) with a rapid temperature change during the reproductive phase (-PC2), and clearly separates the central European and Ethiopian highland areas (Clusters 1, 2) from the remaining Mediterranean collection sites (Fig. 1c).

SYNTHESIS AND IMPLICATIONS

The old world lupins have been collected from distinct habitat types differing in stress intensity, particularly in terms of early cold, terminal drought, rainfall amount and distribution, and varying daylength. Up to 2,000,000 years of selection pressure imposed by these disparate habitats since the beginning of the Quaternary Period (Gladstones, 1998) is likely to have led to ecotype formation. Our work with *L. luteus* certainly supports this conclusion (Berger *et al.* 2008a). As habitats become more stressed or disturbed, ruderal reproductive strategies, based on the exploitation of intermittently favourable habitats, became increasingly conservative, following the predictions of Grime's (1979) triangle. In *L. luteus* this was demonstrated by the earlier phenology, lower leaf node number and higher growth rate in germplasm collected from terminally drought stressed habitats (Cluster 3 versus Cluster 2) (Berger *et al.* 2008a). Ongoing germplasm evaluation in *L. angustifolius*, *L. luteus* and *L. albus* at CSIRO supports the ecotype hypothesis, with significant differences in phenology, vernalisation response, plant growth and productivity.

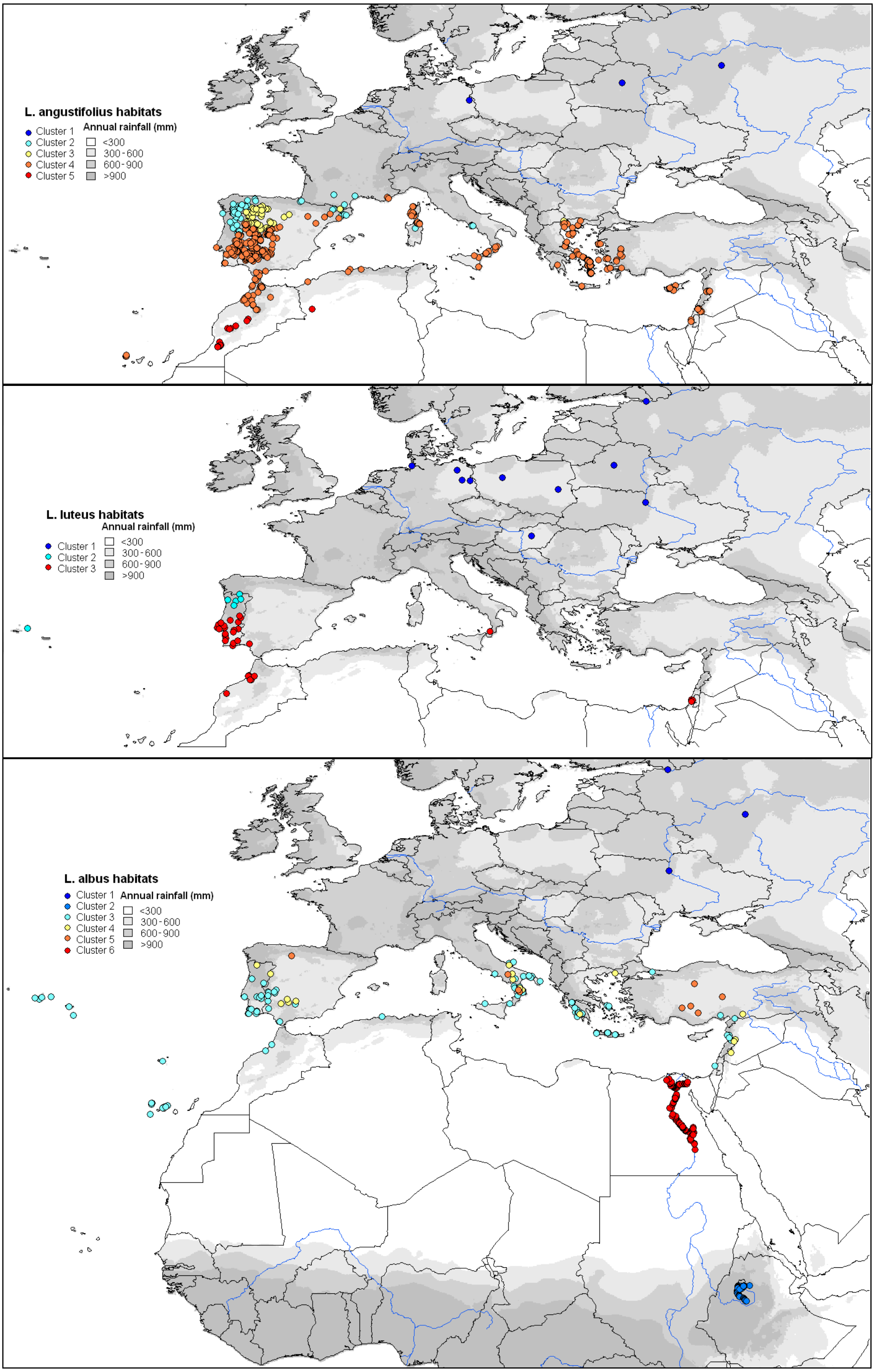

Fig. 2. Map of: a) *L. angustifolius,* b) *L. luteus,* and c) *L. albus* collection sites clustered using Ward's method.

Table 1. Habitat types of *L. angustifolius*, *L. luteus* and *L. albus* sampled in the Australian Lupin Collection, sorted from low to high terminal drought stress.

Cluster	Description	Alt.	Frost	Temperature (°C)			Precipitation (mm)				Daylength
		(m)	(days/ season)	Veg phase	Rep phase	Rep phase slope (°C/day)	Pre-season	Veg phase	Rep phase	Coeff. of Var. (%)	Veg phase
	L. angustifolius										
1	Central Europe: long day, short season, no term drought	116	14	10.8	17.1	-0.02	283	110	212	52.2	14.2
2	Nth Med: med alt, cool frosty veg phase, low term drought	457	60	8.9	16.2	0.09	61	536	309	72.0	10.1
3	Nth Med: high alt, cold, v. frosty veg phase, med term drought	903	95	6.4	16.5	0.11	42	303	153	77.7	10.4
4	Central Med: av. climates, med term drought	319	26	12.4	16.2	0.10	33	321	227	88.7	10.3
5	North Africa: high alt, variable rain, high term drought	1033	30	11.1	16.0	0.09	27	147	129	140.6	10.6
	L. luteus										
1	Central Europe: long day, short season, no term drought	138	12	10.8	17.6	0.01	281	96	212	51	13.9
2	Northern Iberia: med alt, cool frosty veg phase, v. low term drought	437	33	11.1	15.4	0.07	71	618	450	69	10.2
3	Central-southern Med: med term drought	143	5	13.6	16.2	0.08	48	354	224	80	10.3
	L. albus										
1	Central Europe: long day, short season, no term drought	146	12	11.6	17.7	-0.03	299	119	226	52	14.3
2	Ethiopian highlands: warm, wet veg phase, v. low term drought	2061	9	17.3	16.7	-0.01	753	672	52	107	12.2
3	Central Med: coastal areas, wet veg & rep phases, low term drought	434	16	13.0	15.3	0.08	62	438	357	79	10.4
4	Central Med: med alt, cool frosty veg phase, med term drought	596	50	8.7	15.9	0.12	37	377	225	81	10.3
5	Cent Med: inland, high alt, cold, v. frosty veg phase, med term drought	1093	91	5.6	16.6	0.11	36	394	140	70	10.6
6	Nile Valley: irrigated, rapidly rising rep temps, high term drought	58	12	17.7	19.2	0.13	1	4	6	315	10.7

In the absence of specific adaptation among commercial cultivars (Berger *et al.* 2008b) we can use ecotypic differences to gain an insight into how lupins adapted to stresses encountered across the natural distribution range. These insights may suggest new directions that enable breeders to develop horses for courses, and finally maximise the regional potential of the species. Lupin ecophysiology-the study of adaptive strategies in response to environmental selection pressures, is in its infancy. To date we have focused largely on plant growth, productivity and phenology in large scale field evaluations. In 2008 we are applying these principles to more controlled experiments to investigate physiological differences in response to terminal drought stress using contrasting ecotypes of *L. angustifolius* and *L. luteus*.

The ALC is particularly compatible for lupin ecophysiology because of the high quality of the passport data. When site coordinates are accurate, our confidence in clustering germplasm on extracted site characteristics, and then interpreting plant behaviour in the field is high, because the likelihood of misclassification is low. This is in contrast with some of the extensive CGIAR germplasm collections, where passport data may be entirely lacking, or limited simply to a regional name. While the ALC is well described, there are significant collection gaps in all 3 species. Perhaps the most important omission is in *L. luteus*. Gladstones (1998) maps a coastal Mediterranean distribution including southern France, Italy, Corsica, Sardinia, the Aegean, southern Levant and northern Algeria, none of which are sampled in the ALC. While some of these habitat types are likely to be represented in the existing collection, there are no strongly terminally drought stressed environments for *L. luteus* in the ALC (Table 1). Given that there is an existing breeding program attempting to introduce the species to SW Australia, where the grainbelt experiences considerably greater drought stress than the *L. luteus* habitats found in the ALC (Berger *et al.* 2008a), collection from North Africa and the southern Levant should be a priority. While there are significant gaps in both *L. angustifolius* (southern France, Italy, western Greece, southern Turkey) and *L. albus* (southern France, northern Italy, the Balkans, western Turkey, northern Africa), the diversity of habitat types already sampled in the ALC make more collection a less pressing case for these species.

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