

POLYMORPHISMS FOR CYANOGENESIS IN LOTUS AUSTRALIS
ANDR. FROM COASTAL DUNES AT KALBARRI, WESTERN AUSTRALIA

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ABSTRACT

Immature and adult stages of a Lotus australis population from coastal dunes at Kalbarri, Western Australia all contained an identical percentage of cyanogenic phenotypes. The population contained low Ac and high Li allelic frequencies primarily by the absence of the glycosidic (Aci) phenotype and a large proportion of the enzymatic (acLi) phenotypes. This was almost the reverse of a population of the same taxon at Greenough (140 km further south). No satisfactory explanation could be given for the differential selection of the Ac gene.

Introduction

Populations of Lotus australis Andr. from coastal dunes at Greenough Flats, Western Australia, have been shown to be strongly cyanophoric and polymorphic for cyanogenesis at certain locations and stages of development (Foulds, 1982).

Seasonal variation in the expression of cyanogenic phenotypes has been demonstrated in leaves of Trifolium repens L. and L. corniculatus L. (Armstrong et al 1913; Askew 1933; Corkill 1942; de Waal 1942; Jones 1962) and flowers of L. corniculatus (Zhukovski 1956). The variations are often due to the presence of both fixed and changeable individuals (Ellis et al 1977). Within the population at Greenough cyanogenic and acyanogenic stable phenotypes existed along with all forms of unstable types. All adults at the coastal site were cyanogenic probably owing to all seedlings developing glucoside and B-glucosidase. However, at the inland dune site 14% of the adults remained acyanogenic from the seedling stage. This developmental inhibition of glucoside and enzyme was thought to be a response to either soil moisture stress, salt stress or trampling by sheep (Foulds 1982).

Materials and Methods

The population collected was one of the group 4 morphological variant (Larson and Zertova 1965) which is a very robust race identical to populations surveyed at Greenough . As it was

reasonably close to this location with similar environmental conditions and the only other large coastal population found, plants were collected to test if similar cyanogenic frequencies were present. The individuals were collected from calcareous sand dunes at Kalbarri (lat. $27^{\circ} 43'S$, long. $114^{\circ} 10'E$) within 50 m of the sea.

The randomly collected seedlings (no flowers), young adults (less than one year old plants containing flowers) and adults (large specimens with copious flowering stems) were stored at $0^{\circ}C$ from September 1984 until they were eventually all tested in March 1985.

The four phenotypes were distinguished using the picric acid paper technique using three young leaves and the addition of linamarin and linamarase. The pairs of bottles were incubated for 24 h at room temperature. The plants were scored for presence of cyanoglucosides with enzyme (AcLi), glucosides without enzyme (Acli), enzyme without glucosides (acLi) and absence of cyanoglucosides and enzyme (acli).

Results

The Kalbarri coastal region has a dry Mediterranean climate with an average annual rainfall of 416 mm. The rain falls mostly in the winter (65%). The winters are mild (mean temperatures: maximum $20.8^{\circ}C$, minimum 10°) and the summers are warm to hot (mean temperatures: maximum $32^{\circ}C$, minimum $18.7^{\circ}C$).

The nutrient content of the soil surface is given in Table 1. The chloride content at Kalbarri is low (e.g. Greenough and other fore dunes average ca. 200 ug g⁻¹ along the Western Australian coast) but this may be due to the fact that the location was protected by relatively high fore dunes and that the samples were collected after very heavy rain. The soil reaction was, as usual for dunes, alkaline, while the soil was relatively rich in P, K and nitrate-nitrogen.

TABLE 1

Surface soil parameters (ug g⁻¹) of the coastal dunes at Kalbarri and Greenough.

Data was collected from the surface 20 cm in September 1984. Each value quoted is the mean of 5 measurements.

	pH	Chloride	Phosphorus	Potassium	nitrate-nitrogen
Kalbarri	9.0	38	8	14	66
Greenough coastal	9.2	197	15	24	25
Greenough inland	9.0	41	10	19	30

The proportion of cyanogenic to acyanogenic phenotypes is not significantly different between any of the three stages of development (Table 2). Nor is there any significant difference

in the frequency of the cyanoglucoside (Ac) or B-glucosidase (Li) genes in the three stages. However, there is a significant difference in the proportion of the dominant genes (Ac and Li) to the recessive genes (ac and li) in all three groups and the population as a whole (P 0.001).

TABLE 2

Phenotypic and gene frequencies of a population of L. australis from coastal dunes at Kalbarri, W.A.

Stage of development	Phenotypic frequencies				Gene frequencies		
	N	AcLi	Acli	acLi	acli	Cyanogenic glucoside Ac	B-glucosidase Li
Seedlings	101	0.188	0.000	0.772	0.039	0.099	0.961
Adult (1yr)	114	0.210	0.000	0.781	0.009	0.111	0.991
Adult (1yr)	103	0.223	0.000	0.699	0.078	0.119	0.721
Total	318	0.208	0.000	0.751	0.041	0.110	0.797

AcLi, glucoside and enzyme; Acli, glucoside no enzyme; acLi, enzyme no glucoside; acli, neither glucoside nor enzyme.

Discussion

The population of L. australis at Kalbarri was similar to those 140 km further south at Greenough Flats in as much as they were strongly cyanophoric. However, they were different in most other respects (Table 3). Unlike the Greenough populations almost 80% remained acyanogenic from the seedling through to the adult phase, (only 12% of inland and no coastal individuals remained acyanogenic). The greatest majority of these acyanogenic populations in both regions is the enzymic (acLi) phenotype; constituting 94.8% of the acyanogenic phenotypes at Kalbarri and 80.3% at Greenough.

TABLE 3

Phenotypic and gene frequencies of a population of L. australis from Greenough* and Kalbarri coastal dunes.

Stage of development	Phenotypic frequencies				Gene frequencies		
	Cyanogenic		acyanogenic		Cyanogenic glucoside	B-glucosidase	
	N	AcLi	AcLi	acLi	acLi	Ac	Li
Greenough *							
A Coastal seedlings	220	0.864	0.004	0.105	0.027	0.964	0.982
B Inland seedlings	200	0.860	0.010	0.125	0.005	0.964	0.988
C Coastal adults	274	1.000	0.000	0.000	0.000	1.000	1.000
D Inland adults	114	0.877	0.000	0.105	0.018	0.965	0.987
E Coastal (pooled data)	494	0.939	0.002	0.047	0.012	0.976	0.988
F Inland (pooled data)	314	0.866	0.006	0.118	0.010	0.964	0.987
G Total	797	0.924	0.003	0.062	0.011	0.973	0.988
K Kalbarri (pooled data)	318	0.208	0.000	0.752	0.040	0.110	0.798

While the enzyme gene frequency is only significantly different from Greenough populations that include coastal adult data (p 0.05 0.01) the glycosidic gene frequency is significantly different from all Greenough populations (p 0.001). Furthermore there are no Acli individuals at all in the seedling or adult stages, and the frequency of the cyanogenic phenotype (AcLi) is also low.

There are many possible reasons for the differences in the two populations which includes both developmental flexibility, the probable cause of selection at Greenough, and gene selection.

If the Kalbarri population consisted of unstable and stable individuals as did the more southern population the high proportion of acLi phenotypes might be explained by the low soil chloride content (Table 1). However as the samples were collected after rain this low chloride value may be seasonally artificial and not the necessary cause of the failure to "switch on" the Ac genes leaving 80% of the population unstable acyanogenic individuals. No other substratum or climatic difference (established factors influencing flexible genes, Ellis et al 1977; Keymer and Ellis 1978; Foulds 1982) between the two regions can be found except a high nitrate-nitrogen content in the soil at Kalbarri. Thus it may be that this relatively high substrate nitrogen value inhibits cyanoglucoside production which is known to be metabolised along with other nitrogen compounds to compensate for low soil $\text{NO}_3\text{-N}$ or $\text{NH}_4\text{-N}$ in L. arabicus and L. tenuis (Abrol and Conn 1966).

The exceptionally low proportion of Ac individuals at Kalbarri could be explained in terms of gene selection. Such selection of single genes or their linked modifiers has been proposed in many taxa (Daday 1965; Foulds and Grime 1972). Recent studies (Till 1983) also suggest that differences in allelic configuration might influence differential responses of T. repens towards some environmental factors. Of the factors possibly selecting the Ac gene differentially at Kalbarri maximum summer temperatures of 46°C (reaching 70° C at the surface) are little different to those at Greenough.

There is also severe drought (only 22mm of rain falls in 3 summer months) but again similar conditions occur further south and the seedlings develop in milder, wetter winter months.

Therefore, in order to demonstrated whether the reason for the low Ac gene frequency at Kalbarri is a developmental response to environmental conditions, or a fitness selection against the Ac allele by one or more external factors, seedlings from the Kalbarri population should be grown in a variety of controlled environmental conditions and phenotypic changes carefully monitored.

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