

ON THE POLYMORPHISM OF CYANOGENESIS IN *LOTUS CORNICULATUS*

V. DENMARK

DAVID A. JONES

Department of Genetics, University of Birmingham, Birmingham B15 2TT, England

Received 7.vii.72

SUMMARY

Cyanogenic *Lotus corniculatus* occurs as a contaminant of the low grade grass seed used to sow the verges of newly constructed roads in Western Europe. The effect of the new environment on these alien plants is being examined. The influence that the aliens may have on the genetical structure of the native acyanogenic populations of Denmark is being studied.

1. INTRODUCTION

A SURVEY of the distribution of the cyanogenic form of *Lotus corniculatus* L. in Western Europe is in progress (Jones, 1972*a, b*), and some anomalous results have been obtained from samples collected in Denmark. It is the purpose of this report to present the evidence obtained and to outline both the significance of the results and possible long-term studies which could be made.

2. MATERIALS AND METHODS

The methods of testing leaves for cyanogenesis have been given in detail elsewhere (Jones, 1966). Plants have been scored unambiguously for (1) the presence of cyanogenic β -glucosides (linamarin and lotaustralin) and the degradative β -glucosidase, and (2) the presence of the glucosides but no β -glucosidase. No attempt was made to distinguish between the other two phenotypic classes; that is (3) no glucosides, no enzyme and (4) no glucosides but with enzyme.

Except where otherwise stated, established plants growing in their natural habitat have been sampled and thus I have examined plants which have withstood the rigours of germination and establishment.

3. RESULTS

Visits to Jutland were made in 1969 and 1970 and the frequencies of the cyanogenic form found in various localities are presented in table 1. It is clear that while the great majority of plants are acyanogenic, the cyanogenic form is not completely absent. In a few samples the plants are almost exclusively cyanogenic. All these anomalous samples were of plants growing on roadside verges. The plants at Sundstrup (plate I) were amongst the first to be tested in 1969 and it was noticed that the cyanogenic plants growing at the roadside had a markedly different morphology from the native plants (plate II*a, b*) and could therefore have been aliens. Enquiries in

Viborg and Randers revealed that the grass seed had been obtained from a local merchant (*Trifolium Frø*, of Randers) and that the seed had been sown on the verges of the new road after 15th June 1967. Thus if these large *L. corniculatus* plants were contaminants of this grass seed, the variety had survived the winters of 1967-68 and 1968-69, but whether as seed or as plants could not be determined at that time.

TABLE I
Cyanogenic Lotus corniculatus in Denmark

Locality	Cyanogenic phenotype			N
	++	+-	-?	
<i>1969 (July/August)</i>				
1. Viborg (roadside east of town)	0	0	50	50
2. Sundstrup (roadside south-east of village)	49	1	0	50
3. Sundstrup (field adjacent to above)	3	8	83	94
4. Sundstrup (area in front of restaurant)	0	1	17	18
5. Sundstrup (roadside east of 2 above)	22*	1	4	27
6. Sundstrup (roadside west of 2 above)	20*	0	6	26
7. Skals (roadside)	8*	4	50	62
8. Mønsted (field near road)	0	2	56	58
9. Virksund (field near road)	2	1	25	28
10. Skive (plantation to north-east of town)	0	8	60	68
11. Herrup (gravel pit)	2*	0	19	21
12. Herrup (field to north of A16 road)	6	3	41	50
13. Tim (several homogeneous samples)	5	8	111	124
14. Torsminde (dunes)	0	0	125	125
15. Hennestrand (dunes)	0	0	29	29
16. Sig (roadside)	1	4	67	72
17. St Darum (roadside)	0	1	29	30
18. Gram (roadside south of town)	4	4	20	28
19. Lakolk. Rømø (dunes)	0	1	45	46
20. Havneby, Rømø (roadside)	1	0	24	25
21. Ballum (roadside)	10*	1	0	11
22. Hjerpsted (sandy shore)	4	5	32	41
23. Elum (field)	0	4	20	24
24. Søvang (roadside)	3	4	42	49
<i>1970 (September)</i>				
25. Sundstrup (as field sample 3 of 1969)	5	23	144	172
26. Sundstrup (as roadside sample 2 of 1969)	57*	1	1	59
27. Sundstrup (field to south-west of field of 1969)	0	5	39	44
28. Sundstrup (north of road to east of original site)	0	5	22	27
29. Sundstrup (east of above locality)	0	0	7	7
30. Sundstrup (south of road opposite site 28)	0	3	10	13
31. Hobro (roadside west of town)	0	0	14	14
32. Barde (fields near road)	1	5	50	56
33. Snede (roadside)	5*	2	23	30
34. Hellevad (pasture)	3	13	24	40

* Scored for tall upright phenotype before tests for cyanogenesis were set up. All the fields sampled were either pasture or old ley. ++ Cyanogenic glucosides and enzyme; +- glucosides, but no enzyme; -? no glucosides, enzyme phenotype not known.

Subsequently, large plants have been found in several other roadside habitats, but always beside new roads. Additional to those in table 1 are plants found near Monmouth, at Rubery, and on the Clent Hills in England; near the villages of Rahden and Bargum in West Germany; west of Sneek and east of Tiel in the Netherlands. Attempts to locate the exact source of the grass seed used on these roadside verges have been abortive, but there are

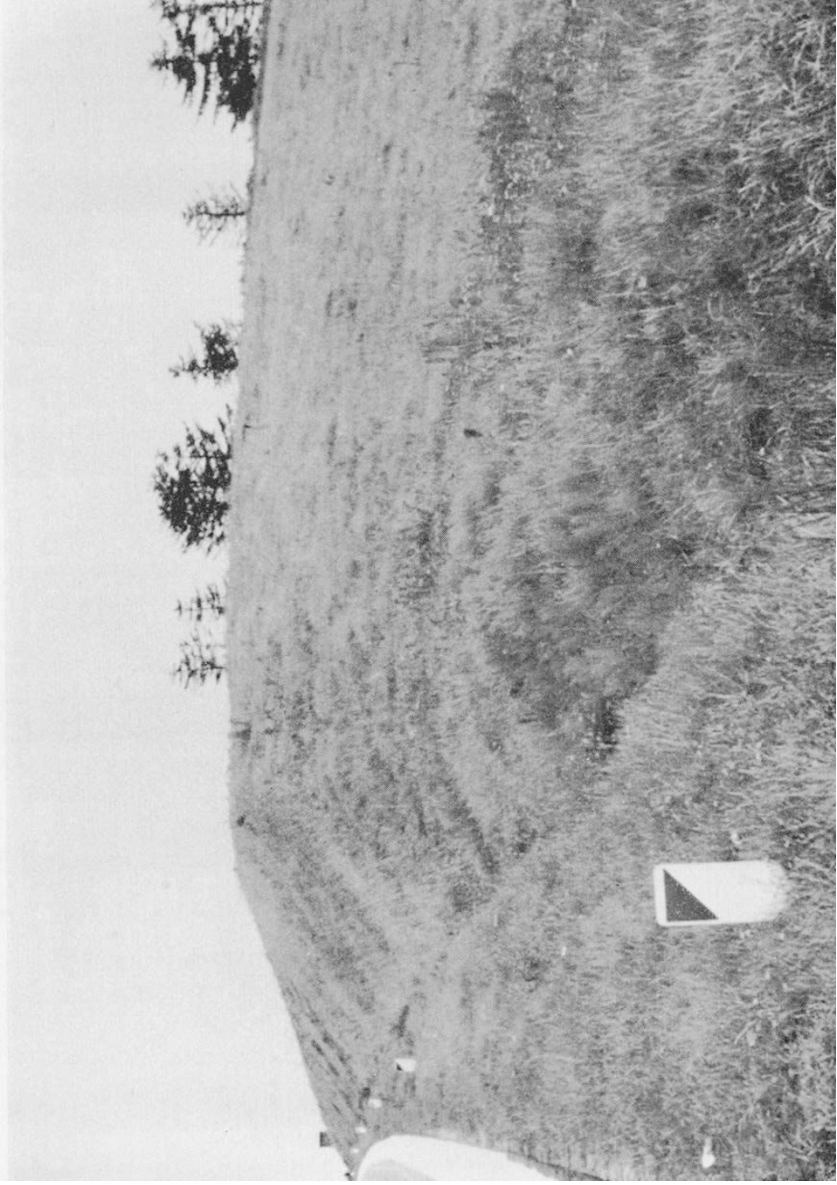


Plate I

The roadside verge and the adjacent field near the village of Sundstrup, Denmark. (September 1970.)

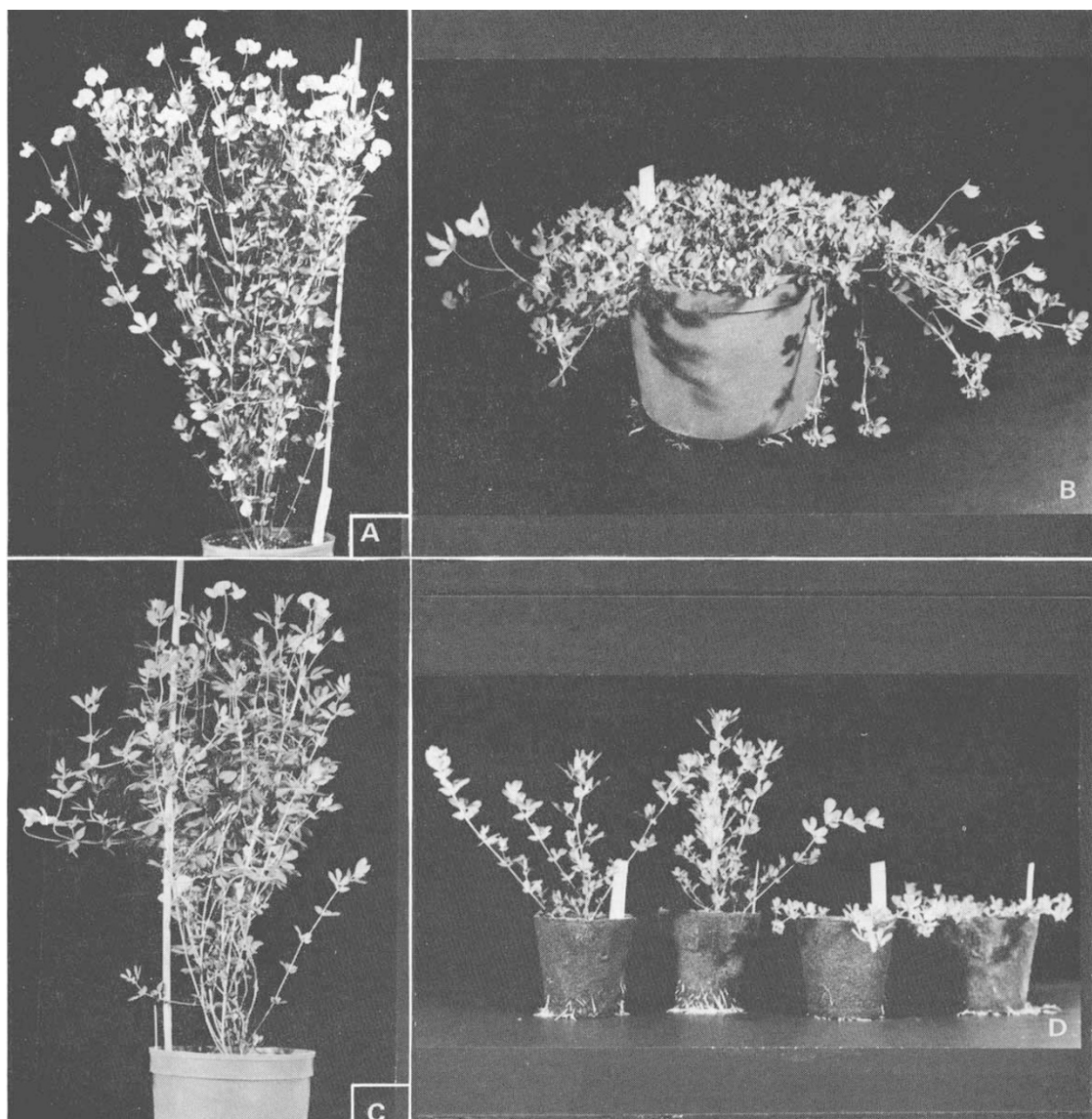


Plate II

- A, B. The growth habit of the alien (A) and native (B) *Lotus corniculatus* at Sundstrup.
 C. The growth habit of *Lotus corniculatus* from the Tuscany hills, Italy.
 D. The growth habit of four seedlings produced by seeds obtained from a plant of native morphology growing amongst alien plants at Sundstrup.

records (Robinson, 1947) that the erect, broad-leaved types originate in Switzerland and Italy. Certainly, plants which I have grown from seed collected by Dr Paolo Talamucci on the Tuscany hills and mountains in Italy have a phenotype very similar to the plants growing at Sundstrup (plate IIc), and it seems justified at present to conclude that these contaminants are of Southern European origin.

Both the Danish alien and the Italian plants have $2N = 24$ and both will cross with native Danish and native British *L. corniculatus*. There is reasonable evidence also that the upright growth and large leaves are dominant over prostrate growth and small leaves, but formal analysis is still in progress. In 1970 seeds were collected from an aglucosidic plant of native morphology growing amongst the alien plants at Sundstrup. A photograph of four of the seedlings obtained is given as plate II d. Scoring the morphology and the cyanogenic nature of these seedlings (Table 2) shows that the upright habit

TABLE 2
Progeny of an aglucosidic plant (-?) growing amongst cyanogenic aliens

Growth habit	Cyanogenic phenotype			Total
	++	+ -	- ?	
Tall	23	5	1	29
Semi-tall	3	0	1	4
Semi-prostrate	2	3	6	11
Prostrate	2	32	39	73
Total	30	40	47	117

and cyanogenesis came in from the pollen parents and thus that these were likely to have been the cyanogenic aliens growing in the immediate vicinity.

4. DISCUSSION

Extensive reconstruction of the road system is a feature common to most of the countries of Western Europe and this has necessitated the production and supply of seed mixtures for sowing on the disturbed edges of these new roads. In time past, *Lotus corniculatus* was included in seed mixtures for a ley crop and for pasture, but this species is now little used outside the United States. The seeds of this plant, however, are of a size comparable with those of various grasses and when sieves are used for segregating off smaller seeds the seeds of *L. corniculatus* can be regarded as mimicking the grass seed. The form of the mimicry is very much cruder than that described by Rowlands (1959) for the *Vicia sativa* L. contamination of *Lens esculenta* L. crops in central Europe, but it is sufficiently effective for *L. corniculatus* seeds to be retained in low grade seed mixtures.

Lotus corniculatus seeds remain viable for many years. Seeds harvested in Finland in 1957 have a germination rate of approximately 10 per cent. while seeds I collected myself in England and in Sweden in 1961 gave over 50 per cent. germination when they were sown this year (1972). It is therefore necessary to ponder whether these anomalous plants could have arisen from seed which had been in the soil for several years and were stimulated to germinate by soil movement during the road construction. Another point

to consider is whether the seeds were contaminants of the soil brought in from elsewhere to build up the verges of the roads. If this were so, it would be reasonable to expect that these large plants would be found mainly on embankments and rarely in cuttings. There is, however, no such pattern of distribution of these plants, while in other disturbed areas, for example outside the restaurant near Sundstrup and at several localities in the Netherlands and in England, these large plants do not occur.

Clearly, it is unlikely that plants of essentially the same morphology, yet markedly different from the native forms, could arise concurrently in several different countries by either of these means. There is, however, independent evidence on the rarity of *L. corniculatus* seeds in pasture and arable soils. Jensen (1969) makes no mention of this species in his extensive survey of arable soils in Denmark while similar results were obtained previously in England by Champness and Morris (1948). In pastures they found that buried seeds of this species are not particularly common but can, where they occur, consist of up to 16 per cent. of the total seed population. Chippindale and Milton (1934) showed that *L. corniculatus* seeds were normally confined to the top 8 cm. of old pastures. The seeds were rarely found in grassland soils which had previously been arable even when the pastures had been established for 50 years or more. It would appear, therefore, that the plant has difficulty over dispersal into new habitats or of re-establishing in old ones, and this fortifies the hypothesis that the alien plants found on roadside verges are contaminants of the grass seed.

Some very useful information on the processes of natural selection has been gained by introducing species or forms into new or different habitats. The experiments began by Sheppard (1951) and continued by Sheppard and Cook (1962) with *Panaxia dominula* L. and the reciprocal transplantation experiments with the plant *Potentilla glandulosa* Lindl. by Clausen and Hiesey (1958) are examples of this technique. In Denmark there has, fortuitously, been the introduction of the dominant allele for cyanogenic β -glucoside synthesis in *L. corniculatus* into what is essentially an acyanogenic native population. This gives a splendid opportunity for a long-term study of the effect of the cyanogenic allele on the native plants and the effect of the local environment of 56° 36' North on what must be regarded as a disadvantageous allele in that habitat.

A start has been made by testing native plants growing in the adjacent field (fig. 1) using a systematic sampling technique. Belt transects were taken parallel to the road. Seeds have been collected in the same systematic way and the results of two years samples are given in table 3 and summarised in table 4.

The first point to notice is that the frequency of the glucosidic form appears to be higher amongst the seed samples than amongst their female parents. This is most marked with belt 2 in 1969. There is also a good correspondence in belt 2 between the frequency in seeds in 1969 and the frequency in plants in 1970.

It is reasonable to expect a higher frequency of cyanogenic forms near the alien plants, and although this is shown by the seed samples, the established plants do not correspond. Only further work would reveal whether there is selection against the cyanogenic form in the field or that the short time since 1967 was insufficient for the cyanogenic allele to break into the relatively closed community.

There is little doubt that the aliens on the Clent Hills and at Rubery are surviving the English winter. The plants die right back to ground level in the autumn and the mode of growth the following spring is clearly different from that of a seedling. Individual plants on the Clent Hills have been marked, and although some plants have failed to survive the winter, the emergence of new growth in the spring corresponds as well as can be expected with the labels. The locations in Denmark and the Netherlands have not been visited in the spring and so it is not yet possible to assert that the same pattern of winter survival occurs there as well.

TABLE 3

The frequency of the cyanogenic form of Lotus corniculatus amongst plants and seed samples collected in the field at Sundstrup adjacent to alien cyanogenic plants growing on the roadside verge. Belt 1 is nearest the road

Phenotype	Transect							
	Belt 1		Belt 2		Belt 3		Belt 4	
	1969	1970	1969	1970	1969	1970	1969	1970
Plants								
++	1	0	0	5	1	0	1	0
+-	1	5	0	11	3	4	4	3
-?	28	36	25	71	20	24	10	13
Seeds								
++	5	5	7	1	0	1	0	1
+-	4	19	5	15	10	11	5	5
-?	40	19	19	19	22	11	16	5

TABLE 4

A summary of the data given in table 3

	Cyanogenic phenotypes			Percentage frequency	
	++	+-	-?	Cyanogenic form	Glucosidic form
Plants 1969	3	8	83	3.2	11.7
Seeds 1969	12	24	97	9.0	27.1
Plants 1970	5	23	144	2.9	16.3
Seeds 1970	8	50	54	7.1	51.8

There are now several examples of differential evolution in closely adjacent groups of plants. Perhaps the most elegant study to date is that of McNeilly (1968) with copper tolerant *Agrostis tenuis* L. He has been able to demonstrate selection in two contiguous habitats and consequently explain the distribution of the tolerant and non-tolerant plants. With *Lotus corniculatus*, however, there are three known selective agents acting on the polymorphism of cyanogenesis (Jones, 1962, 1972*a, b*; Foulds and Grime, 1972), and it is now necessary to determine the role of these in situations such as the one found at Sundstrup.

Acknowledgments.—I am grateful to the Science Research Council (B/SR/6494) for the financial support of this work. I also thank Dr T. J. Crawford-Sidebotham for his help with sampling the plants in 1969, and Professor Kai Larsen for his suggestions about suitable habitats in Jutland.

5. REFERENCES

- CHAMPNESS, S. S., AND MORRIS, K. 1948. The population of buried viable seeds in relation to contrasting pasture and soil types. *J. Ecology*, 36, 149-173.
- CHIPPINDALE, H. G., AND MILTON, W. E. J. 1934. On the viable seeds present in the soil beneath pastures. *J. Ecology*, 22, 508-531.
- CLAUSEN, J., AND HIESEY, W. M. 1958. Experimental studies on the nature of the species. IV. Genetic structure of ecological races. Carnegie Institution of Washington Publication, 615.
- FOULDS, W., AND GRIME, J. P. 1972. The influence of soil moisture on the frequency of cyanogenic plants in populations of *Trifolium repens* and *Lotus corniculatus*. *Heredity*, 28, 143-146.
- JENSEN, H. A. 1969. Content of buried seeds in arable soil in Denmark and its relation to the weed population. *Dansk. Botanisk Arkiv*, 27, No. 2, 56 pp.
- JONES, D. A. 1962. Selective eating of the acyanogenic form of the plant *Lotus corniculatus* L. by various animals. *Nature*, 193, 1109-1110.
- JONES, D. A. 1966. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. Selection by animals. *Canad. J. Genet. Cytol.*, 8, 556-567.
- JONES, D. A. 1972a. "Cyanogenic glycosides and their function", pp. 103-124 in *Phytochemical Ecology* (edited by J. B. Harborne). Academic Press, London.
- JONES, D. A. 1972b. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. IV. The Netherlands. *Genetica*, 43, 394-406.
- MCNEILLY, T. 1968. Evolution in closely adjacent plant populations. III. *Agrostis tenuis* on a small copper mine. *Heredity*, 23, 99-108.
- ROBINSON, D. H. 1947. *Leguminous Forage Plants*, 2nd Ed. Edward Arnold, London.
- ROWLANDS, D. G. 1959. A case of mimicry in plants—*Vicia sativa* L. in lentil crops. *Genetica*, 30, 435-446.
- SHEPPARD, P. M. 1951. A quantitative study of two populations of the moth *Panaxia dominula* L. *Heredity*, 5, 349-378.
- SHEPPARD, P. M., AND COOK, L. M. 1962. The manifold effects of the *medionigra* gene in the moth *Panaxia dominula* and the maintenance of a polymorphism. *Heredity*, 17, 415-426.