THE INFLUENCE OF SOIL MOISTURE ON THE FREQUENCY OF CYANOGENIC PLANTS IN POPULATIONS OF *TRIFOLIUM REPENS* AND *LOTUS CORNICULATUS*

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1. Introduction

Populations of *T. repens* L. and *L. corniculatus* L. frequently contain cyanogenic and acyanogenic plants (Dawson, 1941; Daday, 1954). HCN is released from cyanogenic plants by the action of a β-glucosidase on two cyanogenic glucosides, linamarin and lotaustralin. A selective force helping to maintain the polymorphism of cyanogenesis in *L. corniculatus* appears to be the differential predation of the cyanogenic form by various animals (Jones, 1966), whereas in *T. repens* the greater fitness of the cyanogenic form at higher winter mean temperatures has been demonstrated by Daday (1965). Daday (1954) found no correlation between the frequencies of the glucoside and enzyme alleles of *T. repens* and the rainfall in the region in which they existed. Corkill (1952), however, using a highly cyanogenic strain of white clover (New Zealand Number 1) found that it only occurred in local areas where there was a high soil moisture content. An acyanogenic strain (New Zealand " Dutch "), on the other hand, was predominant in areas exposed to severe desiccation. Foulds and Grime (1971) subjected the four phenotypes (glucoside and enzyme; glucoside and no enzyme; enzyme and no glucoside; neither glucoside nor enzyme) to droughted conditions and found that the vegetative growth of these phenotypes was not affected differentially. They recorded greater numbers of fatalities, on the other hand, in the two phenotypes containing the cyanogenic glucoside (*Ac*) in severely droughted soil.

This experimental work predicts that *T. repens* plants containing the cyanogenic glucoside should be infrequent in droughted habitats. The results following show that the prediction is upheld for wild populations of *T. repens*, but that the polymorphism of cyanogenesis in *L. corniculatus* is not affected by drought conditions in the same way.

2. Methods and results

Samples of populations were collected from two moist and two droughted habitats in Derbyshire. Site selection was based on the appearance of the vegetation, degree of rock and soil exposure and the relative importance of therophytes in the list of species present (none were found at moist sites and 40 per cent. or more on the dry limestone outcrops). All sites were adjacent to permanent pasture which excludes the possibility of contamination by cyanogenic phenotypes of agronomic strains of *T. repens*.

The four phenotypes were distinguished by using the modified picric acid paper technique (Daday, 1954).

Table 1 shows that the populations from similar environments were not

TABLE 1

Phenotype frequencies of populations of T. repens *and* L. corniculatus *from moist and droughted habitats*

	Phenotypes				Phenotypic frequencies	
	AcLi	*Acli*	*acLi*	*acli*	Cyanogenic glucoside	β-glucosidase
T. repens						
Moist						
Monsaldale	6	30	0	14	0·7200	0·1200
Littondale	5	32	0	13	0·7400	0·1000
Droughted						
Monsaldale	3	13	0	28	0·3636	0·0681
Dovedale	5	21	3	21	0·5200	0·1600
L. corniculatus						
Moist						
Littondale	42	7	0	1	0·9800	0·8400
Dudley	12	4	1	0	0·9412	0·7647
Droughted						
Monsaldale	27	23	0	0	1·0000	0·5400
Dovedale	28	20	0	2	0·8160	0·5600

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

TABLE 2

Values of χ^2 *for* 2 × 2 *contingency tables of the raw data used to construct table 1*

Habitat		χ^2	P
T. repens			
Moist	*Ac* × *ac*	0·00	N.S.
Droughted	*Ac* × *ac*	1·72	N.S.
Droughted × Moist	*Ac* × *ac*	14·94	< 0·001
Moist	*Li* × *li*	0·00	N.S.
Droughted	*Li* × *li*	1·12	N.S.
Droughted × Moist	*Li* × *li*	0·01	N.S.
L. corniculatus			
Moist	*Ac* × *ac*	0·00	N.S.
Droughted	*Ac* × *ac*	0·00	N.S.
Droughted × Moist	*Ac* × *ac*	0·01	N.S.
Moist	*Li* × *li*	0·11	N.S.
Droughted	*Li* × *li*	0·00	N.S.
Droughted × Moist	*Li* × *li*	11·92	< 0·001

significantly different. The χ^2 values for the 2 × 2 contingency tables are given in table 2.

The glucoside (*Ac*) phenotypes of *T. repens* on droughted soils were significantly less frequent than on the moist soils. There was, however, no difference in the frequency of the phenotypes, with glucosidase present.

The results for *L. corniculatus* are antithetical to those of *T. repens* in that no differences were apparent between moist and droughted populations for the *Ac* phenotypes but fewer glucosidase (*Li*) plants were found in the droughted populations.

3. Discussion

There is evidence that soil moisture stress is acting as a selective force in the two species. The reduction in frequency of *Ac* phenotypes of *T. repens* in droughted habitats is in agreement with the differentially high mortality of these plants when subjected to severe drought (Foulds and Grime, 1971). Furthermore, the effect is independent of whether the *Li* allele is present or not and so there is further support for the view of Daday (1965) that the locus concerned with cyanogenic glucoside production may be genetically linked to genes concerned with fitness responses to environmental factors.

In contrast to those for *T. repens*, the results for *L. corniculatus* gave no evidence of an effect of the glucoside gene (*Ac*) on survival at the sites subjected to droughting. Although the *T. repens* and *L. corniculatus* populations were taken from the same sites in most cases, it cannot be assumed, however, that they are subject to the same degree of selection, because the two plants may exploit the environment in a different way. They may effectively occupy different niches, because of differences in phenology, root structure and growth rate.

Up to now we have been concerned with the four phenotypes separately, but a different approach is to consider the ability of plants to produce HCN when they are damaged. It appears that the cyanogenic phenotype is disadvantageous under droughted conditions and yet although the genetic polymorphism is the same for both the enzyme and the cyanogenic glucosides in both species, the response to the selection has been different. In *T. repens* a low frequency of cyanogenic plants has been achieved by reduction in the requency of *Ac* while in *L. corniculatus* there has been a reduction in the frequency of *Li*.

This is not the first example of essentially the same genetic polymorphism in two closely related organisms responding to the same selection in different ways. It has been known for several years that the snails *Cepeae nemoralis* L. and *C. hortensis* Müll. (Clarke, 1960) showing the same genetic polymorphism for shell colour and banding patterns have responded to selection in dark uniform habitats by becoming pink unbanded or brown unbanded, and yellow with fused bands respectively.

More extensive studies are essential to confirm and elucidate soil moisture stress as a selective agent.

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THE USE OF HIGHER DEGREE STATISTICS TO ESTIMATE THE NUMBER OF LOCI WHICH CONTRIBUTE TO A QUANTITATIVE CHARACTER

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O'DONALD (1971) showed how second, third and fourth cumulants of the distribution of genetic values could be used to estimate the number of loci responsible for the variation in a quantitative trait. He outlined the influence on estimated number of loci of variation between loci in gene frequency and magnitude of gene effect.

We have made use of similar theory in an attempt to detect the presence of genes of large effect on abdominal chaetae number segregating in panmictic populations of *Drosophila melanogaster* (Hammond and James, 1970). From this work, data are available on cumulants of full-sib family means which can be used to estimate number of loci by O'Donald's method. In terms of the g statistics for skewness and kurtosis (Fisher, 1948), O'Donald's estimate is, for full-sib family mean statistics,

$$\hat{n} = 1/2(g_1^2 - g_2).$$

The factor 2 disappears if cumulants of individual values are used.

This estimate has been made for both males and females from the five parts of our data, using the values given in section 2 (ii) of table 2 in our paper, as well as the pooled estimates given in section 4.2 (ii) of that table. The results were as follows:

	Estimated number of loci	
Part	Males	Females
A	0·8	8·2
B	2·0	0·4
C	0·3	0·5
D	0·2	−454·5
E	−1·3	1·0
Pooled	1·0	

All estimates are low, one being extremely low indeed!

This outcome is to be expected, since O'Donald's estimate is biased downwards. Though an exact value for the bias would be extremely difficult

to derive, the presence of bias is obvious from the presence of g_1^2 in the denominator. The variance of g_1 will tend to make the denominator too large, and hence the estimate too small. Since the variances of g_1 and g_2 are of the order of 6/N and 24/N, where N is the number of families tested, sampling variations will be large in relation to the expected value of $g_1^2 - g_2$ when n is in fact large, and the value of $\hat{n}$ will depend almost wholly on sampling errors.

Our pooled data give 1·0 as our best estimate of the number of gene loci in the Canberra population which affect number of abdominal chaetae. This estimate is based on data from 437 full-sib families. However, neither g_1 nor g_2 differs significantly from zero, so the results are consistent with both third and fourth cumulants being zero, which implies that the number of gene loci approaches infinity. This illustrates the inherent inaccuracy of the method.

It seems to us that, unless an extraordinarily large body of data is collected for the purpose, such estimates of numbers of gene loci are valueless.

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