

ON THE POLYMORPHISM OF CYANOGENESIS IN *LOTUS CORNICULATUS* L.

VIII. ECOLOGICAL STUDIES IN ANGLESEY

W. M. ELLIS, R. J. KEYMER and DAVID A. JONES
Unit of Genetics, University of Hull, Hull HU6 7RX, England

Received 27.ix.76

SUMMARY

The environmental factors influencing a steep morph-ratio cline in a maritime population of *Lotus corniculatus* have been studied in detail. The frequency distribution of cyanogenic plants in the cline has remained stable for 16 years, plants very near the sea being predominantly acyanogenic, whereas 200 m inland 70 per cent of the plants are cyanogenic. Analyses of the biotic, edaphic and microclimatic environment of this population showed that an exposure gradient (wind and windborne salt) and the distribution of known selective herbivores were the only factors which were consistently associated with the cline.

On the basis of these results and the hypothesis that cyanogenesis is a protection against herbivores it was predicted that other sites along the coast, which showed similar environmental variation, should also show a similar distribution of selective herbivores and of cyanogenic plants. It was confirmed that at sites exposed to wind and windborne salt the selective herbivores were rare and the frequency of cyanogenic plants was low. At sites which were less exposed, the numbers of selective herbivores and the frequency of cyanogenic plants were both higher.

1. INTRODUCTION

THERE is now much evidence to show that there is no simple cause-and-effect relationship controlling the distribution of cyanogenic and acyanogenic phenotypes of *Lotus corniculatus* L. and *Trifolium repens* L. in natural populations (Jones, 1977). Three selective agents have been implicated. For both species, differential or selective grazing by herbivores (Jones, 1962; Crawford-Sidebotham, 1972*b*; Whitman, 1973; Angseesing, 1974) and the effect of soil moisture stress (Foulds and Grime, 1972; Jones, 1973) have been described, and Daday (1965) has shown that cold temperature stress is important in *T. repens*. We know of no quantitative data on the action of these factors in natural populations of *L. corniculatus*.

This plant is a tetraploid, outbreeding perennial species and it shows very great morphological and physiological variation (Jones, 1962, 1970; de Vries, 1968; Seaney and Henson, 1970; Borsos, 1976). Some of it has been shown to have a genetic basis (*e.g.* Seaney and Henson, 1970). The genes determining the various morphs of the polymorphism of cyanogenesis must be expressed, therefore, against very variable genetic backgrounds in variable environments.

This report presents the ecological evidence which may explain the frequency distribution of cyanogenic plants in natural populations of *L. corniculatus* on Holy Island, Anglesey. We have deliberately chosen a

population of *L. corniculatus* in which there have been consistent differences in frequency over a short distance for several years and attempted to describe the ecology of the habitat in as much detail as we could. Some aspects of the environmental variation in Anglesey had already been analysed by Goldsmith (1973) and these suggested some components which we should investigate. Our study attempted to assess the roles of selective agents already recognised and to measure other environmental factors which might interact to maintain the cline. Necessarily, this involved collecting information about the environment as a whole and, by a process of elimination, we assessed the importance both of particular factors and of the interaction of these factors as selective agents. Field studies of this kind raise more questions than they answer and subsequent experimental work on samples of plants collected from these populations will be reported in a later paper (Keymer and Ellis, in preparation).

2. THE STUDY AREA AND OBJECTIVES

The initial study was carried out around the bay at Porthdafarch, Holy Island, Anglesey, and this was later extended to the whole south-west coast of Holy Island, from South Stack to Rhoscolyn. It is a rocky dissected coastline with steep cliffs rising to over 100 m, interrupted by coves, all exposed to strong south-westerly winds (Goldsmith, 1973). Our most detailed work was carried out at Porthdafarch, and analysis of these results led to an hypothesis of possible selective factors in that environment. Our subjective assessment was that these factors applied along the coast and by extension we anticipated that we could predict the distribution of cyanogenic plants from South Stack to Rhoscolyn. The hypothesis could then be verified by measuring the environmental factors and by testing plants for cyanogenesis.

The methods of testing leaves for cyanogenesis have been given in detail elsewhere (Jones, 1966). Plants were scored for presence of β -cyanoglucosides with β -glucosidase (+ +), presence of cyanoglucosides without glucosidase (+ -) and absence of cyanoglucosides with or without glucosidase (- ?). Only plants scoring + + are cyanogenic.

3. PORTHDAFARCH

At Porthdafarch, there are three clearly defined areas: the cliff, the hollow and the mound (plate I). These areas are topographically distinct and each has a characteristic vegetation. In general terms, the cliff has an exposed short turf, the hollow a sheltered tall turf and the mound an open heath habitat. The distance from the cliff to the mound is approximately 200 m and the cliff is about 10 m above mean sea level.

(i) *The frequency of cyanogenic plants*

In 1959 Jones (1962) tested samples of plants at Porthdafarch and found a decrease in acyanogenic plants, working inland from the cliff. He suggested that the frequency of acyanogenic plants rises with increased exposure in the habitat, but emphasised that there was little evidence to support this. Subsequent tests over the next 16 years showed that the

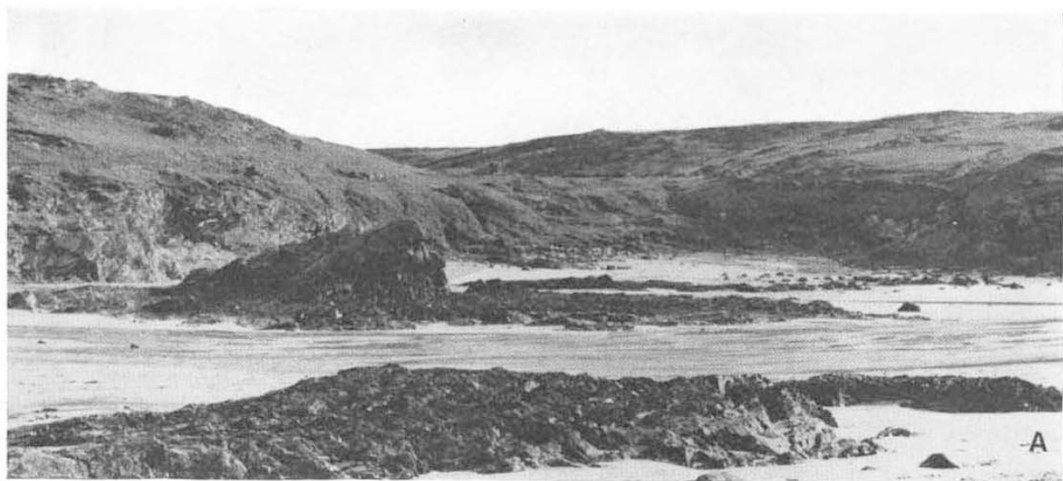


PLATE 1.—The habitats at Porthdafarch Bay. A. The mound is on the left and the hollow is in the centre of the photograph. B. The cliff habitat; the left end of this photograph overlaps the right end of photograph A. C. The cliff and part of the hollow viewed from the mound.

frequency distribution of cyanogenic plants was relatively stable (table 1). The largest deviations from the means occurred in May 1975 and it is most likely that they were due to the small sample sizes at that time. None of the samples exceeded 100 plants because that would have involved over-intensive sampling of a small area with the danger of sampling several clones more than once. To ensure that each plant tested was a separate individual, samples were collected from discontinuous clumps over the entire area is clumped and never continuous it is reasonable to assume that by this method we sampled individuals.

TABLE 1

The frequency of cyanogenic plants at three sites in Porthdafarch, Anglesey from 1959 to 1975

Year	Cliff			Hollow			Mound		
	++	N	%	++	N	%	++	N	%
1959 Jul.	5	20	25	27	40	68	15	20	75
1963 Sep.	14	53	26	26	35	74	45	61	74
1966 Apl.	14	51	27						
1970 Aug.	14	50	28	37	50	74	52	74	70
1974 May	27	83	33	45	58	78	31	51	61
1975 May	5	41	12	17	20	85	10	18	56
1975 Oct.	29	100	29	33	50	66	36	50	72
Means	15.43	56.86	25.71	30.83	42.17	74.17	31.50	45.67	68.00

++ = number of cyanogenic plants, N = sample size, % = frequency of cyanogenesis.

(ii) *Vegetation analyses*

(a) *Methods*

To obtain an objective description of the vegetation we collected species presence and absence data for the three sites and classified them by normal and inverse association analyses (Williams and Lambert, 1959, 1962), which were then amalgamated into a nodal analysis (Lambert and Williams, 1962). The data were collected at each site from 50 cm x 50 cm quadrats arranged 5 m apart in grids. The quadrat size was large in relation to the vegetation, and grids were used because systematic sampling over the entire area was required.

(b) *Results*

Two species were present in every quadrat, *L. corniculatus* and *Festuca rubra* L., and consequently neither of these species influenced the vegetation analyses. Species which occurred in 5 per cent or less of the quadrats were omitted from the analyses. The normal association analysis produced 12 significant quadrat groupings and the inverse association analysis gave nine species groupings whose ecological preferences were similar. Amalgamating the two analyses resulted in a two-way table, the normal groups of quadrats arranged along the top and the inverse species groups down the left margin. The coincidence parameters are the species or quadrats on which divisions were made, and the cells defined by a single coincidence parameter sub-noda (Lambert and Williams, 1962). Rearrangement of this table (fig. 1) shows five clearly defined vegetation units which describe the variation from the cliff to the mound in ecologically significant groups.

	C & M 1	C & M 3	M 2	M 4	M & H 7	H 5	H 8	H 10	C 11	C 8	C 9	C & H 12
A Lotus corniculatus Festuca rubra Agrostis stolonifera Scilla verna Trifolium repens Plantago maritima												▨
G Anthyllis vulneraria Sedum anglicum Asteria maritima Mosses Lichens										■	▨	
H Leontodon autumnalis Plantago coronopus Lolium perenne									■			
B Plantago lanceolata							▨					
C Galium verum Poa pratensis Ranunculus bulbosus Dactylis glomerata Daucus carota Ononis repens Carex flacca					▨	▨	■	▨				
D Bellis perennis Taraxacum laevigatum Senecio jacobaea							▨					
E Erica cinerea Calluna vulgaris Carex nigra Thymus serpyllum Hieracium pilosella Anthoxanthum odoratum Luzula campestris	■	▨	▨	▨								
F Koeleria cristata Agrostis tenuis Centaurea nigra Potentilla erecta Viola riviniana	■											
I Plus 7 species												

FIG. 1.—Rearrangement of the two-way table to give vegetation units of noda (■) and sub-noda (▨) which describe the variation from the cliff to the mound. C = cliff, H = hollow, M = mound.

Three of the vegetation units are characteristic of the cliff and they represent a gradient of increasing soil depth. The group with *Anthyllis vulneraria* L. and *Sedum anglicum* Huds. is typical of the very thin soils on rock outcrops on the cliff. Most of the cliff is covered with a short turf of *Festuca rubra*, with *Scilla verna* Huds. and *Plantago maritima* L. interspersed, and these species form a sub-nodum which is also found in two quadrats in the hollow close to the beach. The third unit, defined by the presence of *Leontodon autumnalis* L., is found on the fairly deep clay loam on the landward end of the cliff.

The hollow is characterised by the unit including *Galium verum* L., a typical grassland species. The vegetation in the hollow quadrats is a tall *Festuca* sward with a large number of associated species, all typical of neutral to calcareous grassland.

The last vegetation unit was formed by the division on the presence of *Erica cinerea* L., which is a typical inhabitant of dry heaths and is indicative of acid conditions. The quadrats in this unit all occur on the mound except for three on the landward end of the cliff.

These analyses provide a description of the coastal vegetation which agrees very well with the work of Goldsmith (1973). The area we are considering fits in the middle of the range he described, from the upper cliff zone to the heath. In an environmental ordination based on 65 stands, Goldsmith recognised two major groups of environmental factors, a salinity group and a soil wetness/humus development group, which were associated with the species succession. The correlation between soil salinity and the distribution of maritime species reflects an indirect relationship. If inland species are susceptible to the saline environment, from spray on the foliage or through the high osmotic potential of the soil water, their competitive ability is reduced in saline situations, thus permitting the growth of the maritime species. The latter species are more salt tolerant than inland species, but do not necessarily have a salt requirement. The low competitive ability of cliff species under mesophytic conditions is related to their slow-growing, perennial nature. At Porthdafarch, we defined a short, open salt-tolerant turf on the cliff with few associated species, giving way to a taller turf with more salt-sensitive species typical of calcareous grassland in the hollow and a taller, salt-sensitive heath on the mound. This succession may be correlated with the change from 25 per cent to 75 per cent in the frequency of cyanogenic plants. Therefore, "Is salt tolerance associated with cyanogenesis in *L. corniculatus*?"

(iii) *Soil analyses*

On Holy Island Goldsmith (1973) showed that a soil wetness/humus development group of factors was associated with the species succession. Furthermore, Foulds and Grime (1972) have demonstrated an association between cyanogenesis and fitness under soil moisture stress in *L. corniculatus*. Therefore, it was a logical step in this study to undertake some measurement of the variation in edaphic factors.

(a) *Methods*

The nutrient status of the soils in the three areas was compared from analyses of samples of soil taken at a depth of 8 cm below the soil surface from 21 cliff, 10 hollow and 10 mound quadrats. The variables measured were conductivity, pH and exchangeable calcium, magnesium, sodium and potassium. Conductivity and pH were measured on portable meters on constant volume soil samples diluted with 2.5 volumes of deionised water. Exchangeable cations were determined according to the methods of the "Perkin-Elmer Cookbook" (1971), calcium and magnesium using a Perkin-Elmer atomic absorption spectrophotometer and sodium and potassium on an EEL flame photometer. The means of each variable for the three areas (table 2) all differed significantly from one another, $P < 0.001$ on heterogeneity F tests.

(b) *Results*

Following Hughes and Lindley (1955), the results were assessed by discriminant function analysis using the ICL Statistical Package programme which optimises the separation of *a priori* defined groups. The programme also computes Mahalanobis's D^2 , a distance statistic which takes into account the total variation in the spatial separation of the three groups of data.

Clearly the nutrients of the hollow soils are quite different from those of the cliff and the mound (fig. 2) and the D^2 values serve to emphasise these differences. The direction and magnitude of the contribution of each variable to the discriminant functions show (fig. 3) that the most important distinguishing factor in these soil analyses was calcium. However, the variation in calcium did not correspond in any way with the variation in the frequency of cyanogenesis and so it could be discounted. Of the other variables which did correspond, sodium was not the most important, magnesium and potassium making larger contributions to the discriminant function analysis (fig. 3). This may be because the variance of sodium

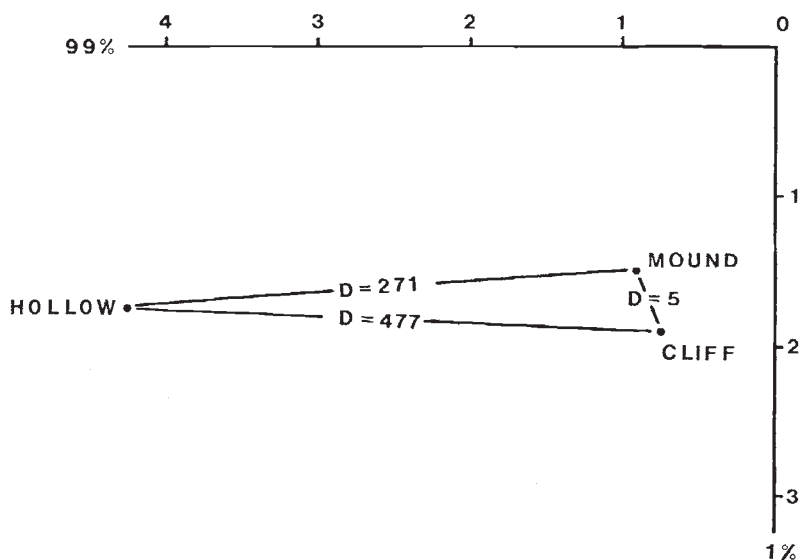


FIG. 2.—Summary of discriminant function analysis of soil nutrient status at Porthdafarch.
 D = Mahalanobis's D^2 .

values for the cliff quadrats was very large following the inclusion of quadrats from the sea *and* landward ends of the cliff. Overall the mean value of sodium (table 2) on the cliff was about five times as high as those of the hollow and the mound.

As nutrient status analysis showed edaphic variation which was not parallel to the change in frequency of cyanogenesis, field measurements were made of soil depth, available soil moisture and temperature. The soils of the cliff and the mound were rather variable, but shallow, the mean depth on the cliff being 16.73 ± 7.94 cm and on the mound 15.03 ± 7.85 cm. The soil in the hollow was more than 30 cm deep.

(iv) *Soil water*

Soil water availability and soil temperature were measured using Wescor soil psychrometers attached to a microvoltmeter. Analyses of variance of measurements made over the 3 days showed some significant

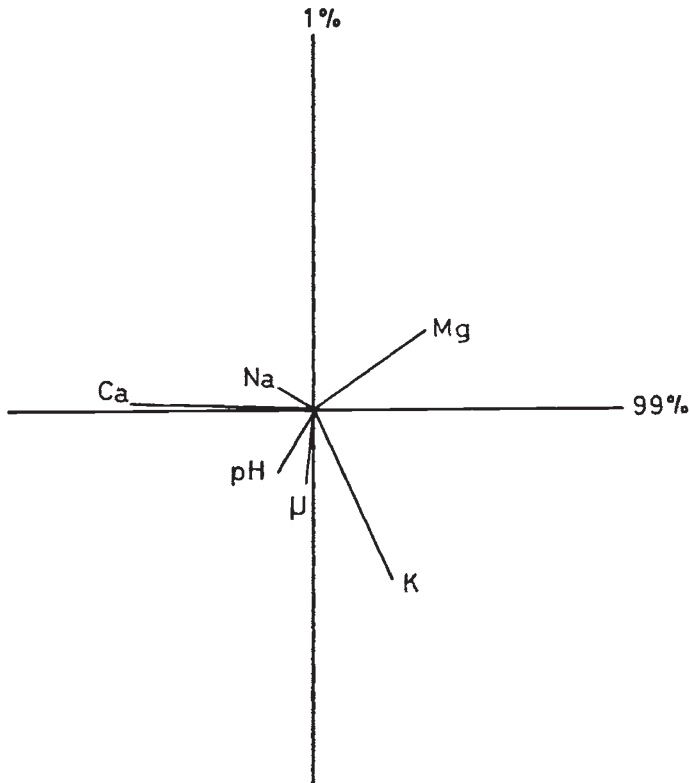


FIG. 3.—The direction and magnitude of the contribution of each variable to the discriminant functions. μ = conductivity.

TABLE 2

The means of soil nutrient variables measured on the cliff, the hollow and the mound

Variable	Cliff	Hollow	Mound
Conductivity μ mos	0.395	0.082	0.108
pH	5.72	8.44	5.59
Calcium μ g/g	1209.0	8798.7	945.8
Magnesium μ g/g	1236.4	314.3	470.1
Sodium μ g/g	2048.5	279.8	493.0
Potassium μ g/g	517.8	96.7	237.9

differences between the cliff and the inland sites for soil moisture, while temperature did not vary very much (table 3). The inland sites, associated with a high frequency of cyanogenic plants, appear to have drier soils than the cliff site. It is interesting that these results would not fit the hypothetical distribution of a lower frequency of cyanogenic plants on drier soils (Foulds and Grime, 1972) and it seems unlikely that soil moisture is an important selective agent in the Anglesey environment.

Some caution is necessary in interpreting our results for soil parameters, which are based on rather small numbers of measurements. It appears,

however, that the soil in the hollow is a deep, dry, basic sand, quite different from the thin, acid, peaty soils of the cliff and the mound. These differences cannot be satisfactorily related to the change in the distribution of cyanogenic plants, with the possible exception of the gradient in exchangeable sodium, magnesium and potassium ions. The soil analyses thus raised the same question as the vegetation analyses, "Is salt tolerance associated with cyanogenesis in *L. corniculatus*?"

TABLE 3

Results of analyses of variance of measurements of soil moisture and temperature on three days. Sample size is shown in brackets beside the mean

	Cliff	Hollow	Mound	F	P
29 Oct 1975					
Moisture	13.05 (35)	25.69 (15)	8.66 (7)	7.11	< 0.005**
Temperature	13.28 (27)	14.98 (12)	—	5.69	0.025*
13 May 1976					
Moisture	27.53 (15)	—	24.78 (13)	0.46	> 0.5
Temperature	16.05 (15)	—	14.16 (15)	3.72	0.10—0.05
14 May 1976					
Moisture	9.68 (18)	—	19.32 (18)	11.60	0.005**
Temperature	12.73 (18)	—	12.66 (18)	0.03	> 0.75

Moisture = bars, temperature = T°C.

(v) *Microclimate analyses*

Jones (1962) noted that the frequency of cyanogenesis decreased as "exposure" increased and our vegetation analyses support this observation. Measurement of the microclimate is one of the more obvious ways of trying to assess "exposure". Microclimate measurements should establish characteristic values of the meteorological parameters for a particular place and, for practical purposes, it is possible that even only one parameter is needed to estimate special features of the weather in a particular area (Geiger, 1971). Apart from the usual difficulties associated with the collection of such data (Wadsworth, 1968), we also had the problems of working in a very popular recreational area. Notwithstanding the difficulties, we selected three sites of decreasing "exposure" and measured three micro-meteorological variables at each site in May, July and October 1975.

(a) *Methods and results*

Relative humidity, temperature and wind speed were measured simultaneously on the seaward and landward ends of the cliff, and in the hollow. Relative humidity was measured using wet and dry-bulb psychrometers constructed according to Long (1968), temperature using Startronic thermistors and wind speed with AVM heat transfer probes made by Prosser Scientific Instruments Ltd. All the instruments were placed 2 to 5 cm above ground level and were connected to multiscrpt recorders through a sequential scanning timer, so that every 11.25 minutes a complete set of measurements was made for each area. Missing data were the result of instrument failures.

The only valid comparisons are between sites on days when simultaneous measurements were made, because day-to-day variation and diurnal

fluctuations are so large. The differences between the areas for each day's measurements were assessed by analyses of variance of the three variables.

In May, the measurements were made on 4 days from 0900 to 2000 hours. The results (table 4) show highly significant differences between the cliff and the hollow, where it was generally more humid, warmer and

TABLE 4

Means and the analyses of variance of microclimate data recorded in May 1975. The arc sine transformation was used for the humidity data

Date	Variable	Cliff A	Cliff C	Hollow	d.f.	F	P	R.A.F. Valley data	
								Mean daily wind m/sec	Direction
4	RH%	55.73 (68%)	50.21 (59%)	57.72 (71%)	2,87	2.87	0.05	2.34	NW
	T°C	13.7	14.27	15.37	2,87	1.17	0.10		
	W m/sec	4.9	3.09	4.61	2,87	17.08	0.01**		
5	RH%	46.51 (53%)	44.92 (50%)	48.91 (57%)	2,141	5.47	0.01**	5.46	NE
	T°C	16.81	16.48	19.67	2,141	18.28	0.001***		
	W m/sec	8.13	4.73	2.05	2,140	68.36	0.001***		
6	RH%	50.43 (59%)	46.89 (53%)	47.49 (54%)	2,123	9.25	0.001***	6.63	NNE
	T°C	17.02	17.81	17.02	2,123	4.06	0.05*		
	W m/sec	14.0	7.05	3.61	2,123	390.82	0.001***		
7	RH%	55.61 (68%)	52.55 (63%)	60.42 (76%)	2,104	15.71	0.001***	7.80	NNE
	T°C	15.17	14.31	15.78	2,105	4.02	0.05*		
	W m/sec	12.19	15.53	4.37	2,105	177.25	0.001***		

sheltered from the strong northerly winds, except on the 4th when the wind was north-westerly. In July, the measurements were made at night from 2100 to 0200 hours when mollusc activity was being scored. Again the differences were highly significant; the hollow was drier and colder than the cliff, but relatively sheltered from the wind (table 5). Wind direction

TABLE 5

Means and the analyses of variance of microclimate data recorded in July 1975. The arc sine transformation was used for the humidity data

Date	Variable	Cliff A	Cliff C	Hollow	d.f.	F	P	R.A.F. Valley data	
								Mean daily wind m/sec	Direction
27	RH%	88.65 (99%)	87.63 (99%)	76.52 (94%)	2,90	39.17	0.001***	2.80	WNW
	T°C	8.45	9.75	8.26	2,90	3.47	0.05*		
	W m/sec	1.21	0.64	1.19	2,89	17.89	0.001***		
29	RH%	—	71.47 (90%)	51.54 (61%)	1,38	2379.65	0.001***	7.90	S
	T°C	14.85	14.93	14.5	2,57	17.17	0.001***		
	W m/sec	5.12	3.01	3.07	2,57	42.44	0.001***		
30	RH%	—	70.44 (89%)	49.74 (58%)	1,44	788.69	0.001***	4.59	SSW, N
	T°C	12.43	10.70	—	1,44	124.21	0.001***		
	W m/sec	4.32	2.28	2.67	2,66	13.34	0.001***		
31	RH%	—	77.08 (95%)	50.50 (60%)	1,32	203.20	0.001***	4.44	NNE
	T°C	14.94	15.00	14.79	2,48	0.63	0.5		
	W m/sec	5.88	2.64	2.36	2,48	54.39	0.001***		

changed over the 4 days and wind speed in the hollow was only as high as on the cliff when the wind was from the north-west (27th July). The October data were more difficult to collect because of the very strong southerly winds. On the 26th the instruments were set up from 1830 to 2300 hours, but the wind and humidity interfered with the flow of the paper charts in the recorders so that only a few sets of measurements were recorded. The data were collected from 1000 to 1815 hours on the 29th and the results show that

the hollow was more humid and sheltered from south-easterly winds while the cliff was most exposed (table 6).

(b) *Discussion*

The only variable which gave consistent results parallel to our subjective exposure gradient was wind speed which was always lower in the hollow, *i.e.* inland, than on the cliff, except when the wind came from the north-west. The mean daily wind speeds and directions recorded at 10 m above mean sea level at the meteorological office at R.A.F. Valley (9 km south-east of Porthdafarch) have been included in tables 4, 5 and 6 as points of reference to the macroclimate of the area. Less than 20 per cent of all winds in the area come from the north-west, whereas 50 per cent come from the south and south-west, so that for most of the year the hollow is less exposed with respect to wind.

TABLE 6

Means and the analyses of variance of microclimate data recorded in October 1975. The arc sine transformation was used for the humidity data

Date	Variable	Cliff A	Cliff C	Hollow	d.f.	F	P	R.A.F. Valley data	
								Mean daily wind m/sec	Direction
26	RH%	70.63 (89%)	39.23 (40%)	73.29 (92%)	2,27	646.70	0.001***	9.18	S
	T°C	13.45	13.55	13.50	2,27	0.12	0.75		
	W m/sec	5.33	—	4.44	1,16	9.13	0.01**		
29	RH%	43.55 (48%)	52.31 (63%)	71.74 (90%)	2,126	207.97	0.001***	3.82	SE
	T°C	15.61	16.44	11.00	2,129	37.99	0.001***		
	W m/sec	2.45	—	0.41	1,84	119.12	0.001***		

The effects of winds from the sea on cliff-top vegetation have been discussed by Edwards and Claxton (1964) and Malloch (1972). The main influence of wind involves the transportation of salt water droplets and they showed that there was a decrease in salt deposition as distance inland increased and also that the effects of salt on the vegetation were less obvious at lower wind speeds. The quantity of salt water droplets available to be carried by the wind depends only partly on local winds. Surf may be produced by waves which have travelled great distances and heavy surf on the south-west coast of Anglesey can originate under the influence of meteorological conditions south of Ireland. Thus it is impossible from the available data to estimate the frequency of surfs heavy enough to produce salt spray over the cliffs or to predict local weather conditions which would affect the amount of salt carried inland. It is probable, however, that only relative values of aerial salt matter with respect to the effect on the biota and these can be estimated.

(vi) *Salt spray*

(a) *Methods*

Measurements of the wind-borne salt on a transect from the cliff inland at Porthdafarch were made using salt-collecting apparatus (impactors) built to the design of Edwards and Claxton (1964). Seven salt impactors were set up at ground level, two on the seaward end of the cliff, three across the landward end, one in the hollow and one on the mound. They were left for 5 days in May and 2 and 3 days in October 1975. The double sample in October was taken because, quite fortuitously, we were there during a period of heavy surf and strong southerly winds, so that after 2 days

the filter papers were very damp and fragile and needed to be replaced. No attempt was made to set up salt impactors in July when the numbers of tourists made work rather difficult.

Two filter papers per impactor were collected and analysed in the laboratory for sodium content. The papers were soaked in 10 ml deionised water for 24 hours and the sodium contents of the solutions were then determined on an EEL flame photometer, the results being expressed as mean $\mu\text{g Na/cm}^2/\text{day}$ (table 7).

A rough estimate of the amount of salt deposited on the vegetation during the period of heavy surf and strong winds was also made. On 3 days, 23, 24 and 29 October, 10 measurements each on the cliff and in the hollow were made on clippings of the *Festuca* sward. Care was taken to select areas of turf which were very similar in the height and density of grass growth.

TABLE 7
Concentration of aerial salt collected on salt impactors at Porthdafarch in May and October 1975

Site	$\mu\text{g Na/cm}^2/\text{day}$		
	May	October 1	October 2
Seaward end cliff 1	0.32	23.94	4.72
2	0.42	10.89	2.05
Landward end cliff 1	0.30	5.17	1.09
2	0.26	14.58	2.52
3	0.14	4.54	1.44
Hollow	0.10	1.32	0.14
Mound	0.08	4.13	0.97

Quadrats, 10 cm \times 10 cm, were cut to ground level and the clippings were shaken in 50 ml deionised water for 1 minute. The conductivity of the solutions was then measured. On the cliff the mean values were 680, 1293 and 1343 μhmhos and in the hollow 219.5, 352 and 392 μhmhos , the cliff values being more than three times as high.

(b) *Results*

Under quite different local weather conditions, the gradient of salt in the air remained the same, highest concentrations on the cliff, decreasing inland (table 7). As might be expected, the mound which is more exposed to wind than the hollow, had slightly higher values of windborne salt but the values on the cliff were approximately four times the inland values.

Thus the special features of the microclimate at Porthdafarch which may be related to the change in the frequency of cyanogenesis in *L. corniculatus* appear to be wind and wind-borne salt. Whether these parameters influence the polymorphism directly or indirectly depends on the wind and salt tolerance of the different phenotypes of *L. corniculatus* as well as the possible effects of wind and salt in the environment on herbivores of this plant.

(vii) *Herbivore activity*

Because selective grazing by molluscs is a known agent affecting the polymorphism of cyanogenesis (Jones, 1962; Crawford-Sidebotham, 1972*b*), data on mollusc numbers and activity in the three areas were obtained by collecting shells, counting numbers per quadrat and scoring activity by the

catch per unit effort method. The most numerous species of molluscs found at Porthdafarch were *Arion ater* (L.), *Agriolimax reticulatus* (Müller), *Cepaea nemoralis* (L.), *Helix aspersa* (Müller), *Cochlicella acuta* (Müller) and *Helicella itala* (L.). All but the last two have been shown to eat, differentially, acyanogenic *L. corniculatus* (Crawford-Sidebotham, 1972b).

The empty shells collected on 28th October 1975 (table 8), occurred in groups near rock outcrops, but as rock outcrops are common, except in the hollow, there is no reason to suppose that birds would bring snails to the mound from the cliff. The most abundant species in the quadrats (table 8) were *C. acuta* and *H. itala* and they occurred in their highest numbers in the hollow.

TABLE 8

Estimates of Mollusc populations at Porthdafarch. The empty shells were collected on one day in October 1975. The quadrat data has been expressed as the mean number of individuals of each species found in the quadrats in September 1974 and in May 1975

	Species	Cliff	Hollow	Mound
Empty shells, October 1975	<i>H. aspersa</i>	0	0	17
	<i>C. nemoralis</i>	0	0	119
Quadrats, September 1974	<i>H. aspersa</i>	0	0	0
	<i>C. nemoralis</i>	0	1	2
	<i>C. acuta</i>	5	236	1
	<i>H. itala</i> and <i>H. virgata</i>	2	37	3
	<i>A. ater</i>	0	0	2
	<i>A. reticulatus</i>	0	0	0
Quadrats, May 1975	<i>H. aspersa</i>	0	1	2
	<i>C. nemoralis</i>	0	6	11
	<i>C. acuta</i>	5	59	0
	<i>H. itala</i> and <i>H. virgata</i>	2	38	8
	<i>A. ater</i>	0	0	2
	<i>A. reticulatus</i>	2	17	14

Mollusc activity was estimated by scoring numbers seen by torchlight along set paths during 15-minute periods on five, four and five nights in July and October 1975 and May 1976. As mollusc activity is positively correlated with temperature and vapour pressure deficit (Crawford-Sidebotham, 1972a), our estimates were weather-dependent and an exceptionally warm, dry summer in 1975 reduced the amount of data that it was possible to collect. There are clear differences, however, from the cliff to the hollow and the mound (table 9). Moreover, even when the hollow was drier and colder than the cliff in July (table 5), there were still many more molluscs present. Although there were fluctuations in the numbers of some species at the inland sites, for example *C. nemoralis* and *H. aspersa*, these species were never recorded on the cliff. Only a few *C. acuta* occurred consistently on the cliff and this species was found much more frequently in the hollow from May 1974 until May 1976, when the population numbers were greatly reduced. *H. itala* normally occurs in discontinuous colonies in sandy hollows (Malan, 1973) and this clumped distribution must be considered in any overall hypothesis of the effect of molluscs as selective agents. Slugs were not as common as snails at Porthdafarch and the results are

somewhat misleading, because the two *A. reticulatus* which were found on the cliff were seen at the landward end only.

The relative distribution of these mollusc species correspond very well with the distribution of cyanogenic plants of *L. corniculatus* and is good circumstantial evidence that they act as selective agents on the polymorphism at Porthdafarch.

TABLE 9

Mollusc activity at Porthdafarch scored as catch per unit effort (15 minutes)

Species	Cliff	Hollow	Mound
July 1975, 5 units of effort			
<i>H. aspersa</i>	0	0.8	1.6
<i>C. nemoralis</i>	0	0.6	3.0
<i>C. acuta</i>	3.4	69.2	0.8
<i>H. itala</i>	0	15.4	2.2
<i>A. ater</i>	0	0.8	1.6
<i>A. reticulatus</i>	1.0	0.8	0.2
October 1975, 3 units of effort			
<i>H. aspersa</i>	0	0	0
<i>C. nemoralis</i>	0	2.33	16.33
<i>C. acuta</i>	12.67	178.0	35.33
<i>H. itala</i>	0	111.67	124.0
<i>A. ater</i>	0	0.67	1.67
<i>A. reticulatus</i>	0.67	2.0	0
May 1976, 5 units of effort			
<i>H. aspersa</i>	0	8.60	11.20
<i>C. nemoralis</i>	0	8.00	46.20
<i>C. acuta</i>	1.6	2.00	0.40
<i>H. itala</i>	0	33.80	55.00
<i>A. ater</i>	0	0	0
<i>A. reticulatus</i>	0.2	1.80	0.40

(viii) *Recreational pressure*

The bay at Porthdafarch is very popular with tourists and from May to September there are hundreds of visitors picnicking on the beach and walking out to the cliff via the hollow, although very few people climb the mound. Quite fortuitously we were able to estimate the trampling on the three areas. In May 1974, 41 cliff, 20 hollow and 18 mound quadrats were marked with bamboo canes 10 cm long, driven into the ground leaving approximately 2 cm above ground. In May 1975, as many of these as possible were relocated, six on the cliff, one in the hollow and 16 on the mound. Assuming that the loss of the bamboo-cane markers can be used as an index of trampling, clearly the cliff and the hollow are heavily trampled, while the mound is rarely walked on. However, canes might be more easily lost in the deep sand of the hollow and it is not strictly comparable to the shallow peaty soils of the cliff and the mound. Thus, excluding the hollow, the change in frequency of cyanogenesis could also be associated with a difference in recreational pressure.

4. HYPOTHESIS

These analyses of the environment at Porthdafarch showed gradients in vegetation, salinity, wind speed, mollusc activity and recreational pressure,

all associated with the change in the frequency of cyanogenesis. Most of these factors could be related to one another under the general term of exposure: the cliff is more exposed than the inland sites. Each factor may of course directly influence the fitness of the cyanogenic phenotype, and laboratory studies are necessary to determine such effects. The integrated effects can be assessed in the field, however, by choosing similar sites and predicting cyanogenesis frequencies which can then be tested. We predicted that on the cliffs which were exposed to high levels of salt spray and strong winds, the vegetation would be short *Festuca* turf, there would be few molluscs and the percentage of cyanogenic *L. corniculatus* plants would be low. Inland from these cliffs, as the levels of salt in the air dropped and wind speed was lower, the vegetation would become a heath, the numbers of molluscs would increase and the percentage of cyanogenic *L. corniculatus* plants would be high.

5. SOUTH STACK TO RHOSCOLYN

Six sites to the west and six to the east and south-east of Porthdafarch were selected for study. At each site a sample of 50 *L. corniculatus* plants from the exposed cliff tops and a sample of 50 plants from a region 100 to 300 m inland were tested for cyanogenesis. In addition, two sites more than 500 m inland were sampled, one at the camp-site (C) and one at Pwllpillo (P). Our experience at Porthdafarch allowed us to rank subjectively, "more" exposed and "less" exposed habitats using the two criteria of cliff height and aspect in relation to the prevailing wind. All the sites were sampled in May or July and resampled in October 1975. The mean frequencies of cyanogenesis in the two tests were plotted as histograms on a map of the area (fig. 4).

The conditions of our hypothesis were satisfied on sites with a southerly aspect, that is at sites West 1, 2 and 3 and East 1, 2 and 3. The cliff tops were covered with a short *Festuca* turf and the frequency of cyanogenesis was low, less than 20 per cent; inland the vegetation gave way to stable heath communities and the frequency of cyanogenesis increased to more than 35 per cent. Apart from the site at South Stack which is exceptional, the remaining sites were all more exposed with respect to westerly and south-westerly winds and the overall frequency of cyanogenesis at these sites was much lower, though still in the same relative proportion. West 4 inland had recently been burnt, the heath was very poorly developed and it is unlikely that the population of *L. corniculatus* had reached a stable equilibrium in that environment at the time of sampling. Because these sites, particularly on the Rhoscolyn Peninsula, appeared to be more exposed, we sampled further inland. At Pwllpillo, more than 500 m inland on a sheltered pasture, the frequency of cyanogenesis was over 90 per cent. A comparable site more than 500 m inland of Porthdafarch, at the camp-site, showed a frequency of over 75 per cent.

Only one site appeared to be an exception to our hypothesis and it was so in all aspects, salt spray, per cent cyanogenesis and mollusc distribution. That was at South Stack. There the cliffs are very high, over 150 m, the vegetation on the cliff is mixed, a tall *Festuca* sward with heath on flatter areas and on rocky outcrops some common cliff species, such as *Silene maritima* With., *Armeria maritima* (Mill.) Willd. and *Anthyllis vulneraria*, occur

whereas inland it is a typical heath of *Calluna vulgaris* (L.) Hull. and *Ulex europea* L. The frequency of cyanogenic plants both on the cliff (150 m) and inland (200 m) was about 50 per cent, which led us to suppose that salt spray rarely reached these heights and, therefore, to expect larger numbers of molluscs on this cliff than elsewhere.

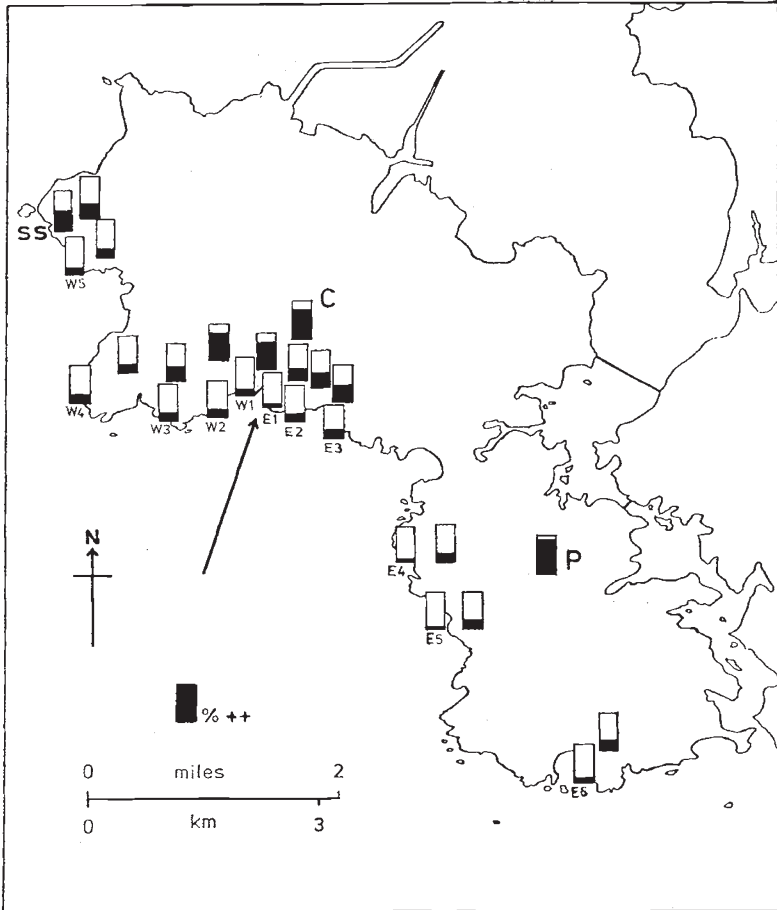


FIG. 4.—The distribution of cyanogenic *Lotus corniculatus* along the south-west coast of Holy Island, Anglesey. The arrow points to Porthdafarch Bay. SS, South Stack; C, camp-site; P, Pwllpillo; W1 to W5, E1 to E6 and SS are the sites sampled after the main work at Porthdafarch was completed.

In order to compare the relative amounts of aerial salt at South Stack and other sites, salt impactors were set up for 5 days on the cliffs and inland from them at four sites in May 1976—Porthdafarch, South Stack, West 4 and East 5. Unfortunately one impactor was lost over the cliff, but the results for the other seven are shown in table 10. At Porthdafarch, the concentrations of aerial salt were almost identical to those measured previously in October 1975 (table 7) and at East 5 the situation was similar, although the inland value was almost twice the value for the mound. At

South Stack the concentration of aerial salt both on the cliff and inland was as low as inland at East 5. Using the same technique as before a further estimate of the amount of salt deposited on the vegetation was carried out comparing Porthdafarch and South Stack. Five quadrats at each site on 3 days were measured and the mean values are shown in table 11. The two inland sites were very similar, but there was considerably less salt deposited on the cliff at South Stack than at Porthdafarch. Therefore, our supposition appeared to be correct; that on high cliffs, such as those at South Stack, there is less salt in the air and deposited on the vegetation than on the lower cliffs along the rest of the coast.

TABLE 10

*Concentration of aerial salt collected on salt impactors
at four sites in May 1976*

Site	$\mu\text{g Na/cm}^2/\text{day}$
Porthdafarch, cliff	4.26
Porthdafarch, mound	0.91
East 5, cliff	4.57
East 5, inland	1.74
West 4, cliff	lost
West 4, inland	1.67
South Stack, cliff	1.71
South Stack, inland	1.53

TABLE 11

Conductivity of solutions of deionised water in which clippings of the Festuca sward had been shaken to estimate the amount of salt deposited on the vegetation at cliff and inland sites at Porthdafarch and South Stack in May 1976

Site	Conductivity μmhos		
	13th May	14th May	15th May
Porthdafarch, cliff	490	738	1384
Porthdafarch, mound	249	170	244
South Stack, cliff	356	696	552
South Stack, inland	202	181	182

The last test of our hypothesis was the distribution of molluscs. Mollusc activity was estimated by the catch per unit effort method as before, in July and October 1975 and May 1976 at several sites: South Stack, West 1, 3 and 4 and East 1. The warm dry summer of 1975 was not favourable for mollusc activity so that all the scores are low (table 12) and it is possible that it will take the mollusc populations some time to recover (Potts, 1975). It is significant, however, that none of the known differential herbivores of *L. corniculatus* occurred on the cliff sites *except at South Stack*. Daytime searches during and after heavy rain were added to these scores and they showed the same distributions. The only species which occurred on cliff sites were a few individuals of *Cochlicella acuta* and a lower number of *Helicella virgata*. *H. itala* only occurs in clumped colonies in sandy hollows (Malan, 1973) such as the hollow at Porthdafarch and does not occur on the cliffs. Incidentally, we predicted that *H. itala* would be a differential or selective herbivore (Keymer *et al.*, in preparation) and that where colonies occur the frequency of cyanogenesis in the population of *L. corniculatus* would be high.

TABLE 12

Mollusc activity scored as catch per unit effort at sites along the coast from South Stack to Rhoscolyn

Species	South Stack		West 1		West 3		West 4		East 1	
	C	I	C	I	C	I	C	I	C	I
July 1975, 2 units of effort										
<i>H. aspersa</i>	0.5	—	—	—	—	—	—	—	—	—
<i>C. nemoralis</i>	—	3.5	—	—	—	—	—	—	—	—
<i>C. acuta</i>	—	—	—	—	—	—	—	—	9.0	—
<i>H. virgata</i>	—	—	—	—	—	—	—	—	—	—
<i>A. ater</i>	—	—	—	—	—	—	4.5	—	—	3.5
<i>A. reticulatus</i>	1.0	—	—	—	—	—	—	—	—	1.5
October 1975, 2 units of effort										
<i>H. aspersa</i>	—	—	—	—	—	—	—	—	—	—
<i>C. nemoralis</i>	0.5	—	—	0.5	—	—	—	—	—	—
<i>C. acuta</i>	—	—	0.5	—	—	—	—	—	2.0	—
<i>H. virgata</i>	—	7.0	1.5	—	—	—	—	—	—	—
<i>A. ater</i>	1.0	—	—	—	—	1.0	—	—	—	1.0
<i>A. reticulatus</i>	1.0	—	—	—	—	1.0	—	—	—	1.0
May 1976, 2 units of effort										
<i>H. aspersa</i>	2.0	—	—	—	—	—	—	—	—	—
<i>C. nemoralis</i>	—	6.0	—	3.0	—	—	—	—	—	0.5
<i>C. acuta</i>	—	—	—	—	—	—	—	—	0.5	—
<i>H. virgata</i>	—	—	0.5	—	—	—	—	—	—	—
<i>A. ater</i>	—	0.5	—	—	—	—	—	—	—	5.0
<i>A. reticulatus</i>	—	—	—	—	—	—	—	—	—	—
Daytime search after heavy rain May 1976, 3 units of effort										
<i>H. aspersa</i>	0.67	0.33	—	—	—	—	—	—	—	—
<i>C. nemoralis</i>	1.0	11.67	—	8.0	—	—	—	—	—	—
<i>C. acuta</i>	—	—	0.33	—	—	—	—	—	2.0	—
<i>H. virgata</i>	—	—	—	—	—	—	—	—	—	—
<i>A. ater</i>	1.67	1.33	—	—	—	7.0	—	3.5	—	2.0
<i>A. reticulatus</i>	1.0	—	—	—	—	—	—	—	—	1.0

C = cliff, I = inland.

In May 1976 a sample of 50 *L. corniculatus* plants was collected from each of Malan's *H. itala* sites at Newborough Warren. The percentages of + plants were 72 and 44, as high as most of the inland sites tested on Holy Island.

6. DISCUSSION

In a previous paper in this series (Jones, 1977) it was shown that the morph ratio clines for cyanogenesis in *T. repens* and *L. corniculatus* appeared to exist at three levels related to the distances over which phenotype frequencies changed. These levels range from continental to very local environmental effects.

The present paper has been concerned with differences in frequency over very short distances (metres) and so it is to be expected that comparisons would be made with similar situations which occur in other organisms. It is only with *Anthoxanthum odoratum* (Antonovics and Bradshaw, 1970) and

Avena barbata (Hamrick and Allard, 1972), and with *Cepaea* (e.g. Sheppard, 1951) in the Oxford district, that the selective agents involved in maintaining the clines have been recognised and are tolerably well understood. Attention will therefore be concentrated on the two plant examples.

Knowing that heavy metal contamination of soil exerts very strong selection for tolerant plants, Antonovics and Bradshaw (1970) looked at an array of morphological characters in *Anthoxanthum odoratum*. They collected samples along a transect crossing the boundary between the lead and zinc mine at Trelogan (Wales) and the surrounding pasture. They found, for example, that the plants on the mine flowered earlier, were more often self-fertile, were shorter, and produced fewer tillers than the plants on the uncontaminated soil. At the mine boundary the plants were of intermediate phenotype for these characters. Because the founders of the mine group of plants were small in number there was some correlated response to the edaphic selection, but the clines were of varying steepness showing that this association was being eroded.

In a population at Nada Valley, California, Hamrick and Allard (1972) found spectacular changes in allele frequency in *Avena barbata* over a distance of 120 m at six of the eight loci they examined. The habitat conditions change from mesic to xeric over this short distance and in the xeric region the plants are essentially monomorphic. In other Californian populations of *A. barbata* growing in xeric conditions the plants are monomorphic for exactly the same genotype. This is one of the reasons why Hamrick and Allard interpret the variability as resulting from natural selection. It should be noted that Hamrick and Allard chose to work with this population in the Nada Valley because the transition from mesic to xeric vegetation types occurred within the one population of *A. barbata*. Thus, the choice of population for study, like Antonovics and Bradshaw (1970), was based on habitat and not on the known variation in the population. In other words, like the early work with *Cepaea nemoralis* (Cain and Sheppard, 1950), the studies on *A. odoratum* and *A. barbata* involved looking at variation within different, but defined, habitats which were easy to distinguish without recourse to instruments.

The work on the polymorphism of cyanogenesis in *L. corniculatus* has been approached from the opposite direction, essentially because it has not been possible to identify a predominant selective agent. It is more comparable, therefore, with the later work on *Cepaea*, particularly on area effects (e.g. Cain and Currey, 1968). Although previous work with *L. corniculatus* has implied that selective eating (Jones, 1966; Crawford-Sidebotham, 1972b) and soil water stress (Foulds and Grime, 1972; Jones, 1973) could be important, it was not possible to explain the variation in those terms.

In this paper we have deliberately chosen a population of *L. corniculatus* in which there have been consistent differences in frequency over a short distance for several years and attempted to describe the ecology of the habitat in as much detail as we could.

Our objective was to answer the question: "Why is there an increase in the frequency of cyanogenesis in populations of *L. corniculatus* inland from the cliff at Porthdafarch?". Because this was a stable biological situation (table 1) the field work was designed to distinguish any consistent relative differences between the cliff-top and the inland heath communities which

might account for this increase. We collected sets of data during the growing season of *L. corniculatus* from May to October over 2 years and analysed each set separately.

A vegetation analysis was undertaken because, in contrast to short-term measurements, the vegetation reflects the effect of the interaction of various environmental factors experienced over preceding seasons. There are two methods, ordination and association analysis. Comparisons of these methods have been made by Gittins (1965) and Bunce (1968), who obtained satisfactory correspondence between the two methods, but Goldsmith (1973), working on sea-cliff data, favoured ordination, although the data took much longer to collect. Those comparisons did not take association analysis to its ultimate development, nodal analysis (Lambert and Williams, 1962), which we used here. This method produced a description of the vegetation which corresponds well to the work of Goldsmith (1973) and fits in the middle of the range he described, from the short *Festuca* turf on the cliff to the heath inland (fig. 1).

Soil and microclimate measurements were more difficult to make and less satisfactory than those of the vegetation. In the short-term measurement of these parameters, larger differences were found at each site on different days than between sites on the same day (tables 3-7), but only the comparison of values recorded simultaneously can lead to the isolation of significant relative differences between sites, that may parallel the change in exposure found over the area. On the other hand, the small amount of quantitative data it was possible to collect is more valuable because the vegetation analyses are there to support it. The three analyses together show an exposure gradient in the environment which is mainly caused by the strong prevailing south and south-westerly winds and wind-born salt. The gradient in wind and salt can be related to the change in the frequency of cyanogenesis. On the exposed cliffs where wind speed and salt concentration are high, the per cent cyanogenesis is low; inland, wind speed and salt concentration are lower and the per cent cyanogenesis is higher. Whether these parameters influence the polymorphism directly or not depends on the wind and salt tolerance of the different phenotypes of *L. corniculatus*. Indirect effects may occur through the influence of these parameters on the activities of herbivores.

Of those herbivores which selectively graze acyanogenic *L. corniculatus* plants, molluscs were the most obvious to study in this habitat. Small mammal trapping at such a popular tourist resort was impossible and anyway it is unlikely that small mammals occur in these rather exposed habitats. No evidence for their presence, such as runs, nest or faeces, were found. Rabbits, sheep and cattle occur sporadically along the cliffs and inland, but they are probably non-selective grazers. There are grasshoppers and crickets on the cliffs, but their selective grazing has yet to be demonstrated (Miller *et al.*, 1975).

It seems likely that the gradient in exposure directly affects the numbers and activities of molluscs at Porthdafarch and indeed along the whole coast from South Stack to Rhoscolyn. The most reasonable explanation is that the wind-borne salt is responsible for the distribution of the molluscs—it is common knowledge that the activity and distribution of many garden gastropods can be restricted with NaCl—so that where mollusc numbers are high, the percentage cyanogenesis is also high and vice versa. If this is true

then we have clear evidence for the defensive role of cyanogenesis in natural populations (Ellis *et al.*, 1977).

A further implication is that cyanogenic plants are at some disadvantage on exposed cliff sites. We have observed that cliff-top plants are generally of prostrate habit with short internodes, which may indicate a slow growth rate, compared with the more upright, straggly habit of plants with long internodes growing in heath. The plants retain these growth forms when grown in uniform glasshouse conditions and, therefore, it is possible to correlate a slow growing, prostrate habit with the acyanogenic phenotype. The correlation does not imply any genetic linkage between the two, although selection in the Anglesey environment may act to keep these phenotypes positively associated. Two possible selective agents in this environment are salt and trampling and experimental work to determine whether they act differentially on the cyanogenic phenotypes of *L. corniculatus* will be reported in a later paper.

Acknowledgments.—We are most grateful to the Science Research Council (B/RG/33539, W. M. E. and D. A. J.) and to the Natural Environment Research Council and the Sir Philip Reckitt Educational Trust (research studentship, R. J. K.) for the financial support of this work. It is a pleasure to thank Mr A. K. Kemp and the Meteorological Officers at R.A.F. Valley for data on the macroclimate of the area and for their helpful comments. We would like to thank especially Mr E. Wright for putting together our portable "weather station". The constructive comments of Professor A. J. Cain on an earlier draft of this paper are gratefully acknowledged.

7. REFERENCES

- ANGSEESING, J. P. A. 1974. Selective eating of the acyanogenic form of *Trifolium repens*. *Heredity*, 32, 73-83.
- ANTONOVICS, J., AND BRADSHAW, A. D. 1970. Evolution in closely adjacent plant populations. VIII. Clinal patterns at a mine boundary. *Heredity*, 25, 349-362.
- ASTON, J. L., AND BRADSHAW, A. D. 1966. Evolution in closely adjacent plant populations. II. *Agrostis stolonifera* in maritime habitats. *Heredity*, 21, 649-664.
- BORSOS, O. 1976. Aszarvas kerep. *Lotus corniculatus* L. *Cultural Flora of Hungary*, Volume 3. Adadémiai Kiado, Budapest.
- BUNCE, R. G. H. 1968. An ecological study of Ysgolion Duon, a mountain cliff in Snowdonia. *J. Ecol.*, 56, 59-75.
- CAIN, A. J., AND CURREY, J. D. 1968. Studies on *Cepaea*. III. Ecogenetics of a population of *Cepaea nemoralis* (L.) subject to strong area effects. *Phil. Trans. Roy. Soc. B*, 253, 447-482.
- CRAWFORD-SIDEBOTHAM, T. J. 1972a. The influence of weather upon the activity of slugs. *Oecologia*, 9, 141-154.
- CRAWFORD-SIDEBOTHAM, T. J. 1972b. The role of slugs and snails in the maintenance of the cyanogenesis polymorphisms of *Lotus corniculatus* and *Trifolium repens*. *Heredity*, 28, 405-411.
- DADAY, H. 1965. Gene frequencies in wild populations of *Trifolium repens* L. IV. Mechanism of natural selection. *Heredity*, 20, 355-365.
- EDWARDS, R. S., AND CLAXTON, S. M. 1964. The distribution of air-borne salt of marine origin in the Aberystwyth area. *J. Applied Ecol.*, 1, 253-263.
- ELLIS, W. M., KEYMER, R. J., AND JONES, D. A. 1977. The defensive function of cyanogenesis in natural populations. *Experientia*, 23, 309-311.
- 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.
- GEIGER, R. 1971. *The Climate Near the Ground*. Harvard University Press, Cambridge, Massachusetts.
- GITTINGS, R. 1965. Multivariate approaches to a limestone grassland community. III. A comparative study of ordination and association analysis. *J. Ecol.*, 53, 411-425.

- GOLDSMITH, F. G. 1973. The vegetation of exposed sea cliffs at South Stack, Anglesey. I. The multivariate approach. *J. Ecol.*, 61, 787-818. And II. Experimental studies. *J. Ecol.*, 61, 819-829.
- HAMRICK, J. L., AND ALLARD, R. W. 1972. Microgeographical variation in allozyme frequencies in *Avena barbata*. *Proc. Nat. Acad. Sci.*, 69, 2100-2104.
- HUGHES, R. E., AND LINDLEY, D. C. 1955. Applications of biometric methods to problems of classification in ecology. *Nature*, 175, 806-807.
- JONES, D. A. 1962. D.Phil. Thesis, Oxford.
- JONES, D. A. 1966. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. I. Selection by animals. *Can. J. Genet. Cytol.*, 8, 556-567.
- JONES, D. A. 1970. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. III. Some aspects of selection. *Heredity*, 25, 633-641.
- JONES, D. A. 1973. Co-evolution and cyanogenesis. In *Taxonomy and Ecology*, ed. V. H. Heywood, pp. 213-242. Academic Press.
- JONES, D. A. 1977. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. VII. The distribution of the cyanogenic form in Western Europe. *Heredity* 39, 27-44.
- LAMBERT, J. M., AND WILLIAMS, W. T. 1962. Multivariate methods in plant ecology. IV. Nodal analysis. *J. Ecol.*, 50, 775-802.
- LONG, I. F. 1968. Instruments and techniques for measuring the microclimate of crops. In *The Measurement of Environmental Factors in Terrestrial Ecology*, ed. R. M. Wadsworth. Blackwell Scientific Publications, Oxford.
- MALAN, M. A. 1973. M.Sc. Thesis, University College of North Wales.
- MALLOCH, A. J. C. 1972. Salt-spray deposition on the maritime cliffs of the Lizard Peninsula. *J. Ecol.*, 60, 103-112.
- MILLER, J. D., GIBSON, P. B., COPE, W. A., AND KNIGHT, W. E. 1975. Herbivore feeding on cyanogenic and acyanogenic white clover seedlings. *Crop Science*, 15, 90-91.
- POTTS, D. C. 1975. Persistence and estimation of local populations of the garden snail *Helix aspersa* in unfavourable environments. *Oecologia*, 21, 313-334.
- SEANEY, R. R., AND HENSON, P. R. 1970. Birdsfoot Trefoil. *Adv. in Agron.*, 22, 120-157.
- SHEPPARD, P. M. 1951. Fluctuations in the selective value of certain phenotypes in the polymorphic land snail *Cepaea nemoralis* (L.). *Heredity*, 5, 125-134.
- DE VRIES, W. 1968. Oecologische variabiliteit van *Lotus corniculatus* L. Thesis, Instituut voor Systematische Plantkunde, Utrecht.
- WADSWORTH, R. M. 1968. *The Measurement of Environmental Factors in Terrestrial Ecology*. Blackwell Scientific Publications, Oxford.
- WHITMAN, R. J. 1973. Herbivore feeding and cyanogenesis in *Trifolium repens* L. *Heredity*, 30, 241-244.
- WILLIAMS, W. T., AND LAMBERT, J. M. 1959. Multivariate methods in plant ecology. I. Association analysis in plant communities. *J. Ecol.*, 47, 83-101.
- WILLIAMS, W. T., AND LAMBERT, J. M. 1962. Multivariate methods in plant ecology. III. Inverse association analysis. *J. Ecol.*, 49, 717-729.