Variation in the colour of the keel petals in *Lotus corniculatus* L.

5. Successional differences in the distribution of dark-keeled plants

S. G. Compton*,
S. G. Beesley† and
David A. Jones‡‡

* Department of Zoology and Entomology,
Rhodes University, Grahamstown 6140, South Africa.

† Department of Plant Biology and Genetics,
University of Hull, Hull HU6 7RX.

Using individual plants of *Lotus corniculatus* as the focus of 1 m² sampling units the plant species associated with *L. corniculatus* of known phenotype for the colour of their keel petals have been recorded in the chalk quarry at Wharram, North Yorkshire. Multivariate analyses revealed that there was a negative association between the frequency of dark-keeled plants and the percentage of bare ground within each sampling unit and almost no association with any particular plant species. Thus dark-keeled plants were commoner than the light-keeled form in the denser, more mature grassland vegetation. An increase in the frequency of dark-keeled plants in the more mature vegetation was observed between 1980 and 1984. This was not associated with any selective damage by herbivores to petals, buds, pods or leaves nor were there any differences between the morphs for infestation by seed herbivores. While there was more damage to pods in the areas with less mature vegetation there was no evidence of any differences between the morphs in the production of good, viable seed.

INTRODUCTION

In the United Kingdom *Lotus corniculatus* L. is almost exclusively polymorphic for the genetic polymorphism of dark and light-keel petals, (Jones, 1977; Jones and Crawford, 1977; Abbott, 1981; Crawford and Jones, 1986) dark keels being dominant (Ramnani and Jones, 1984). The dark-keeled plants are commoner in the north east of England and in eastern Scotland than elsewhere and there is a very marked west to east cline between latitudes 53°30'N and 54°30'N in England (Crawford and Jones, 1986).

We have no evidence that colonization by flower feeding insects is influenced by petal phenotype, nor do pollinating bees appear to discriminate consistently between different keel colours while foraging (Jones *et al.*, 1986). There is some evidence, however, that the bumble-bee *Bombus lapidarius* L. preferentially forages dark-keeled plants in cloudy conditions and that the dark-keeled plants (in one population at least) produce more viable seed overall (Jones *et al.*, 1986).

While accumulating data on the distribution of the dark-keeled morph we have obtained evidence of local small scale variation. A particularly good example is among plants growing in and around Wharram Quarry (National Grid Reference SE 860653, latitude 54°04'N, 131 m above sea level) in the Yorkshire Wolds (fig. 1). Some of the pastures to the west and south of Wharram Quarry are ancient domains associated with the deserted medieval village of Wharram Percy. In these pastures the frequency of the dark-keeled morph of *L. corniculatus* ranges from 53.9 to 82.6 per cent (Samples 1, 3, 4, 5, 7 and 11 in table 1), whereas in the quarry random samples from different parts of the quarry and on different occasions had frequencies ranging from 36.9 to 51.0 per cent (including samples 8, 9 and 10 in table 1). Two areas near the quarry have frequencies of 50.0 and 51.0 per cent (samples 2 and 6 in table 1), but these were known to have been disturbed during the past 50 years. The land to the north and west of the quarry is arable and for at least the past decade it has grown cereals.

There is *prime facie* evidence, therefore, that the dark-keeled morph is less common in the more disturbed areas in and around Wharram Quarry,

‡ Reprint requests to D. A. Jones.

Figure 1 Wharram Quarry, North Yorkshire and environs. The pies show the locations of groups of *Lotus corniculatus* sampled outside the quarry, the size of the black section indicating the proportion of dark-keeled plants in the sample. The data and map references are in Table 1

and we can ask whether there is an increase in the frequency of the dark-keeled form as the grassland matures. Clearly the selective pressures acting on plants during the establishment phase are likely to be different from those which operate during the later stages of succession. In this paper we examine whether these differences are reflected in the frequency of dark-keeled individuals in vegetation at different stages of a successional sequence.

LOCALITY, MATERIALS AND METHODS

The plants used during this work were exactly the same individuals as those tested for petal and leaf cyanogenesis (Compton *et al.*, 1986). Thus the detailed description of the habitat in Wharram

Quarry will not be repeated here. It is necessary to explain, however, that the flat, dry quarry floor is a mosaic of bare chalk, chalk rubble and cinders supporting a rich calcicolous flora. It has been deliberate management policy to scrape off the top soil layer from different parts of the quarry from time to time so that different stages in the successional sequence are always available for teaching and research purposes.

Sampling took place in the section of the quarry floor to the north and west of the main entrance, extending over an area of 100×125 m. Metal pipes were inserted into the chalk at 25 m intervals to form a grid from which random coordinates could be determined. The *L. corniculatus* individual nearest to each coordinate, together with the vegetation in the surrounding m^2 formed the basic sampling unit of which 207 (one incomplete) were collected on fourteen dates between April and September 1980. This type of sampling conforms to that recommended by Heltshe and Ritchey (1984) for detecting clumping, although we were allowing for patterns in the vegetation types rather than of patterns in the distribution of individual plants of particular species.

The following procedure was carried out with each sample:

1. From each plant three flower umbels were removed for phenotype determinations. If conflicting phenotype scores were obtained the sample was assumed to have been based on two or more individuals and was discarded.
2. Any remaining flowers and seed pods were collected for scoring of damage. Usually each flower and pod was examined individually, but with some very large plants it was necessary to extrapolate from sub-samples.
3. The area of ground covered by the plant was determined by recording the length of twenty equally spaced radii extending from its centre. A modified dart-board "spider" was used for this purpose. Surface area was calculated from the mean length of the radii.
4. Five leafy stems were removed from different parts of the plant to provide an estimate of leaf damage. The terminal bud and first four leaves (20 leaflets) were examined, with damage to each leaflet being scored as nil (0), nominal (1), less than 25 per cent removed (2), greater than 25 per cent removed (3), or totally removed (4). The values in parentheses were used to produce an overall damage score for each plant. Extensively chewed leaflets were given a weighting equal to that of totally removed leaflets because large amounts of chewing were considered to

Table 1 The frequency of the dark-keeled form of *Lotus corniculatus* in and around Wharram Quarry, North Yorkshire. All the sites are in the 10 km square SE(44)

Site	Date	Map reference	No. of plants in sample	Percentage with dark keels
1	13.6.1978	856 654	52	71.2
2	27.6.1978	857 654	50	50.0
2	14.8.1987		67	50.7
repeated				
3	2.7.1978	858 641	116	66.4
4	15.7.1979	858 651	39	53.9
5	2.7.1978	859 640	50	64.0
6	27.6.1978	859 654	101	50.5
7	12.6.1978	859 652	65	40.0
7	27.6.1978		139	41.7
repeated				
8*	13.6.1978	859 653	147	51.0
9†	12.6.1978	859 653	139	35.3
9	27.6.1978		252	36.9
repeated				
10	15.6.1979	860 645	60	66.7
11	14.8.1987	862 648	31	67.7
12	14.8.1987	862 647	96	51.0
13	13.6.1978	863 646	46	82.6

* Large plants

† Random sample of plants of all sizes

be particularly indicative of those plants which were highly palatable to herbivores.

5. Cover estimates were produced for the plant species growing in the surrounding m², together with an estimate of the extent of any bare ground. Grass species were not recorded individually, but were combined as "total Gramineae". Other reasons for treating the grasses in this way are given in Compton *et al.* (1986).

The same area was also sampled systematically by removing a single flower umbel from plants growing at least one metre apart. The characteristics of this sampling method have been discussed elsewhere (Jones, 1977). Supplementary information on the distribution of the keel-colour phenotypes was obtained from large collections in the same section of the quarry on 5 June 1984 and 29 June 1984.

Phenotype determinations were carried out shortly after collection. The colour of the keel tips was scored as light or dark, depending on the presence or absence of any red-brown pigmentation. "Pale" keels (Jones and Crawford, 1977) were included in the dark keel category. Leaves and petals were tested for cyanogenesis using the standard tests (*e.g.*, Jones, 1977; Tantisewie *et al.*, 1969).

The names of plants follow Clapham, Tutin and Warburg (1981).

RESULTS

The proportions of dark-keeled and cyanogenic individuals that formed the basis of the sampling units are given in table 2(i). As the plants were monomorphic for petal cyanogenesis this character will not be considered further. Not all plants were in flower at the time of sampling and this is reflected in the number of plants that were scored for keel petal colour. The combination of phenotypes for keel colour and leaf cyanogenesis are given in table 2(ii). There is no indication of any relationship between the expression of keel colour and cyanogenesis.

An ordination of the vegetation in the 1 m² quadrats was carried out using the correspondence analysis (reciprocal averaging) programme in the GENSTAT Macro library (Alvey *et al.*, 1982). This technique provides a means of plotting both plant species and individual quadrats in the same co-ordinate system. The uses of correspondence analysis have been reviewed by Gauch (1982) who concluded that for most analyses of community data sets this method has advantages over principal components analysis. Bare ground 'cover' and total Gramineae were included as variables in the ordination, but not the rarer plant species, nor *Ononis repens*, which proved to be an extreme outlier when

Table 2 Phenotype frequencies in the randomly selected *L. corniculatus* plants at Wharram Quarry 1980
(i) Phenotype frequencies

	Phenotypes		Totals
	cyanogenic	acyanogenic	
1. Leaf cyanogenesis	165	41	206
2. Petal cyanogenesis	136	0	136
3. Keel colour	Dark 52	Light 83	135

(ii) Combinations of phenotypes

		Leaf		Totals
		cyano-genic	acyano-genic	
Keel Colour	Dark	44	8	52
	Light	67	16	83
		111	24	135

$$\chi^2_{(1)} = 0.33, P > 0.05.$$

included in preliminary analyses. Eigen values for the first three axes were 0.3812, 0.1746 and 0.1174 respectively. Figs 2-3 illustrate the distribution of the plant species and the sample quadrats in the first two ordination axes. The quadrats and plant species are displayed separately in order to improve the clarity of the presentation. The amount of bare ground present appears to be the most indicative of the variables contributing to the separation of the quadrats in the first axis (fig. 4), with *Thymus praecox* (and correlated species) equally indicative in the second (see Compton *et al.*, 1986).

Comparison of the distribution of dark and light-keel centred quadrats along the first two ordination axes suggests that their relative proportions vary in different sections of the orientation space (fig. 3). Although light-keel centred quadrats are less frequent at the lowest values on the first axis, in the section where bare ground was rare or absent (fig. 4), this is not statistically significant (table 3).

The vegetation in the 1 m² quadrats was separated into discrete groupings using a cluster analysis programme (CLUSTAN, Wishart, 1975—Ward's method) on the same data set. Two primary groups were distinguished (designated A and B, with 142 and 65 quadrats respectively) and one major subdivision (A1 and A2, with 20 and 122 quadrats). Most of the quadrats that comprise vegetation group B are concentrated in the section of the ordination space where bare ground was rare or absent. The proportions of dark-keeled petals in the three vegetation groups were 15.4 per cent (A1), 39.8 per cent (A2) and 43.6 per cent (B). Analysis based on the original numbers again shows no significant variation between groups ($\chi^2_{[2]} = 3.41$, $P > 0.05$) although dark-keeled plants are commoner in the type B vegetation.

Fig. 5 illustrates the distribution of the vegetation groups on the floor of Wharram Quarry. Vegetation of type B was recorded mainly in three distinct "islands" (one of which is bisected by a path) and around the periphery of the quarry. These three areas (separated by diagrammatic boundary lines in fig. 5) are clearly recognisable in the field, being composed of a tall grass sward which is often abruptly demarcated from the surrounding more open sections which comprise vegetation group A. Most examples of type A1 vegetation were widely scattered (fig. 5) and the subdivisions of the type A vegetation are not readily distinguishable in the field. Stepwise discriminant function analysis (Mahal method) was used to determine the best combinations of factors for distinguishing the vegetation groups defined by the cluster analysis (cf. Mathews, 1979). Grass

Figure 2 Ordination of plant species in 1m² quadrats surrounding *L. corniculatus* plants at Wharram Quarry on RA axes 1 and 2.

Abbreviations

AA	<i>Anagallis arvensis</i>
AM	<i>Achillea millefolium</i>
BG	Bare ground
BP	<i>Bellis perennis</i>
CA	<i>Cirsium arvense</i>
CB	<i>Cerastium arvense</i>
CC	<i>Crepis capillaris</i>
CF	<i>Carex flacca</i>
CL	<i>Galium cruciata</i>
CM	<i>Crataegus monogyna</i>
CN	<i>Centaurea nigra</i>
CR	<i>Campanula rotundifolia</i>
CU	<i>Carlina vulgaris</i>
CV	<i>Cirsium vulgare</i>
EA	<i>Chamerion angustifolium</i>
EO	<i>Euphrasia officinalis</i>
GA	<i>Gentianella amarella</i>
GR	Total Gramineae
HP	<i>Hieracium pilosella</i>
HR	<i>Hypochaeris radicata</i>
HS	<i>Heracleum sphondylium</i>
LC	<i>Linum catharticum</i>
LH	<i>Leontodon hispidus</i>
LO	<i>Lotus corniculatus</i>
LP	<i>Lathyrus pratensis</i>
ML	<i>Medicago lupulina</i>
OA	<i>Ophrys apifera</i>
PE	<i>Picris echinoides</i>
PV	<i>Prunella vulgaris</i>
RS	<i>Rosa</i> sp.
RR	<i>Ranunculus repens</i>
SJ	<i>Senecio jacobaea</i>
TD	<i>Thymus praecox</i>
TF	<i>Tussilago farfara</i>
TP	<i>Trifolium pratense</i>
TR	<i>Trifolium repens</i>
VC	<i>Veronica chamaedrys</i>
VS	<i>Vicia sepium</i>

Figure 3 Ordination of 1 m² sample quadrats surrounding dark-keeled (D), light-keeled (L) and non-flowering plants (·) at Wharram Quarry.

Figure 4 Ordination of 1 m² sample quadrats displaying the area of bare ground in each quadrat. 0 = <1 per cent bare ground, 1 = 1–10 per cent, 2 = 11–25 per cent, 3 = 26–50 per cent, 4 = 51–75 per cent and 5 = >75 per cent.

Table 3 The distribution of dark and light keeled individuals among randomly selected *L. Corniculatus* at Wharram Quarry in relation to the area of bare ground in the surrounding 1 m² (1980 data)

Phenotype	Number of plants in 1 m ² quadrats	
	10 per cent or less bare ground	Greater than 10 per cent bare ground
Dark	15	37
Light	17	66

$$\chi^2_{(1)} = 1.24, P > 0.05.$$

cover was found to be the major factor separating group A and B, with *Crepis capillaris* and *Ranunculus repens* important in distinguishing A1 and A2. (Appendix 1).

Keel-colour frequencies were re-scored in 1984. The sampling was based on the two major vegetation types and because of the difficulty of distinguishing the A1 and A2 types reliably in the field this subdivision was not made. Phenotype frequencies were found to vary between the two major vegetation types, with plants that had dark-keeled flowers occurring more frequently in the grassy type B areas (table 4(i)). An increased proportion of dark-keeled plants was evident in all three "islands" of type B vegetation, which did not differ significantly in their keel colour frequencies (table 4(ii)). The 206 plants sampled in 1980 also showed a high proportion of dark-keeled plants in type B vegetation, but it was only when the two large samples collected independently in 1984 were combined that the 5 per cent level of significance was reached in the χ^2 analysis. Notice, also, that plants of both phenotypes flower later in the type B vegetation ($\chi^2_{(1)} = 168.1, P \ll 0.001$). Of particular interest is the significant change in frequency of the dark keeled form in type B vegetation overall from 43.6 per cent in 1980 to 56.0 per cent in 1984 (table 4(iii)). The three sub-samples of type B vegetation taken in 1984 show that all three are homogeneous in this change in frequency (table 4(ii)).

Table 5 summarises comparisons of the size and reproductive output of the *L. corniculatus* individuals in relation to their background vegetation and phenotypes. The plants growing in the more open (type A) plant assemblages were generally smaller than those in the closed sward (type B) and, in relation to their size, produced more flowers (table 5(i)). Despite the differences in their surroundings the density of the plants (measured as dry weight/ground area covered) was not different in the two areas (Mann-Whitney U =

Figure 5 The distribution of the three vegetation types in the study area at Wharram Quarry. (Vegetation type A1 = 1, A2 = 2, B = 3).

4274.9, $n_1 = 141$, $n_2 = 65$, $P > 0.05$). Flower production by plants growing in the extremely open type A1 areas was not greater than that recorded from the rest of the type A assemblage (table 5(ii)). No differences in the size of plants of different phenotype are evident, but light-keeled plants had marginally more flowers than dark-keeled ones (table 5(iii)).

Comparisons of leaf, terminal bud, flower and seed pod damage scores are given in tables 6–8. Leaf damage scores based on leaf chewing did not differ between assemblages A and B, nor between plants with different keel colour phenotypes (table 6). Terminal bud damage did not vary significantly between phenotypes or assemblages (table 7). Damage to the seed pods was infrequent, particularly in the type B vegetation areas and no between-phenotype differences in seed pod damage were detected (Table 8).

DISCUSSION

Lotus corniculatus has been used as a model species for several ecological studies. Usher (1975) chose the plant from among those studied by Burden (1970) in Wharram Quarry because “it appeared to be one of the first colonizers of bare chalk, and nitrogen fixation in its root nodules, and hence a local nitrogen availability, may act as a stimulus

for other plant species to become established”. Reader and Southwood (1981) surprisingly did not regard the plant as an example of an early successional species, rather including it in their mid successional assemblage.

Davis *et al.* (1985) studied the effect on the development of the vegetation of introducing *L. corniculatus* (and *Brachypodium sylvaticum*) into a sparsely vegetated area (<10 per cent) of a limestone quarry. The species was chosen, again, because it is one of the most widespread, early and persistent herb colonists of chalk and limestone quarries throughout England (Davis, 1983) and because of the long term beneficial effects of the legumes on soil nitrogen. In none of these studies has the known genetical variation in *L. corniculatus* been taken into account, even when it was directly relevant. Reader and Southwood (1981), for example, ignored the polymorphism for cyanogenesis when they were assessing the palatability of the plant to herbivores when it was already known that one of the animals they used, *Agriolimax (Deroceras) reticulatus*, was a selective herbivore of acyanogenic *L. corniculatus* (Jones, 1962a, b, 1966). Furthermore, it was not part of any of these studies to observe any interactions between the variation in *L. corniculatus* and other vegetation in the habitat.

Starting with studies in the Netherlands (Ellis, Keymer and Jones, 1976) we have developed a

Table 4 The frequency of keel colour phenotypes of *L. corniculatus* in vegetation zones on the floor of Wharram Quarry, June 1984

(i) Vegetation groups A and B

Sampling dates	Group A		Group B		$\chi^2_{[1]}$	<i>P</i>
	Light	Dark	Light	Dark		
5.6.84	119	112	28	44	3.50	>0.05
29.6.84	74	64	154	188	2.91	>0.05
Totals	193	176	182	232	5.44	<0.05

Heterogeneity $\chi^2_{[1]} = 0.97$, $P > 0.05$.

(ii) "Islands" of group B vegetation

Sampling dates	"Island" B1		"Island" B2		"Island" B3		$\chi^2_{[2]}$	<i>P</i>
	Light	Dark	Light	Dark	Light	Dark		
5.6.84	10	14	8	5	10	25	4.45	>0.05
29.6.84	46	54	53	61	55	73	0.36	>0.05
Totals	56	68	61	66	65	98	2.03	>0.05

Heterogeneity $\chi^2_{[1]} = 2.78$, $P > 0.05$.

(iii) The numbers of plants of each phenotype in type B vegetation in 1980 and 1984

Sampling dates	Light	Dark	$\chi^2_{[1]}$	<i>P</i>
1980	32	23	3.96	>0.05
1984	182	232		

novel and unique approach to predictive ecology based on the known variation within a plant species, the most successful study being on Holy Island, Anglesey (Ellis, Keymer and Jones, 1977; Compton, Beesley and Jones, 1983). Here we have shown, beyond reasonable doubt, that selective grazing by molluscs is the predominant agent determining the distribution of cyanogenic *L. corniculatus* on the west coast of Holy Island. At Wharram Quarry we have used individual *L. corniculatus* plants as the focus of sampling the surrounding vegetation both for plant and animal species. It was only after the data were collected for each quadrat sampled that the focal plant was tested for leaf and petal cyanogenesis (Compton, Beesley and Jones, 1986). That study showed a clear association between a more mature stage in the succession in the quarry and the occurrence of leaf cyanogenic plants although it was not possible to determine, at that time, why this should be. The most likely explanation is that under certain conditions leaf cyanogenic *L. corniculatus* has a faster rate of root growth than the acyanogenic form (Keymer and Ellis, 1978). This might provide an important advantage when root competition is intense, and so allow cyanogenic plants to persist for longer periods under these conditions.

At the same time that these *L. corniculatus* plants at Wharram Quarry were tested for cyanogenesis they were also scored for the colour of the keel petals and this means that exactly the same data set was available for two analyses, one based on cyanogenic/acyanogenic plants and the other on dark/light-keeled plants.

The A1, A2 and B vegetation assemblages at Wharram represent stages in a successional sequence which broadly correspond to groups A, B and C in Usher's (1979) description of succession at the quarry. They can be described as pioneer, chalk grassland and meadow communities. *Arrhenatherum elatius*, a tall grass which is common at Wharram, was noticeably rare in the quadrats centred on *L. corniculatus*, suggesting that the presence of this species may be indicative of the stage in the succession when *L. corniculatus* is eliminated.

The data collected in 1984 show a significant excess of dark-keeled plants in the more mature parts of Wharram Quarry (table 4(i)). This distribution of the keel colour phenotypes shows an interesting parallel with that for leaf cyanogenesis in the quarry (Compton *et al.*, 1986), cyanogenic plants also occurring more frequently in the areas of dense grassland. The expression of the keel

Table 5 Mann-Whitney U test analysis of the reproductive effort, size and surroundings of *L. corniculatus* at Wharram Quarry (1980)

(i) Comparisons between vegetation groups A and B

	Group A		Group B		U	P
	n	Mean rank	n	Mean rank		
Bare Ground in m ²	142	129.88	65	47.45	939.5	<0.001
Grass cover in m ²	142	80.04	65	156.35	1212.5	<0.001
Area of central <i>L. corniculatus</i>	142	92.32	65	129.52	2956.5	<0.001
Flowers present	142	109.37	65	92.27	3852.5	>0.05
Flowers/unit area	142	112.71	65	84.96	3377.5	<0.01
Flowers/unit mass*	118	85.62	42	66.12	1874.0	<0.05

(ii) Comparisons between vegetation groups A1 and A2

	Group A1		Group A2		U	P
	n	Mean rank	n	Mean rank		
Bare Ground in m ²	20	102.47	122	66.42	600.5	<0.001
Grass cover in m ²	20	56.15	122	74.02	913.0	>0.05
Area of central <i>L. corniculatus</i>	20	72.87	122	71.27	1192.5	>0.05
Flowers present	20	65.17	122	72.54	1093.5	>0.05
Flowers/unit area	20	65.08	122	72.55	1091.5	>0.05
Flowers/unit mass*	13	52.38	90	51.94	580.0	>0.05

(iii) Comparisons between keel colour phenotypes

	Light		Dark		U	P
	n	Mean rank	n	Mean rank		
Bare Ground in m ²	82	70.37	52	64.22	1961.5	>0.05
Grass cover in m ²	83	63.16	52	75.72	1756.5	>0.05
Area of central <i>L. corniculatus</i>	83	71.42	52	62.55	1874.5	>0.05
Flowers/unit area	83	72.09	52	61.47	1818.5	>0.05
Flowers/unit mass*	62	51.87	40	50.92	1217.0	>0.05

* Includes only plants with a diameter of less than 25 cm.

colour and cyanogenic phenotypes does not appear to result from genetic linkage, suggesting that separate factors are acting to influence their distribution. In the case of keel colour we have no direct information about which factors may be involved, but at Wharram Quarry *L. corniculatus* plants growing in the grassy areas commence

flowering somewhat later than those growing in the more open areas.

It was noticeable that the plants growing among the more mature vegetation were larger than those in the more open sward, but they produced fewer flowers per unit area. Although it was possible to distinguish subdivisions of the more open sward during the analysis, it was not easy to pick these out on the ground. Bare ground was the major discriminating feature, but it was difficult to make subjective judgements in the field in 1984 and so the distinction was not made. The problem was made more difficult by the fact that the vegetation was four years more mature and there was less bare ground than previously. The distinction between the A type and B type vegetation was clear, however, and there was no doubt that dark-keeled plants were commoner in the type B than in the type A vegetation.

Table 6 Analysis of variance of the leaflet damage sustained by *L. corniculatus* at Wharram Quarry. Angular transformed damage scores are based on samples of 100 leaflets (5 stems, 4 nodes 5 leaflets per node) from each plant. Maximum damage score attainable was 400 (1980 data)

Keel colour phenotypes	df	ms	P
Between groups	1	15.285	>0.05
Within groups	133	31.270	
Total	134		

Mean damage scores: light keel, 87.99, dark keel, 90.76.

Table 7 The levels of terminal bud damage sustained by *L. corniculatus* at Wharram Quarry. Five terminal buds were examined on each plant (1980 data)

Keel colour phenotypes							
Phenotype	No. of damaged buds recorded per plant						$\chi^2_{[5]}$
	0	1	2	3	4	5	
Light	18	16	30	13	3	3	3.95
Dark	7	12	16	12	4	1	

Mean damaged buds per sample: light keel, 1.71, dark keel, 1.94.

As the vegetation in the quarry has been allowed to develop naturally it is most likely that the *L. corniculatus* plants in the quarry were derived, ultimately, from plants that grew in the surrounding pasture. In these old pastures the frequency of dark-keeled plants is consistently higher than in the pioneer vegetation in the quarry. If we assume that the higher frequencies in the old pastures are near to equilibrium, which is

not unreasonable as the area is at the high frequency end of the cline across northern England (Crawford and Jones, 1988), we have to conclude that light keeled plants have an advantage in the pioneer population in the quarry.

There is independent evidence from two sources that the frequency of the dark-keeled form increases as the vegetation matures. Table 4(i) shows that the frequency of the dark-keeled form is significantly higher in the type B vegetation than in the type A while a significant increase in the frequency of the dark-keeled form in type B vegetation over the four years 1980–84 is shown in table 4(iii).

As yet we have no direct evidence to explain why this has occurred. Jones *et al.* (1986) found no significant differences in the production of healthy seed between a premarked sample of light- and dark-keeled plants at Wharram Quarry in 1982. Furthermore there was no difference between the morphs in infestation by seed herbivores. In July 1986, however, there was a significant excess of Thysanoptera adults in dark as opposed to light

Table 8 The levels of petal and seed pod damage sustained by *L. corniculatus* at Wharram Quarry in 1980

(i) Flower petal damage
(a) Wharram vegetation groupings

Group	Damaged flowers	Undamaged flowers	$\chi^2_{[1]}$	P	Per cent damaged
A	314	1458	0.13	>0.05	17.72
B	97	472			17.05

(b) Keel colour phenotypes

Phenotype	Damaged flowers	Undamaged flowers	$\chi^2_{[1]}$	P	Per cent damaged
Light	268	1366	2.52	>0.05	16.40
Dark	115	82			19.27

(ii) Seed pod damage
(a) Wharram vegetation groupings

Group	Damaged pods	Undamaged pods	$\chi^2_{[1]}$	P	Per cent damaged
A	44	846	9.13	<0.01	4.94
B	4	332			1.19

(b) Keel colour phenotypes

Phenotype	Damaged pods	Undamaged pods	$\chi^2_{[1]}$	P	Per cent % damaged
Light	33	633	0.63	>0.05	4.95
Dark	8	211			3.65

keeled flowers, but as this was the only significant χ^2 value in 13 similar tests the effect may well be the result of chance. Tables 6, 7 and 8 in this paper show an absence of any selective leaf, bud, petal and pod damage by herbivores although a higher proportion of the pods in type A vegetation were damaged than in type B. Because all the plants tested had cyanogenic petals the data in tables 7 and 8(i) are not complicated by cyanogenesis. The plants were polymorphic for cyanogenesis in their leaves and thus there was confounding with petal colour in table 6. As it happens there is no significant difference between the two petal-colour types and so cyanogenesis is not a complication in this particular set of data.

We have observed a change in the frequency of the dark-keeled form in the type B vegetation between 1980 and 1984 and the situation is being studied further. The management policy in Wharram Quarry is to clear to bare chalk parts of the quarry floor on a regular and planned basis and this means that there are regions of known stages in a successional sequence. In the long-term, therefore, it should be possible to monitor the changes in the frequency of the dark-keeled form in the quarry both over time and space and to study the effects of severe droughting as occurred, for example, in 1975 and 1976.

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APPENDIX 1

Discriminant function analysis separating the vegetation groupings obtained by cluster analysis of cover values in 1 m² quadrats at Wharram Quarry

1. Vegetation groups A and B

Step	Variable entered	Standardised discriminant function coefficient
1	Total <i>Gramineae</i>	-0.476
2	<i>Centaurea nigra</i>	-0.236
3	<i>Hieracium pilosella</i>	0.287
4	<i>Leontodon hispidus</i>	0.414
5	<i>Thymus praecox</i>	0.281
6	'Bare Ground'	0.227
7	<i>Rosa</i> sp.	-0.263
8	<i>Picris echioides</i>	-0.250
9	<i>Veronica chamaedrys</i>	-0.159
10	<i>Euphrasia officinalis</i>	-0.208
11	<i>Hypochaeris radicata</i>	0.146
12	<i>Achillea millefolium</i>	-0.168
13	<i>Heracleum sphondylium</i>	-0.184
14	<i>Vicia sepium</i>	-0.165
15	<i>Prunella vulgaris</i>	0.152
16	<i>Ophrys apifera</i>	0.162
17	<i>Senecio jacobaea</i>	0.124
18	<i>Ranunculus repens</i>	0.143
19	<i>Bellis perennis</i>	0.142
20	<i>Cerastium arvense</i>	0.118
21	<i>Tussilago farfara</i>	0.103

Discriminant function group mean for assemblage A = 0.955.
Discriminant function group mean for assemblage B = -2.09.
93.2 per cent of the quadrats were "correctly" classified by the function.

2. Vegetation groups A1 and A2

Step	Variable entered	Standardised discriminant function coefficient
1	<i>Crepis capillaris</i>	1.018
2	<i>Ranunculus repens</i>	1.193
3	<i>Cirsium arvense</i>	0.923
4	<i>Anagallis arvensis</i>	0.727
5	<i>Senecio jacobaea</i>	0.656
6	<i>Tussilago farfara</i>	0.462
7	<i>Chamerion angustifolium</i>	0.393
8	<i>Rosa</i> sp.	-0.284
9	'Bare Ground'	-0.246
10	<i>Leontodon hispidus</i>	-0.173
11	<i>Linum catharticum</i>	-0.151
12	<i>Campanula rotundifolia</i>	-0.110

Discriminant function group mean for assemblage A1 = 6.341.
Discriminant function group mean for assemblage A2 = 1.040.
100 per cent of the quadrats were "correctly" classified by the function.