Variation in the colour of the keel petals in *Lotus corniculatus* L. 3. Pollination, herbivory and seed production

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The response of pollinators to the pseudo-cryptic polymorphism of dark/light keel colour in Lotus corniculatus has been studied experimentally. Some evidence was obtained that the bumble-bees Bombus lapidarius and B. terrestris forage more dark-keeled flowers in dull and overcast conditions than in bright sunlight.

The distribution of herbivorous insects within the flowers of L. corniculatus suggests that they do not distinguish between the keel colour phenotypes.

The entire seed production of pre-marked plants growing at two sites was recovered. Analysis of the data obtained from one site showed that the phenotypes did not differ significantly in any of the following characteristics: pods produced, seeds produced, seeds per pod, seed herbivores, seed herbivores per pod, damaged seeds, and germination patterns. At the second site no germination trials were effected, but even more closely similar values for seed production and overall seed herbivory of the two keel colour phenotypes were obtained.

At the latter site the herbivorous wasp *Eurytoma platyptera* was commoner in the pods of light-keeled plants and light-keeled plants produced more seeds than the dark-keeled plants later in the season even though light-keeled plants tend to flower earlier.

INTRODUCTION

Most European populations of the legume Lotus corniculatus L. are polymorphic for the character of keel petal colour, with some individuals producing flowers that have entirely yellow ("light") keel petals and the remainder having red-brown ("dark") keel tips. The inheritance of this character is tetrasomic, with dark keels dominant (Hart and Wilsie, 1959). This has been confirmed in British material (Ramnani and Jones, 1984b). Many L. corniculatus populations are also polymorphic for the presence or absence of cyanogenesis by the leaves and/or petals, but there is no evidence of a relationship between the expression of the cyanogenic and keel colour phenotypes in natural populations (Jones and Crawford, 1977; Compton et al., 1983a, b).

Dark-keeled plants are more numerous in the north-east than elsewhere in Britain (Jones and

Crawford, 1977; Abbott, 1981; Crawford and Jones, 1986). A steep cline in keel colour frequencies has also been recorded in the Jostedal Valley (Norway), where dark-keeled plants are found to be less numerous in the peri-glacial northern populations (Compton et al., 1983a). Despite being genetically dominant, the dark-keel phenotype only occasionally forms a large majority in natural populations, and in the coastal regions of mainland Europe it is light-keeled individuals which are most frequently encountered (Jones and Crawford, 1977). Inland the dark-keel phenotype is commoner and a clear association between the frequency of the dark-keeled form and altitude has been described for the Auvergne, the Pyrenees and the Haute-Savoie in France and also in Austria (Baker and Jones, 1986). This implies that in many areas dark-keeled plants are at some disadvantage relative to their light-keeled neighbours.

The relative fitness of the phenotypes has been examined by Jones and Crawford (1977), who failed to detect any differences in winter survival or in seed production under experimental conditions. These studies did suggest that dark-keeled plants may begin flowering later than the lightkeeled plants and this is examined further in this report. The main purpose, however, is to describe the behaviour of pollinator bees in experimental plots containing mixtures of the two keel colours, and to compare seed production and herbivore loads of plants growing in natural conditions.

METHODS AND RESULTS

Localities

Natural populations of *L. corniculatus* were sampled at the following U.K. locations: Eppleworth Quarry, E. Yorks (TA 023327); Wharram Quarry, N. Yorks (SE 859653); Cottingham/Walkington, E. Yorks (TA 020257) and Cauldon Lowe, Staffs (SK 083486). Pollination studies were carried out using experimental populations established at the Hull University Botanic Gardens, Cottingham.

Sequential sampling of keel colour frequencies

L. corniculatus populations were monitored at intervals during the flowering season in order to determine whether the peak flowering periods of the two keel colour phenotypes are different. Sections of the populations at Eppleworth and Wharram were sampled systematically by removing a single flower umbel from plants growing at least one metre apart. L. corniculatus clones readily and this sampling method aims to reduce the probability of repeat sampling of the same individual (Jones, 1977).

The variation recorded for keel colour frequencies is given in table 1. In each sequence the first sample collected contained a lower proportion of the dark-keeled morph than in subsequent samples although correlation analysis on the 1978 and 1981 sets of data failed to show an association between the proportion of dark-keeled plants flowering and the sampling date. These data indicate that sequential sampling should begin much earlier in the flowering season. Data collected recently at other sites at approximately the same latitude show clearly the earlier flowering of the light-keeled plants (Crawford and Jones, in preparation).

- **Table 1** Number of plants with dark and light keel petals observed during sequential sampling of *L. corniculatus* populations. Plants were sampled at 1 m plus intervals
- (a) Eppleworth Quarry, 1978

	Keel colour		
Date	Light	Dark	– % Dark
20.7.78	30	15	33.3
27.7.78	12	14	53.8
15.8.78	18	19	51.4
30.8.78	15	16	51.6
4.9.78	9	9	50.0
19.9.78	6	11	64.7

 $r_{[4]}$ arc sine transformed per cent dark keels with date = 0.740, P > 0.05.

(b) Wharram Quarry, 1974 and 1981

	Keel colou		
Date	Light	Dark	% Dark
12.6.74	65	35	35.0
6.8.74	106	94	47.0
13.8.74	136	100	44.7
9.6.81	89	57	39.0
25.7.81	70	66	48.5
11.8.81	103	107	51.0
18.8.81	144	136	48.6
28.8.81	99	70	41.4

 $r_{[3]}$ arc sine transformed per cent dark keels with date (1981 data) = 0.501, P > 0.05.

Pollinator responses to keel colour polymorphism

Cultivars of L. corniculatus display varying degrees of self-incompatibility and insects are required for successful pollination to take place (Tome and Johnson, 1945; Giles, 1949; Seaney, 1964). Furthermore, Morse (1958) observed that between 12 and 25 visits per flower are required for maximum seed set. With British material collected in the wild all seven plants we have tested so far are self-sterile (Ramnani and Jones, 1984a, b). The UV reflectance spectrum is such that bees certainly should be able to distinguish between the two types of keel. With dark keels we have found that the reflectance is uniformly low between 222 and 695 nm, a sharp increase occurring at the longer wavelength. Thus to a bee dark keels should appear uniformly black (or grey). Light keels, and also wing and standard petals, show a marked increase in reflectance at 490 nm, reaching a maximum at 625. There is a subsequent minimum at 695 nm, although this is not as low as with dark keels. The pattern for longer wavelengths is essentially the same as for dark keels. Light keels should, therefore, be "beeyellow" (Daumer, 1956). The difference between the dark and light keels appears to be based on different concentrations of anthocyanin rather than on any qualitative differences (J. B. Harborne, personal communication, and P. Kakes, personal communication), but more work needs to be done on the chemistry of the pigments involved.

There appears to be no definitive list of natural pollinators of *L. corniculatus* in the U.K., but there is positive evidence that honeybees (McLellan, 1976), *Bombus monticola* Smith, syn. *B. lapponicus* (F.) (Yalden, 1982) and *B. lucorum* (L.) (Yalden, 1983) collect pollen from the plant. Whether small insects are effective pollinators remains to be determined, but Compton (1983) has found that the flowers often contain *Meligethes erythropus* (Marsham) and *Odontothrips loti* (Haliday), for example, and as these insects move from flower to flower seeking pollen we are not prepared to exclude them from a list of possible pollinators.

Two experimental plots were established 3 m apart, each containing mature plants grown from the progeny of a single cross (self-sterile clones A13 and C27). One plot consisted of 71 plants (35) light keel, 36 dark) arranged at random in a 12×6 matrix, 0.5 m between plants. The other plot had the same orientation and consisted of 54 plants (27 light keel, 27 dark) arranged systematically in a 9×6 matrix. Each plant (except those at the edges) was surrounded by four light and four dark keeled plants, with two plants of each phenotype forming the "diagonal" neighbours. Monitoring of bees entering the plots was carried out during July 1980. Bees were not recognisable individually and some may have had several of their visits to the plots recorded. The plants were in flower throughout the observation period, but it was not possible to count the number of flowers available to the pollinators at any given time.

The flight of individual bees was recorded on an audio-taperecorder and the information was later transcribed to data sheets. By this means it was possible to follow the travels of individual bees without difficulty. For example, one bee foraged the plants in the random plot for 37 minutes, visited 50 of the 71 plants (27 dark, 23 light) and foraged in 262 dark and 117 light flowers. The position of a plant was identified by its grid reference and the number of flowers visited on that plant was recorded. The data for the two plots were collected separately. On many days two people watched the plots, one scoring activity in the systematic plot and the other recording from the random plot. Scorers were assigned to the plots at random. Two types of *visiting* were recorded. Sometimes a bee would inspect a flower but not enter it. This has been scored as *inspecting*. A visit, involving pollen and/or nectar gathering was recorded as *foraging*.

When a bee left a plant and visited a flower on a different plant the movement was counted as a single *pollinator flight* (*e.g.*, as in table 2).

Two species of bees regularly foraged both areas (*Bombus lapidarius* L. and *Bombus terrestris* L.), while a third species, the solitary *Andrena* sp., was only numerous on the random plot. Comparisons of the numbers of flights made between plants indicate that none of the species showed a preference for flying to plants of the same phenotype as the one from which they had left, nor to plants of the alternative phenotype (tables 2 and 3).

In the random plot the bee species were not homogeneous in their foraging behaviour (table 4). As there were 35 light and 36 dark-keeled plants in the plot we have a basis for testing the preference of the bees. Clearly *Bombus terrestris* and the *Andrena* sp. show no preference, whereas *B. lapidarius* does show a preference for dark-keeled plants ($\chi^2_{11} = 9.61, 0.01 > P > 0.001$). A similar test of significance cannot be used on the number of individual flowers foraged because the total number of flowers of each phenotype was not known, but the pattern is the same as for the foraging of plants.

This pattern was not repeated in the systematic plot, where both *Bombus* species foraged similar numbers of light and dark-keeled flowers (table 4b).

The prevailing weather conditions were noted at the beginning of each recording sequence so allowing the flights to be grouped as occurring in either "sunny" or "overcast" conditions. Flights during periods of intermediate or variable weather conditions have been excluded from the analysis. During overcast conditions *B. lapidarius* made a higher proportion of foraging flights to dark-keeled plants than it did in sunny conditions (random plot only), $\chi^2_{[1]} = 10.55$, P < 0.001 (based on 35 light and 36 dark-keeled plants), and foraged more dark-keeled flowers (table 5a). Neither of the other species gave any indication that their flight activity in the random plot was being influenced by the weather.

The behaviour of the two *Bombus* species in the systematic plot shows that while they were not foraging on more dark-keeled plants during overcast conditions they were foraging more flowers **Bombus** lapidarius

		Random plot				Systematic plot				
	L ¹		D^1			L		D^1		
Bee	L ²	D ²	L ²	D ²	Bee	L ²	D^2	L ²	D^2	
1	1	8	8	8	18	10	6	6	8	
2	13	24	26	27	19	10	10	11	10	
3	1	2	2	3	20	9	6	5	7	
4	4	1	2	5	21	7	10	10	10	
5	8	10	11	20	22	2	3	3	4	
6	7	7	7	9	23	1	2	2	4	
7	7	8	7	3	24	1	1	2	2	
8	8	6	7	13	25	6	7	6	6	
9	0	4	5	2	26	7	8	8	3	
10	2	4	4	3	27	20	13	13	19	
11	3	6	6	11	28	3	2	3	2	
12	3	4	5	5	29	2	2	3	3	
13	16	13	12	11	30	9	6	6	9	
14	7	4	3	7	31	11	10	10	17	
15	4	7	8	11	32	2	8	8	3	
16	5	3	4	5	33	1	1	1	1	
17	11	10	10	6	34	10	14	13	7	
					35	15	13	13	7	
	100	121	120	149		126	122	123	122	

 Table 2(a)
 The frequency of pollinator flights between plants of L. corniculatus for 35 bees

Random plot: $\chi^2_{[11]}$ for total values = 0.07, P > 0.05; Heterogeneity $\chi^2_{[16]} = 21.55$, P > 0.05. Systematic plot: $\chi^2_{[12]} = 0.18$, P > 0.05; Heterogeneity $\chi^2_{[17]} = 17.63$, P > 0.05. L¹ = Light keeled plant from which flight began. D¹ = Dark keeled plant from which flight began. L² = Light keeled plant on which flight ended. D² = Dark keeled plant on which flight ended.

on each dark-keeled plant under these conditions (table 5b).

Flower production

The numbers of flowers present on the umbels of light and dark-keeled plants growing at Eppleworth Quarry were compared. Collecting by eye at 1 m plus intervals is not an appropriate sampling method in this case because umbels with large numbers of flowers are liable to be chosen preferentially. As an alternative, small quadrats were placed at random over *L. corniculatus* plants and all the flower umbels inside were removed and scored. The results (table 6) suggest that the light and dark-keeled plants produce umbels which bear similar numbers of flowers.

Colonisation of flowers by phytophagous insects

L. corniculatus flowers support a well developed community of phytophagous insects, some of which can cause considerable damage to the petals and immature seeds (Compton, 1983). The distribution of these insects was investigated to determine whether they showed a preference for one or other of the keel colour phenotypes. Flower umbels were sampled systematically at intervals of at least one metre, then stored individually prior to freezing and subsequent dissection.

Three species of beetles (Coleoptera) regularly feed in the flowers of L. corniculatus in northern England. Apion loti Kirby larvae develop inside the seed pods of the plants, where they feed on the ovules and immature seeds. Hypera plantaginis (DeGeer) larvae feed on the outside of the pod, devouring seeds, pods and petals. The third species, *Meligethes erythropus* (Marsham) appears to be less destructive as both the larvae and adults are pollen feeders. Several species of thrips (Thysanoptera) also occur in the flowers, of which the commonest are Odontothrips loti (Haliday) and Aeolothrips tenaicarnis Bagnall. Both species feed on the surface tissues of the immature seed pod, but the latter may also be a facultative predator of small arthropods and other thrips (Ward, 1973). The aphid Acyrthosiphon loti (Theobald) is less frequently encountered. Its feeding habits were not observed.

Table 2(b)	Comparisons	of the f	requency	of p	pollinator	flights	between	plants o	of <i>L</i> .	corniculatus	for 24	Bombus	terrestris	and	14
Andrena	a sp.					-									

Bombus terrestris

			Random pl	ot		Systematic plot				
	L1		D ¹			L'	L ¹		1	
Bee	L ²	D^2	L ²	D^2	Bee	L ²	D^2	L ²	D^2	
1	2	4	4	2	12	6		10	11	
2	3	4	5	0	13	8	11	10	11	
3	4	4	4	4	14	5	3	3	4	
4	3	1	1	2	15	9	7	8	8	
5	9	12	13	16	16	2	1	1	3	
6	4	11	11	10	17	7	7	6	6	
7	23	14	14	8	18	7	13	12	10	
8	4	1	2	2	19	3	4	4	3	
9	18	11	12	9	20	1	3	2	4	
10	0	4	3	2	21	11	8	8	10	
Í1	2	3	3	4	22	7	10	9	9	
					23	13	12	11	9	
					24	1	2	1	5	
	—	—	_			_	_		—	
	72	69	72	59		80	92	85	93	

 $\chi^2_{[1]}$ for total values = 0.41, P > 0.05; Heterogeneity $\chi^2_{[10]} = 13.46$, P > 0.05. $\chi^2_{[1]} = 0.05$; P > 0.05. Heterogeneity $\chi^2_{[12]} = 5.85$, P > 0.05.

Andrena	sp.			
		Ran	dom plot	
	L ¹		D	1
Bee	L^2	D ²	L ²	D ²
1	2	1	0	0
2	2	1	2	4
3	5	5	4	11
4	2	2	2	3
5	6	9	9	4
6	10	3	4	2
7	5	2	2	0
8	7	4	5	8
9	12	13	13	14
10	3	5	4	7
11	16	13	13	10
12	4	5	5	11
13	8	10	11	6
14	4	3	3	4
	—			
	86	76	77	84

 $\chi^{2}_{[1]}$ for total values = 0.89, P > 0.05. Heterogeneity $\chi^{2}_{[13]} = 8.56$, P > 0.05.

The distributions of the insects within the flowers of *L. corniculatus* suggest that they do not distinguish between the keel colour phenotypes (table 7). On one occasion an excessive number of adult thrips was found in dark-keeled flowers, but this result was not repeated in the other samples and was clearly atypical.

Seed production and herbivory

The most accurate measure of the success of a plant in evolutionary terms is the number of successfully reproducing offspring which it produces during its lifetime. This value is generally unobtainable particularly for a long-lived cloning

	L^1		\mathbf{D}^{1}	1		
	L^2	D^2	L ²	D^2		
Bombus lapidarius	100	121	129	149		
Bombus terrestris	72	69	72	59		
		76	77	84		
Andrena $\chi^2_{[7]} = 10.11, P > 0.05$ (b) Systematic plot	86 5 (a 2×2×	3 conting	ency χ^2)			
Andrena $\chi^2_{[7]} = 10.11, P > 0.05$ (b) Systematic plot	$\frac{86}{10}$	3 conting	$\frac{\gamma}{2}$	1		
Andrena $\chi^2_{[7]} = 10.11, P > 0.05$ (b) Systematic plot	$\frac{86}{1000}$	70 3 continge D ²	$\frac{(1)^{2}}{D^{2}}$	D ²		
Andrena $\chi^2_{[7]} = 10.11, P > 0.05$ (b) Systematic plot Bombus lapidarius	$\frac{86}{16} = \frac{1}{L^2}$ $\frac{L^2}{126}$	76 3 continge D ² 122	$\frac{D}{L^2}$	D ²		

 Table 3
 The frequency of pollinator flights between plants of L. corniculatus. Between species comparisons

perennial and in most cases viable seed production provides the best available estimate of relative fitness. Comparisons of the seed production of light and dark-keeled L. corniculatus were obtained by harvesting the entire seed production of premarked plants growing at Cauldon Lowe and at Wharram Quarry. Animals feeding in the pods were also identified and counted, together with any of their parasites that were present. Phytophagous species recorded inside the pods were Apion loti, Eurytoma platyptera (Walker), (Hymenoptera) and Cydia compositella (F), (Lepidoptera). The biology of these species, and their associated parasite complex, has been described elsewhere (Compton, 1983). Any insects which had completed their development and vacated the pods would not have been detected easily by this sampling regime. Normally it is only adult A. loti and prepupal larvae of C. compositella that can escape from undehisced pods. The head capsules of A. loti larvae were recovered wherever possible and counted.

In June 1973 the seeds of the 1972 collection from Cauldon Lowe were examined, counted (using the Decca Mastercount) and placed on damp filter paper in petri dishes. In this way it was possible to distinguish three groups of seeds: a few seeds germinated immediately (year 1 germination); a large number of hard seeds did not imbibe water; the remainder swelled, but did not germinate. This last group included seeds damaged by insects, immature seeds and seeds inviable for other reasons. It was not possible to examine all 31,704 seeds individually for evidence of damage

Table 4	The foraging patterns of pollinators given a choice
betw	een dark and light-keeled L. corniculatus. These data
diffe	r from those in table 2 in two ways: (1) the first plant
forag	ged is included and (2) plants inspected, but not
fora	ged are excluded

(a) Random plot		No. of pla	inte forgrad	
	No. of		ints totaged	
Species	observations	Dark Keel	Light Keel	
Bombus lapidarius	17	230	163	
Bombus terrestris	11	108	118	
Andrena	14	135	134	
$\chi^2_{[2]} = 8.11, P < 0.05$				
	NK 6	No. of flo	wers foraged	
Species	No. of observations	Dark Keel	Light Keel	
Bombus lapidarius	17	943	603	
Bombus terrestris	11	368	349	
Andrena	14	405	470	
$\chi^2_{[2]} = 53.03, \ P < 0.001$				
(b) Systematic plot				
		No. of pla	ants foraged	
Species	No. of observations	Dark Keel	Light Keel	
Bombus lapidarius	18	198	188	
Bombus terrestris	13	156	146	
$\chi^2_{[1]} = 0.01, P > 0.05$				
		No. of flo	wers foraged	
Species	No. of observations	Dark Keel	Light Keel	
Bombus lapidarius	18	824	704	
Bombus terrestris	13	552	465	
$\chi^2_{[1]} = 0.04, P > 0.05$				

by herbivores. From our knowledge of the feeding behaviour of the seed herbivores and their parasites found in these pods (*e.g.*, Compton, 1983) we were able to estimate the minimum number of seeds likely to have been damaged by the insects.

The hard seeds were rescued, washed and dried and in 1974 they were scarified and the germination test was repeated. The overall germination rate (year 2 germination) among these seeds was $84 \cdot 10$ per cent which compares favourably with formal genetical experiments requiring a high germination rate (*e.g.*, Ramnani and Jones, 1984a).

The data obtained are presented in table 8. Analysis of variance after relevant transformations showed that the phenotypes did not differ significantly in any of the following characters: pods produced, seeds produced, seeds per pod, seed

KEEL PETAL COLOUR IN LOTUS

7

11

72

47

4

3

(a) Random plot				No. of	f plants forag	ed		
	No. of flights		Overcast		Sunny			
Species	Overcast	Sunny	Dark	Light	Dark	Light	$\chi^{2}_{[1]}$	Р
Bombus lapidarius	10	7	153	98	77	65	1.69	>0.02
Bombus terrestris	4	7	17	22	91	96	0.33	>0.02
Andrena	3	11	16	14	123	120	0.08	>0.02
				No. of	flowers forag	ed		
	No.	of flights	Over	cast		Sunny	_	
Species	Overcast	Sunny	Dark	Light	Dark	Light	$\chi^2_{[1]}$	Р
Bombus lapidarius	10	7	684	372	305	256	16.70	<0.001

52

45

(b) Systematic plot

Bombus terrestris

Andrena

				NO. 01	plants forage	:d		
Species	No. of flights		Overcast			Sunny		
	Overcast	Sunny	Dark	Light	Dark	Light	$\chi^{2}_{[1]}$	Р
Bombus lapidarius	7	11	69	51	129	137	2.68	>0.05
Bombus terrestris	4	9	39	49	117	97	2.68	>0.02
				No. of flowers foraged				
	No.	of flights	Over	rcast		Sunny		
Species	Overcast	Sunny	Dark	Light	Dark	Light	$\chi^{2}_{[1]}$	Р
Bombus lapidarius	7	11	333	167	491	537	48.04	<0.001
Bombus terrestris	4	9	220	150	332	314	6.17	<0.02

herbivores, seed herbivores per pod, damaged seeds and germination patterns. A similar survey carried out at Wharram Quarry in 1982, which did not include germination studies, obtained even more closely similar values for the seed production and seed herbivory of the two keel colour phenotypes (table 9). For this survey, when a parasite was found it was scored as its host (Compton, 1983).

Note, however, the higher incidence of *E.* platyptera in the pods of light-keeled plants and the larger number of seeds produced by the light keeled plants in the second collection at Wharram Quarry, even though the light-keeled plants tend to flower earlier. The two collections are significantly different from each other ($\chi^2_{[1]} = 54.42$, $P \ll 0.001$).

DISCUSSION

296

367

297

421

2.73

0.67

>0.05

>0.05

Variation in the colouration of the keel petals of *Lotus corniculatus* provides an intriguing example of a polymorphic character of indeterminate role maintained by selective processes that have yet to be discovered. The results presented here indicate that keel colour does not influence pollinator foraging behaviour, nor colonisation by flower-feeding insects, and we have no evidence of the phenotypes differing in terms of (maternal) reproductive output.

The importance of flower colouration as an isolating mechanism has been reviewed by Kevan (1978), Kay (1978) and others. Different colour forms of the same species are known to differ in their attractiveness to pollinators (*e.g.*,

Table 6 A comparison of the number of flowers per umbel on light and dark-keeled L. corniculatus at Eppleworth Quarry. All the flowers within randomly placed quadrats were recorded

	17 1		Flow				
Period	colour	1	2	3	4	$\chi^{2}_{[3]}$	Р
1977	Light	20	6	8	7		
	Dark	8	6	3	0	5.41	>0.02
1978	Light	19	25	16	32		
	Dark	15	13	16	13	4.29	>0.02
Total	Light	39	31	24	39		
	Dark	23	19	19	13	4.11	>0.02

Heterogeneity $\chi^2_{[3]} = 5.59$, P > 0.05. Mean flowers per umbel: light 2.59, dark 2.32.

Table 7 The colonisation of L. corniculatus flowers by insects. One umbel per plant removed at metre plus intervals

(a) Roadside verge between Cottingham and Walkington, 5th July 1978

	Number of insects present		Dark		Light			
Insect	Dark (195)*	Light (87)	present	absent	present	absent	$\chi^2_{[1]}$	Р
Apion loti	27	10	19	65	5	27	0.69	>0.05
Hypera plantaginis	30	14	17	67	8	24	0.31	>0.05
Thysanoptera larvae	59	35	26	58	10	22	0.001	>0.05
Thysanoptera adults	68	16	36	48	10	22	1.31	>0.05
Meligethes erythropus	27	18	19	65	13	19	3.76	>0.05
Acyrthosiphon loti	20	11	15	69	3	29	1.27	>0.02

(b) Wharram Quarry, 9th June 1981

	Phenotype and number of flower umbels containing insects							
	present		Dark		Light			
Insect	Dark (206)	ark (206) Light (312)		absent	present absent		$\chi^2_{[1]}$	Р
Apion loti	213	334	48	6	78	7	0.32	>0.02
Hypera plantaginis	9	21	8	46	16	69	0.37	>0.02
Thysanoptera larvae	66	84	24	30	32	53	0.63	>0.02
Thysanoptera adults	25	34	21	33	25	60	1.34	>0.05

(c) Wharram Quaryy, 25th July 1986

	Number	of insects		Phenotype a umbels c	nd number of ontaining inse	flower		
	present		Dark		Light			
Insect	Dark (83)	Light (101)	present	absent	present	absent	$\chi^2_{[1]}$	Р
Apion loti	61	58	21	7	26	9	0.01	>0.02
Thysanoptera larvae	66	64	21	7	26	9	0.01	>0.05
Thysanoptera adults	92	60	25	3	23	12	4.76	>0.02

* The number of individual flowers examined.

Table 8Seed production by L. corniculatus at Cauldon Lowe.The entire seed production of each plant was harvested
during September/October 1972. Germination techniques
are described in the text

(a) Seed pro	oduction							
Keel colour phenotype	Plan	ts Po	ds	Se	eds	Se	eds/pod	
Dark	45	281	13	17	,213	6.1	2	
Light	48	260)7	14	,491	5.5	6	
(b) Seed he	rbivores (i	ncluding	; their	para	asites)			
Keel colour phenotype	r Apion loti	Euryto platypi	oma tera	<i>А</i> . /р	<i>loti</i> od	<i>E</i> . /p	<i>platyptera</i> od	
Dark	589	124		0.2	21	0.0)4	
Light	413	108		0.16		0.0	0.04	
(c) Seed ge	rmination							
Keel colour phenotype	Insect damaged seeds	Failed soft seeds	Germ ated Year	in- 1*	Gern ated Year	nin- 2	Failed hard seeds	
Dark	1597	7599	126		6711		1180	
	9.28%	44.15%	0.73%	6	38.99	9%	6.86%	
Light	1141	6530	143		5541		1136	
	7.87%	44.06%	0.99%	6	38.24	4%	7.84%	

* Seeds germinating immediately, without requiring scarification.

Dronamraju, 1960; Lloyd, 1969; Kay, 1976; Levin and Schaal, 1970; Waser and Price, 1981; Brown and Clegg, 1984), while variation in the preferences shown by different pollinators towards Cirsium palustre (L.) colour forms may be responsible for altitudinal differences in morph frequencies (Mogford, 1974a, b). Other studies have yet to consider the role of pollinators in the maintenance of a flower colour polymorphism-e.g. the purpleviolet/white perianth and deep orange/cream white to white stigmata in Crocus scepusiensis (Rehm. et Wol.) Borb. in Poland (Rafinski, 1979). There are also examples where the differential attractiveness of the different colour morphs has been ruled out (e.g., in Platystemon californicus Benth., Hannan, 1981) and others where assertions to this effect have been made (e.g., for Anemone coronaria, Horovitz, 1976).

When a bee forages a *Lotus corniculatus* flower the wing petals are depressed and the keel is revealed. After the visit the wing petals slowly return to their original position, the whole process taking approximately 15 minutes. If the visible keel petals were a signal to a bee that a flower had recently been visited—and presumably been denuded of nectar—no bee would visit a flower with an obvious keel. Frequently during our observations we noticed bees visiting flowers recently visited by other bees and on at least five separate occasions a bee was seen to return to a flower that it had itself foraged only a few minutes previously.

The data and analyses in tables 2 and 3 show that the pollinator flights between plants are essentially at random with respect to keel colour. The observation that B. lapidarius shows a preference for dark-keeled plants in overcast conditions only in the random plot does not make much sense. Obviously assortative mating would result, but the relevance of this behaviour to natural populations-where the distribution of the phenotypes will be neither random nor systematic-cannot be determined. If there were evidence that the darkkeeled plants produced more seeds than lightkeeled plants then we could begin to formulate some testable hypotheses, but the evidence in tables 8 and 9 is equivocal. Overall the two pod collections made at Wharram Quarry show no difference in healthy seed production between the two morphs (10,425 from 49 dark and 10,696 from 50 light-keeled plants). At Cauldon Lowe the numbers of viable seeds are 6837 for the dark and 5684 for light-keeled plants. Based on an expected ratio of 45:48, $\chi^2_{[1]} = 193.79$, $P \ll 0.001$ showing a marked excess of viable seed from the dark-keeled plants. The analysis of variance based on the seed production of individual plants, however, shows no significant difference between the phenotypes! $(VR_{[1,91]} < 1.)$ The reason for this apparent discrepancy is the very large variation in seed production between individual plants of the same phenotype. Effectively what is happening is that a few individuals are producing a very large number of seeds, so biasing the total seed production of one morph. Unfortunately we have no means of testing whether this is an atypical result nor yet whether it is biologically meaningful in our attempts to explain the genetic polymorphism. Thus there is too much "noise" in the system and we must conclude either that our experimental and observational work has been done on too small a scale or has been looking at the wrong candidates for selective agents or that the selective forces are so finely balanced that genuine differences are beyond the resolution of statistical tests of significance.

There are, however, several features of the keel colour polymorphism in *Lotus corniculatus* that may well be unique. Firstly, the keel petals are not visible until a flower has been foraged by a bee.

8.82)					
ion					
Plants	Pods	Seeds	Seeds/pod	Chewed seeds	Healthy seeds
49 50	361 302	2614 2091	7·24 6·92	386 (14·8%) 234 (11·2%)	2228 (85·2%) 1857 (88·8%)
res (including the	eir parasites*)				
Apion loti	Eurytoma platyptera	Cydia compositella	A. loti/pod	E. platyptera/pod	C. compositella/pod
166 146	4 56	15 12	0·460 0·483	0·011 0·185	0·042 0·040
-15.9.82)					
ion					
Plants	Pods	Seeds	Seeds/pod	Chewed seeds	Healthy seeds
49 50	1523 1623	9183 9731	6·03 6·00	986 (10·7%) 892 (9·2%)	8197 (89·3%) 8839 (90·8%)
res (including the	eir parasites)				
Apion loti	Eurytoma platyptera	Cydia compositella	A. loti/pod	E. platyptera/pod	C. compositella/pod
800 739	9 27	31 38	0·525 0·455	0·006 0·017	0·020 0·023
	3.82) ion Plants 49 50 res (including the <i>Apion loti</i> 166 146 15.9.82) ion Plants 49 50 res (including the <i>Apion loti</i> 800 739	B.82)ionPlantsPods4936150302res (including their parasites*)Apion lotiEurytoma166414656.15.9.82)tionPlantsPlantsPods491523501623res (including their parasites)EurytomaApion lotiPlants91523501623res (including their parasites)EurytomaApion lotiplatyptera800973927	BaselPodsSeeds49 361 2614 50 302 2091 res (including their parasites*)Apion lotiEurytoma platypteraCydia compositella1664151465612.15.9.82)PlantsPodsSeeds91835016239731res (including their parasites)Apion lotiEurytoma compositella800931 38800931 38	Basel Pods Seeds Seeds/pod 49 361 2614 7·24 50 302 2091 6·92 res (including their parasites*) Eurytoma platyptera Cydia compositella A. loti/pod 166 4 15 0·460 146 56 12 0·483 .15.9.82)	Basel ion Plants Pods Seeds Seeds/pod Chewed seeds 49 361 2614 7·24 386 (14·8%) 50 302 2091 6·92 234 (11·2%) res (including their parasites*) Apion loti Eurytoma platyptera Cydia compositella A. loti/pod E. platyptera/pod 166 4 15 0·460 0·011 146 56 12 0·483 0·185 ·15.9.82)

Table 9	Seed production by L.	corniculatus at	Wharram Q	uarry. The	entire pod	production o	f each plan	was	harvested	on two
occa	isions									

* Each parasite indicates that a herbivore of a particular species would have been there had it not been parasitized (Compton, 1983).

The character is, therefore, partly cryptic. It was for this reason that the behaviour of bees in response to the keel colour variation had to be studied. But, approximately 15 minutes later the keel is no longer visible because the keel petals have returned to their original position. Does it take this length of time for the flower to recharge the nectaries?

Secondly, each flower requires between 12 and 25 active visits by bees for full seed set (Morse, 1958). Thus flowers must remain sufficiently attractive to bees for repeated foraging visits to be made. Thirdly, we now have records from nearly 800 populations of *Lotus corniculatus* in the U.K. and we have exceedingly few that are monomorphic for keel colour. None of Abbott's (1981) samples from 61 locations in Scotland is monomorphic. In the majority of other examples of flower colour polymorphism most populations are monomorphic and only a few are polymorphic. Such evidence as there is with respect to pollinator behaviour is that they tend to discriminate against

a rare morph and this tends to produce monomorphism (e.g., Levin, 1972; Waser and Price, 1981).

We are not prepared to accept that the spectacular west to east cline in the U.K., that is most noticeable between latitudes 53°30' N and 54°30' N and approximately 210 km long (Crawford and Jones, 1986), is the result of chance effects. In the west the frequency of dark-keeled plants is almost zero whereas at Flamborough Head the frequency is close to 100 per cent. The pattern of the distribution of the dark-keeled plants in England, Wales and Scotland suggests that strong selection has acted on the polymorphism in the past. Kay (1978) has remarked that older populations of Chrysanthemum coronarum in Greece are essentially monomorphic for petal colour whereas "populations growing in temporarily available roadside and ruderal habitats are heterogeneous with great changes in morph frequency over distances of a few metres". Lotus corniculatus is a common early successional plant particularly on roadside verges, in quarries and on waste land where the pH of the soil is above 5.0. Thus many populations of L. *corniculatus* will be in a state of dynamic flux and polymorphism is only to be expected. On the other hand, well established populations in old pastures and on chalk grassland are always polymorphic.

A few populations in the Netherlands and in France appear to contain only light-keeled individuals (Jones and Crawford, 1977) and no population consisting exclusively of dark keeled plants has been recorded. This is in contrast to the polymorphism of leaf cyanogenesis in L. corniculatus where approximately 25 per cent of the recorded populations are monomorphic (Jones, 1977). This may be indicative of frequency dependent selection contributing to the stability of the keel colour polymorphism, with whichever phenotype that is rarer in a population being placed at an increased advantage. But what is the mechanism? It is clearly the case that most of the studies reported here would be unlikely to detect frequency dependent selection because the morphs have occurred at intermediate frequencies. We need to look at natural populations where one or the other morph is rare. A start has been made at Porthdafarch, Anglesey (Compton et al., 1983b), where the dark morph increased in frequency from 5.4 per cent (June 1979) to 20 per cent (May 1982) in one group of plants and from 10.7 per cent (June 1979) to 12.9 per cent (May 1982) in a closely adjacent group of plants. On both occasions the damage to the flowers was recorded, but no evidence of differential herbivory was obtained. Neither the behaviour of pollinators nor seed production was examined.

Many of the ovules produced by L. corniculatus fail to develop into mature seed, despite abundant pollination (Stephenson, 1984). Selective abortion of immature seeds is commonly based on the order in which ovules are produced (Stephenson, 1981) and in this situation pollen competition is likely to be intense. Keel colour variation may have a direct influence here with any dark pigment increasing the heat absorbing properties of the petals surrounding the stigma and style and thereby influencing pollen physiology. Unfortunately, some Lotus species are monomorphic for dark keels (e.g., L. cytisoides L. and L. creticus L.) and other, sympatric species, are monomorphic for light keels (e.g., L. halophilus Boiss. and Spruner in Boiss. and L. ornithopodioides L.). Without detailed comparative information on the pollen physiology and pollination biology of these related species no simple explanation can be based on temperature effects.

In the work reported above we have examined the more obvious fitness characters of seed production, herbivores and the interaction between the flower colour polymorphism and pollinators and not found any differential effects. We have now begun to examine whether there are any pleiotropic effects of keel colour or germination, establishment, resistance to herbivores and survival in a maturing grassland community.

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