VARIATION IN THE COLOUR OF THE KEEL PETALS IN *LOTUS CORNICULATUS* L.

I. THE POLYMORPHISM IN WESTERN EUROPE

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SUMMARY

The distribution of the dark keeled form of *Lotus corniculatus* in Western Europe is described. Although there is an association between the frequency of the dark keeled form and latitude in England and Wales, no differences between the dark keeled and light keeled forms in the winter survival or in seed production were found under experimental conditions. Furthermore, there appears to be no association between cyanogenesis in the leaves and keel colour, either on a per plant or on a population basis.

1. INTRODUCTION

DURING studies on the geographical distribution of the cyanogenic form of *Lotus corniculatus* L. (Jones, 1972, 1973) plants have also been scored for the colour of their keel petals. Because these plants clone readily it was essential to have a phenotypically stable marker to distinguish between individual plants growing closely together. Yellow versus brown-red keel colour provided a convenient marker. The difference is determined by a pair of alleles inherited in a tetrasomic manner, brown-red keel being dominant (Hart and Wilsie, 1959). In cultivated material, however, Buzzell and Wilsie (1963) and Bubar and Miri (1965) have reported that at least one locus which is involved in the control of cyanogenesis in leaves is also involved in the expression of keel colour. Plants homozygous for a recessive allele at a suppressor locus are acyanogenic and also have a yellow keel tip. We might expect, therefore, that the frequency of cyanogenic plants would be correlated with the frequency of dark keeled plants and also that we would find an excess of cyanogenic dark keeled and acyanogenic light keeled plants over the other two phenotypic combinations in natural populations.

Populations in Western Europe are usually polymorphic, the brown-red keel phenotype being less common than the yellow. Although Harborne (1969) has identified two flavonols in the flowers of *L. corniculatus*, the exact chemical difference between light and dark keels has yet to be determined. This report presents population data and shows the lack of relationship between the characters of keel colour and cyanogenesis in the wild.

2. METHODS

Systematic plant sampling has been used for this work (Jones, 1977) and individual plants were often scored both for their keel colour and for

cyanogenesis. Seed samples were collected using the method described previously (Jones, 1977). When sampling plants one flowering head is collected from each plant and the sample is usually scored immediately after collection. If necessary, flowers can be kept in a "polythene" bag at room temperature for 2 or 3 days, and for even longer at 4°C with no apparent deterioration in their keel colour. There is no difficulty over distinguishing yellow (light) from brown-red (dark) keels, but a subjective division of the latter class into pale and deep brown-red is possible. This distinction was made at the beginning of the work and reference to standard plants growing in the glasshouse was made for keels of doubtful classification. For the purposes of this report we shall be concerned primarily with the yellow/brown-red distinction and not with the sub-division of the brown-red class. Repeated examination of several thousands of individual plants growing in the glasshouse or experimental field has led us to the conclusion that the character is phenotypically stable. No plant has been recorded as having changed phenotype either from dark to light or vice versa.

3. RESULTS AND DISCUSSION

The full population data are presented in tables 1a to 1d and a selection of them is plotted on the map of fig. 1. The details about the location and the altitude of the population samples have been given in earlier papers on cyanogenesis in *L. corniculatus* (e.g. Jones, 1977).

There is strong evidence that the frequency distribution of the dark keeled form in England and Wales is clinal, there being a significant regression ($b = 0.039$) of frequency on latitude (table 2a), V.R. [1,56] = 11.95, $P = 0.001$. On the other hand this does not appear to be a general phenomenon because it is not paralleled in other parts of Europe. Thus, with the exception of samples from Groote Keeten and Callantsoog the frequency of the dark keeled form in the coastal dunes of the Netherlands is small and independent of latitude. The data from Jutland, however, do give a positive association between dark keel frequency and latitude although it is not significant (table 2b), V.R.[1,23] = 4.01, $P > 0.05$.

(i) *The fitness characters of longevity and seed production*

Although there has been continued interest in seed production of *L. corniculatus* in the United States (e.g. Seaney and Henson, 1970) none of this work is related, even indirectly, to particular phenotypic variation within the species. Salisbury (1942) makes no reference to the reproductive capacity of the species, whereas the work of Albrechtsen (1965) and Bader and Anderson (1962) is confined to cultivars.

Experiments on winter survival and seed production by plants of the four basic phenotypes associated with cyanogenesis have been described previously (Jones, 1970). The plants used for those experiments were chosen at random from 995 plants grown from seed collected in Britain and in Scandinavia. They had also been scored for keel colour before the experiment began and so it is legitimate to re-analyse the data for keel colour alone, ignoring cyanogenesis. These plants have been classified into the three phenotypes of deep, pale and light keel colour for the analysis of variance for winter survival on the one hand and overall survival on the

other (September 1966 through April 1968) given in table 3. With phenotypes fixed and habitats regarded as random for this analysis, it can be concluded that there are no effects either of the phenotype or of the origin of the plants on survival.

FIG. 1. The frequency distribution of the dark-keeled form of *Lotus corniculatus* in England and Wales.

It will be noticed that the mean square for habitats in this table is not the same as that published previously (Jones, 1970). Because the data comprise disproportionate numbers, a weighted interaction item is calculated and the main effects sum of squares corrected accordingly (Snedecor and

Cochran, 1967). Consequently the habitat sum of squares will be different and, indeed, with respect to overall survival it is not significant on this occasion.

In table 4 the analysis of variance of seed production is presented in three different ways. Again, there is no significant effect of origin or of keel phenotype on seed production of these plants. Although the interaction mean squares are not significantly larger than the between plant mean squares, their variance components do constitute a large proportion of the total variation. Pooling what are admittedly heterogeneous data gives table 5. These data suggest that an interaction between keel phenotype and the origin of the plants may genuinely exist. Clearly the possibility should be explored further.

TABLE 1a

The frequency of dark keeled plants and of cyanogenic plants at various locations in Western Europe. Further details about the dates and locations are given in Jones (1977). Within each State the localities have been arranged in order of latitude with the most northerly first

Location	Sample size	Percentage with dark keels	Percentage cyanogenic	International UTM-Grid Northing
<i>Denmark</i>				
Sundstrup	49	18.4	3.2	6260
Sundstrup (cultivars)	50	8.0	98.0	
Virksund	26	30.8	8.9	6260
Skive	63	28.6	0.0	6260
Viborg	50	50.0	0.0	6250
Ravenstrup	24	33.9	0.0	6240
Mønsted	50	10.0	0.0	6240
Herrup	48	20.8	12.0	6240
Torsminde	49	18.4	0.0	6240
Husby	39	35.9	7.3	6230
Ørnhøj	44	31.8	0.0	6220
Rinkøping	37	10.8	0.0	6210
Hennesstrand	26	19.2	0.0	6170
Hodde	69	14.5	2.3	6170
St Darum	29	20.7	0.0	6140
Lakolk	60	26.7	0.0	6110
Ellum	21	38.1	0.0	6090
Hjerpsted	37	21.7	9.8	6090
Søvang	48	16.7	6.1	6090
<i>West Germany</i>				
Dagbüll	21	14.3	0	6060
Arlewatt	44	15.9	0	6040
Solwitt	30	6.7	0	6040
Luiden	75	8.0	—	6020
Weddingstedt	46	13.0	7.5	6000
Büsum	242	2.1	84.5	6000
Meldorferrhafen	29	3.4	69.2	5990
Itzehoe	42	23.8	0	5970
Lentförhrden	61	14.8	1.6	5960
Barmstedt	23	17.4	0	5960
Warstade	17	5.9	17.6	5940
Bucholz	24	25.0	0	5910
Rethem	47	83.0	79.6	5840

Table 1a—(continued)

				National Grid* Northing
<i>England and Wales</i>				
Dunstanstead (Northumberland)	29	72.4	95.1	620
Sandside (Cumbria)	100	9.0	99.0	480
Fountains Fell (N. Yorks)	22	72.7	100.0	470
Burdale Warren (N. Yorks)	103	60.2	96.0	460
York (N. Yorks)	48	68.7	100.0	450
Acaster (N. Yorks)	120	71.7	99.0	440
Strines (Cheshire)	102	24.5	100.0	390
Parys Mountain (Gwynedd)	77	6.5	100.0	390
Bwrdd Arthur (Gwynedd)	50	8.0	100.0	380
Porthdafach (Gwynedd)	55	27.2	27.0	370
Newborough Warren (Gwynedd)	120	10.8	95.8	360
Grindon (Staffs)	43	30.2	86.0	350
Ballidon 1 (Derby)	50	38.0	57.1	350
Ballidon 2 (Derby)	50	28.0	86.4	350
Newton Grange (Staffs)	87	42.5	92.2	350
Blackheath 1 (Staffs)	20	40.0	95.0	340
Blackheath 2 (Staffs)	29	65.5	23.3	340
Cauldon Lowe (Staffs)	154	60.4	98.2	340
Froghall (Staffs)	108	31.5	91.7	340
Hunstanton (Norfolk)	37	64.9	84.3	340
Breedon Hill (Leics)	41	68.3	88.9	320
Ashby de la Zouch (Leics)	60	70.0	87.0	310
Swaffam (Norfolk)	85	16.5	13.9	300
Glaston (Camb)	130	40.8	100.0	300
Rounds Green (W. Midlands)	118	24.6	84.0	280
Windmill Hill (Hereford-Worcs)	1302	37.9	54.1	280
Kings Norton (W. Midlands)	31	32.3	84.8	270
Wawensmoor (Warks)	19	31.6	58.8	260
Wilmcote (Warks)	29	17.2	78.9	250
Leominster (Hereford-Worcs)	40	20.0	94.2	250
Eltisley (Camb)	52	38.5	98.1	250
Rhydd Green 1 (Hereford-Worcs)	72	16.6	63.0	240
Rhydd Green 2 (Hereford-Worcs)	43	37.2	73.9	240
Taf Fechan (Powys)	100	34.0	100.0	210
Cwm Taf (Mid. Glam)	77	26.0	100.0	200
Mynydd Aberdar (Mid. Glam)	69	37.7	87.0	200
Branscombe (Devon)	29	3.5	91.4	080

* These entries refer to the nearest 10 km grid line south of the site of the population sampled.

Whether or not there is a differential effect of the origin of the plants on the seed production of dark and light keeled plants, it is clear that the large differences between plants within a phenotype/habitat class indicate that the plants have not been classified, for this analysis, in a way which can explain the major causes of differential seed production. On the other hand, there is some evidence, in cultivated material, of genetic differences between plants for the fitness character of seed production (Albrechtsen, 1965). As with the data presented previously (Jones, 1970), the within-clone mean squares are consistent over the three analyses. Thus the plants which did not produce seeds were a random sample of the total number examined.

There is independent evidence that the putative interaction between keel colour and origin may be worthy of further study. All 995 of the original plants from Britain and Scandinavia were scored for keel colour.

TABLE 1b

The frequency of dark keeled plants and of cyanogenic plants at various locations in Western Europe

Location	Sample size	Percentage with dark keels	Percentage cyanogenic
<i>The Netherlands</i>			
<i>Coastal Dunes</i>			
Groote Keeten	54	13.0	3.6
Callantsoog	59	11.9	0.0
Hargen	59	1.7	1.7
Bergen 1	47	0.0	6.0
Bergen 2	25	4.0	0.0
Egmond Binnen	50	0.0	0.0
Castricum	50	0.0	4.0
Noordwijk	35	0.0	22.9
Katwijk	50	4.0	16.0
Groote Berkheide	46	2.2	44.0
Zwarte Pan	48	0.0	48.0
Wassenaar 1	46	0.0	79.5
Wassenaar 2	63	3.2	79.3
Monster	51	0.0	76.5
Goeree	22	0.0	96.1
<i>The Netherlands</i>			
<i>East of Coastal Dunes</i>			
Oude Mirdum	141	7.1	0.0
Kleine Sluis	50	4.0	8.5
Joppe	45	33.3	64.0
Drempt	57	47.4	77.2
Heelsum	33	39.4	93.9
Wageningen	50	40.0	97.0
Veenendaal	49	49.0	0.0
Slijk Ewijk	51	43.1	98.2
Zaltbommel	292	39.0	100.0
Beers	50	60.0	90.0
Odiliapeel	40	27.5	63.4
<i>Austria</i>			
Kössen (Kaisergebirge)	50	82.0	40.0
Walchsee (Kaisergebirge)	62	66.1	24.3
Nockberg (Oberdrautal)	25	48.0	0
Weissensee (Oberdrautal)	37	43.2	0
<i>France, Savoie</i>			
Col de la Forclaz	35	61.5	2.9
Crêt de Châtillon	44	65.9	0.0
Synclinal de Bisanne	18	66.7	12.5
<i>Spain</i>			
Orion (Santander)	25	12.0	100.0
Fuente Dé (Picos de Europa)	48	29.2	41.7
Argelaguar (Gerona)	52	0	0
Collado de Corbet (Gerona)	49	2.0	0

It is clear from table 6 that the frequency of the dark form is significantly greater amongst the Scandinavian than amongst the British plants.

In some cases both flower and seed samples have been taken from the same group of plants at the same time. It is noticeable that in all four examples where the seed progenies have been scored (table 7) there is a

TABLE 1c

The frequency of dark keeled plants in various locations in Sweden and Britain. Plants grown from seeds

Location	Sample size	Percentage with dark keels	Percentage cyanogenic	National Grid Northing
<i>Britain</i>				
Aysgarth (N. Yorks)	81	70.4	96.9	480
Llanidloes (Powys)	49	16.3	29.4	280
Blenheim Park (Oxon)	79	27.9	45.2	210
Long Hanborough (Oxon)	68	10.3	72.5	210
Forest Hill (Oxon)	48	27.1	80.0	200
Newbury (Berks)	90	60.0	93.5	160
Denbies (Surrey)	30	36.7	90.0	150
Nare Head (Cornwall)	61	29.5	94.2	030
Portscatho (Cornwall)	102	25.5	99.0	030
St Anthony's Head (Cornwall)	72	19.4	96.3	030
Kynance Cove (Cornwall)	43	23.3	50.9	010
<i>Sweden</i>				
Sannas	41	61.0	0	
Orust	77	48.1	0	
Halleviksstrand	258	84.5	0.8	
Haverstessund	140	59.3	0.7	

TABLE 1d

*The frequency of dark keeled plants in various locations in France and Britain. (Plants not tested for cyanogenesis.) *Data of Dr Mary Ellis*

Location	Sample size	Percentage with dark keels	National Grid Northing
<i>France</i>			
Concarneau (Brittany)	50	12.0	
Cognac	50	0	
St George de Didonne	51	0	
<i>England and Wales</i>			
*Wharram Quarry (N. Yorks)	246	44.7	460
Rudyard Reservoir (Staffs)	57	28.1	350
West Heath (W. Midlands)	44	20.5	270
Stratford (Warks)	37	32.9	250
Llandovery (Dyfed)	32	21.9	230
Enstone Quarry (Oxon)	100	42.0	220
Beacon Hill (Hants)	62	21.0	150
Box Hill (Surrey)	52	30.8	150
Dover (Kent)	75	32.0	140
Poldhu (Cornwall)	50	22.0	010

TABLE 2a

Analysis of variance of regression of the frequency of the dark keeled form in England and Wales on the National Grid Northing. Angular transformation of the percentage data was used

	d.f.	M.S.
Regression	1	1462.34***
Residual	56	122.34

TABLE 2b

Analysis of variance of regression of the frequency of the dark keeled form in Jutland on the international UTM-grid

	d.f.	M.S.
Regression	1	169.37
Residual	23	41.99

TABLE 3

Analysis of variance of survival of Lotus corniculatus

	d.f.	Survival of winter 1966-67 M.S.	Overall survival 1966-68 M.S.	
Habitats	1	0.028	10.228	Random effect
Phenotypes	2	0.166	2.888	Fixed effect
H × P	2	0.035	0.341	
Between plants	36	0.382	4.927	
Within clones	200	0.109	*0.824	

* 0.024 in the previous publication (Jones 1970) was a printing error.

TABLE 4

Analysis of variance of seed production

	Plants which produced seed		Plants which could have produced seed		All plants	
	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Habitats	1	12.776	1	3.143	1	10.741
Phenotypes	2	10.436	2	12.263	2	19.147
H × P	2	48.767	2	40.570	2	26.760
Between plants	27	82.351	35	60.690	36	36.777
Within clones	97	5.385	157	5.282	200	4.575

TABLE 5

Mean seed production according to phenotype and origin of seed parent (plants which did produce seeds)

	Mean No. of seeds		No. of seed parents	
	Dark	Light	Dark	Light
British	1441	3710	31	48
Scandinavian	4090	3375	25	28

TABLE 6

Numbers of dark keeled plants produced from seed samples

	Dark	Light	Total	% Dark
Britain	172	273	445	38.7
Scandinavia	371	179	550	67.5

$$\chi^2_{[1]} = 82.3***$$

higher frequency of dark keeled plants amongst these seed progenies. Buzzell and Wilsie (1963) also obtained an excess of dark keeled plants when they backcrossed duplex with nulliplex plants. In our experiments germination and establishment is approximately 95 per cent of the seeds sown and thus the excess of dark keeled progeny is unlikely to result from selection in favour of dark keeled plants at the seedling stage. Allowing that the development of some flower pigments is temperature sensitive it is possible that the warmer conditions in the glasshouse in which *L. corniculatus* plants were raised may have altered the manifestation of keel colour in some plants. This cannot be the explanation for the increase in the frequency of the dark keeled form in successive seed samples collected at Rounds Green (table 8) because all the plants raised from these seed samples were grown in the same glasshouse and at the same time and, as has been stated before, the character is phenotypically stable. There is another and more important conclusion to be drawn from table 8. The excessive production of seeds which give rise to dark keeled plants should lead to a marked increase in the frequency of the dark keeled plants in this population. Over the 4-year period 1966-70

TABLE 7

Comparison between the frequency of the dark keeled form in plant and seed samples taken from the same population at the same time

Location	Plants		Seeds	
	Sample size	Percentage of dark keels	Sample size	Percentage of dark keels
Rounds Green (Dudley)	299	22.4	176	33.0
Concarneau	50	12.0	94	24.2
St George de Didonne	51	0.0	92	3.3
Windmill Hill (Hereford-Worcs)	902	38.0	321	40.8

the frequency of dark keeled plants rose from 22.4 to 24.6 per cent, which is clearly much less than expected. The reason is not known.

L. corniculatus is an outbreeder (*e.g.* Dawson, 1941) and seed yield depends markedly on bees that collect pollen rather than nectar (Bader and Anderson, 1962). The results of the experiments on seed production suggest that the character of keel colour has little if any effect on the overall behaviour of pollinating insects. Studies on the role of pollinating bees will be discussed in a later paper on keel colour (Jones *et. al.*, in preparation).

(ii) *Keel colour and cyanogenesis*

It was suggested in the introduction to this paper that there should be a correlation between the frequency of cyanogenic and dark keeled plants (Bubar and Miri, 1965) and that, within a population, there should be an excess of cyanogenic dark keeled and acyanogenic light keeled plants over the other two phenotypic combinations. The work of Ellis, Keymer and Jones (1977) has shown, however, that any correlation must be interpreted with more than the usual caution because they found that some plants have fixed and others have variable cyanogenic phenotypes. Inspection of the data in table 1 suggests that the most likely samples to show a correlation are those collected inland from the dunes in the Netherlands. Using arc

sine transformation of the percentage data the correlation coefficient is $r_{[9]} = 0.59$ ($P > 0.05$), and so we can conclude that the frequency of the dark keeled form is not correlated with the frequency of the cyanogenic form at the time that the plants were tested for cyanogenesis. On the other hand, we cannot exclude the possibility that at other times of the year there may be a correlation, because of the variable expression of the cyanogenic phenotype. In an earlier paper Bubar (1958) described the growth conditions of his experimental plants. These were raised in field nurseries during the summer and transferred to glasshouses during the intervening winters, but he makes no mention of the time of year when he tested plants

TABLE 8

Frequency of dark keeled plants amongst successive plant and seed samples taken in the same year from the same group of plants growing at Rounds Green, Dudley (West Midlands). Two trials of each seed sample were grown; these have been labelled A and B

Type of sample	Date	Sample size	Percentage with dark keels	
	1966			
Plants	5th August	101	20.8	} 22.4 $\chi^2_{[1]} = 0.23$
Plants	8th August	198	23.2	
Seeds	5th August	A 114	36.0	} 33.0 $\chi^2_{[1]} = 1.33$
		B 62	27.4	
Seeds	8th August	A 57	31.6	} 33.8 $\chi^2_{[1]} = 0.23$
		B 73	35.6	
Seeds	8th September	A 107	40.2	} 43.7 $\chi^2_{[1]} = 1.64$
		B 51	50.9	
Plants	1970	118	24.6	

The frequency of the dark keeled plants grown from the seed samples collected in 1966 was, in all comparisons, significantly greater than the frequency amongst the plants.

First seeds and plants	$\chi^2_{[1]} = 6.36^*$
Second seeds and plants	$\chi^2_{[1]} = 6.18^{**}$
Third seeds and plants	$\chi^2_{[1]} = 22.36^{***}$
First seeds and third seeds	$\chi^2_{[1]} = 4.05^*$
Third seeds and 1970 plants	$\chi^2_{[1]} = 10.76^{**}$

An independent sample of 69 plants was transferred to the glasshouse on 5th August 1966. The frequency of the dark keeled form was 23.2 per cent.

for cyanogenesis and so direct comparison between his work and ours is hazardous.

The within-population comparisons are more difficult to make. It is clear that any sample which is not polymorphic for one or other of these characters contributes no information at all. The criteria used for including data in table 9 were (a) that the sample size should be at least 45 and (b) that the rarest morph should occur at a frequency of at least 20 per cent. Analysis of χ^2 (table 9) shows that there is no association between keel colour and cyanogenesis within populations on an individual plant basis and there is no evidence that the populations differ in this respect, $\chi^2_{[5]} = 1.35$ ($P > 0.90$).

Note that to be included in table 9 a plant must have been scored both for keel colour and for cyanogenesis. Some plants that were tested for cyanogenesis were not flowering and consequently the frequency of cyano-

genic plants calculated from the data in table 9 will not necessarily be the same as that in table 1.

In an earlier paper (Jones, 1977) it was argued that the cline for cyanogenesis in the Netherlands could not be compared with clines in other organisms because the latitudinal effect was confined to the Netherlands. With the cline in England and Wales for keel colour the same argument applies, although two other examples have been described in detail. Unfortunately, it is not known whether the clines for papillae on the seeds of *Spergula arvensis* L. (New, 1958) and for spotting on the leaves of *Arum maculatum* L. (Prime, 1955) occur on the mainland of Europe. New obtained experimental evidence that non-papillate seeds germinate more rapidly than

TABLE 9

Populations of Lotus corniculatus for which individual plants were scored both for leaf cyanogenesis and for keel colour. The criteria by which populations were included in this table are given in the text

Location	Light		Dark		Sample size	$\chi^2_{[1]}$ (inc. Yates correction)
<i>Plants</i>	++	-	++	-		
Windmill Hill*	440	369	264	229	1302	0.06
Ballidon	18	13	8	11	50	0.65
Drempt	21	8	23	5	57	0.31
Fuente Dé	13	21	7	7	48	0.18
Joppe	20	10	10	5	45	0.00
Walchsee	7	14	10	31	62	0.20
<i>Seeds</i>						
Blenheim Park	25	32	11	11	79	0.06
Total	104	98	69	70	341	0.05

Heterogeneity $\chi^2_{[5]} = 1.35$

* Because of the very large size of the Windmill Hill sample it has not been included in the heterogeneity χ^2 test.

papillate seeds at 13°C whereas the reverse is true at 21°C. Non-papillate-seeded plants produce a lower proportion of fertile capsules than papillate-seeded plants when grown at 21°C. Non-papillate-seeded plants wilt and die at the higher temperature and low humidity (55 per cent), whereas papillate-seeded plants grow normally under these conditions. Prime (1955) has shown that the var. *immaculatum* of *Arum maculatum* is at a selective disadvantage with respect to seed output and yet var. *immaculatum* is by far the commoner form in England and Wales. He suggests that differences in vegetative reproduction may account for the greater frequency, but he found very great variation between corms in this respect quite irrespective of leaf spotting.

The cline for keel colour cannot be compared directly with either of these examples, but the work by New strongly suggests that cloned plants of *L. corniculatus* should be subjected to different temperature regimes to determine whether there is a differential effect on seed production and seed viability.

In the INTRODUCTION it was explained that this work arose out of studies

of cyanogenesis in the leaves of *L. corniculatus*. It has been known for many years, however, that the petals of *L. corniculatus* may be cyanogenic (Guérin, 1929). We have found that the four phenotypes relating to cyanogenesis in leaves can be cross-classified with the same array of phenotypes in flowers. In a large group of plants growing near Birmingham (England) all 16 phenotypic combinations occur (table 10), but not at the frequencies

TABLE 10

Cyanogenesis in the leaves and flowers of a sample of Lotus corniculatus collected at Windmill Hill, Rubery (West Midlands) in 1971

Flowers	Leaves			
	++	+-	-+	--
++	434	143	76	39
+-	23	13	34	24
-+	2	7	46	20
--	3	3	18	15

$$\chi^2_{[9]} = 290.5***$$

expected from the product of the frequencies of the respective leaf and flower phenotypes. Outside the laboratory there is, unfortunately, no reliable method for testing petals for cyanogenesis. Consequently it has not been possible to test whether there is an association between cyanogenesis in flowers and keel colour in natural populations. Clearly this work needs to be done.

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