3. The model is an adequate description of some experimental data which show apostatic selection.

4. The exactitude of fit of the model to the data suggests that there is not, as often thought, a high variance in the apostatic selection imposed by individual predators.

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7. References

ALLEN, J. A., AND CLARKE, B. 1968. Evidence for apostatic selection by wild passerines. *Nature*, *220*, 501-502.

CLARKE, B. 1962. Balanced polymorphism and the diversity of sympatric species. In *Taxonomy and Geography* (ed. D. Nicholls), *Syst. Ass. Publ. No. 4.*

COOK, L. M. 1965. A note on apostasy. *Heredity*, *20*, 631-636.

FINNEY, D. J. 1964. *Statistical methods in biological assay* (2nd ed.). Griffin, London.

POPHAM, E. J. 1941. The variation in the colour of certain species of *Arctocorisa* (Hemiptera: Corixidae) and its significance. *Proc. zool. Soc. Lond. A.*, *III*, 135-172.

TINBERGEN, L. 1960. The natural control of insects in pinewoods. I. Factors influencing the intensity of predation by songbirds. *Arch. Neerl. Zool.*, *13*, 265-336.

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

III. SOME ASPECTS OF SELECTION

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1. Introduction

BUBAR AND LAWSON (1959) have shown that different varieties, strains and ecotypes of *Lotus corniculatus* L. exhibit differences in their ability to survive winter conditions in Canada. Bubar (1964, personal communication) has indicated that he has no evidence to suggest that acyanogenic plants are either more or less subject to winter injury than cyanogenic plants. This report contains the results of a formal investigation of (1) the problem of winter survival, (2) the production of seed by plants of different phenotype, and (3) the reanalysis of some data presented by Dawson (1941) and by Daday (1954).

2. Survival of mature plants

During the early summer of 1966 clones were established from 42 plants of *L. corniculatus*. In late summer the plants were arranged in the experimental field using a randomised block, spaced plant design. The original plants were grown from seed and represented the four possible phenotypes with respect to cyanogenic glucosides and β-glucosidase production and in most cases each clone was represented by 6 replicates. A grand total of 242 experimental plants were used and these were derived from 15 which

contained both glucosides and enzyme, 16 which contained neither, 6 had only the glucosides while 5 had only the enzyme. The limits on the smaller classes were imposed by the number of plants available for cloning. The original seed samples came from England and Wales on the one hand and from Denmark and Sweden on the other and the data have been partitioned as British/Scandinavian accordingly.

TABLE 1

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	0·371	23·656*
Phenotypes	3	0·431	5·484
H×P	3	0·056	3·228
Between plants	34	0·373	4·649
Within clones	200	0·109	0·024

The analysis is based on 242 plants. Forty-four plants died during the winter 1966-67, while 115 plants died during the period September 1966 to April 1968.

* Significant mean square at the 5 per cent. level for differences between habitats.

On several occasions during the winter of 1966-67 the plants were scored, but the deadline for winter survival was taken as 1st May. This date was chosen because no plant which was alive in March had died by 1st May and yet sufficient time in early summer was needed to ensure that dormant plants had adequate chance to produce shoots above ground. Nearly a dozen plants were in this category.

By 1st May, 18·2 per cent. of the plants originally put in the field had died. By the following 9th November a total of 42·5 per cent. of the plants had died while a further 5 per cent. died during the second winter.

TABLE 2

Pattern of survival of plants in relation to their source

	Died between 14.9.66 and 1.5.67	Died between 1.5.67 and 9.11.67	Died between 9.11.67 and 23.4.68	Survived to 23.4.68	Total
British	23	16	4	78	121
Scandinavian	21	43	8	49	121
Total	44	59	12	127	242

Use was made of the scheme for disproportionate numbers in an R × 2 table outlined by Snedecor and Cochran (1967) in preparing the two-way analysis of variance presented in table 1. There is no evidence of an effect of habitat or of phenotype on the ability of plants to survive the first winter. There is, however, a marked effect of the source of the plants on the overall survival from September 1966 through April 1968. Closer examination of the data (table 2) shows that 43 per cent. of the Scandinavian plants remaining in the spring of 1967 died during the following summer, while only 16·3 per cent. of the British plants failed to survive until November 1967. Phenotype, therefore, does not appear to influence the overall survival of the plants in Birmingham during the period 1966-68.

3. Seed production

During 1967 all the seed produced by the plants used in the survival experiments were collected. Care was taken to ensure that the seed was ripe before harvesting so that errors due to the stimulation of succession flowering would be minimal. No restriction was placed on the source of pollen and ripe seed was collected weekly from June through October.

Plants varied in their seed output from zero to over 18,000 and the results were analysed by the same technique as the one used for survival. For computation the seed numbers were reduced by a factor of 10^3 so this means that all the mean squares in table 3 should be multiplied by 10^6 to give their true values. The adjustment of scale makes no difference to the variance ratio test.

Table 3

Analysis of variance of seed production by Lotus corniculatus

	Plants which produced seeds		Plants alive at the end of the harvest period		All plants	
	d.f.	M.S.	d.f.	M.S.	d.f.	M.S.
Habitats	1	89·319	1	8·824	1	3·045
Phenotypes	3	50·742	3	28·334	3	23·450
H×P	3	13·955	3	16·378	3	14·186
Between plants	27	36·618	32	38·755	34	38·401
Within clones	97	5·385	133	5·053	200	4·575

See the comment on scale in the text.

Clearly not all the plants used in the survival experiments were alive to produce seeds and even some of those which were alive throughout the harvesting period produced no seeds at all. This means that there are three ways in which the data can be grouped. Firstly, we may include only those plants which produced seeds; secondly, we can consider those plants which were alive during the harvesting period, whether they produced seeds or not; and thirdly, there is the overall seed production of all 242 plants used in the experiment.

The analysis of variance in table 3 reveals that the effect of difference between plants is greater than differences between habitats or phenotypes, and even when we consider only those plants which produced seeds the mean square for habitats, although large, is not significant. This evidence suggests that the cyanogenic phenotype and the source of the plant material has little effect on seed production in the conditions under which the plants were grown. It is noticeable how consistent are the mean squares for within clones and between plants, showing that the individuals which died early and those which did not produce seed were a random sample of all the plants.

4. Developmental viability of *Lotus corniculatus*

In his paper on the inheritance of cyanogenesis in *L. corniculatus*, Dawson (1941) has listed both the number of seeds sown and the number of seeds germinated in addition to the results of reciprocal crosses. The percentage germination was high (not less than 95 per cent.), but there was considerable

mortality between germination and the age at which the plants were tested for cyanogenesis. This breeding data can be used, therefore, to examine whether there are any differences between the reciprocal crosses with respect to segregation at the glucoside locus during the maturation of the plants.

TABLE 4

A summary of the 13 crosses Aaaa × aaaa *and their reciprocals* (Data of Dawson, 1941)

	Dead	Scored		Dead	Scored
$5^{9} \times 5^{40}$	47	73	$5^{45} \times 5^{40}$	11	69
	45	35		13	67
$4^{16} \times 5^{40}$	29	51	$5^{32} \times 5^{17}$	9	71
	28	52		10	70
$5^{38} \times 5^{40}$	50	30	$4^{23} \times 5^{40}$	13	27
	35	45		7	33
$4^{18} \times 5^{34}$	24	96	$5^{32} \times 5^{40}$	12	28
	44	76		23	17
$5^{43} \times 5^{34}$	10	41	$5^{1} \times 5^{19}$	17	23
	47	113		11	29
$4^{2} \times 4^{11}$	6	74	$5^{31} \times 5^{19}$	5	35
	9	71		9	31
$4^{13} \times 4^{11}$	14	66			
	18	62			

TABLE 5

2 × 2 contingency χ^2 *analysis of reciprocal crosses between* Aaaa *and* aaaa

	d.f.	χ^2	
Between reciprocals	1	2·699	0·2 > P > 0·1
Between crosses	12	32·146	0·01 > P > 0·001

TABLE 6

The progeny of plant 5^{40} (aaaa) *when used as seed and pollen parent in crosses with plants of* Aaaa *genotype*

Other parent	5^{40} ♀			5^{40} ♂		
	Dead	Tested	Total	Dead	Tested	Total
5^{9}	45	35	80	47	73	120
4^{16}	28	52	80	29	51	80
5^{38}	35	45	80	50	30	80
5^{45}	13	67	80	11	69	80
4^{23}	7	33	40	13	27	40
5^{32}	23	17	40	12	28	40
Total	151	249	400	162	278	440
	Heterogeneity $\chi^2_5 = 42{\cdot}49$ P < 0·001			Heterogeneity $\chi^2_5 = 42{\cdot}40$ P < 0·001		

Table 4 is a summary of the 13 crosses *Aaaa* × *aaaa* and their reciprocals. When these reciprocal crosses and the pooled data are examined as 2 × 2 contingency tables the results listed in table 5 are obtained. Thus there is no significant departure from contingency in the pooled data, but the crosses

are highly heterogeneous. Analysis of χ^2 reveals that 4 of the 13 reciprocal crosses are significant. Thus in 9 of the crosses and their reciprocals there is no evidence of a consistent effect (either maternal or paternal) on survival to maturity. On the other hand, 4 matings do show a significant difference between the reciprocal crosses, but note that plant 5^{40} is the *aaaa* parent in three of these matings. The behaviour of this plant both as seed parent and pollen parent in the 6 crosses in which it was used was, however, also heterogeneous and so the progeny of plant 5^{40} did not behave in a rational way (see table 6).

TABLE 7

Analysis of χ^2 *for segregation of* A *from* a *in the reciprocal crosses* Aaaa × aaaa

	d.f.	χ^2	
Between reciprocals	1	0·062	P > 0·80
Between crosses	12	8·460	P > 0·70

Furthermore, Dawson has shown that the overall data are not heterogeneous for the segregation expected from a cross *Aaaa* × *aaaa* (table 7), and therefore it can be concluded that the differential survival from seedling to maturity is unlikely to be associated with the character of cyanogenesis, when the plants are grown under experimental conditions.

5. SEED SAMPLES FROM WILD POPULATIONS OF *Trifolium repens* AND *Lotus corniculatus*

Daday (1954) lists the phenotypes of *T. repens* plants grown from seed samples collected from various localities in Europe. This data has been reworked using angular transformation of the phenotype frequencies and not the supposed allele frequencies. The analysis of variance in table 8 reveals that the regression of the frequency of the enzymatic form on the glucosidic form is highly significant.

TABLE 8

Analysis of variance of regression of the frequency of the enzymatic form on the frequency of the glucosidic form of Trifolium repens. (Data of Daday, 1954)

	d.f.	M.S.	
Regression	1	18123·42	P < 0·001
Residual	47	83·45	

The reasons for using the phenotype frequencies will be discussed in the fourth paper of this series.

Similar data have now been obtained for *Lotus corniculatus* and they are presented in table 9. A population was sampled by collecting one pod from each of at least 20 plants. All the seeds from one population were mixed together. Samples of these seeds were sown and the phenotype frequencies in the resultant plants were determined.

The analysis of variance of regression in table 10 again shows a highly significant correlation between the frequency of the two forms of *Lotus corniculatus*. There is no doubt, therefore, that the interaction between the

enzyme and the glucosides is very similar in each species. The evidence also points to the production of HCN being an important function of the enzyme substrate system in both species.

TABLE 9

Phenotypic frequencies of plants of Lotus corniculatus *grown from seeds collected in fourteen different locations*

	Phenotype				Phenotypic frequencies	
	++	+−	−+	−−	Cyanogenic glucoside	β-glucosidase
Britain						
1 Aysgarth, Yorks	93	2	1	0	0·9895	0·9792
2 Llanidloes, Radnor	32	50	6	20	0·7592	0·3519
3 Rounds Green, Staffs.	112	3	1	1	0·9829	0·9658
4 Forest Hill, Oxon.	28	6	1	1	0·9444	0·8056
5 Blenheim Park, Oxon.	36	19	11	16	0·6707	0·5732
6 Long Hanborough, Oxon.	38	18	0	0	1·0000	0·6786
7 Newbury, Berks.	86	5	1	0	0·9891	0·9457
8 Denbies, Surrey	27	1	2	0	0·9333	0·9667
9 Kynance Cove, Cornwall	28	8	14	3	0·6792	0·7924
Sweden						
10 Eskilstuna	92	5	32	7	0·7132	0·9118
11 Sannas	0	0	6	43	0·0000	0·1224
12 Hallevikssstrand	2	17	8	192	0·0868	0·0457
13 Orust	0	0	5	62	0·0000	0·0746
Denmark						
14 Åbenrå	0	0	4	29	0·0000	0·1212

++ glucoside and enzyme; +− glucoside, no enzyme; −+ enzyme, no glucoside; −− neither glucoside nor enzyme. Percentage germination in excess of 95 per cent.

TABLE 10

Analysis of variance of regression of the frequency of the enzymatic form on the frequency of the glucosidic form of Lotus corniculatus

	d.f.	M.S.	
Regression	1	6914·73	$P < 0{\cdot}001$
Residual	12	158·12	—

Data in table 9.

6. DISCUSSION

Cyanogenesis in *Trifolium repens* and *Lotus corniculatus* is determined by the action of a β-glucosidase on two cyanogenic glucosides, linamarin and lotaustralin. It appears that the biochemical systems are very similar in the two species (Butler, 1965), but study of the enzymes is still at an early stage (Hughes, 1968). Evidence has been presented by Jones (1966) that the selective eating of the acyanogenic form of *L. corniculatus* by certain molluscs is likely to be important in the dynamics of the polymorphism and he suggested that selective eating might be important in *T. repens* also. On the other hand, Bishop and Korn (1969) obtained no evidence of selective

eating of *T. repens* by *Agriolimax reticulatus* Müller and *Helix aspersa* Müller in their elegant experiments even though mention has been made on several occasions that selective eating may be important in this species (Corkill, 1952; Daday, 1955; W. Ellis Davies, 1967, personal communication).

Previously, Daday (1965) was able to demonstrate by direct experimentation that the acyanogenic form of *T. repens* was at a selective disadvantage in a coastal environment in New South Wales (January mean temperature 22° C., July mean temperature 13° C.) whereas the cyanogenic form was a disadvantage in an alpine environment in N.S.W. where the January mean temperature is 12° C. and the July mean temperature −2° C. He suggests that it is not the cyanogenic character *per se* which is responsible for the advantage of such plants at high winter mean temperature, but that the locus concerned with cyanogenic glucoside production is genetically linked to genes concerned with fitness responses to temperature. His evidence, that plants which contain cyanogenic glucosides but no enzyme (phenotypically acyanogenic) are as fit as acyanogenic plants, supports this hypothesis. Obviously the suggestion could be proved or disproved quite simply by the appropriate breeding programme.

The work reported earlier in this paper suggests that the cyanogenic phenotype has little or no effect on survival by *L. corniculatus* of either winter or summer conditions in England.

On the surface, therefore, it appears that the selective agents are different in the two species and yet the polymorphisms of cyanogenesis are essentially the same. This is not to say that selective eating of *T. repens* and effects of temperature on *L. corniculatus* are always unimportant; it merely indicates that these selective agents are relatively trivial in the experiments which have been carried out so far.

If winter temperature is the sole or major selective agent in *T. repens* it is difficult to understand why the populations remain polymorphic. It is more likely that the selection is cyclical. For example, in habitats where the mean temperature is high in summer cyanogenic plants have an advantage because of physiological superiority and perhaps lack of predation. In winter, on the other hand, the acyanogenic form may be at an advantage because of frost resistance. In regions where the summer temperature is low, the selective advantage associated with the cyanogenic form could well be lower than in the warmer regions and so the net effect of the cyclical selection may favour the acyanogenic forms.

From the evidence presented earlier this explanation is unlikely to hold for *L. corniculatus*. Survival of winter and summer does not apparently depend upon the cyanogenic phenotype nor yet on genes genetically linked to the glucoside locus. In the fourth paper of this series (Jones, in preparation) evidence will be presented showing that the distribution of the cyanogenic forms of *L. corniculatus* in Europe does not parallel that of *T. repens*, although there are similarities. This result also suggests that winter temperature is of much less importance in determining the distribution of the cyanogenic form of *L. corniculatus*.

The apparent paradox in the response of the various cyanogenic and acyanogenic forms of the two species to selection by temperature is, therefore, simply resolved. In *L. corniculatus*, the genes concerned with temperature response are not genetically linked to the glucoside locus while in *T. repens* they are. In *T. repens* there is, consequently, a correlated response of the

glucoside locus to selection and this is reflected in the frequency distribution of the cyanogenic form. Why there should be this difference in genetic architecture between the two species is a problem which requires further investigation. Whatever explanation there is for these differences it must also accommodate the interactions between the species which were reported previously (Jones, 1968).

As far as the selective eating of the acyanogenic form of *L. corniculatus* is concerned it is now necessary to determine the distribution and abundance of molluscs in relation to the distribution of cyanogenic plants. This should reveal whether selective eating is important or trivial in natural populations. Further population studies and selection experiments are in progress.

In both *T. repens* and *L. corniculatus* there is a high correlation between the frequency of plants containing the cyanogenic glucosides and the frequency of plants containing the β-glucosidase. HCN is not evolved by plants containing both the glucosides and the enzyme unless the plant is damaged, in which case the production of HCN begins immediately (Corkill, 1942). There can be little doubt, therefore, that the production of HCN is a response to plant damage and the HCN, being highly toxic, could be a device for preventing further damage by grazing animals. This does not in any way rule out the probability that individuals or species may be partly or wholly resistant to HCN in the quantities evolved by these plants.

7. Summary

1. The character of cyanogenesis appears to play little or no part in determining whether mature *Lotus corniculatus* plants survive either winter or summer conditions.

2. Scandinavian plants appear to be less suited to summer conditions in England than do British plants.

3. There is no difference between cyanogenic and acyanogenic plants in their ability to survive from seed to maturity under horticultural management.

4. A strong correlation between the frequency of the allele determining cyanogenic glucosides and the allele determining the β-glucosidase has been demonstrated for populations of *Lotus corniculatus*.

5. The role of temperature in the maintenance of the polymorphism of cyanogenesis in *Trifolium repens* and *Lotus corniculatus* is discussed and an explanation of the differences observed is suggested.

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8. References

BISHOP, J. A., AND KORN, M. E. 1969. Natural selection and cyanogenesis in white clover, *Trifolium repens* L. *Heredity, 24*, 423-430.

BUBAR, J. S., AND LAWSON, N. G. 1959. Note on inheritance of ability to survive winter-killing conditions in Birdsfoot Trefoil. *Canad. J. Pl. Sci., 39*, 125-126.

BUTLER, G. W. 1965. The distribution of the cyanoglucosides linamarin or lotaustralin in higher plants. *Phytochemistry, 4*, 127-131.

CORKILL, L. 1942. Cyanogenesis in white clover (*Trifolium repens* L.). V. The inheritance of cyanogenesis. *N.Z. J. Sci. Tech., 23 B*, 178-193.

CORKILL, L. 1952. Cyanogenesis in white clover (*Trifolium repens* L.). VI. Experiments with high glucoside and glucoside free strains. *N.Z. J. Sci. Tech.*, *34A*, 1-16.
DADAY, H. 1954. Gene frequencies in wild populations of *Trifolium repens* L. 1. Distribution by latitude. *Heredity*, *8*, 61-78.
DADAY, H. 1955. Cyanogenesis in strains of white clover (*Trifolium repens* L.). *J. Brit. Grassland Soc.*, *10*, 266-274.
DADAY, H. 1965. Gene frequencies in wild populations of *Trifolium repens* L. IV. Mechanism of natural selection. *Heredity*, *20*, 355-365.
DAWSON, C. D. R. 1941. Tetrasomic inheritance in *Lotus corniculatus* L. *J. Genetics*, *42*, 49-72.
HUGHES, M. A. 1968. Studies on the β-glucosidase system of *Trifolium repens* L. *J. Exp. Bot.*, *19*, 427-434.
JONES, D. A. 1966. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. Selection by animals. *Canad. J. Genet. Cytol.*, *8*, 556-567.
JONES, D. A. 1968. On the polymorphism of cyanogenesis in *Lotus corniculatus* L. II. The interaction with *Trifolium repens* L. *Heredity*, *23*, 453-455.
SNEDECOR, G. W., AND COCHRAN, W. G. 1967. *Statistical Methods*. 6th Edition, Iowa State University Press, Ames.

LIGHT AND TEMPERATURE EFFECTS ON THE GERMINATION OF WILD OATS

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1. INTRODUCTION

Avena fatua L. and *Avena ludoviciana* Dur. are the wild oats of English agriculture. *A. ludoviciana* tends to occur on heavy land where winter cereals are grown, and the caryopses (seed or grain) of this species germinate mainly in autumn. *A. fatua*, on the other hand, has a wider distribution and is particularly associated with spring cereals (Thurston, 1954); the grain of this species show some germination in autumn but a considerably greater frequency of germination occurs in spring (Thurston, 1951). Reports indicate considerable variation in the degree and periodicity of dormancy both between and within samples of *A. fatua* from different localities (Toole and Coffman, 1940; Sexsmith, 1967).

The genetic control of dormancy in oats has been considered by Garber and Quisenberry (1923), who found that delayed germination was recessive in crosses involving *A. fatua* and *A. sativa*. Johnson (1935*b*), using similar material, suggested that the observed variation could be explained on a three-locus basis in which the triple recessive genotype showed greatest dormancy.

Purely genetic models invoked to explain germination characteristics ignore the very marked effects of environmental factors during grain development. Thus, both the position in the spikelet at which the seed develops and the storage conditions markedly affect germination behaviour (Johnson, 1935*a*; Kommedahl, DeVay and Christensen, 1958; Thurston, 1963*a*). Environmental effects also cause variation during germination (Black and Naylor, 1957). Cumming and Hay (1958) found an inhibitory effect of