

## THE BIOLOGY OF THE LEAF MARK POLYMORPHISM IN *TRIFOLIUM REPENS* L.

### 1. DISTRIBUTION OF PHENOTYPES AT A LOCAL SCALE

M. G. CAHN\* and J. L. HARPER

*School of Plant Biology, University College of North Wales, Bangor, Gwynedd*

Received 18.xi.75

#### SUMMARY

The distribution of the white leaf mark polymorphism of *Trifolium repens* L. was studied in a lowland pasture in North Wales and in a series of 1 m<sup>2</sup> quadrats elsewhere in Britain. Each quadrat was subdivided into 10 × 10 cm quadrats to allow the study of pattern. The number of morphs found in a 10 cm square was in the range 1.5-6.0 with a mean value of 4.16. In a 5-year-old seeded ley the mean was 2.75. The populations consist of a large number of morphs intimately mixed. The frequency distribution of morphs per quadrat differs strikingly from the frequency distribution of species suggesting that some selective force operates to prevent any morphs from dominating the sward even on a local scale. The frequency of morphs bearing leaf marks decreased with increasing grass length suggesting a relationship with grazing. There is a seasonal pattern in the expression of the leaf mark phenotypes and the floristic diversity of such a pasture is therefore represented at three levels, (a) the species present, (b) the genotypes present within each species and (c) the various phenotypic expressions of these genotypes.

#### 1. INTRODUCTION

WITHIN plant populations diversity appears at three levels, (a) the diversity of species present in the area, (b) the genetic diversity within each species and (c) phenotypic diversity represented by ages and plasticity of the genotypes present. Populations of white clover (*Trifolium repens*) are characteristically polymorphic for white leaf marks. The white marks appear as bands on the laminae as a result of air spaces within the palisade tissue. Two linked groups of genes control leaf marking in *T. repens* (Corkill, 1971); the white leaf marks are controlled by multiple alleles at a locus in one of these groups (Brewbaker, 1955; Carnahan *et al.*, 1955). Seven major alleles are commonly present in natural populations and genotypes can often be identified by the specific pattern of the phenotypic white leaf mark provided that this is fully expressed (see table 1). These leaf marks, together with other leaf characters have been used to identify clones of *Trifolium repens* in the field (Harberd, 1963).

In a study of this polymorphism over the natural range of the species (partly based on herbarium material) Pusey (1965) found that the frequencies of the alleles showed gentle clines, the major one being from north Scotland to Afghanistan. However, the existence of clines does not explain the existence of polymorphism. It is doubtful indeed if any extensive survey

\* Present address: c/o County Planning Officer, West Glamorgan County Council, Planning Department, 12 Orchard Street, Swansea, West Glamorgan.

procedure can reveal the nature of the real factors that balance the survivorship of morphs in a balanced polymorphic system particularly in perennial species. More detailed analysis, with or without perturbation, may be needed to isolate the real events that determine the life and death of individuals.

TABLE 1  
*Classification of leaf mark used in this paper*

Leaf mark	Abbreviation used
(1) Unmarked	O
(2) Marked	
(a) Simple mark	L
(b) Point*	L
(c) Double simple mark	LL
(d) Broken Yellow	Y
(e) Broken Yellow/Simple mark	YL
(f) Basal	Ba
(g) Basal/Simple mark	BaL
(h) Filled	F
(i) Filled/Simple mark	FL
(j) Filled/Broken Yellow mark	FY
(k) Broken	B
(l) Distal	X

\* The "point" phenotype has been aggregated with the "simple mark" phenotype since simple marks frequently appear identical to "point" marks in the field.

This paper describes the intra-specific genetic diversity of some populations of *Trifolium repens* set in the context of the diversity of species and the phenotypic variation within a localised area.

## 2. MATERIALS AND METHODS

Most of the studies reported in this paper were made on a field of permanent grassland at Henfaes, Aber, Gwynedd in N. Wales. The history and management of this pasture have been described in Sarukhán and Harper (1973). Some additional surveys were made on permanent grasslands in other parts of Britain for comparison with the Henfaes pasture.

### (i) *Toe-point survey*

A survey was made to discover what variation in the frequency of phenotypes occurred within the field at Henfaes and to determine if this was correlated with grass length or grazing intensity. Samples were taken every three paces across the field and every five paces along the field. The results were then grouped into areas (Cahn, 1976) which are described in table 2. Sampling (which followed the procedure of Pusey, 1965) involved finding the clover leaf nearest to a mark on the shoe and this was determined at each sample point. If there was no clover leaf within  $7\frac{1}{2}$  cm radius no sample was taken.

Stolons of marked plants occasionally produced unmarked leaves so, when a phenotypically unmarked leaf was found, the stolon was followed backwards

and the other leaves checked for the presence of a mark. The field had recently been grazed by sheep and cattle. The grass length at each sample point was recorded on a 4 point scale: 1, well grazed < 7.5 cm; 2, lightly grazed 7.5-15.0 cm; 3, ungrazed (more than 15 cm); 4, within 15 cm of a thistle (*Cirsium arvense*).

TABLE 2  
*Characters of areas in Henfaes field, Aber, Gwynedd*

Area	Character	Topography
A	Short grass	Level
B	Short grass	Level, dips near railway
C	Long grass + <i>Cirsium arvense</i>	Level
D	Long grass + <i>Cirsium arvense</i>	Level
E	Short grass	Level
F	Short grass	Dips near stone
G	Long grass + <i>Cirsium arvense</i>	Level
H	Long grass + <i>Cirsium arvense</i>	Level
J	Short grass	Level
K	Intermediate	Ridge
L	Intermediate-Short	Hollow
M	Long grass	Level

(ii) *Detailed mapping of permanent quadrats*

The distribution of clover morphs was studied in 12 permanent 1 m sq quadrats (A1-G1, A2-E2) in the field between August 1971 and August 1972, and an analysis of the floristics of these quadrats was made in 1973. Five of the 12 quadrats were sited in lightly grazed and seven in heavily

TABLE 3  
*Sites of mapping quadrats*

Site	No. of quadrats (1 m <sup>2</sup> )	Quadrat titles	Habitat/management	Last cultivated	No. of clones/10 cm <sup>2</sup>
Cae Cwrtiau, Aber	2	CC1-2	Ley	1968	2.75
Lugg Meadows Hereford	2	LM1-2	Common land. Grazed Sept.-Dec. Cut of hay June-July	Before 1750	1.61
Walland Marsh, Rye, Sussex	3	R1-3	Paddock on sheep farm. Intermittent grazing	Never in living memory	2.81
Port Meadow, Oxford	4	PM1-4	Common land. Grazed water meadow	Before 1086	3.54
Henfaes, Aber, Gwynedd	12	A1-G1 A2-E2	Paddock. Intermittent grazing	Never in memory	4.16

grazed areas of the field. At each site four pieces of steel electric conduit tubing (1.9 × 30 cm) were sunk vertically into the ground. These allowed accurate placement of a large quadrat (1.20 × 1.20 m) which had, at its corners, supports made of pieces of conduit tube (1.6 × 40 cm) which fitted tightly into the buried tubes. The quadrats were removed after each mapping operation and the sunken tubes marked with pieces of coloured

TABLE 4a  
*The number of leaves of different leaf mark phenotypes collected in the initial survey at Henfaes, Aber, Gwynedd, and grouped according to position in the field*

Area	Leaf mark phenotypes													Total (rare mks)	(1) $\chi^2_1$	(2) $\chi^2_2$
	L	O	LL	Y	YL	Ba	BaL	F	FL	FY	B	Total				
A	95	23	4	7	2	1	1	1	—	—	—	134	25.90***	17.48***		
B	91	33	1	3	—	1	1	—	1	—	—	131	4.23	4.37*		
C	98	40	—	2	1	1	—	—	—	—	—	142	2.50	1.94		
D	71	60	1	3	—	—	—	—	—	—	1	136	7.71**	7.71**		
E	85	45	—	1	1	—	—	—	—	1	—	133	0.79	0.01		
F	65	48	1	5	1	—	—	—	—	—	—	120	3.68	2.55		
G	72	50	1	2	—	1	—	—	—	—	—	126	2.76	2.43		
H	56	48	—	2	—	3	2	1	—	—	—	112	10.54**	4.84		
J	79	44	2	2	—	—	—	—	—	—	—	127	1.70	0.10		
K	58	42	—	2	—	—	—	—	—	—	—	102	3.46	2.97		
L	86	27	3	—	—	—	—	—	—	—	—	116	12.24**	5.81*		
M	65	37	2	—	2	—	—	2	—	—	—	108	0.03	0.03		
Total (all areas)	921	497	15	29	7	7	4	2	3	1	1	1487		$\chi^2_{23} = 69.25***$		

(1) Refers to Simple mark:0:other phenotypes ratio.

(2) Refers to Total marked:0 ratio.

marine twine. Each quadrat was subdivided into 100 10 × 10 cm sub-quadrats. The technique allowed accurate re-siting of the quadrats at each time of observation and permitted repeated study of the same sites without seriously disturbing the vegetation of the quadrat: nothing except the marking string protruded above the ground that might affect the behaviour of grazing animals. The marking string disappeared on occasions and the buried tubes were then relocated with a metal detector. For accurate mapping of individual plants a small 10 cm sq quadrat was suspended from the large quadrat.

The number of morphs present was recorded in each of the 10 cm<sup>2</sup> quadrats. The density of clover was recorded on a three-point scale and records were also made of flowers, seedlings and any obvious signs of disease.

(iii) *Analysis of the distribution of the leaf morphs present in five grassland sites in other parts of England and Wales*

During late June 1973 the pattern of distribution of leaf mark morphs was studied in a variety of grassland fields listed in table 3 in order to set the results found at Henfaes in a wider context.

(iv) *The growth of plants of different leaf mark phenotypes in a cool greenhouse*

Core soil samples 2.5 cm diameter were collected in November 1971 from the corners of each of the 12 permanent quadrats in the field at Henfaes. The stolons of *T. repens* in the vegetation covering the core were propagated in a heated greenhouse and classified into leaf mark phenotypes.

### 3. RESULTS

(i) *Toe-point survey-*

The results of this survey are given in tables 4 and 5. Chi-squared values were determined from 2 × n contingency tables. The proportions of the

TABLE 4b  
*Leaf mark phenotypes in long and short grass areas at Henfaes, Aber, Gwynedd*

	L	LL	Y & YL	Other marks O	
Long grass areas (C, D, G, H, K, M)	409 (449.66)	11 (7.32)	22 (17.58)	7 (8.79)	277 (242.65) 726
Short grass areas (A, B, E, F, J, L)	512 (471.34)	4 (7.68)	14 (18.42)	11 (9.21)	220 (254.35) 761
Total	921	15	36	18	497 1487

$$\chi_4^2 = 23.18^{***} \text{ (expected numbers in brackets).}$$

different morphs present differed significantly in different areas of the field—largely because of local high frequencies of the rarer phenotypes (table 4a). This indicates that the leaf mark phenotypes scored are not distributed at random in the sample area. When all those leaves bearing marks were grouped together and the ratio of unmarked leaves was considered, significant differences also appeared between positions in the field (table 4a, column 2).

TABLE 5a  
*The relationship between leaf mark phenotype and grazing régime at Henfaes (areas within field)*  
 Grazing régime (i.e. length of grass at sample points)

Area	1 (<7.5 cm)			2 (7.5-15.0 cm)			3 (> 15.0 cm)			4 (within 15 cm of <i>Citrium arvense</i> )		
	Marked	Unmarked	$\chi^2$	Marked	Unmarked	$\chi^2$	Marked	Unmarked	$\chi^2$	Marked	Unmarked	$\chi^2$
A	58 (52.3)	13 (18.7)	2.73	25 (18.8)	5 (11.2)	5.75*	2	1	—	1	1	—
B	70 (63.3)	16 (22.7)	3.18	18 (16.9)	9 (10.1)	0.20	5	4	—	5	4	—
E	71 (75.8)	32 (27.2)	1.44	11 (12.6)	9 (7.4)	0.55	1	1	—	5	2	—
F	18 (19.9)	9 (7.1)	0.72	26 (32.7)	26 (19.3)	4.10*	27 (22.1)	12 (16.9)	3.01	1	1	—
J	42 (40.5)	13 (14.5)	0.25	27 (28.9)	19 (17.1)	0.36	4 (6.8)	8 (5.2)	2.75	10 (8.9)	4 (5.1)	0.39
L	34 (31.7)	9 (11.3)	0.70	44 (32.7)	8 (19.3)	11.83***	8 (9.6)	9 (7.4)	0.56	3	1	—
C	21 (21.3)	8 (7.7)	0.03	27 (21.4)	7 (12.6)	4.31*	22 (17.0)	8 (13.0)	3.90*	32 (31.2)	17 (17.8)	0.09
D	1	5	—	16 (18.8)	14 (11.2)	1.24	26 (30.0)	27 (23.0)	1.50	29 (29.9)	18 (17.1)	0.11
G	12 (17.6)	12 (6.4)	6.99**	33 (36.4)	25 (21.6)	0.98	19 (15.3)	8 (11.7)	2.35	12 (0.8)	5 (6.2)	0.39
H	19 (20.6)	9 (7.4)	0.50	24 (27.6)	20 (16.4)	1.42	13 (15.3)	14 (11.7)	0.87	7	6	—
K	27 (26.5)	9 (9.5)	0.04	29 (31.4)	21 (18.6)	0.57	4 (8.5)	11 (6.5)	5.78*	0	1	—
M	29 (28.0)	9 (10.0)	0.15	26 (27.6)	18 (16.4)	0.29	16 (14.7)	10 (11.3)	0.29	0	0	—
Whole field	402	144	17.32+	306	181	28.15+***	147	113	16.83+	105	60	1.39+

Total contingency  $\chi^2$  of Areas  $\times$  Marked/Unmarked with degrees of freedom as follows: grazing régime 1 = 10; grazing régime 2 = 11; grazing régime 3 = 9; grazing régime 4 = 4. For all others,  $\chi^2$  degrees of freedom = 1.

TABLE 5b

*The relationship between leaf mark phenotype and grazing at Henfaes (whole field)*

Grazing régime	Marked	Unmarked	$\chi^2$	} Total contingency $\chi^2$ (3 d.f.) = 27***
1	402 (359.5)	144 (186.5)	23.51***	
2	306 (320.7)	181 (166.3)	2.95	
3	147 (171.2)	113 (88.8)	12.18**	
4	105 (108.6)	60 (56.4)	0.43	

Expected values in brackets.

TABLE 5c

*The relationship between morph frequency and the nature of the area at different localised grazing intensities (expected values in brackets)*

Character of the area	Grazing régime ( <i>i.e.</i> length of grass at sample point)							
	1		2		3		4	
	Marked	Unmarked	M	U	M	U	M	U
Short grass sites (A, B, E, F, J, L)	293 (283.5)	92 (101.5)	151 (142.6)	76 (84.4)	47 (46.4)	35 (35.6)	25 (24.2)	13 (13.8)
Long grass sites (C, D, G, H, K, M)	109 (118.5)	52 (42.5)	155 (163.4)	105 (96.6)	100 (100.6)	78 (77.4)	80 (80.8)	47 (46.2)
$\chi^2$ (1 d.f.)	$\chi^2 = 4.13^*$		$\chi^2 = 2.49$		$\chi^2 = 0.04$		$\chi^2 = 0.10$	

Totals for all grazing régimes

	Marked	Unmarked
Short grass sites	449 (483.4)	277 (242.6)
Long grass sites	541 (506.7)	220 (254.3)

 $\chi^2$  (1 d.f.) = 14.27\*\*\*.\* =  $P < 0.05$ .\*\* =  $P < 0.01$ \*\*\* =  $P < 0.001$ .

When the long grass areas (A, B, E, F, J, L) and short grass areas (C, D, G, H, K, M) were summed separately, there was clear evidence ( $P < 0.001$ ) of a significant difference between the two groups. The significant chi-squared value in table 4b largely represents an excess of unmarked leaves in long grass areas and of marked leaves in the shorter grass.

At each toe sample the height of grass had been determined in four categories. The frequency of marked and unmarked leaves could therefore be compared with the grass lengths (table 5). The ratio of marked to unmarked leaves (values summed over areas) changed from 2.79 in grass less than 7.5 cm high, to 1.69 in grass 7.5-15.0 cm high and to 1.30 in grass more than 15 cm high. The ratio close to thistles, which tend to protect against grazing, was 1.65.

(ii) *Detailed maps of permanent quadrats*

The preparation of detailed maps of morphs was complicated by the frequent failure of leaves to express their mark phenotype. Unmarked clones

were recorded in a high proportion of the quadrats. The clarity of marks varies through the year—they are clearest between April and July and least expressed in December and January. An estimate of the seasonal variation in expression was obtained by comparing the frequency with which phenotypes were recorded through the year within the 10 cm<sup>2</sup> sub-quadrats of a 1 m sq quadrat. These values are shown for an annual cycle in fig. 1. The maximum number of clones was usually distinguishable in May, June or July followed by a sudden decline to a lower figure. A comparable cycle in the expression of the leaf marks occurred when plants were grown in a cool

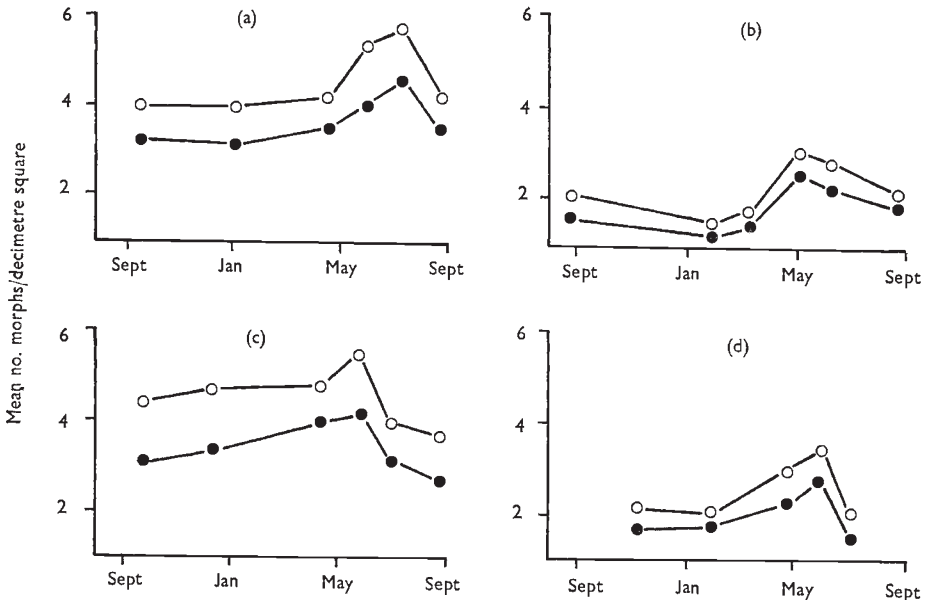


FIG. 1.—Seasonal variation in the mean number of phenotypes recognisable per 100 cm<sup>2</sup> quadrat at Henfaes, Aber, Gwynedd. ○ = maximum estimate; ● = minimum estimate (see text).

greenhouse. Not all the phenotypes followed a similar cycle in the field—different morphs varied in the time of year at which they were clearest and most readily recognised (fig. 2).

The periodicity of expression of leaf marks introduced some problems in identifying clones and two sets of results were therefore produced—maximum and minimum estimates. In the maximum estimate all the phenotypes that might represent separate morphs were counted separately. In the minimum estimate only those phenotypes were counted for which there was indubitable evidence (difference in leaf mark) that they belonged to separate clones. The difference between the two estimates was greater at Henfaes than at the other sites.

An estimate was made of the number of morphs present in different sizes of quadrat. The resulting morph-area curves are plotted in fig. 3 (see Cahn, 1976). The accuracy with which clones had been identified in the field was checked against the identification of clones from the soil cores which had been propagated in the greenhouse. Wholly accurate identification of these





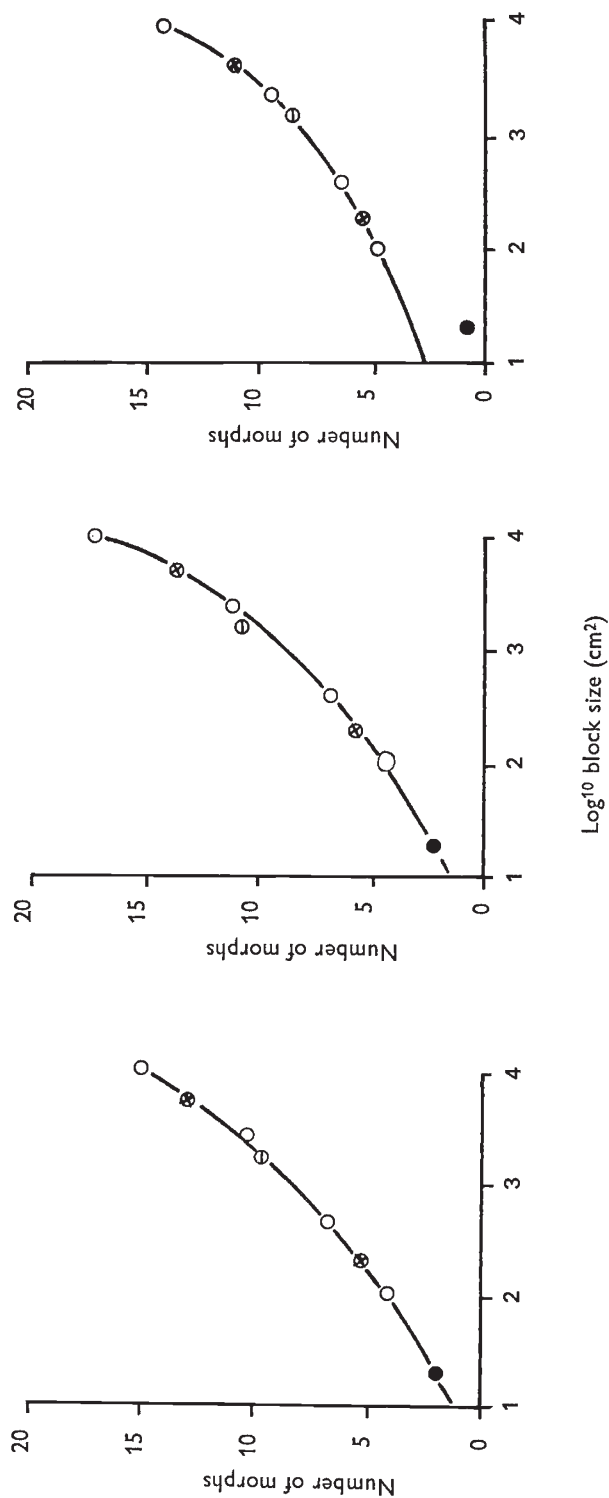


FIG. 3.—The relationship between block size and the number of morphs present at sites at Henfaes, Aber, Gwynedd. ○ = square quadrats; ⊗ = 2 × 1 rectangular quadrats; ● = soil core samples; ⊖ = square quadrats—samples within 1 m<sup>2</sup> quadrats.

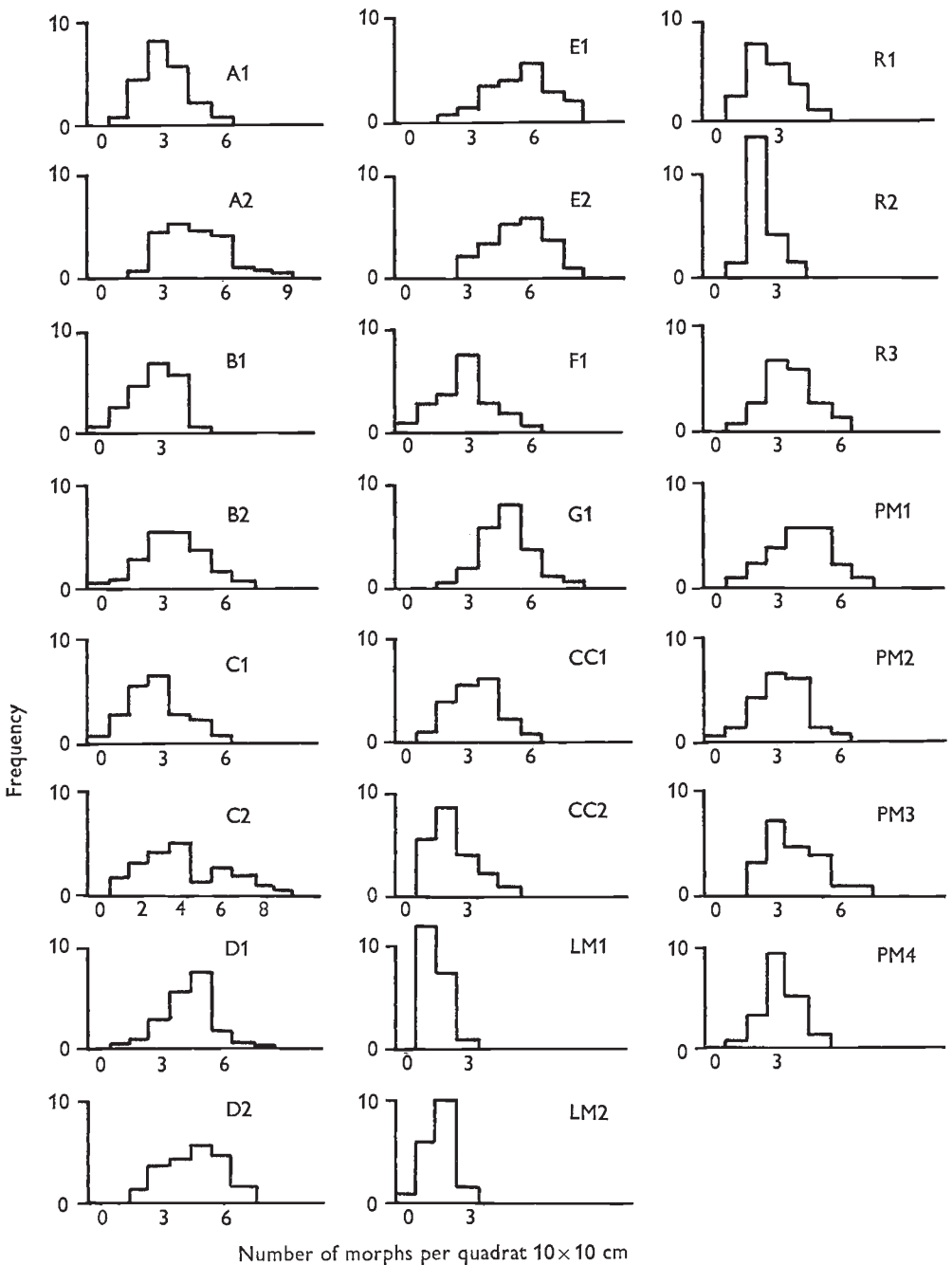


FIG. 4.—The frequencies of 10 cm<sup>2</sup> quadrats containing given numbers of morphs. A-G, Henfaes, Aber, Gwynedd. CC, Cae Cwrtiau, Aber, Gwynedd. LM, Lugg Meadow, Hereford. R, Walland Marsh, Rye, Sussex. PM, Port Meadow, Oxford.

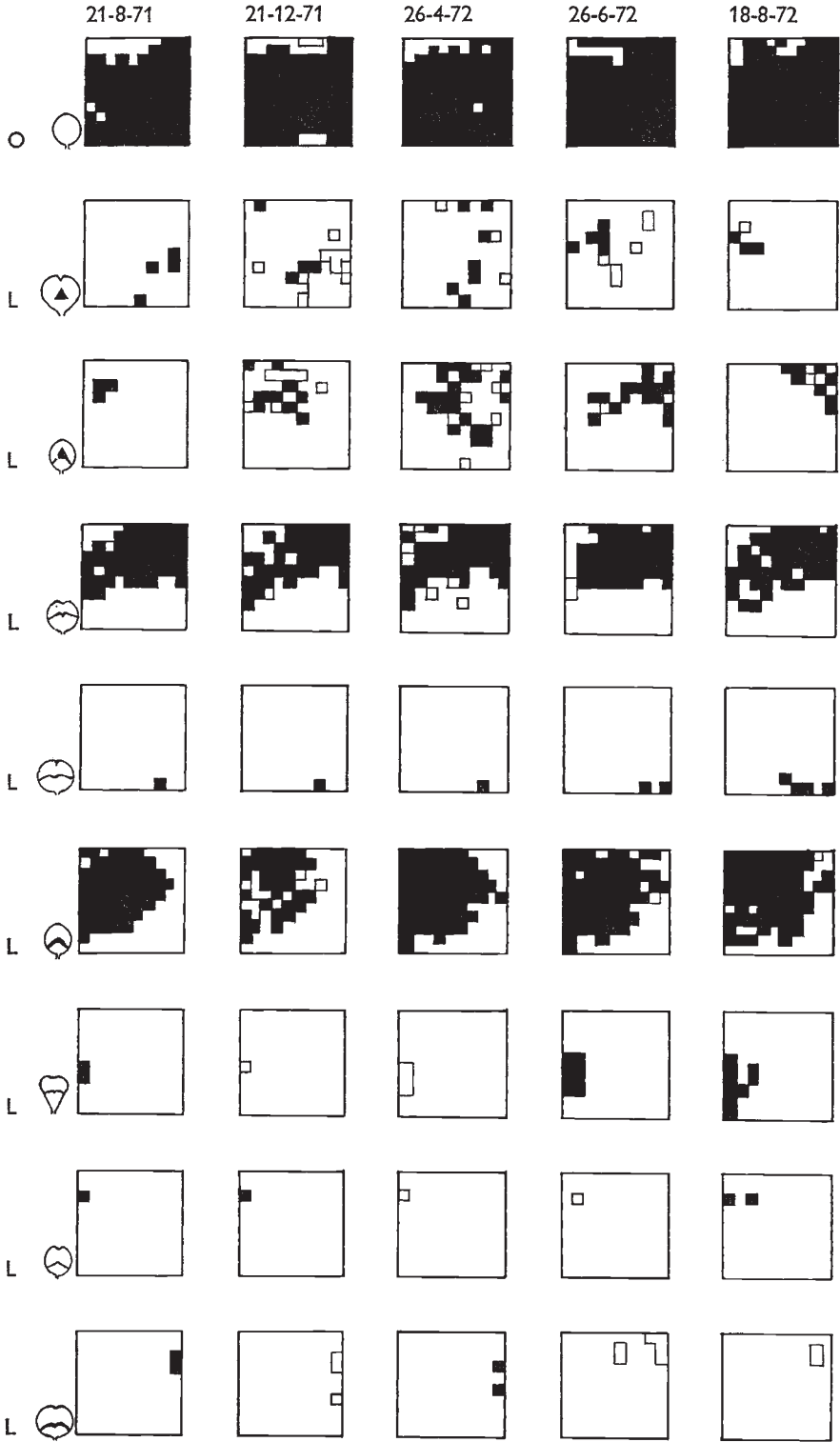




FIG. 5.—Maps of the distribution of clover leaf marks at site A1, Henfaes, Aber, Gwynedd indicating the distribution of each morph on the dates shown. Uncertain classifications are indicated by open squares. A picture of a leaflet of each morph is given to the left together with the corresponding phenotype abbreviation from table 1.

clover-patches in areas of otherwise low clover density—it is likely that clover density was low in the core samples.

The frequency distribution of morphs in the hundred 10 cm<sup>2</sup> sub-quadrats within each quadrat is shown in fig. 4. A surprisingly high number of recognisable morphs was present within the sub-quadrats. The frequency distributions of morphs per sub-quadrat have (with the exception of C2) a single mode, suggesting that there is reasonable homogeneity in leaf mark interspersion. C2 was unusual in that it contained a markedly high density of clover and intensity of grazing in one corner of the quadrat.

No obvious differences could be detected in the growth patterns of the different morphs in the field. Some of the individual clones were very extensive and presumably very old. One distinctive clone was present at two quadrats, E1 and E2, 10 m apart. This compares with a clonal spread of 18 m reported for *T. repens* growing on sand dunes (Harberd, 1963.) Some of the clones were very disjunct in their distribution while some occupied a restricted area with well-defined edges. However, the clonal spread of individual clones of the rare morphs (Broken Yellow (Y), Filled (F) and

Basal (Ba)) was significantly smaller than that of the common ones (unmarked (O) and a simple mark (L)) (Cahn, 1976).

Maps of the clones found at one site in Henfaes field are given in fig. 5.\* The size and pattern of distribution of the clones was similar in the different areas (Cahn, 1976).

#### 4. DISCUSSION

Plants that spread clonally have potentially infinite life and may be expected to prolong the process of competitive elimination of the unfit. Within a relatively stable community composed largely of clonally spreading species, the number of genotypes present might be expected to decline

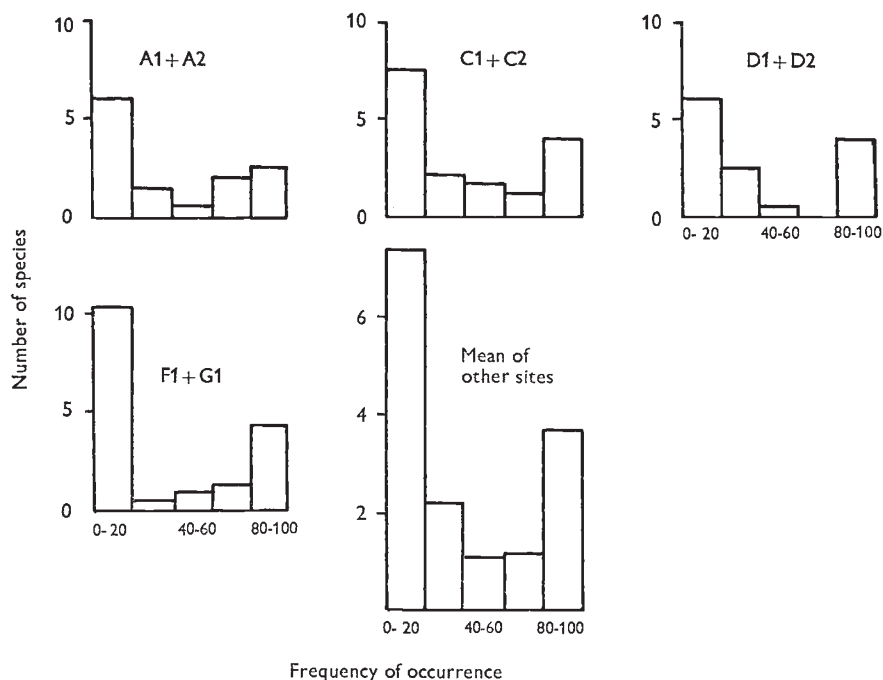


FIG. 6.—Raunkiaer diagrams for species at the various sites at Henfaes, Aber, Gwynedd. The horizontal axis is the percentage frequency in 1 m<sup>2</sup> quadrats (20 per cent intervals).

continuously as the community ages. This has been shown clearly for grasses in sown pastures (Charles, 1964) and in an experimental study of populations of *Lolium perenne* (Kays and Harper, 1974) while Charles (1968) has shown that this occurs with *T. repens* in the establishment phase of a grass-clover ley. In permanent grassland the input of new genotypes through seedling establishment appears to be extremely rare for many perennial species, e.g. *Rumex acetosa* and *R. acetosella* (Putwain and Harper, 1968), *Plantago* spp. (Sagar and Harper, 1960). Within the studied quadrats only six seedlings of *T. repens*

\* A limited number of copies of maps of the clones found at all the sites studied are obtainable from M. G. C.

were observed and these all died before forming established plants. Establishment of new plants of *T. repens* from seed was only observed to occur on mole hills. These represent small local uninhabited islands for new invasions.

In the absence of frequent input of new genotypes, local dominance by a few successful clones might be expected and this is indeed the situation found

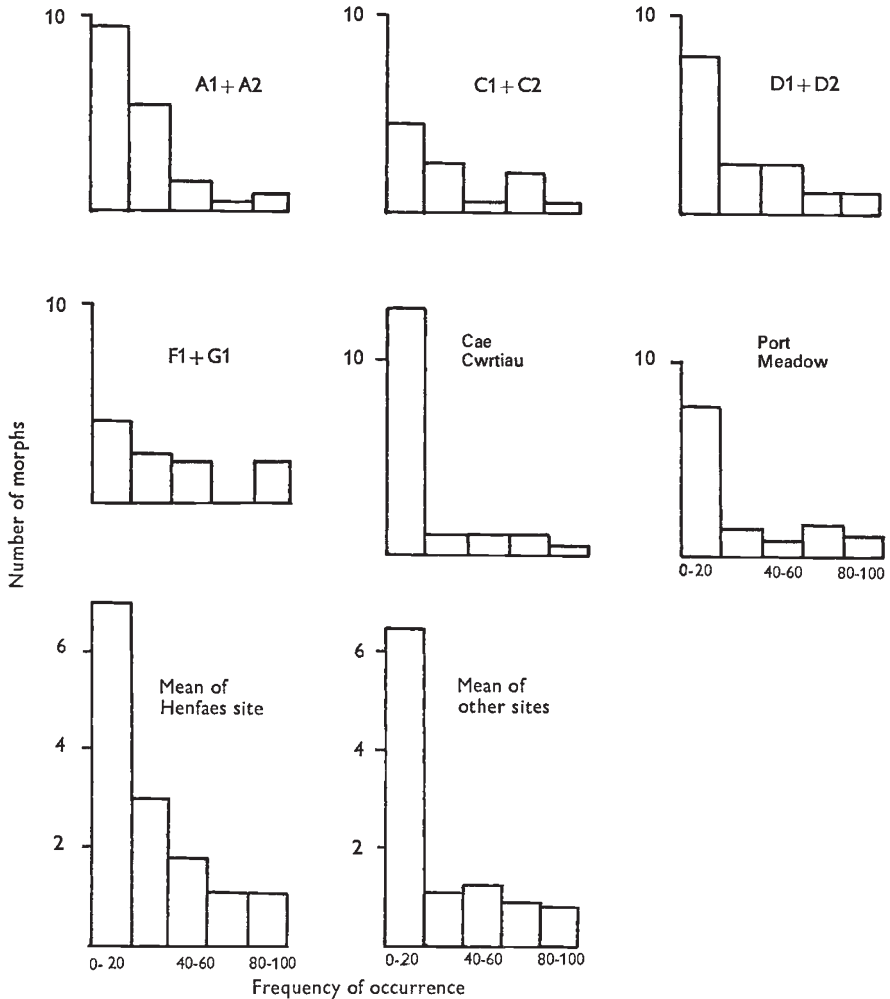


FIG. 7.—Raunkiaer diagrams for leaf mark morphs of *T. repens*, at Henfaes, Aber, Gwynedd. The same 1 m<sup>2</sup> quadrats were used for Henfaes sites as in fig. 6. The minimum estimates of morphs are used (see text).

with some other species e.g. *Erica* spp. (Webb, 1954, 1958), *Festuca ovina* (Harberd, 1962) and *Holcus mollis* (Harberd, 1967). Populations of *Lysimachia nummularium* in Britain are believed to be derived from a very few incompatible clones (G. E. Marks, pers. comm.).

However, there is evidence that in some fertile lowland pastures a high diversity of clones may be maintained in some species. At Henfaes at the

time of maximum mark clarity we found a mean frequency of 4.16 morphs per 10 cm<sup>2</sup> and a mean frequency of 2.75 at Cae Cwrtiau, a 5-year-old clover/rye grass ley (table 3). Harberd and Owen (1969) found an average of 5.2 clones of *Festuca rubra* per 15 × 15 cm quadrat in a water meadow at Barden Bridge, Yorks. Such a level of diversity is comparable to that reported for *T. repens* in the present paper. Pusey (1965) had found 1.99 clones of *T. repens* per 10 × 10 cm quadrat in a 3.0 × 1.2 m area of pasture near Oxford. There is, however, an important difference between the situation in *Festuca rubra* and in *Trifolium repens*. Harberd and Owen (1969) found that the lowest number of clones of *Festuca rubra* occurred in the areas of highest density of the species: this finding is compatible with a process of competitive exclusion of genotypes. By contrast the intermingling of the leaf marks of *T. repens* appeared in the present study to be greatest where clover density was high. The linear regression of morph number per quadrat against clover density on a five-point scale is positive ( $P < 0.01$ ,  $t = 14.54$ ) in the Henfaes field.

It is interesting to compare the diversity of morphs with the diversity of species—the contrast between intra- and interspecific diversity. A classical way of describing diversity within a plant community uses the Raunkiaer diagram (Raunkiaer, 1913, 1918). The procedure involves dividing the total number of species (or, in this case, morphs) within a community into classes according to their frequency of occurrence. When the percentage occurrence of species is plotted against frequency class, a characteristic J-shaped curve is usually obtained. This procedure has been criticised (Kylin, 1926; Greig-Smith, 1957) because the frequency classes do not represent equal ranges of density. Indeed, the highest frequency class includes a wider range of possible densities than the other frequency classes. This criticism is, however, irrelevant for the present purposes; what is important is that the procedure demonstrates a marked difference between the behaviour of species and that of morphs. Figs. 6 and 7 show Raunkiaer diagrams calculated for morphs of *T. repens* and for species in the Henfaes pasture. The species curves have the characteristic form of a Raunkiaer “Law of Frequencies” with the expected J-shaped curve: morph frequency is quite different and, instead of being J-shaped, shows a continuous decline in the percentage of morphs present in the classes of successively higher frequency. There is under-representation of morphs that occur at high frequency compared with a similar analysis made on species. There is less obvious dominance shown between clones than between species. In only one of the 12 sites at Henfaes (C2) was one clone clearly dominant. The general picture is of a number of intimately intermingled clones of *T. repens*, even in the extremely old (900 years) grassland at Port Meadow, Oxford.

There is a clear relationship between the frequency of marks and grass length. In the toe-point samples the data can be classified both according to the nature of the area (table 1) and by the length of the grass at the collection point. In Table 5c, 2 × 2 contingency tables are shown for each grazing régime and it is clear that a significant difference in the marked/unmarked ratio can be found only at grazing regime 1, *i.e.* well-grazed areas.

Since the frequency of marked leaves is higher in short, well-grazed pasture, this suggests that marks provide some protection from grazing. If this is the case it might be expected that in ungrazed pastures there would be a few dominant clones and a low frequency of morphs. This hypothesis is



difficult to test because if grassland is not regularly defoliated *T. repens* rapidly disappears. At Henfaes, site C2, the only site with a single dominant clone, grazing was relatively slight; there are relatively few clones at the Lugg Meadows site which is only grazed in the autumn when marks are not clearly expressed. In mown pastures or lawns where defoliation is not selective, but has been long continued, a convenient comparison could be made with the grazed condition. There seem, however, to be exceptionally few situations in which a regularly mown grassland has not been treated with selective herbicide. A subsequent paper describes experiments that suggest that the selective grazing of sheep may be sufficient to balance the leaf mark polymorphism of *Trifolium repens* in pastures and account for the fine ecological grain of the phenomenon.

*Acknowledgments.*—We are grateful to Mr David Machin, Mr E. R. B. Oxley and Mr M. O. Hill for help and advice in statistical and computer handling of the data, and also to the staff of College Farm, Aber, Gwynedd and Mr Pilcher of North Farm, East Guldeford, Rye, Sussex on whose land some of the work was done. This work was done while M.G.C. was in receipt of a National Environment Research Council Studentship.

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