THE GENETIC CONTROL OF LEAF DEVELOPMENT IN *LOLIUM*

1. ASSESSMENT OF GENETIC VARIATION

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1. INTRODUCTION

THE ultimate efficiency of a pasture, provided water and nutrients are not limiting, may well be expressed as the proportion of the incoming light energy utilised by the sward. This will depend on the area of photosynthetic tissue exposed to the light, usually expressed as the leaf area index (*i.e.* the leaf area per unit area of ground), and also on the photosynthetic efficiency per unit area of leaf, usually measured as the net assimilation rate (Watson, 1956; Donald and Black, 1958).

Growth analyses have shown that in ryegrass, as in several annual field crops (Watson, 1956), there are varietal differences in dry matter accumulation in the early seedling stage, up to a leaf area index of about 2; these depend largely on variation in leaf area, there being little difference between varieties in net assimilation rate. At a later stage when shading has started and the leaf area index has risen to 3-4, a more complex picture is revealed, with varietal differences in both leaf area index and net assimilation rate (Edwards, unpublished). Selection for rapid increase in leaf area, especially in the seedling stage or after defoliation, might, therefore, be a useful approach to the problem of breeding for yield, bearing in mind that the density dependence of net assimilation rate may become important in later stages of growth.

The possibilities of selection for any character depend on the amount of genetic variation available and on its heritability. Earlier studies on date of ear emergence in *Lolium* (Cooper, 1959*c, d*) showed that each outbreeding population contained considerable genetic variation for this character, the heritability was high (over 0.7) and rapid immediate response to selection occurred. Furthermore, most plants were highly heterozygous and continued selection resulted in wide response well outside the range of the original population.

Work has been started, therefore, to see whether similar response to selection is possible for other agronomic characters, especially those determining production in the sward. The present series deals with leaf development and the accumulation of leaf area in the seedling

stage, but the work is being extended to the production of dry matter under controlled defoliation and competition.

The present paper surveys the general pattern of leaf and bud development in the seedling stage, and estimates the amount of genetic variation for seedling characters. Later papers will deal with the response to selection for these leaf components, including correlated responses in other characters, and with the developmental basis of these differences.

2. PATTERN OF LEAF AND TILLER DEVELOPMENT

The pattern of leaf and tiller development in *Lolium* has been described in detail by Mitchell (1953*a, b*) and Patel (1958). The present observations agree with these accounts and only a brief outline will be given here as an introduction to the analysis of the genetic variation.

In the embryo, the growing point carries the coleoptile, and 3-4 leaf primordia arranged alternately on the shoot apex. Following germination these leaf primordia expand in sequence, and a continuing succession of new leaf primordia are formed on the apex. During early vegetative growth the rate of leaf appearance on the main shoot is linear, so long as environmental conditions remain constant.

New vegetative tillers arise from tiller buds in the leaf axils. The first tiller may arise from the axil of the coleoptile, the first leaf or the second leaf, depending on the genotype and environment. Tiller expansion in any axil does not usually begin until the leaf above is fully expanded. The rate of tiller appearance on the main shoot is thus linear until competition or floral initiation occurs. New leaves and tillers are produced at a linear rate on the secondary tillers also, so that in the absence of competition the increase in total number of tillers or leaves is exponential. The total number of leaves and tillers per plant will thus depend primarily on the rate of leaf appearance, but will also be influenced by the level at which the first tiller is produced, and by the rate of expansion of available tiller buds.

Leaf size is determined by length and width, but both these can vary independently. In the young seedling the width and length of successive leaves increase regularly, and there is a high correlation between the dimensions of successive leaves on the main tiller and between these and corresponding leaves on side tillers. The total leaf area per plant is thus influenced by leaf size, by rate of leaf appearance per tiller and by number of tillers; these components may not be independent. There is considerable variation between and within varieties in all these components, resulting in large differences in total leaf and tiller number, in leaf area per plant and hence in dry matter production.

The first step in the present work, therefore, has been to compare the pattern of leaf and tiller development in contrasting ryegrass varieties and to decide on certain standard measurements which would

adequately specify the variation in this pattern. These measurements should be easily and rapidly recordable, should show good repeatability within the genotype, and should relate to the accumulation of total leaf area on the plant.

(i) *Materials and methods*

Three contrasting ryegrass varieties were studied:

Italian ryegrass (*L. multiflorum* Lam.), a biennial commercial variety, with large leaves and thick tillers, grown in Northern Ireland:

Irish perennial ryegrass (*L. perenne* L.), a perennial commercial variety grown in Northern Ireland, with smaller leaves and tillers than the Italian:

Hunsballe perennial ryegrass (*L. perenne* L.), a perennial variety, bred at Holstebro, Denmark, with larger leaves and tillers than the Irish, and a more erect habit.

All varieties were sown on 16th September 1957 in a warm glass-house in four blocks and the seedlings thinned out to a 3 inch spacing, and the following measurements were recorded on 20 seedlings of each variety:

Date of appearance of successive leaves on the main shoot and on the first three side-tillers.

Date of appearance of the tillers in the axil of the coleoptile and of the first four leaves on the main shoot.

The length, median width and area of the first six leaves on the main shoot, and of corresponding leaves on the first three side-tillers. All leaf measurements were taken after the leaf blade had finished expanding. The median width was measured using a Beck illuminated magnifier ($\times 10$), and the area calculated as the length $\times$ median width. This gives a good estimate of leaf area with an error of less than 5 per cent. The equation of closest fit, derived from 50 leaves of widely differing sizes is $A = 11 + 0.95 L \times B$ (in mm.²).

From the above records the rate of leaf and tiller appearance on each shoot, the total number of leaves and tillers, and the total leaf area 34 days after sowing were calculated for each seedling.

A further tiller count was made on 19th December 1957 (94 days after sowing) when all the seedlings had produced an expanded 6th leaf, and each plant was transplanted to an 8 inch pot and placed outdoors. On 27th February 1958 leaf length, width and area were measured on the youngest expanded leaf of three tillers of each plant to detect any differential effect of outdoor conditions on these characters.

Further sowings of these varieties were made in the glasshouse on 25th August 1958, 14th April 1959 and 19th August 1959, to detect any sowing date effects and variety/sowing-date interactions.

(ii) Varietal differences

Varietal differences for the individual seedling characters are shown in tables 1 and 2; sowing-date effects and sowing-date/variety

TABLE 1
Seedling measurements in ryegrass varieties
Main sowing, 16th September 1957

	Italian		Irish		Hunsballe		Sig. diff. at 5 per cent.
	Mean	Var.	Mean	Var.	Mean	Var.	
Rate of leaf app. (4th to 7th leaf) (days/leaf)	7.37	0.90	7.74	1.71	8.75	0.60	0.65
Plants with coleoptile tiller	80 per cent.		35 per cent.		15 per cent.		Not available
Rate of tiller app. (1st to 4th tiller) (days/tiller)	6.97	1.04	6.90	1.04	7.60	0.48	0.58
Tiller no. (20 October)	4.58	0.37	3.95	0.68	3.35	0.24	0.41
Tiller no. (19 December)	38.26	74.11	23.85	54.26	20.05	38.79	4.72
Total leaf no. (20 October)	13.6	4.67	11.25	3.89	9.5	3.74	1.28
Length of sixth leaf (mm.)	268	1,551	226	1,180	242	593	20.2
Width of sixth leaf (mm.)	5.65	0.20	3.88	0.46	3.56	0.21	0.34
Area of sixth leaf (mm. ²)	1,505	81,276	880	44,776	868	29,288	1.44
Total leaf area/plt. (cm. ²) (20 October)	57.8	112.32	27.2	118.26	22.6	33.48	5.9

Block differences not significant.

All measurements refer to main shoot except where otherwise stated.

interactions in table 2, while location (outdoor *versus* glasshouse) effects are in table 3.

The repeatability of each character was calculated from the analysis of components of variance (table 4). Estimates were obtained from (a) successive measurements of the same character along the main shoot; (b) measurements of the same character on different tillers at the same date, both indoors and outside; and (c) measurements of the same character indoors in the autumn and outdoors in the spring.

Rate of leaf appearance. The rate of leaf appearance was markedly influenced by sowing date, being much slower in the August 1958 sowing. Hunsballe produced leaves more slowly than the other

TABLE 2
Varietal and sowing-date comparisons, and variety/sowing-date interaction

	Varieties			Sowing dates			Sig. diff. at 5 per cent.	Components of variance			
	Italian	Irish	Hunsballe	25/8/58	14/4/59	19/8/59		V _v	V _s	V _i	V _E
Rate of leaf app. (days/leaf) .	7.5	6.7	7.0	8.3	6.5	6.4	0.71	0.10	1.13**	0.03	2.07
Leaf length (mm.) .	250	203	227	293	174	213	25	510*	3,623***	51	2,169
Leaf width (mm.) .	5.4	3.8	3.9	4.7	4.1	4.3	0.42	0.828***	0.094*	0.016	0.480
Leaf area (mm. ²) .	1,400	799	932	1,427	740	965	344	91,719*	114,999*	20,089***	94,754
Tiller no. (4 weeks) .	5.7	4.5	4.0	3.5	4.8	5.8	1.03	1.25**	0.65*	0.12	2.58
Dry weight/plant (gm.) .	0.61	0.51	0.63	0.49	0.70	0.56	0.10	0.0038*	0.0106*	-0.0034	0.0712
Dry weight/tiller (mgm.) .	43	27	33	47	27	28	n.s.	37	96	87***	131

	Sample analysis: leaf length			
	dF	S.S.	M.S.	Expectation
Varieties	2	99,141	49,570*	V _E +30 V _i +90 V _v
Dates	2	659,501	329,750***	V _E +30 V _i +90 V _s
Interaction	4	14,785	3,696	V _E +30 V _i
Within var. dates . .	8	773,427		
	261	566,223	2,169	V _E
Total	269	1,339,650		

* P = 0.05-0.01

** P = 0.01-0.001

*** P = <0.001

varieties in the main sowing, but no significant varietal differences could be detected in the later sowings, nor was there any variety/sowing-date interaction.

The repeatability of this character is only moderate (0.38-0.47) when assessed from successive individual leaf intervals on the main shoot, but much higher figures were obtained from the mean interval

TABLE 3
Comparison of seedling measurements in the glasshouse and outdoors

	Glass-house	Outdoors	Components of variance				
			V _V	V _G	V _L	V _{VL}	V _E
<i>Leaf length (mm.)</i>							
Italian . . .	250	197	682***	425***	2,970***	251***	127
Irish . . .	214	128					
Hunsballe . .	224	127					
<i>Leaf width (mm.)</i>							
Italian . . .	5.1	5.9	0.93***	0.22***	0.19***	0.02*	0.07
Irish . . .	3.6	4.0					
Hunsballe . .	3.4	4.1					
<i>Leaf area (mm.²)</i>							
Italian . . .	1,256	1,164	97,745***	14,754***	19,050***	4,715***	5,694
Irish . . .	830	539					
Hunsballe . .	748	520					

Sample analysis. Leaf length

	dF	S.S.	M.S.	Expectation
Varieties	2	40,710	20,355***	$V_E + 12 V_{VL} + 2 V_G + 24 V_V$
Seedlings within varieties . .	33	32,251	977***	$V_E + 2 V_G$
Location	1	110,059	110,059***	$V_E + 12 V_{VL} + 36 V_L$
Var./location inter.	2	6,268	3,134***	$V_E + 12 V_{VL}$
Residual error	33	4,201	127	V_E
Total	71	193,489		

of three corresponding leaves on different tillers, and there is evidently considerable interplant variation within each variety.

Tillering. The node at which the first tiller appears was recorded in the main sowing and in April 1959. At both sowings Italian had a higher proportion of plants with the coleoptile tiller than had Irish, while Irish was higher than Hunsballe.

Once tillering has started, the rate of tiller appearance on the main shoot is correlated with the rate of leaf appearance ($r = 0.74^{***}$ for Italian, 0.57^{**} for Irish and 0.71^{***} for Hunsballe), and in the main sowing is thus slower for Hunsballe than for the other varieties.

As a result of these differences in position of the first tiller and rate of tiller appearance, the total number of tillers also varies. Thirty-four days after the main sowing Italian had more tillers than Irish

TABLE 4
Repeatability of leaf measurements in ryegrass varieties

	Successive leaves on main shoot: glasshouse		Corresponding leaves on different tillers: glasshouse		Corresponding leaves on different tillers: outdoors		Glasshouse versus outdoor measurements	
	V_G	$\frac{V_G}{V_G + V_E}$	V_G	$\frac{V_G}{V_G + V_E}$	V_G	$\frac{V_G}{V_G + V_E}$	V_G	$\frac{V_G}{V_G + V_E}$
<i>Rate of leaf app. (days/leaf)</i>								
Italian . . .	0.63	0.38†	0.60	0.72*	...	...	...	...
Irish . . .	1.07	0.46†	0.65	0.63*	...	...	...	...
Hunsballe . .	0.45	0.47†	0.38	0.67*	...	...	...	...
<i>Leaf length (mm.)</i>								
Italian . . .	1,062	0.87	1,216	0.88	468	0.65	409	0.70
Irish . . .	747	0.88	900	0.89	493	0.82	548	0.76
Hunsballe . .	371	0.82	469	0.78	250	0.55	289	0.72
<i>Leaf width (mm.)</i>								
Italian . . .	0.118	0.72	0.198	0.80	0.321	0.81	0.150	0.55
Irish . . .	0.267	0.77	0.374	0.94	0.220	0.79	0.270	0.87
Hunsballe . .	0.111	0.64	0.174	0.92	0.197	0.83	0.186	0.87
<i>Leaf area (mm.²)</i>								
Italian . . .	43,744	0.81	83,903	0.93	37,453	0.69	27,510	0.72
Irish . . .	22,845	0.82	33,890	0.88	14,242	0.81	18,747	0.77
Hunsballe . .	14,770	0.77	25,101	0.89	11,272	0.69	14,283	0.92

* Derived from means of three leaf intervals.

† Derived from individual leaf intervals.

Sample analysis. Leaf length of Italian. Glasshouse

	dF	S.S.	M.S.	
Genotypes . . .	16	70,439	4,406***	$V_E + 4 V_G$
Leaf position . .	3	101,143	33,714***	$V_E + 17 V_L$
Error . . .	48	7,633	159	V_E
Total . . .	67	179,215		

probably because of its earlier start, and Irish had more than Hunsballe, because of its faster rate of tiller appearance. By 19th December (94 days), however, Hunsballe had caught up with Irish, though both had fewer tillers than Italian.

In the later sowings also, Italian had a higher tiller number than the perennial varieties, presumably due to tiller development from

lower nodes, as there were no differences in rate of leaf appearance. There is no significant variety/sowing-date interaction, but marked differences between sowing dates, related largely to differences in rate of leaf appearance.

Total number of leaves. The total number of leaves per plant was recorded only in the main sowing. It is determined by tiller number and by rate of leaf appearance on each tiller. On 20th October there was little difference in leaf number per tiller, and total leaf number was closely related to tiller number, with Italian high, followed by Irish and Hunsballe in that order.

Leaf size. Leaf size and its two components, length and width, show large differences between the Italian and the perennial varieties, Italian having slightly longer and much wider leaves in all sowings. There were no significant differences between Irish and Hunsballe in the main sowings, but in the later sowings, Hunsballe had longer leaves than Irish though they were not significantly greater in area.

There are marked sowing date effects which influence length more than width, the leaves being both larger and wider at the August 1958 sowing. No interaction occurred individually for leaf length or width, but there was a high interaction for leaf size possibly scalar in origin.

Highly significant variety/location interactions between indoor and outdoor material were found for leaf length and leaf size, and less so for leaf width (table 3). Transfer outdoors had a differential effect on leaf width and length. Width was slightly increased, while leaf length decreased considerably, resulting in a large reduction in leaf size. The relative decrease in length was, however, much less in Italian than in the perennial varieties.

The repeatabilities of leaf dimensions are high (usually over 0.7) whether assessed from successive leaves on the main shoot, from corresponding leaves on different tillers, or between outdoor and indoor material, and considerable interplant variation occurs within the varieties.

Total leaf area per plant. The total leaf area per plant was measured only at the main sowing. Italian with more and larger leaves had a much greater area than the perennial varieties, but these did not vary among themselves.

The measurement of total leaf area per plant is rather laborious but it has proved possible to obtain an accurate estimate from the total leaf number $\times$ area of the 6th leaf on the main shoot. This estimate gives a correlation of over 0.8 with the actual leaf area for the range of material studied here, but the exact equation of estimation must be calculated from a sub-sample of each variety at each harvest date.

(iii) Choice of measurements

The two most important components of total leaf area in the seedling stage are number of leaves (determined by rate of leaf appearance

and rate of tillering) and leaf size. These components of course may not be independent in development, and the genetic and environmental correlations between them are discussed later (p. 76). In view of the high repeatability of individual leaf size, it was decided to take the dimensions of the 6th leaf on the main shoot as a standard index. Rate of leaf appearance shows a somewhat lower repeatability as assessed from individual leaf intervals, so the average of three leaf

TABLE 5
Estimation of genotypic variation from out-breeding and self-fertilising material of Lolium

	Sixth leaf						Leaf app. (4th-7th leaf) days/leaf	
	Length (mm.)		Width (mm.)		Area (mm. ²)		Mean	Var.
	Mean	Var.	Mean	Var.	Mean	Var.		
<i>Outbreeding varieties</i>								
<i>L. perenne</i>								
Irish Ba 6650 . . .	205	1,669	3.9	0.157	797	36,186	6.5	1.97
Irish Ba 6738 . . .	178	918	3.5	0.119	635	16,697	6.2	1.10
Hunsballe Ba 6652 . . .	211	2,826	3.8	0.222	811	35,539	6.2	0.73
Mean	198	1804	3.7	0.166	748	29,474	6.3	1.27
<i>Self-fertilising lines</i>								
<i>L. remotum</i> Ba 3078 . . .	293	760	4.9	0.060	1,432	2,533	6.7	0.87
<i>L. temulentum</i> Ba 3084 . . .	338	474	7.3	0.214	2,454	6,626	6.8	0.49
Ba 6902	270	449	6.7	0.083	1,817	3,664	6.5	0.67
Ba 6137(6)	230	1,127	7.0	0.182	1,542	7,987	5.8	0.51
Ba 6137(11)	252	770	7.4	0.134	1,870	7,462	6.3	0.97
Ba 6139(7)	269	508	6.4	0.082	1,718	3,706	6.3	0.46
Mean	275	681	6.6	0.126	1,806	5,330	6.4	0.66
$\frac{\text{Var.}_{\text{Out}} - \text{Var.}_{\text{Self}}}{\text{Var.}_{\text{Out}}}$		0.62		0.24		0.82		0.48
Corrected using coefficient of variation (see text)		0.79		0.77		0.97		0.48

intervals (from the appearance of the 4th to 7th leaf on the main shoot) has been used to provide a more precise measurement. The tiller number at 4 weeks from sowing has also been recorded to obtain a measure of independent variation in tiller production. These measurements will be used as standards in subsequent genetical work.

(iv) Variation within populations

Although regular varietal differences have been detected for leaf size and tiller production, considerable plant-to-plant variation occurs within each variety, and the high repeatability of these components within the plants suggests that much of this variation may be genetic.

Some indication of the total genotypic variation can be obtained from a comparison of the variances of outbreeding and homozygous material. The variation within two samples of Irish perennial ryegrass and one of Hunsballe was, therefore, compared with that of a number of homozygous lines of the self-fertilising *L. remotum* and *L. temulentum*. All the material was sown on 4th February 1959, and standard measurements of rate of leaf appearance and leaf dimensions were taken on 40 seedlings of each lot (table 5).

The mean variance of all characters is higher in the outbreeding varieties than in the homozygous lines and the difference between these mean variances can be used as a rough measure of the genotypic variation within the outbreeding varieties. Quite high estimates were obtained for leaf length (0.62) and leaf area (0.82) but the figures for leaf width (0.24) and for leaf appearance (0.48) were lower. There is, however, a strong correlation between the mean and the variance for leaf dimensions in this material, *L. remotum* and *L. temulentum* having much larger leaves than *L. perenne*. Correction for this effect by the use of the squared coefficient of variation increases the estimates for leaf dimensions to over 0.7 for all characters, but does not alter that for rate of leaf appearance.

3. ESTIMATES OF HERITABILITY

Although there is evidently appreciable genotypic variation for leaf characters within these ryegrass varieties, its exact estimation and separation into additive and non-additive portions can only be made by a breeding test. In the present work provisional estimates of additive genetic variation and heritability have been obtained from a small number of crosses within each variety.

(i) *Material and methods*

The seedlings of Italian, Irish and Hunsballe ryegrass measured for leaf characters in autumn 1957 were crossed in pairs in summer 1958 using positive assortative mating for leaf size, as the families were to be used as the first generation of selection for leaf size. Eight crosses were made within each variety using the automatic pollination method of Jenkin (1931) without emasculation. Little selfing occurs in this material, and would not be expected to influence the selection results unduly (Cooper, 1959*b*).

The original varieties and the progenies were sown in a warm glasshouse in 5 blocks on 25th August 1958 and standard measurements of leaf size and rate of leaf appearance were taken on 30 seedlings of each lot. A tiller count was made on 4 November (71 days from sowing), the plants were cut to ground level, and dry weights per plant and per tiller were recorded for each seedling. Six seedlings from each family were also preserved for developmental studies of cell size and number in the shoot apex and expanded leaf. Further sowings

were made of four families of each variety in April and August 1959, to detect any family/sowing-date interactions for these leaf characters.

Heritability estimates were obtained in two ways: first, from the analysis of variance of full-sib families, and second, from the regression of progeny means on mid-parent values (Falconer, 1960). Such estimates are liable to large sampling errors, especially if the number of families is small. In the present instance, which serves as the first generation of a selection experiment, a small number of families (8) but a large family size (30 plants) was used to give a larger selection differential within families. The analysis of full-sib families thus gives much greater precision than does parent/progeny regression. It also avoids any effects of genotype/environment interaction between parents and progeny, but, of course, includes part of the variation due to dominance and any common maternal environmental effects.

Phenotypic, genetic and environmental correlations were calculated for the following pairs of characters:

rate of leaf appearance	×	leaf length
”	”	”
”	”	”
”	”	”
”	”	”
leaf length	×	leaf width
leaf area	×	dry weight per tiller
tiller number	×	dry weight per tiller

All heritabilities and genetic correlations were corrected for assortative mating according to Reeve (1953).

(ii) *Heritability estimates*

Both the heritability and the amount of genetic variation (V_G) differ with the character and the variety (table 6). The estimates are rather lower than those obtained earlier for date of ear emergence and other ear characters (Cooper, 1959*c*, 1960). Leaf area, for instance, gives a figure of 40-50 per cent. in Italian, but rather less for Irish and Hunsballe. There were, however, encouragingly high estimates for tiller number, dry weight per plant and per tiller in most varieties.

Because of the small number of families the errors of estimation are high, and the estimates from parent/progeny regression are of little value. The precision of those from full-sib families is rather greater, and 16 out of 21 were significantly positive, while significant family differences were obtained in 19 out of 21. It must be remembered that these estimates are derived from only a small sample and may not represent the variety as a whole. Even so, there is evidently significant additive genetic variation even within these small samples.

Preliminary results (Cooper and Edwards, 1960) suggest that a heritability of more than about 0.35 gives a rapid immediate response

to selection, and this value is attained for many of the present characters. Furthermore, the variation within each F_1 progeny indicates considerable heterozygosity of the parents, hence the possibility of continued response to selection.

TABLE 6
Heritability estimates for leaf characters
Main sowing 25th August 1958

	Mean	V_G	From full-sib families			From parent/progeny		
			h^2	significance of		correlation r	regression b	
				estimate	family diffs.			
<i>Rate of leaf app. (days/leaf)</i>								
Italian . . .	9.6	3.6	0.73 ± 0.31	*	***	0.86**	1.40 ± 0.34	**
Irish . . .	7.9	2.5	1.16 ± 0.27	**	***	0.93**	1.44 ± 0.24	***
Hunsballe . . .	8.1	1.6	0.62 ± 0.25	*	***	0.13	0.18	N.S.
<i>Leaf length (mm.)</i>								
Italian . . .	292	1,216	0.42 ± 0.20	*	**	0.73	0.63 ± 0.24	*
Irish . . .	259	606	0.40 ± 0.19	*	**	0.28	0.26	N.S.
Hunsballe . . .	290	232	0.15 ± 0.11	N.S.	*	-0.31	-0.18	N.S.
<i>Leaf width (mm.)</i>								
Italian . . .	5.8	0.25	0.38 ± 0.18	*	***	0.64	0.57	N.S.
Irish . . .	4.1	0.10	0.34 ± 0.17	*	**	0.15	0.12	N.S.
Hunsballe . . .	4.3	0.13	0.41 ± 0.19	*	***	0.58	0.43	N.S.
<i>Leaf area (mm.²)</i>								
Italian . . .	1,711	89,356	0.41 ± 0.19	*	**	0.54	0.51	N.S.
Irish . . .	1,069	12,014	0.18 ± 0.12	N.S.	N.S.	0.06	0.03	N.S.
Hunsballe . . .	1,249	21,852	0.26 ± 0.15	N.S.	**	0.36	0.26	N.S.
<i>Tiller No.</i>								
Italian . . .	10.7	4.38	0.43 ± 0.20	*	**	...	...	...
Irish . . .	13.0	12.68	0.79 ± 0.28	**	***	...	...	...
Hunsballe . . .	13.4	9.64	0.56 ± 0.24	*	***	...	...	...
<i>Dry weight/plant (gm.)</i>								
Italian . . .	0.62	0.026	0.57 ± 0.24	*	**	...	...	...
Irish . . .	0.46	0.016	0.46 ± 0.21	*	**	...	...	...
Hunsballe . . .	0.59	0.010	0.28 ± 0.16	N.S.	N.S.	...	...	...
<i>Dry weight/tiller (mgm.)</i>								
Italian . . .	57	229	0.67 ± 0.26	**	***	...	...	...
Irish . . .	33	22	0.27 ± 0.15	N.S.	**	...	...	...
Hunsballe . . .	42	48	0.51 ± 0.22	*	***	...	...	...
			$n = 240$			$n = 8$		

(iii) Family/sowing-date interaction

Even though useful additive genetic variation can be detected at a single sowing date, response to selection may be limited by genotype/environment interaction between parents and progeny.

Family/sowing-date interactions have, therefore, been assessed from four families of Italian and four of perennial ryegrass at three

different sowing dates (table 7). The analysis for perennial ryegrass includes *two* families of Irish and *two* of Hunsballe, as the same four families of Irish and Hunsballe were not sown at all dates.

Significant differences were obtained between families and between sowing dates for all characters, except dry weights per plant and per

TABLE 7
Family/sowing-date interactions for leaf characters

	Italian (4 families)				Irish + Hunsballe (4 families)			
	V _F	V _S	V _I	V _E	V _F	V _S	V _I	V _E
Rate of leaf app. (days/leaf)	1.17***	2.51***	-0.03	2.33	0.44**	1.70***	0.10**	1.22
Leaf length (mm.)	700**	3,134***	128*	2,208	1,166***	2,249***	20.9	1,248
Leaf width (mm.)	0.09**	0.04**	0.00	0.41	0.08**	0.03*	0.02**	0.22
Leaf area (mm. ²)	47,547**	124,451***	6,879*	134,067	40,173***	53,406***	2,494*	48,758
Filler No. . . .	1.44*	3.45**	0.54***	2.0	0.80*	1.62***	0.45***	1.8
Dry weight/plant (gm.)	0.0127	-0.0001	0.0017	0.0279	0.0001	0.0087**	0.0021*	0.0195
Dry weight/tiller (mgm.)	93	264**	15	223	34	17	25**	105

Sample analysis. Leaf length of Italian

	dF	S.S.	M.S.	Expectation
Families . . .	3	207,177	69,069***	V _E + 30 V _I + 90 V _F V _E + 30 V _I + 120 V _S V _E + 30 V _I
Sowing dates . . .	2	764,360	382,180***	
Interaction . . .	6	36,300	6,050*	
Within-Family S.D. . .	11 348	1,007,837 768,549	... 2,208	V _E
	359	1,776,386		

tiller, and significant interactions could be detected for leaf length, leaf area and tiller number in Italian, and for all characters except leaf length in perennial ryegrass. This interaction, however, represents the statistical deviation from purely additive effects of family and sowing-date and does not necessarily imply reversal of the relative order of families. It is only for tiller number and dry weight per tiller that the interaction is high enough to decrease markedly the overall differences between families. Significant heritabilities were recorded for both these characters at the main sowing date (table 6).

Such family/sowing-date interactions may become more important over a wider range of environments, or when selection has reduced the additive genetic variation.

(iv) *Genetic and environmental correlations*

The estimates of heritability give an indication of the probable response to selection for each character independently, but correlated responses in other characters may also occur.

TABLE 8
Genetic and environmental correlations within ryegrass varieties

	From original varieties r_P	From first-generation families		
		r_P	r_G	r_E
<i>Rate of leaf app. $\times$ leaf length</i>				
Italian	+0.21	+0.10	-0.63 $\pm$ 0.19***	+1.11
Irish	+0.28	-0.05	-0.43 $\pm$ 0.19*	+0.42
Hunsballe	-0.32	+0.08	-0.74 $\pm$ 0.17***	+0.48
<i>Rate of leaf app. $\times$ leaf width</i>				
Italian	+0.14	+0.41***	+0.22 $\pm$ 0.30	+0.71
Irish	+0.57***	+0.34***	+0.38 $\pm$ 0.21	+0.41
Hunsballe	+0.08	+0.56***	+0.11 $\pm$ 0.30	+1.01
<i>Rate of leaf app. $\times$ leaf area</i>				
Italian	+0.60***	+0.26**	-0.25 $\pm$ 0.29	+0.99
Irish	+0.47**	+0.15	-0.17 $\pm$ 0.27	+0.49
Hunsballe	-0.15	+0.13	-0.19 $\pm$ 0.33	+0.36
<i>Leaf length $\times$ leaf width</i>				
Italian	+0.51**	+0.55***	+0.93 $\pm$ 0.05***	+0.31
Irish	+0.67***	+0.12	-0.27 $\pm$ 0.32	+0.29
Hunsballe	+0.18	+0.56***	+0.35 $\pm$ 0.36	+0.66
	$n = 30$		$n = 240$	
<i>Rate of leaf app. $\times$ tiller number</i>				
Italian	+0.24	+0.33***	+0.23 $\pm$ 0.30	+0.52
Irish	+0.61***	+0.60***	+0.61 $\pm$ 0.13***	+0.55
Hunsballe	+0.46*	+0.49***	+0.67 $\pm$ 0.16***	+0.26
<i>Leaf area $\times$ dry weight/tiller</i>				
Italian	+0.63***	+0.69***	+0.99 $\pm$ 0.01***	+0.40
Irish	+0.77***	+0.69***	+0.65 $\pm$ 0.22**	+0.71
Hunsballe	+0.58**	+0.65***	+0.75 $\pm$ 0.16***	+0.63
<i>Tiller number $\times$ dry weight/tiller</i>				
Italian	-0.41*	-0.33***	-0.53 $\pm$ 0.22*	-0.10
Irish	-0.07	+0.08	-0.27 $\pm$ 0.29	+0.51
Hunsballe	+0.09	-0.09	-0.53 $\pm$ 0.22*	+0.43
	$n = 24$		$n = 192$	

The simple phenotypic correlations may be of genetic or environmental origin, and they do not necessarily predict the correlated response to selection. Separate genetic and environmental correlations have, therefore, been calculated (table 8).

In the present work, the rate of leaf appearance has been measured in terms of days per leaf, and hence rapid leaf appearance is indicated by a low numerical value. Since, however, rapid leaf appearance is an expression of vigour, for more convenient comparison with the

other seedling characters, it has been regarded in these correlations as a *positive* deviation from the mean.

The phenotypic correlations, as derived from either the original varieties or the first generation families, show no significant relation between rate of leaf appearance and leaf length, but rate of leaf appearance is *positively* correlated with leaf width in all varieties and with leaf area in Italian and Irish. Leaf width and length are usually, but not always, *positively* correlated. As expected, tiller number usually shows a positive correlation with leaf appearance, while dry weight per tiller is closely correlated with leaf area, but although most of the correlations between tiller number and dry weight per tiller are negative, only one of these is significant.

The separation into genetic and environmental correlations gives a much clearer picture. There is a strong *positive* environmental correlation between nearly all the seedling characters, suggesting a common environmental effect on vigour, possibly acting in part through seed size. The genetic correlations also show uniform trends for most characters. The sampling errors of genetic correlations are high (Falconer, 1960), but the uniform direction of the correlation for most pairs of characters, either positive or negative, is encouraging.

The genetic correlation between rate of leaf appearance and leaf length is always high and *negative*, while that between leaf appearance and leaf width is always *positive*, although individual values are not significant. These two combined result in a *negative* but low genetic correlation between leaf size and rate of leaf appearance which may explain the marked inverse correlated response between leaf size and rate of leaf appearance (Cooper and Edwards, 1960). Similarly, there is usually a *negative* genetic correlation between tiller number and dry weight per tiller.

The genetic correlations between leaf length and leaf width are not so straightforward, being *positive* and high in Italian and Hunsballe, but negative, though not significant, in Irish, possibly because of the lack of genetic variation for these characters in Irish. The genetic correlation between leaf area and dry weight per tiller is high and positive in all varieties, while that between rate of leaf appearance and tiller number is also *positive* in all varieties, but low and not significant in Italian, presumably a result of independent variation in tiller production.

The developmental basis of these genetic correlations and their value in predicting correlated responses to selection will be discussed in later papers.

4. DISCUSSION

(i) *Genetic structure*

The genetic situation for these leaf components in *Lolium* is evidently similar to that for date of ear emergence and other ear characters (Cooper, 1959*d*, 1960); additive genetic variation exists within each

variety, and most plants are highly heterozygous. The estimates of heritability, however, are rather lower than for date of ear emergence. This may be the result of greater environmental variation, less genetic variation, or both. Developmentally, ear initiation is a response to a regular environmental threshold of photoperiod. The rate of subsequent development, although modified by spring temperature, is not greatly affected by previous nutrition and management. By contrast, most seedling components are strongly influenced by small fluctuations in light and temperature, by nutrition, especially nitrogen, and by conditions of ripening and harvesting, possibly acting through seed weight. They might, therefore, be expected to show greater environmental variation.

The amount and type of genetic variation will also be influenced by past selection history as pointed out by Breese and Mather (1960) in *Drosophila*. Where there has been strong directional selection for the extreme expression of a character, as for fertility in ryegrass, (Cooper, unpublished), little additive genetic variation may be left. On the other hand, for a character which has not been selected intensively, or where stabilising selection has operated for an intermediate expression, as in date of ear emergence (Cooper, 1959c), appreciable additive genetic variation may be maintained.

The Italian and Irish ryegrass used in the present work were both obtained from commercial stocks grown in Northern Ireland. The selection history of Irish perennial ryegrass has been discussed earlier (Cooper, 1959b), and a similar system of management has been adopted for Italian ryegrass (Beddows, 1953). The grass seed is sown early in May under a cover crop of oats, the sward may be grazed leniently in the autumn after the oats are harvested and a seed crop is taken the following summer. Selection has thus been primarily for the maximum seed yield in the first harvest year, necessitating a fairly uniform date of heading, and the maximum number of fertile tillers. Any selection for vegetative characters would be indirect; individual components of leaf and tiller production would be selected only in so far as they contribute to competitive ability in the sward or to a large number of flowering culms in the seed crop.

Hunsballe, on the other hand, is a Danish bred variety. The parents were presumably selected as single spaced plants, although their progeny may have been assessed under sward conditions, and the variety must have passed the official State trials, based on a system of hay and aftermath cuts. However, in spite of the presumably narrower genetic base of this variety, and the more stringent selection of the parents for uniformity of vigour and growth type, there is no regular decrease in the amount of additive genetic variation compared to Italian and Irish (table 6).

Although only small samples of these varieties were studied, some additive genetic variation was detected not only in rate of leaf

appearance and leaf size, but also in tiller number, dry weight per plant, and dry weight per tiller. It may well be that none of these seedling components contribute appreciably to competitive ability and final tiller number in the seed crop, and, therefore, are not under strong selection pressure. This is borne out by an earlier study of the selective changes during seed multiplication of S.23 perennial ryegrass (Cooper, 1959a) where, although a regular advance in earliness of ear emergence was apparent, no corresponding changes in seedling components could be detected.

A further possible explanation for this maintenance of genetic variation lies in the fact that selection operates on the end product, in the present case, on competitive ability and seed yield, rather than on individual components. As we have seen, leaf size and rate of leaf appearance show a negative genetic correlation, and can compensate for each other, and the same is true for tiller number and dry weight per tiller. As Falconer (1960) points out, if two characters are selected simultaneously, as in components of yield, a negative genetic correlation is likely to develop between them. This may in part explain the present findings.

(ii) *Agronomic implications*

It only remains to discuss the implications of these results in selecting for yield under sward conditions. The relation between leaf area increase and dry matter production in ryegrass has been studied by Brougham (1956, 1958) and Davies (1960), and the general sequence in the forage grasses and legumes has been reviewed by Donald and Black (1958).

The rate of increase of dry matter follows a sigmoid growth curve. In the early seedling stage, while only part of the incoming light is being intercepted and before a complete ground cover has been achieved, both the tiller number and the total leaf area are increasing exponentially. Differences in dry matter production are a reflection of differences in leaf area index, with little or no variation in net assimilation rate (leaf efficiency). During this stage of exponential growth, a plant capable of the maximum rate of increase of leaf area is most productive, and the intrinsic rate of tillering is therefore important. Although appreciable genetic variation may exist within a variety for such components as leaf size and rate of leaf appearance, selection for these independently does not necessarily produce corresponding changes in total leaf area or in dry weight (Cooper and Edwards, 1960), partly because of the inverse correlation between leaf appearance and leaf size, and partly because of independent changes in tiller number. Selection for dry matter production at this state must evidently be made on the basis of total leaf area rather than on individual components.

As the leaf area increases, shading of the lower leaves occurs and

eventually light interception is complete. The leaf area index at which all the light is intercepted varies with the species and the degree of insolation. The efficiency of utilisation will be greatly influenced by leaf angle or leaf disposition. A sward composed of erect narrow leaves, as in ryegrass, will absorb more light than a flat reflecting surface, as in white clover (Mitchell and Calder, 1958). Similarly, Brougham (1958) in New Zealand finds the optimum leaf area index for perennial and short-rotation ryegrass to be 7.1, but for white clover only 3.5. At this stage, tiller number has become more or less stable, although some seasonal fluctuation occurs (Langer, 1958, 1959), and the intrinsic rate of tillering is not so important as the production of dry matter per tiller. Thus leaf size and rate of leaf production per tiller become important, and both these characters are differentially affected by shading and defoliation (Mitchell and Coles, 1955).

Once the optimum leaf area index has been attained, the sward is intercepting all the available light, and a steady maximum rate of growth is maintained. Further uninterrupted growth, although the leaf area index can increase up to 9-10, may lead to a reduction in the rate of dry matter production, as the lower leaves on each tiller become completely shaded and parasitic on the rest of the shoot. In practice, the crop should never be allowed to reach this stage, but should be utilised while still at the optimum leaf area index.

The above sequence applies to plants growing vegetatively without defoliation. The frequency and level of defoliation superimposed on this primary growth curve will greatly affect dry matter production (Brougham, 1956; Mitchell and Coles, 1955; Langer, 1959). The starting point of the regrowth curve depends on the amount of leaf area left for photosynthesis. With a lenient cut, the sequence is similar to that outlined above, and for maximum yield sufficient herbage should be left for complete light interception; this is 5 inches in ryegrass, according to Brougham (1956). With a severe cut, however, where the initial regrowth has to be made at the expense of reserves, the distribution of carbohydrates between root and shoot may be important in determining recovery after cutting.

The physiological basis of yield even in the vegetative sward is thus complex, and the characters required vary with the stage of the growth curve and the management envisaged, especially the frequency and intensity of defoliation. The rapid attainment of a high leaf area index is important during establishment and after heavy defoliation, but in addition we need to select for a high optimum leaf area index, capable of utilising a large proportion of the incoming light energy. The present results have been obtained from the early seedling stage, while leaf area increase is still exponential, and may not be reflected in a competitive sward. Furthermore, both leaf area and dry matter accumulation are strongly modified by inflorescence development (Langer, 1958, 1959). Direct selection for

a high optimum leaf area index may prove a more useful approach to the problem of increasing yield in the sward.

5. SUMMARY

1. This paper describes the pattern of leaf and tiller development in three contrasting varieties, Italian ryegrass and Irish and Hunsballe perennial ryegrass, and attempts to assess the genetic variation within each variety for such components of leaf area as leaf size, rate of leaf appearance, and tiller number.

2. Italian ryegrass has larger leaves than the perennial varieties and tillering begins at a lower node, resulting in a larger leaf area and greater dry weight in the seedling stage. No regular varietal differences in rate of leaf production could be detected.

3. The repeatability of leaf size is high (0.6-0.9), but that of rate of leaf appearance is lower (0.3-0.4) and the mean of three successive leaf intervals gives greater precision. Considerable genotypic variation for leaf components exists within these outbreeding varieties, compared to that within homozygous lines of *L. remotum* and *L. temulentum*.

4. The heritability of the leaf characters has been estimated from parent/progeny regression and from the analysis of full-sib families. Useful additive genetic variation exists for many leaf components, for tiller number and for dry weight, and most plants are highly heterozygous for genes controlling these characters.

5. There is a positive *environmental* correlation between nearly all the seedling characters, suggesting common environmental effects on vigour, but a negative *genetic* correlation between leaf size and rate of leaf appearance, and between tiller number and dry weight per tiller. Both these negative correlations will decrease the effect of selection for individual components on total yield.

6. The amount of genetic variation for leaf components is discussed in relation to the past selection history of each variety, and it is concluded that selection for individual components is not likely to increase dry matter production in a competitive sward.

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