

Developmental genetics of leaf formation in *Lolium*

3. Inheritance of a developmental complex

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SUMMARY

Using four lines derived from a single base population of *Lolium perenne* by selection for large leaf size (LL), small leaf size (SL), fast rate of leaf appearance (FR), and slow rate of leaf appearance (SR), the inheritance of a number of related characters specifying various aspects of leaf development was studied. F_1 and F_2 generations were produced for all possible crosses between these four lines.

The genetic differences between the selection lines were largely additive for all characters studied and entirely so for rate of leaf appearance, duration of elongation of a single leaf and for the time interval between the maturation of leaf 3 and the unfolding of the next youngest leaf on the same side of the apex, leaf 5. The non-additive variances noted in rate of total leaf area formation, individual leaf size and its components length and width, and in the rate of leaf elongation, were associated with a tendency towards heterosis in these characters. This was quite marked in some crosses and tended to be larger for the more complex characters, rate of total leaf area formation and leaf size, suggesting that the heterosis was, to a considerable extent, due to interactions between genes controlling component characters.

The data confirmed the earlier finding that the negative correlated selection response between leaf size and rate of leaf appearance was due to a basic association between the maturation of a leaf and the unfolding (onset of rapid elongation) of the next youngest leaf on the same side of the apex. Thus an increase in rate of leaf appearance reduces the duration of elongation of a leaf and this in turn will reduce leaf length. However, the basic association, which seems to be controlled by vascular development of the young leaf, is not entirely invariate.

1. INTRODUCTION

The rate of formation of new leaf surface area on a single shoot of *Lolium* can be subdivided into two components: the average individual leaf size and the rate of appearance of new leaves. But these two components show a strong negative genetic correlation in both *Lolium perenne* and *Lolium multiflorum* (Edwards & Cooper, 1963; Edwards, 1967*b*). Selection for either character was accompanied by a negative response in the other.

A detailed developmental study of the various lines which had been selected for either high or low expression of one or other character showed that the negative

association was between leaf length (one of the two components of leaf size, leaf width being the other) and rate of leaf appearance (Edwards, 1967*b*). The developmental 'block' was that the number of leaves actively expanding at any time on any one shoot was limited to two (one on each side of the shoot apex at which the leaves are formed alternately). The onset of the phase of rapid expansion, which is largely an elongation process in the grass leaf, of any leaf coincides with the maturation and cessation of growth of the next older leaf on the same side of the apex. Thus an increase in the rate of leaf appearance involves a reduction in the period between the onset of expansion of successive leaves on the same side of the apex, and this reduces the period of elongation of each leaf and therefore tends to reduce its final length. The association of the timing of these two events in the development of successive leaves appeared to be unchanged in any of the selection lines and it seems that the observed negative genetic correlation between leaf length and rate of leaf appearance was not due to linkage but to pleiotropy.

The negative association between leaf size and rate of leaf appearance was not complete because leaf size could also be influenced by variation in leaf width which did not seem to be highly correlated with rate of leaf appearance. Also, the leaf length/rate of leaf appearance association was not complete because, while the *duration* of elongation of a leaf is associated with the rate of leaf appearance, the final length of the leaf will also be influenced by the *rate* of leaf elongation.

In order to study the inheritance of this complex and the possibility of obtaining recombination between its components, all possible crosses were made between the four selection lines (selected for large leaf size, LL, small leaf size, SL, fast rate of leaf appearance, FR, and slow rate of leaf appearance, SR, respectively) obtained from a single base population of 'Hunsballe' perennial ryegrass.

2. MATERIALS AND METHODS

(a) *Characters studied*

Following earlier papers (Edwards, 1967*a*, *b*) the characters chosen to specify leaf development on the main shoot of the seedling have been basically the dimensions of a standard leaf (length, width and area of leaf 6) and the rate of leaf appearance expressed as the mean number of leaves appearing per week. The product of these two characters is the rate of total leaf area formation per shoot.

The time of 'unfolding' (that is the time at which the leaf switches from slow primordial growth to the main phase of rapid elongation) of leaf 5 was estimated from its rate of elongation at visible appearance of the tip and its length at appearance (Edwards, 1967*a*). Previous results have shown that in the selection lines this event coincides with the maturation of leaf 3 (Edwards, 1967*b*). The interval between the maturation of leaf 3 and the unfolding of leaf 5 is called the interval between leaves. In addition to measuring the rate of elongation of leaf 5, the duration of elongation was also determined from the data on the times of unfolding and maturation of that leaf.

(b) Experimental details and material

The basic parents were the four selection lines obtained from a cultivar of *Lolium perenne* (Hunsballe) after six generations of selection. All F_1 and F_2 generations were produced and in addition the parental lines were maintained by pair crosses within each line on randomly chosen plants. All the plants grown in this experiment were obtained from seeds produced by crosses made in the same season. The technique of automatic pollination without emasculation (Jenkin, 1931) was used and reciprocal crosses were made. Duplicate crosses of each of the parental lines and of each F_1 were made using different randomly chosen parents.

The data were collected in a greenhouse in late summer and early autumn from plants grown in soil in boxes. Each family was represented by duplicate rows randomized within each of the two blocks. Each row was sown with ten seeds which were reduced to six after germination. In some rows fewer than six seeds germinated and data were then collected on the number available. This was rare, however, and unweighted row means were used in the analysis of the results.

3. RESULTS

(a) Variation within parental lines

The parental lines had been subjected to fairly intense selection for six generations but could not, of course, be regarded as highly inbred. In fact, during the selection programme a system of double-first-cousin mating had been used to minimize the inbreeding that occurs with a breeding population size of four plants. The theoretical inbreeding coefficient (F) after six generations of selection using such a mating system is 0.31. While the effects of selection will undoubtedly have been to increase the number of loci fixed over that expected by inbreeding alone, it seems unlikely that all loci will have been fixed even for the characters which have undergone direct selection. Table 1 shows that variation between duplicate crosses existed for both leaf size and rate of leaf appearance, demonstrating that the lines are not fixed and homozygous.

The existence of variability within the parental lines limits the possible genetic analysis. The lines cannot be reliably treated as inbred lines and analysed by the method of Mather (1949) which involves comparing the variances of segregating (F_2 and backcross) and non-segregating (parental and F_1) generations. Nor are the methods of analysis of diallel crosses between inbred lines (Jinks, 1954) appropriate. A diallel analysis on non-inbred material (Dickinson & Jinks, 1955) really requires more than the four lines available. It is clear, however, from Table 1 that considerable differences do exist between the lines, for the variance between lines is very much greater than that between duplicate crosses within lines. To attempt to discover some of the characteristics of this genetic variation between lines an analysis based on the generation means has been used.

Table 1. Variation between duplicate crosses of selection lines

Selection lines	Mating	Mean leaf size (mm ²)			Mean rate of leaf appearance (leaves/day)		
		Duplicate family 1	Duplicate family 2	Mean	Duplicate family 1	Duplicate family 2	Mean
Hybrids	Large leaves	1426	991	1209	0.120	0.130	0.125
	Small leaves	605	390	498	0.152	0.179	0.166
	Fast rate	362	394	378	0.183	0.160	0.172
	Slow rate	741	855	798	0.121	0.125	0.123
Selection lines	Large × Small	1064	1032	1048	0.152	0.147	0.150
	Large × Fast	880	905	893	0.168	0.156	0.162
	Large × Slow	1376	1324	1350	0.124	0.129	0.127
	Small × Fast	668	617	643	0.164	0.170	0.167
	Small × Slow	1044	781	913	0.132	0.140	0.136
	Fast × Slow	823	659	741	0.164	0.151	0.158
V_1	variance between duplicate rows within blocks and families (80 D.F.)	30037			7.183×10^{-5}		
V_2	variance between duplicate crosses within lines or hybrids (10 D.F.)	140996			73.480×10^{-5}		
V_2/V_1		4.69 ($P < 0.001$)			10.23 ($P < 0.001$)		
V_3	variance between lines and hybrids (9 D.F.)	1.472037			580.804×10^{-5}		
V_3/V_2		10.44 ($P < 0.001$)			7.90 ($P < 0.01$)		

NOTE. Each family mean in this table is based on eight rows (two duplicate rows within each block of each reciprocal of each family).

(b) Reciprocal differences

Before turning to an analysis of the generation means the question of differences between reciprocal crosses must be considered. Edwards & Emará (1970) have shown on a study of a population of *Lolium multiflorum* that while leaf-3 dimensions did show maternal effects, there were no reciprocal differences for either rate of leaf appearance or leaf dimensions. In order to detect reciprocal differences between crosses involving the selection lines, these four lines and their six possible F_1 generations were analysed according to Hayman (1954). In this analysis the c and d items represent an analysis of the *differences* between reciprocal crosses, while a and b are mean squares derived from an analysis of the *sums* of reciprocal crosses. Of the two items associated with differences, c measures average maternal effect of each parental line while d measures variation in reciprocal differences not ascribable to c (see table 1 of Hayman, 1954). These items plus the genetic effects a and b are tested against an error based on the interactions of these effects with blocks. With one exception these interaction mean squares were homogeneous within each character and so a single pooled error mean square is presented. The exception is rate of total leaf area formation where the mean squares for interactions with blocks were much larger for c and d than for a and b ; therefore separate error mean squares are presented.

The data presented in Table 2 shows that while small and marginally significant reciprocal effects appeared in leaf width and rate of leaf elongation, large effects occurred only in rate of leaf appearance where they were due to maternal interactions. In general, however, the reciprocal differences mean squares were much smaller than the genetic effects.

(c) Genetic effects

Information on the genetic differences between lines is provided by the a and b items in the Hayman analysis, which analyse the variation between sums of reciprocal crosses. While a measures the variation between the mean effects of parental lines, b measures variation in reciprocal sums not ascribable to a (see table 1 in Hayman, 1954). These two items measure general and specific combining abilities, respectively. Their detailed genetic interpretation depends on the genetic assumptions which are made. If there are no non-allelic interactions and if gene frequencies are equal then a represents additive genetic effects and b dominance. Unequal gene frequencies or multiple allelism complicates a by including a dominance component. Non-allelic interactions will introduce unknown biases into both items but mainly into b . Furthermore, a comparison of the magnitudes of a and b is complicated by the presence in the expectations of a of a coefficient depending on the number of parental lines in the diallel (four in this case) (see table 3 of Hayman, 1954).

For all characters at least one of these components is highly significant. The specific combining ability mean square is not significantly larger than the error item for duration of leaf elongation and the interval between successive leaves, and

Table 2. *Analysis of variance of diallel table of crosses between selection lines (mean squares)*

Item	Degrees of freedom	Rate of leaf area formation	Leaf size	Leaf width	Leaf length	Rate of leaf elongation	Duration of leaf elongation	Rate of leaf appearance	Leaf interval
<i>a</i>	3	6857***	681016***	455.0***	8103***	5051***	1229***	2995***	4.23***
<i>b</i>	6	1931***	74289***	51.1***	1394***	1681***	77	161***	0.59
<i>c</i>	3	378	16965	12.3*	325	619*	119*	16	0.08
<i>d</i>	3	663	15052	13.7*	125	628*	41	174***	0.54
Error	15	91 (i)† 352 (ii)	9900	3.2	182	158	30	14	0.42
Ratio <i>a/b</i>			9.17	8.90	5.81	3.00	15.96	18.60	7.17

* $P < 0.05$. *** $P < 0.001$.† Separate error mean squares. (i) Error for items *a* and *b* based on 9 D.F. (ii) Error for items *c* and *d* based on 6 D.F.

Table 3. Relationships between means of the parental lines and their F_1 hybrids for eight characters representing leaf development

Mating	Family	Rate of leaf area formation (mm ² /day)	Leaf size (mm ²)	Leaf width (mm)	Leaf length (mm)	Rate of leaf elongation (mm/days)	Duration of leaf elongation (days)	Rate of leaf appearance (leaves/day)	Leaf interval (days)
LL × SR	LL	150	1208	5.6	214	22.1	12.8	0.125	2.4
	F_1	165	1301	5.7	227	24.3	13.0	0.127	2.2
	SR	95	770	4.2	178	16.9	13.7	0.123	2.0
LL × SL	LL	150	1208	5.6	214	22.1	12.8	0.125	2.4
	F_1	154	1113	4.7	215	23.0	12.3	0.150	1.5
	SL	75	444	3.3	135	17.4	10.9	0.170	0.1
LL × FR	LL	150	1208	5.6	214	22.1	12.8	0.125	2.4
	F_1	143	892	5.1	174	21.2	11.1	0.162	0.9
	FR	65	372	3.4	107	14.5	9.6	0.174	1.2
SR × SL	SR	95	770	4.2	178	16.9	13.7	0.123	2.0
	F_1	124	913	4.6	196	21.1	12.5	0.136	1.7
	SL	75	444	3.3	135	17.4	10.9	0.170	0.1
SR × FR	SR	95	770	4.2	178	16.9	13.7	0.123	2.0
	F_1	114	725	4.2	165	18.6	11.0	0.155	1.0
	FR	65	372	3.4	107	14.5	9.6	0.174	1.2
SL × FR	SL	75	444	3.3	135	17.4	10.9	0.170	0.1
	F_1	111	642	3.9	165	19.8	10.5	0.173	0.1
	FR	65	372	3.4	107	14.5	9.6	0.174	1.2
S.E.M.		5.8	43.1	0.37	5.3	0.59	0.27	0.002	0.28

it is not larger than the *d* item (which, if significant, may be expected to bias the *b* term) in the third character associated with the timing of leaf development, namely rate of leaf appearance. For the other characters the mean squares for specific combining ability are significant but are smaller than those for general combining ability. Bearing in mind the presence of a coefficient of 4 in the genetic expectation of the latter, it would appear from the ratios *a/b* that additive genetic variance is appreciably larger than the dominance plus interaction effects for leaf size, leaf width and possibly leaf length.

Table 4. Means for 3 types of family

	Parents	F_1	F_2	$\frac{\bar{F}_1 - \bar{P}}{\bar{P}} \times 100$
Rate of leaf area formation (mm ² /day)	96	135	118	+41
Leaf size (mm ²)	699	931	795	+33
Leaf width (mm)	4.1	4.7	4.6	+15
Leaf length (mm)	159	190	169	+19
Rate leaf elongation (mm/day)	17.7	21.3	19.6	+20
Duration leaf elongation (days)	11.8	11.7	11.5	-1
Rate of leaf app. (lvs/day)	0.148	0.151	0.156	+3
Leaf intervals (days)	1.4	1.2	1.2	-14

In summary of the genetic effects, there is appreciable genetic variation between lines for all characters. This is very largely additive for the timing of leaf development but contains considerable dominance and interaction effects for the other characters, especially rate of total leaf area formation and rate of leaf elongation.

The patterns of inheritance are shown in Table 3. The additive nature of the differences for rate of leaf appearance, duration of leaf elongation and interval between leaves is shown by the general intermediacy of the hybrids compared with their parents. For the other characters there is a tendency for heterosis with at least some of the F_1 means being greater than the higher parent. The relationship between the means of all the parents and of the F_1 and F_2 generations in Table 4 shows that the heterosis is largely lost in the F_2 . This also shows that the greatest degree of increase in the mean of all the F_1 families over the mean of all the parental lines occurs for rate of total leaf area formation (41%). This is developmentally the most 'complex' character, being the product of leaf size and rate of leaf appearance. Leaf size itself shows the next largest increase in the F_1 (33%) while its components of width and length have increases of 15% and 19% respectively.

(d) Association between characters

The picture of the relationships which has emerged from a study of the responses to selection is that an advance in the rate of total leaf area formation might be possible if one of the following changes occurred:

- (i) An increase in leaf width independent of leaf length.

(ii) An increase in the rate of leaf elongation independent of the duration of elongation.

(iii) An increase in the number of leaves expanding at any one time.

The data available provide some indication of the possibilities of such changes.

(i) *Leaf width and leaf length*

The correlations between these two characters were computed on family means and also on the individuals within families (Table 5). The correlations are large and positive although, since those on the family means have so few degrees of freedom, only that for the F_2 families is significantly different from zero. If the data from the parental lines and the F_1 families are pooled the combined correlation is 0.88, which is significant at the 0.001 probability level. The correlations based on individuals within families are lower than those for the family means and there is a hint that the correlation within F_2 is lower than that within non-segregating families.

Table 5

(a) Correlation coefficients between leaf length and leaf width

	Parental lines	F_1 families	F_2 families
(a) Family means	0.93 ($n = 4$)	0.73 ($n = 6$)	0.85* ($n = 6$)
(b) Individuals within families	0.57*** ($n = 32$)	0.67*** ($n = 48$)	0.39 ($n = 24$)

(b) Correlation coefficients between rate of leaf elongation and duration of elongation

(a) Family means	0.58 ($n = 4$)	0.82* ($n = 6$)	0.96** ($n = 6$)
(b) Individuals within families	-0.16 ($n = 32$)	-0.47*** ($n = 48$)	-0.10 ($n = 24$)

Correlation coefficients significantly different from zero at:

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

(ii) *Rate and duration of leaf elongation*

The correlations are also presented in Table 5. Again the correlations between family means are generally large and positive. The lower value for the parental lines is due to the slow line, which has a long duration (13.7 days) while having a relatively low rate (16.9 mm/day). If this one line is removed the remaining parental and F_1 lines have a combined correlation of 0.89. The within-family correlations are small and negative, suggesting that it might be possible to select within families for increased rate without increasing duration which in turn affects rate of leaf appearance. Indeed the slow line shows that selection for increased duration may have little effect on rate of elongation.

(iii) *Number of actively expanding leaves*

This is determined by measuring the interval between the time of maturation of leaf 3 and the estimated time of unfolding of leaf 5. A negative value would indicate an overlap. Table 6 presents the various family means and the relevant mid-parental values. It seems that selection for either large leaves (with concomitant slow rate of leaf appearance) or for slow rate of leaf appearance (with increased leaf

size) has tended to create an interval between the timing of maturation of a leaf and the unfolding of the next younger leaf on the same side of the apex. This trend was noted in an earlier experiment (Edwards, 1967*b*) but there it was not statistically different from zero. Here the differences are repeated and are significant. Furthermore the differences between lines appear to be heritable, since the F_1 and F_2 family means show a high correlation to each other and to their respective mid-parental values. In these lines the only heritable differences are in a positive direction, that is in the creation of a lag between the maturation of leaf 3 and the onset of rapid growth in leaf 5.

Table 6. *Interval (days) between maturation of leaf 3 and unfolding of leaf 5*

Parental lines			
	Large	2.4	
	Small	0.1	
	Fast	1.2	
	Slow	2.0	
Hybrids			
	\bar{P}	\bar{F}_1	\bar{F}_2
LL × SL	1.3	1.5	1.2
LL × FR	1.8	0.9	1.1
LL × SR	2.2	2.2	2.7
SL × FR	0.7	0.1	0.2
SL × SR	1.1	1.7	1.7
FR × SR	1.6	1.0	0.4

4. DISCUSSION

The aims of this study were, firstly, to investigate the nature of the genetic differences between four lines selected from within some base population for four different but related criteria of leaf development; and secondly, to attempt to relate the findings on the genetic variation for a number of characters to the developmental relationships between them.

The final product of this complex is the rate of total leaf area development on a single shoot. This character, although not directly selected for in any of the lines, shows considerable differences between the lines, and the inheritance of these differences appears to be largely additive. There is also evidence of an appreciable amount of non-additive genetic variation and of considerable heterosis in some of the crosses. Can a study of the components of this character tell us anything further about the genetic variation?

The two primary components of rate of total leaf area formation are individual leaf size and rate of leaf appearance. Both these show considerable additive genetic variation, which is in line with the conclusions from a study of the base population (Cooper & Edwards, 1961) and from the responses to selection (Edwards & Cooper, 1963). For leaf size the specific combining ability variance is also significant. Furthermore, leaf size shows marked heterosis in some crosses but this is absent for rate of leaf appearance. Thus the heterosis in rate of total leaf area formation

seems to be due largely to heterotic effects in leaf size. The two components of leaf size, width and length, both show some heterosis but to a lesser extent than leaf size.

Of the components of leaf length, only rate of leaf elongation shows any heterosis. This pattern is shown in Table 7 for the two crosses which gave the greatest degree of heterosis, although here the data is presented in terms of deviations from the mid-parental values. Clearly this pattern is very similar to the overall trends shown in Table 3, which were averaged over all families. The superiority of the hybrid over the mid-parental value (which in some crosses leads to true heterosis—that is, superiority over both parents) for rate of total leaf formation is a reflexion of that for leaf size. Leaf width and length both apparently contribute to the F_1 superiority for leaf size, while all the superiority of F_1 families for leaf length is due to rate of leaf elongation and none to duration of elongation.

Table 7. Deviation of F_1 from mid-parental value (as a percentage) for two crosses

Character	Fast \times Small	Small \times Slow
Rate of total leaf area formation	+ 59 %	+ 46 %
Leaf size	+ 57 %	+ 50 %
Leaf width	+ 16 %	+ 23 %
Leaf length	+ 36 %	+ 25 %
Rate of leaf elongation	+ 17 %	+ 23 %
Duration of leaf elongation	+ 2 %	+ 2 %
Rate of leaf appearance	+ 1 %	+ 8 %

While the same general trend appears to exist in all crosses, it is interesting that little true heterosis appears in any of the crosses involving the LL line. Since leaf size, through its components, appears to be sensitive to inbreeding, it may be that only where selection has been directly for large leaves has a homozygous combination been obtained which shows no inbreeding depression. If this is so it is not expected that hybrids involving this line would be superior to it.

If we compare the characters for the proportion of the genetic variance which is non-additive (that is, specific combining ability) there appears to be a general parallel between this proportion and the relative excess of the F_1 families over the mid-parental values. In general the more developmentally complex the character the higher the proportion of non-additive variation and the greater the tendency to show heterosis. Although the relationship is not linear, for example leaf width shows a high proportion of non-additive variance, it does suggest that to a considerable extent the observed heterosis is a consequence of interactions between genes controlling the various component characters, although the accumulation of favourable dominants is also likely to be important.

The discussion of interaction between characters raises the issue of association between characters. Leaf length and leaf width appear to be highly correlated with little potential for selecting independently for width. But rate and duration of leaf elongation, while showing a high positive correlation between families, appear to be uncorrelated within families. This suggests that it might be possible,

by selecting for the rate of leaf elongation, to increase leaf length without causing a reduction in the rate of leaf appearance. Furthermore, a more detailed examination of the parental lines shows that the SR line has responded by an increase in duration of elongation but no change in rate of elongation.

The negative genetic correlations between rate of leaf appearance and leaf size have been due to the association between the rate of appearance and the duration of leaf elongation. This was, in turn, a reflexion of the association between the onset of rapid elongation of one leaf (the unfolding) and the maturation of the next older leaf on the same side of the apex. Further work on the physiology of the relationship (Edwards, in preparation) has shown that its basis seems to be that when the vascular system of a young developing leaf becomes connected to that of the stem this leaf begins to grow rapidly and that this, in turn, inhibits further growth of the older leaf. But although selection has not produced any large disturbance of this association, it has produced small changes and these are heritable. However, the only changes which have been produced are towards creating a gap between the maturation of the older leaf and the onset of growth of the younger. This has occurred in both the LL and the SR lines. In the latter line it is easy to see how direct selection for a slower rate of leaf appearance could have caused both an increase in the duration of elongation and a positive interval between maturation and onset of growth of successive leaves. But in the LL line it is not too easy to see why both have occurred unless there is some unexpected correlation between these two characters.

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