INTER-POPULATION VARIATION IN PERENNIAL RYEGRASS

I. POPULATION MEANS

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1. INTRODUCTION

THE genetic basis for variation between populations of *Lolium perenne* has been investigated by Corkill (1950), Fejer (1955, *1958*, 1959, 1960), Torrie (1957), Breese (1960), Hayward and Breese (1963), Thomas (*1963*, *1965*) and Hayward and Breese (*1966*). Most of these authors used diallel analysis of one kind or another, plus, in some cases, selection experiments (Fejer, 1960). Their general findings were that the characters they considered are under polygenic control, and that heritability, usually high, is attributable to a high level of general combining ability or additivity. In addition, some of the above authors (references in italic) demonstrated significant differences between reciprocal crosses for certain characters.

The present paper deals with the results of analysis of a complete diallel series of crosses between six populations of ryegrass. Estimates of the components of variation, for a number of characters, primarily measures of seedling growth are presented.

2. EXPERIMENTAL METHODS

(i) *Sampling technique involved in diallel cross construction of the F_1 generation*

There is good *a priori* evidence to suppose that there is a high level of heterozygosity within *Lolium* populations since outbreeding is necessitated by a rigid system of self-incompatibility (Jenkin, 1931*b*). Moreover, Cooper (1959) and Corkill (1956) have demonstrated considerable variation within populations and considered that the extent of this could most reasonably be attributed to a high level of heterozygosity. Any attempt to reduce this level of heterozygosity, *i.e.* to approach inbred lines, results in a marked decrease in vigour (Jenkin, 1931*b*, Cooper and Thomas, 1961). Thus the usual method of constructing a diallel series of crosses utilising inbred populations could not be attempted here.

Dickinson and Jinks (1956), however, have suggested that a diallel between outbreeding populations may be constructed from crosses between groups of plants, rather than individuals. Implicit in this suggestion is, of course, the proviso that a reasonable number of plants are included in each group. Thus each group should represent

the range of within population variation. What constitutes a reasonable sample will, of course, depend on the variability of the character being investigated and Dickinson and Jinks (*loc. cit.*) have in fact stated that analysis applied to this construction " will be progressively less accurate the lower the ratio of genetic variance between parental groups to that within groups ". With the above points in mind the diallel crosses between the six populations were made (in the spring of 1962) as described below.

The total number of plants utilised from any one population to construct the diallel cross families was 70. These were allocated at random to seven groups of 10 plants each. Between population families were then constructed from 10 pair crosses between any two groups from separate populations. Having made all possible inter-population crosses, the two remaining groups within each population were also crossed together in 10 pairs to represent the parental populations. The crossing technique used was the method of automatic cross pollination, without emasculation (Jenkin, 1931*a*). Reciprocal plants from each pair cross were separated before the seed was mature and later harvested. Each of the reciprocal F_1 families to be sown out was made up in turn by bulking equal numbers of seed from the group of 10 plants used in crossing. Parental families were constructed by taking equal amounts of seed from the two groups (20 plants) used in within population crosses. Thus a complete 6×6 diallel was made up. All the 420 plants used for crossing were tested for self-incompatibility, using as many aftermath heads as were available. The possibility of selfing on any appreciable scale is ruled out by the fact that the selfed seed averaged 0·2 per head with a range of 0-2 whereas the seeds per head from crosses averaged 30 with a range of 5-200.

(ii) *Extension of the diallel crosses to the F_2 generation*

For reasons already considered it was impossible to raise a F_2 generation from selfed F_1 plants. None the less it was considered essential to raise the equivalent of this generation and this for convenience is referred to as the F_2 and was constructed as described below.

As well as the bulked F_1 diallel, three single F_1 diallels were constructed to form the basis for the F_2 crosses. For any one of these diallels the seed from only one cross (not all 10) between any two populations and one cross within each population, sampled at random from the 10 crosses affected, was taken and a complete F_1 diallel set made up. Four plants (sibs) from each reciprocal F_1 between population and within population family from each of these diallels were potted up in May 1963 and polycrossed together to obtain seed from the F_2. Equal amounts of seeds from corresponding families in those three diallels was then used to form the bulked F_2 diallel. Thus this diallel is based on a much smaller sample of the parental populations than the F_1 diallel, but it probably still constitutes a reasonable sample from the population as the only character measured (dry weight of tops) in this generation has a very high between versus within population ratio of genetic variance.

(iii) *Plant materials and metrics assessed*

The six populations used differ widely in agronomic type, geographic origin and in the degree of past artificial selection applied. They have previously been described by Cooper (1963, 1964), who demonstrated that the wide differences in origin are reflected in differences in a number of continuously varying characters. These populations are: 1, Irish (Ba. 7209); 2, Italian (Ba. 894); 3, Algerian (FAO 3112);

4, Lithuanian (Ba. 7267); 5, New Zealand (Ba. 7209) and 6, S.23 (AB. 59) and will only be referred to henceforth by the prefix 1-6. The figures in brackets indicate the appropriate W.P.B.S. accession numbers.

The complete F_1 diallel was sown out in four randomised blocks in an unheated greenhouse on 10th October 1962. The seeds were planted in steam-sterilised soil, in shallow boxes, containing a modified John Innes compost. To obtain 10 plants per block for each of the 30 reciprocal F_1 families and 20 for each parental family, twice this number of seed was sown. All seeds were weighed individually and the position of each noted during sowing.

Most of the characters observed in this generation were quantitative measures of seedling development. Obviously in grasses the most important character is the amount of green material produced and also in this persistent crop the amount of root—usually both measured as dry weight. Attempts have been made by *e.g.* Mitchell (1953*a*, *b*), Patel (1958), Cooper and Edwards (1961, 1964) and Edwards (1961), to assess plant growth less destructively and this has led to a breakdown into yield components. These components fall into three categories: (1) leaf size, (2) rate of leaf production and (3) rate of tiller production. In the present study, basing measurements on the work of the above authors, the estimate of (1) used was the length, breadth and area of two standard leaves—the third and fifth on the main tiller; the record of rate leaf production was the time in days between the appearance of the third and sixth leaves on the main tiller. Rate of tillering estimates were represented by two metrics—tiller number at third and sixth leaf stages. These measurements were taken on all plants. In addition top and root dry weight of half the plants (taken randomly) of each family in each block was observed 90 days after sowing. The remaining plants were allowed to head outdoors in the summer of 1963 and time of ear emergence (flowering time) recorded.

The F_2 diallel was sown out in exactly the same way as the F_1 in a similar greenhouse environment in October 1963. The only measurement observed in this generation was dry weight of the tops, which was taken in March 1964.

All analysis of these families within each generation for any one character are based on the mean value of all plants in each family within a block.

3. RESULTS

(i) *The F_1 diallels*

The mean values for combinations of crosses (including reciprocals) between and within populations are shown in diallel table form, in table 1A to 1L. These are the mean values over all plants for each family.

The genetic basis for the differences between populations may be examined with reference to the analysis of the diallel cross families (all characters show significant differences between populations as has been demonstrated elsewhere (Thomas, 1965). There is a large number of available analyses for this purpose and these fall into two categories. Firstly, analysis of variance of the diallel table, in which few inferences are made as to the genetic basis for population differences, can be applied, *e.g.* Yates (1947), Hayman (1954*a*). Secondly, depending on the results obtained in the variance analysis, a more sophisticated breakdown into genetic parameters representing *e.g.* dominance and additivity may be attempted, *e.g.* Jinks and Hayman (1953), Jinks (1954), Hayman (1954*b*). In the first instance, the utilisation of these methods will be considered and illustrated with reference to one character—fifth leaf area.

TABLE 1

Diallel table of values for " F1 " characters

	A						B					
	5th leaf length (in mm.)						5th leaf breadth (in mm.)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	131	153	165	137	137	138	2·67	3·24	2·79	2·70	2·61	2·70
2	152	184	160	190	168	151	3·19	4·15	3·38	3·95	3·31	3·26
3	161	163	186	169	155	156	2·67	3·08	2·71	2·73	2·63	2·55
4	141	149	160	135	141	133	2·76	3·05	2·83	2·76	2·79	2·62
5	145	144	162	143	140	128	2·65	2·95	2·86	2·81	2·67	2·52
6	131	141	149	131	129	131	2·56	2·78	2·47	2·70	2·47	2·44
	C						D					
	5th leaf area (in sq. mm.)						Top weight (in milligrammes)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	362	509	463	371	361	375	136	171	188	149	114	137
2	498	772	553	756	564	510	180	292	224	260	199	171
3	437	492	510	463	410	399	115	148	117	134	146	142
4	389	464	460	377	393	352	160	138	146	139	135	129
5	386	438	473	404	374	329	144	136	177	157	151	123
6	334	402	374	361	373	333	129	125	127	116	118	114
	E						F					
	Tiller No. at 6th leaf						Rate of leaf appearance (in days)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	6·13	5·83	6·75	5·45	5·58	5·35	33·7	35·4	33·9	33·6	36·3	32·8
2	5·98	6·28	5·90	5·75	5·75	5·05	32·7	30·8	32·1	33·4	32·1	32·2
3	5·80	5·53	4·13	5·23	5·13	4·63	36·1	33·1	35·6	34·2	32·7	31·9
4	5·78	5·53	5·70	4·85	4·95	4·75	34·1	35·3	35·1	34·2	34·3	33·8
5	5·98	5·00	6·10	5·28	6·10	4·88	34·3	33·9	33·3	33·0	33·1	31·5
6	5·20	4·68	4·30	3·95	4·95	4·15	33·7	33·6	34·4	33·4	32·4	32·4

The results of the first analysis to be applied to this data—the " Hayman (1954*a*) analysis of variance of diallel tables " are shown in table 2. All four main items in this analysis are initially tested against estimates of random variation, each with their particular replicate errors. It can be observed from these tests that both the zygotic items (*a*) (average zygotic effect of each parent) and (*b*)*

* The further subdivision of the (*b*) item, suggested by Hayman (1954*a*), was not considered necessary here for reasons which will be apparent later.

TABLE 1—*continued*

	G						H					
	3rd leaf length (in mm.)						3rd leaf breadth (in mm.)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	93	117	119	98	105	96	2·09	2·43	2·29	2·05	2·16	2·07
2	116	136	111	142	124	113	2·46	3·16	2·47	3·00	2·61	2·54
3	123	110	122	119	107	102	2·17	2·24	2·12	2·16	2·22	2·16
4	110	107	121	103	100	93	2·27	2·25	2·18	2·18	2·29	2·13
5	107	106	116	108	105	88	2·23	2·22	2·40	2·39	2·25	2·10
6	97	96	100	87	90	89	2·06	2·12	2·00	1·94	2·05	2·05
	I						J					
	3rd leaf area (in sq. mm).						Root weight (in milligrammes)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	202	294	273	211	229	198	86·0	102	104	107	86·0	95·0
2	292	435	287	428	323	294	99·0	116	114	129	111	95·8
3	266	250	262	257	238	218	68·5	79·5	71·3	96·5	85·3	96·0
4	248	244	269	226	230	203	105	80·5	113	93·5	117	110
5	240	239	281	257	237	185	113	87·3	96·5	109	114	85·0
6	201	198	204	171	185	185	81·5	66·3	92·8	72·5	93·5	88·3
	K						L					
	Tiller No. at 3rd leaf						Flowering time (in days)					
	1	2	3	4	5	6	1	2	3	4	5	6
1	1·60	1·45	1·85	1·45	1·53	1·45	13·0	24·5	13·5	20·8	11·5	28·8
2	1·63	1·88	1·63	1·55	1·33	1·30	19·3	29·3	29·3	27·0	22·8	30·3
3	1·28	1·50	1·18	1·30	1·40	1·25	14·5	23·5	12·8	16·5	17·0	26·5
4	1·68	1·40	1·58	1·40	1·55	1·35	21·5	29·3	19·3	29·3	22·5	32·5
5	1·78	1·35	1·78	1·70	1·83	1·40	12·8	22·8	16·3	19·3	17·0	28·3
6	1·30	1·28	1·23	1·05	1·40	1·13	28·5	30·8	25·0	35·5	27·5	41·8

(residual zygotic effect) and both items indicating the presence of reciprocal differences, (*c*) (average reciprocal effect of each parent) and (*d*) (residual reciprocal effect), are significant. However, when reciprocal differences are detected in these tests, further, more valid, tests of zygotic effects within the structure of this analysis must be considered (Wearden, 1964). The relevant tests are (*a*) against (*c*), and (*b*) against (*d*)—which are also shown in table 2. Zygotic effects are now only apparent as average effects of the parents. This finding

cannot be considered as a final assessment of the genetic situation, since there has been no independent test of the relative importance of the mean zygotic effect of each parental line compared with the residual zygotic effect. Nor can there be in this analysis in the event of both

TABLE 2

Hayman (1954a) analysis of variance of the diallel table for "5th leaf area"

Item	df.	M.S.	F†	F††
a	5	212,560	50·97***	6·96*
b	15	9,051	2·44*	1
c	5	30,499	15·53***	...
d	10	10,004	3·31**	...
Block	3	11,875	...	...
B×*a*	15	4,170	...	...
B×*b*	45	3,716	...	...
B×*c*	15	1,963	...	...
B×*d*	30	3,017	...	...

F† = Variance ratios of main items to their particular block errors.
F†† = Variance ratios of *a* item against the *c* item, and the *b* item against the *d* item.

(*c*) and (*d*) being significant. An additional variance analysis needs to be carried out on these data as a result of the above findings—namely the "factorial" analysis of the diallel table—a modification of which is used here and has been described by Wearden (1964)—table 3. In this analysis an independent test of the mean zygotic

TABLE 3

Factorial analysis of the diallel table for 5th leaf area

Item	df.	M.S.	F†	F††
Row	5	184,733	54·98***	3·17
Column	5	58,363	17·37***	6·45**
R×C { *b*	15	9,050	2·69**	...
R×C { *d*	10	10,004	2·99**	...
Block	11,894	11,894	3·54	...
Residual	3,360	3,360	...	...

F† = Variance ratios against the residual error.
F†† = Variance ratios of the Row against the Column item and the Column item against the *b* item.

effect of parental lines against residual zygotic variation can be effected—namely the column to (*b*) comparison—which is in fact significant here, indicating that the former is the more important genetic effect. A further advantage of this analysis is that the relative

size of the Row and Column items indicates the origin of the reciprocal differences, being considered maternal when Row is larger and paternal when the reverse occurs. In this instance, Row is the larger and although it is not quite significantly greater at the 5 per cent. level, we can reasonably take these reciprocal differences as indicating maternal effects. In table 3, for the sake of completeness, all main items are tested against the homogeneous replicate interactions, and although all are significant, their interpretation does not add to the knowledge obtained from the above more relevant tests, and they will not be considered further. Thus from the results of tables 2 and 3 we can conclude that maternal effects are present as average effects of parents and as residual variation and zygotic effects only as average parental effects.

In the light of the above findings it would be superfluous to present any more sophisticated analyses such as those of Jinks and Hayman (Jinks and Hayman, 1953; Jinks, 1954; Hayman, 1954*b*), for two main reasons. Firstly, in the absence of any genetic effects such as dominance not ascribable to the mean effects of the parents, these analyses yield no further information, merely confirming the above findings. Secondly, the presence of maternal effects invalidates one of the basic assumptions underlying these further analyses, and although the analyses can be applied, with modifications, the only point they serve is to illustrate maternal and mean genetic effects graphically and this is relatively inessential in view of the conclusions based on analysis already performed.

Analyses of remaining F_1 characters

The diallel tables for the remaining characters measured are analysed using the Hayman (1954*a*) and " factorial " methods in tables 4A-L and 5A-L respectively. These results will not be dealt with in so much detail, and the characters have been for convenience placed in four groups, where within a group the pattern of variation for the character(s) included in that group is substantially different from the pattern for the character(s) in other groups.

Group 1 Characters. Fifth leaf breadth, length and area, third leaf length and tiller number at the sixth leaf stage. For all these characters both zygotic and reciprocal effects occur. Reciprocal effects in each case are apparent as average effects of the parents (significance of (*c*))—which for two characters, fifth breadth and area are also coupled with residual reciprocal effects (significance of both (*c*) and (*d*)). From the results of table 5A, B, C, E and J comes a reasonable justification for terming these (*c*) or (*d*) effects maternal rather than paternal, since the row column ratio of mean squares is always positive though never in fact significant at the 5 per cent. level.

Zygotic effects are present as average effects of parents for all these variables on both the tests of (*a*) against (*c*) and column against (*b*),

TABLE 4

Hayman (1954a) variance analysis of diallel tables for each " F_1 *" character*

Item	df.	A		B		C	
		5th leaf length		5th leaf breadth		5th leaf area	
		F†	F††	F†	F††	F†	F††
a	5	27·36***	10·48**	104·05***	8·89*	50·97***	6·96*
b	15	1·90*	1·16	1·66	<1	2·44*	<1
c	5	4·36*	...	14·24***	...	15·53***	...
d	10	1·13	...	2·60*	...	3·31**	...
Item	df.	D		E		F	
		Top weight		Tiller No. at 6th leaf		Rate of leaf appearance	
		F†	F††	F†	F††	F†	F††
a	5	17·37***	2·31	16·32***	8·58*	5·44**	1·71
b	15	2·32*	1·11	2·84**	2·41	1·79	1·62
c	5	11·51***	...	4·64*	...	2·23	...
d	10	2·10*	...	<1	...	<1	...
Item	df.	G		H		I	
		3rd leaf length		3rd leaf breadth		3rd leaf area	
		F†	F††	F†	F††	F†	F††
a	5	33·30***	7·44*	49·85***	3·90	40·39***	4·32
b	15	3·23**	1·24	6·91***	<1	7·07***	<1
c	5	9·70***	...	11·55***	...	18·14***	...
d	10	1·72	...	2·99*	...	2·81*	...
Item	df.	J		K		L	
		Root weight		Tiller No. at 3rd leaf		Flowering time	
		F†	F††	F†	F††	F†	F††
a	5	2·38	<1	14·90***	2·76	257·90***	262·20***
b	15	1·32	<1	2·94**	1·47	11·43***	2·05
c	5	5·37**	...	4·17*	...	1·62	...
d	10	1·59	...	1·65	...	1·88	...

Note. The mean squares for the main items and also their block interactions are not included here, only the " F " value for certain comparisons.

F† = Ratio of each main effect M.S. to its particular block interaction M.S.

F†† = Ratio of *a* to *c* and *b* to *d*.

TABLE 5

Factorial analysis of diallel tables for each " F_1 " character

Item	df.	A		B		C	
		5th leaf length		5th leaf breadth		5th leaf area	
		F†	F††	F†	F††	F†	F††
Row	5	23·06***	2·19	83·87***	3·28	54·98***	3·17
Column	5	10·54***	6·31**	25·60***	13·68***	17·37***	6·45**
R × C *b*	15	1·67	...	1·87*	...	2·69**	...
R × C *d*	10	1·43	...	2·50*	...	2·99**	...
Block	3	3·29*	...	3·85*	...	3·54*	...
Residual	105	...	...	...	...	...	...
Error variance:		(183)	...	(349)	...	(3,360)	...
Item	df.	D		E		F	
		Top weight		Tiller No. at 6th leaf		Rate of leaf appearance	
		F†	F††	F†	F††	F†	F††
Row	5	29·65***	7·39*	15·98***	1·98	5·59***	1·86
Column	5	4·01**	1·80	8·09***	3·44*	3·00*	1·96
R × C *b*	15	2·22*	...	2·35**	...	1·53	...
R × C *d*	10	1·99*	...	<1	...	<1	...
Block	3	4·20**	...	4·76**	...	4·59**	...
Residual	105	...	...	...	...	...	...
Error variance:		(1,000)	...	(37)	...	(294)	...
Item	df.	G		H		I	
		3rd leaf length		3rd leaf breadth		3rd leaf area	
		F†	F††	F†	F††	F†	F††
Row	5	22·61***	2·18	66·50***	8·65*	58·54***	5·31*
Column	5	10·37***	3·50*	7·69***	1·89	11·03***	2·76
R × C *b*	15	2·96**	...	4·05***	...	4·14***	...
R × C *d*	10	2·38*	...	4·15***	...	4·37***	...
Block	3	2·24	...	24·85***	...	2·17	...
Residual	105	...	...	...	...	...	...
Error variance:		(94)	...	(191)	...	(1,043)	...
Item	df.	J		K		L	
		Root weight		Tiller No. at 3rd leaf		Flowering time	
		F†	F††	F†	F††	F†	F††
Row	5	6·15***	4·36	14·91***	3·15	115·12***	1·08
Column	5	1·41	1·13	4·73***	1·84	106·79***	18·00***
R × C *b*	15	1·24	...	2·57**	...	5·93***	...
R × C *d*	10	1·68	...	1·74	...	2·93**	...
Block	3	1·82	...	3·54*	...	<1	...
Residual	105	...	...	...	...	...	...
Error variance:		(454)	...	(4)	...	(6)	...

F† = Ratio of each item M.S. to residual error M.S.
F†† = Ratio of Row against Column, and Column against *b* mean squares.

and there is no indication, in the test of (*b*) against (*d*), of any residual genetic variation not attributable to this.

Group 2 Characters. Dry weight, third leaf breadth and area, tiller number at the third leaf stage, top and root dry weight. Differences between the populations in average reciprocal effects of parents are evident for all the above characters—the significance of the (*c*) item in table 4D, H, L, J and K. For the first two of these characters residual reciprocal effects are also apparent from the significance of the (*d*) item in the same table. Here again, it seems to be a reasonable assumption that these (*c*) and (*d*) effects are maternal and not paternal in origin, since the Row to Column ratio is always positive and indeed significant except in the case of tiller number at the third leaf, and root weight. There are no indications from table 4D, H, I, J and K of zygotic effects determining any part of the differences between populations for all these characters, since both the (*a*) and (*b*) items are non-significant in the tests against (*c*) and (*d*) respectively. The final proof for the absence of any genetic variation, at least for variation due to the mean parent component, comes from the lack of significance of the column item against (*b*) in table 5D, H, I, J and K.

Group 3 Characters. Flowering time and rate of leaf appearance. Here there is no evidence for either kind of reciprocal effect; the differences between populations being solely accounted for by zygotic effects. In table 4F and L it can be seen from the tests of zygotic effects against their replicate errors that for flowering time both average and residual items are significant, whereas only average effects are apparent for rate of leaf appearance. However, the significance of Column against (*b*) in table 4F indicates that even for " flowering time " mean zygotic effect of parents is the more important genetic component.

The results of more sophisticated analyses are not presented for any variables under study, since under any of the above circumstances they contribute little further information.

The above points will be considered more fully in the discussion, but it is relevant to mention one point here. It is evident that measurements recorded at the younger stages of plant growth are predominantly maternally controlled and that genetic effects generally tend to become apparent only at later stages. This fact is coincident with the circumstance that in a number of investigations in grasses seed weight has been shown to be positively and highly correlated with seedling characters but less highly with adult measurements, *e.g.* Pearce (1953), Rogler (1954). Moreover, it has been shown by Thomas (in preparation) that in the particular populations used, seed weight is controlled by the maternal plant and not by the zygotic genotype—a not surprising fact in view of the seed's very close developmental and physiological dependence on the maternal plant. Thus the possibility exists that these results could be explained by the seedling characters being

dependent on the mother plant, through the effect of seed weight in the early stages of growth, with a gradual wearing away with greater maturity. This possibility could be investigated because the weight of each seed of every plant had been noted. The approach used was to take out the effect of seed weight in a covariance correction of mean squares, for each item in the Hayman analysis for all characters. This produced virtually no change in the results of the analyses, indeed there was no real justification for effecting these corrections since all the regression coefficients used in the corrections were not significant for any character. We must therefore conclude that seed weight has little or no influence on either the maternal or apparent genetic control of the expression of these characters.

(ii) *The F_2 diallel*

The main purpose of raising a further generation from the F_1 diallel was to discover whether the reciprocal effects observed in the F_1 were transmitted through a further sexual cycle. To do this, it was thought necessary to examine only one of the characters analysed in the F_1 generation and top weight was chosen, first of all because of the large maternal control over this character and secondly because it is the primary yield component. The diallel table of values and the results of the Hayman and factorial analysis applied to this character are shown in tables 6, 7 and 8 respectively. From table 7 it can be seen that both average and residual reciprocal effects of the parents are discernible and both are highly significant. This is a rather different situation to that of any character considered in the F_1 generation where it is obvious from tables 4 and 5 that average reciprocal effects, when present, are of greater importance than residual. In this situation, further tests of the average reciprocal effects may be conducted to assess its relative importance to the residual reciprocal effect, *i.e.* the (*c*) item is tested against (*d*). The (*c*) item on this test was found to be non-significant and we must suppose that residual reciprocal effects are the more important. The tests of genetic effects in the Hayman analysis indicate that only average effects of the parents are present. However, this is not substantiated in the test of column against (*b*) in the factorial analysis and we must accept the evidence of the latter, more valid test.

Thus the results of these analyses indicate that reciprocal effects are only apparent as residual variation (*i.e.* not as in the F_1 where average effects of the parents occur) and that genetic effects are not present.

To account for the discrepancy between the F_1 and F_2 analyses results the correlation between the 15 corresponding reciprocal differences in the two generations was calculated—$r = 0{\cdot}133$ (13 df.). This lack of significant correlation might be taken to indicate that the particular reciprocal differences present in the F_1 are not themselves passed into the F_2. However, it must be borne in mind that the

difference in pattern may have been caused by the F_2 having been raised from a smaller sample of the original population than the F_1. Alternatively the discrepancy may be due to the fact that the F_2

TABLE 6

Diallel table of values for the "F_2" character top weight (mean/plant in milligrammes)

	1	2	3	4	5	6
1	127	201	182	176	122	109
2	258	393	245	189	292	179
3	212	204	116	115	193	195
4	159	194	134	112	119	107
5	265	235	228	101	161	133
6	117	191	178	158	130	81

TABLE 7

Hayman (1954a) analysis of variance of the F_2 character—top dry weight

Item	df.	M.S.	F†	F††	F††
a	5	80,885	49·65***	6·14*	...
b	15	14,589	5·76****	1·62	...
c	5	13,183	5·22**	...	1·46
d	10	8,995	5·58***	...	...

F† = Variance ratios of the main items to their particular block errors.
F†† = Variance ratios of the *a* item against *c*, and the *b* against *d*.
F†† = Variance ratio of the *c* item against the *d* item.

TABLE 8

Factorial analysis of variance of the F_2 character—top dry weight

Item	df.	M.S.	F†	F††
Row	5	48,843	25·12***	1·55
Column	5	32,740	16·82***	2·24
R × C *b*	15	14,589	7·49***	...
R × C *d*	10	8,995	4·62***	...
Block	3	6,453	3·33*	...
Residual	105	1,946	...	...

F† = Variance ratios against the residual error.
F†† = Variance ratios of the Row against the Column item and the Column against the *b* item.

environment was slightly different from that of the F_1. Indeed, similar situations have been shown to occur by this author (unpublished) in comparisons between this F_1 diallel sown in early spring rather than late autumn. Reciprocal effects are still present but not necessarily in the same crosses. Thus it might not be pertinent to compare as we

have done the F_1 and F_2 results presented here. On this basis we are able to say that reciprocal differences appear to be transmitted through more than one successive sexual cycle but that in the present circumstances it is not possible to determine definitely whether specific differences present in the F_1 are transmitted intact to the F_2.

4. DISCUSSION

The most interesting point to emerge from the results of the analysis of the F_1 diallel cross families is that differences between reciprocal crosses account for a large proportion of the variation between the six populations assessed for the majority of the characters considered. Indeed, for a number of aspects of seedling growth studied, all the heritable variation is traceable to these reciprocal effects, *e.g.* third leaf breadth and area. For other measurements, genetic as well as reciprocal effects apparently control the heritable variation of these populations, *e.g.* fifth leaf measurements. Only two characters—including the sole adult measurement, flowering time—were entirely under the control of genetic components. These reciprocal effects are invariably expressed as average (or constant) effects of the parents and are attributable to maternal influences. Although these average maternal effects are, for a few characters, found together with residual reciprocal effects, the latter play a secondary role. In cases where they are apparent, genetic effects also appear predominantly as average effects of the parents. These effects are generally taken to indicate general combining ability (g.c.a.) or additivity. Thus, in general, both genetic and maternal influences are exerted as mean effects of the parents and we might say that either g.c.a. (or additive) genetic effects and/or g.c.a. maternal effects control most of the heritable variation for these characters.

As discussed earlier, there seems to be a definite pattern discernible in the relative roles of genetic and maternal effects at different stages of growth. Measurements taken up to the third leaf stages are predominantly maternally controlled, whereas by the fifth leaf stage both maternal and genetic effects are distinguishable and the only adult character measured, flowering time, is genetically and not maternally controlled. At first sight the fact that only maternal effects control the expression of top weight and root weight is a little anomalous in view of the fact that these records are taken some time after the measurements of leaf size, where both genetic and maternal effects occur. However, dry weight is the end-product of all preceeding processes of plant growth and thus is the best integral of the mean control of heritable variation up until time of harvest.

The above findings are in broad agreement with previous published inter-population studies in ryegrass where diallel analysis has been effected (Torrie, 1957; Fejer, 1958; Beddows, Breese and Lewis, 1962; Hayward and Breese, 1966). The first two of these authors analysed adult vegetative characters and demonstrated that the main

genetic control was predominantly in the form of general combining ability and that average maternal effects of parents were also obtained for some (not all) characters. They did not, however, attempt any critical tests of genetic versus reciprocal effects of the kind that have been applied here—an omission which does not apply to the latter two works. Beddows, Breese and Lewis (1962), using the characters seed set and first leaf length, demonstrated that only reciprocal effects were affecting the expression of these characters and that genetic effects were conspicuous by their absence. They further demonstrated that these reciprocal differences were predominantly due to average maternal effects of the parents although residual reciprocal effects were also apparent for the latter character. Hayward and Breese (1966), in contrast, find some male parent influence on seed set. Their findings also deviate to some extent from the results described here, since these authors did not detect average reciprocal effects in the four other seed or seedling characters they considered, the main control being additive for percentage germination, leaf number, tiller number and fifth leaf length. Nevertheless, they found residual reciprocal effects for the first three of these characters; the last could not be tested for this effect due to absence of replication. These residual reciprocal effects were tentatively interpreted as indicating nuclear cytoplasmic interaction. This interpretation is not readily applicable to the results presented here (or to those of the other authors discussed above), since residual reciprocal effects were only detected for a minority of characters and even in these instances were of secondary importance to the generally detectable average reciprocal (maternal) effects of parents. It could well be that the general discrepancy with the work of Hayward and Breese (*loc. cit.*) may be intrinsic to the kind of material used—these authors being unique in utilising very old vegetative clones as parental material. However, sampling methods in constructing the diallels also vary from author to author and could also contribute to differences of results and relatively minor discrepancies should not be pursued in too great detail until further work has been done using standardised construction techniques. Nevertheless, it does seem that some generalisations may already be made at this stage as to the control of heritable variation in *Lolium*. It appears that characters which can be considered either as very early F_1 or even parental characteristics are mainly under maternal control and that the same may be said of a number of seedling measures. However, in the later stages of seedling development genetic effects also become apparent and both genetic and maternal effects may continue to operate in the adult phase for certain characters; in other adult measurements only genetic effects operate.

It is apparent from the diallel analyses of the F_2 character " top weight " that reciprocal differences can still be observed after more than one sexual cycle. The magnitude and direction of these reciprocal effects are, however, dissimilar in the F_1 and F_2 generations described here for top weight as evidenced by the lack of correlation between

co-responding reciprocal crosses from the two generations. However the two generations are not strictly comparable for reasons previously discussed. Thus the question as to whether reciprocal effects are passed intact through further sexual cycles could not be answered here, although the main point that reciprocal effects are capable of emerging after a further sexual cycle is not in doubt.

The maternal influences of the parental populations used here could conceivably be transmitted to their progeny by the maternally controlled seed by the following mechanisms.* Firstly these effects could operate through a quantitative nutritional seed size influence, since these populations differ for seed weight (which in grasses is primarily a reflection of endosperm bulk the embryo being relegated to a small basal portion of the seed).

The second possibility is that the transfer could be brought about by a qualitative nutritional influence of the seeds. Lastly, extranuclear or cytoplasmic inheritance of some maternal factor(s) could be involved. The first possibility as we have shown in the results appears to be unimportant at least in the F_1 generation. Thus we are left with the second or third mechanism, neither of which could be experimentally substantiated here, but evidence from another source does indicate that extranuclear factors are important in ryegrass. Breese, Hayward and Thomas (1965) have demonstrated that within genotypes which had recently undergone a sexual cycle (but not in older clonal material) it was possible to select for the adaptive response of high and low asexual reproduction rates (tillering capacity). Moreover, they put this response down to selection for extranuclear plasmagenes and discount the possibility that the effect could be attributable to gross nutritional differences in the tillers selected since these were reasonably standardised between the two selection lines in any one clone. They further suggest that these plasmagenes which have been artificially selected may also be liable to natural selection and form the basis for the generally observable vegetative plasticity of grass swards. Thus there exist several parallels between the occurrence of plasmagene differences within a genotype and the maternal effects observed here. These parallels do not in any way constitute a basis for regarding these two phenomena as identical. The possibility that adaptive plasmagene effects may be transmitted through a sexual stage and thus give rise to maternal effects must remain a matter of conjecture. Nevertheless, in fungi where extranuclear inheritance has been intensively studied, there is a large number of recorded examples of induced cytoplasmic changes having passed through a sexual stage (Jinks, 1964).

* The possibility that the maternal effects are a reflection of unequal selfing frequencies in the parental populations can be ruled out on three counts:

1. The proportion of self/outcrossed seed is extremely low.
2. There are no differences between populations in this proportion.
3. It would be extremely difficult to imagine a situation where differential selfing results in apparent maternal effects in some characters and not in others, as is the situation here.

The possible importance of these plasmagene effects in allowing adaptation to environmental factors has been fully discussed by Breese *et al.* (*loc. cit.*). Here we will mainly confine ourselves to briefly summarising the broad implications of the maternal effects found in the inter-population studies. The discovery of what may be regarded as maternal care effects, which are found in the early stages of growth, is in itself not surprising, even though it does not seem to be a gross quantitative effect of maternal nutrition. What is surprising is that the effect may well continue after this and may even be transmitted through a further sexual stage. Furthermore, preliminary results obtained by this author indicate that differences between the fifth generation selection lines of *Lolium* (from within populations) for high and low fifth leaf size (Cooper and Edwards, 1961) are due to both genetic (additive) and maternal effects. Thus maternal effects must certainly be taken into account in any consideration of the system of variability in ryegrass, but the relative importance of genetic, maternal and plasmagene effects in controlling the system awaits further investigation.

Although the results of these analyses have been considered in detail we have not yet examined the extremely relevant point of whether the method of construction of the diallel cross families is effective. This appears, superficially, to be a cart-before-horse situation. However, this need not be so since the best criterion of effectiveness, in the present circumstances, can be considered as whether or not analysis of the constructed families has been successful in obtaining a meaningful elucidation of the control of heritable variation. This does appear to be the case here since the results are in broad agreement with those of previous workers (who in the main employed clonal material to represent the parental lines), *e.g.* Fejer (1958), Torrie (1951), Breese (1960) and Hayward and Breese (1963). Moreover, Dickinson and Jinks (1956) have stated that analysis applied to this form of construction " will be progressively less accurate the lower the ratio of genetic variation between as opposed to within groups " (populations). This seems to be the situation here—in variance analysis top weight and leaf size measurements showed the greatest differences between parental populations, rate of leaf appearance, the least and tiller number is intermediate (Thomas, 1965). These differences are reflected in the extent of genetic and/or maternal effects as revealed in the Hayman, and factorial analyses. Thus we can assume that this method of construction is a reliable one.

5. SUMMARY

The investigations of the basis for heritable inter-population variation in ryegrass by means of analysis of a complete diallel series of crosses between six climatic populations has shown:

1. The expression of the number of earlier seedling characters in inter-population (F_1) families is predominantly affected by average or

constant reciprocal effects of parents which are maternal in origin. Thus the effect of the hybrid zygote is not detectable at this stage of growth.

2. By the fifth leaf stage genetic effects as well as maternal effects occur. However, dry weight of the tops and roots which were harvested at a later date than the time when leaf measurements were taken showed maternal effects only. But these are cumulative characters and incorporate the effects of all previous measurements.

3. The only adult character investigated (flowering time) is under genetic control only.

4. The genetic and maternal effects, whether present together or singly are normally apparent as average effects of the parents (*i.e.* general combining ability or additive effects) and not residual variation (due to *e.g.* dominance or genic interaction).

5. The maternal effects in F_1 characters are not a reflection of differences in seed weight of the parental populations. It is, therefore, quite conceivable that they are extranuclear or cytoplasmic in origin.

6. Reciprocal differences can still be observed in the one character studied in the F_2 generation suggesting a further parallel with the behaviour of extranuclear or cytoplasmic effects.

7. The methods of diallel construction adopted to overcome the difficulties of using heterozygous self-incompatible material is based on using groups of plants rather than individuals as samples of the six populations assessed. The particular method utilised here is a reliable one and allows meaningful estimates of the components of variation to be obtained.

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