

# INTER-POPULATION VARIATION IN PERENNIAL RYEGRASS

## 3. INTERACTION OF HERITABLE AND ENVIRONMENTAL VARIATION

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### 1. INTRODUCTION

COOPER, (1963, 1964) has demonstrated that the expression of a number of seedling measurements in climatic races of *Lolium perenne* is subject to population  $\times$  environmental variation. The present work was designed to determine which particular heritable components are responsible for this category of observable interaction in inter-population crosses. Analyses of population means of some seedling characters from a  $6 \times 6$  "F<sub>1</sub>" diallel between climatic populations of ryegrass, grown in three glasshouse environments, are presented.

The parental populations, environments and characters considered here are similar to those described by Cooper *loc. cit.*, and identical to those considered by Thomas (1965) where diallel analysis *within* each of the three environments was effected. Diallel analysis (Thomas *loc. cit.*) indicated that, with only very minor discrepancies, the same pattern of heritable variation of means was apparent for the four characters actually used in this study in all three environments. The more important zygotic control was invariably ascribable to general combining ability and, in addition, reciprocal effects primarily due to average maternal effects of parental populations were present in all cases.

### 2. MATERIALS AND METHODS

The technique of construction of the diallel cross families needs no elaboration since it has been described before and found to be reliable; Thomas, 1965, 1967. The characters used here are fifth leaf length, breadth, area and tiller number at the sixth leaf stage and need no redescription either since their use is now fairly widely recognised. A brief reiteration of the nature of the environments and the experimental design would, however, be valuable since they determine the form of analysis carried out below. All three sowings of the complete diallel series of thirty-six crosses were in four randomised blocks (ten plants per family per replicate) and were located in the same sector of one unheated greenhouse but were planted on different dates: one, in late autumn, 1962; two, in early spring, 1963; three, in late spring, 1963. Consequently, the environments could well be considered as three replicates of the diallel in time—when "replicates" thus defined would physically differ considerably in aspects such as day length, temperature, etc. Thus it is not altogether surprising that Cooper (*loc. cit.*) was able to demonstrate that "his" populations interacted with similar environments since he

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utilised populations whose regions of origin were extremely various with respect to aspects of climate. The actual six populations used here derive from Algeria—3, Ireland—1, Lithuania—4, New Zealand—5 and Wales 2 and 6—referred to below as 1-6 only. Analysis of the diallel families was based on the mean of eight plants in each family within each replicate of each environment.

### 3. RESULTS

The diallel tables of mean values for all four characters, over the four replicates, for each of the three environments is given in the appendix table. Diallel analysis results within these environments are not given here since they have already been described before Thomas (*loc. cit.*) and summarised in the introduction.

To test for interactions of the parental populations with environments for the four characters, the analysis of variance shown in table 1 is carried out. For this analysis the experiment was not treated as, perhaps it strictly should have been, a split plot design, but as a completely factorial experiment. However, the absence of significant replicate  $\times$  environment and replicate  $\times$  population interactions in all four cases does somewhat reassure us that this rearrangement was not entirely unjustified. Differences between the six parental populations are indicated for the four measurements by the highly significant variance ratio in the populations versus residual variation comparison and it is also clear that these differences are apparent over and above their interactions with environments since the  $F$  ratio of the population to population  $\times$  environments items is also significant. Two tests of interaction between populations and environments are available and for three out of the four characters, they are obviously discernible in the first of these tests, namely in the population  $\times$  environments versus residual variation comparisons and all four are significant in the second, admittedly less valid, test of the population  $\times$  environment versus population  $\times$  block comparison. Thus it is quite evident that a high level of parental population  $\times$  environment interaction exists.

A method for detecting the heritable components responsible for genotype  $\times$  environment interactions in diallel crosses has been suggested by Allard (1956). The technique, however, depends on two situations being applicable—the presence of dominance and the absence of maternal effects—neither of which conditions is fully satisfied here as was stated in the introduction. Nevertheless, a simple extension of the Hayman (1954) analysis, diallel tables can usefully be employed to explore this particular situation of table 2. The form of this analysis assumes a factorial arrangement of all items. The main motive for this somewhat debatable procedure is that the more interesting comparisons made below would not be available in a split plot analysis and departure from usual convention is justified on two counts. The first follows from the dialectical point made in the Methods Section—that the environments can be considered as replicates of the diallel system in time—and thus environments, replicates and main effects are “by definition” factorially arranged. Secondly, having applied a completely factorial analysis to the parental populations and having some evidence that the procedure was not unsound why not apply similar analysis to the complete diallel?

TABLE I  
*Variance analysis of parental populations over the three environments*

Item	Df.	Fifth leaf length		Fifth leaf width		Fifth leaf area		Tiller no. at the sixth leaf stage	
		$F_A$	$F_B$	$F_A$	$F_B$	$F_A$	$F_B$	$F_A$	$F_B$
Populations	5	65.55***	6.22**	73.96***	30.43***	154.61***	11.65***	10.60***	11.98***
Environments	2	163.10***		411.79***		207.89***		89.55***	
Blocks	3	1.87		<1		1.18		<1	
Pops. × Environments	10	10.37***	17.61***	2.43*	4.16**	13.27***	24.28***	<1	3.30*
Pops. × Blocks	15	<1		<1		<1		<1	
Environments × Blocks	6	<1		<1		<1		<1	
Residual	30								
Error mean square =		141	214	4183	150				

$F_A$  = ratio of M.S.'s to residual error.

$F_B$  = ratio of Population M.S. to Population × Environment M.S. and ratio of Pop. × Environment M.S. to Pop. × Block M.S.

In table 2 each individual main effect ( $a$ ,  $b$ ,  $c$  and  $d$ ) is initially tested against its particular second order interaction with environments  $\times$  blocks, as are the first order interactions of main effects with Blocks and main effects with environments (test A). The interpretation of the values for main effects will be dealt with first. It is readily observable that for all four characters the two primary zygotic items— $a$  and  $b$  and at least constant reciprocal effects— $c$  are significant. (These constant reciprocal effects can be shown to be maternal in origin in tests not included here and the significance of  $c$  will be interpreted as a maternal effect). In the event of significant maternal effects the mean square values of zygotic items may be inflated and further more valid tests of the latter effects as suggested by Wearden (1964) should be performed. The relevant tests are  $a$  against  $c$  and  $b$  against  $d$  and these are also given in table 2 (test B). It is now apparent that zygotic effects are real since the  $a$  item indicating average effects of parents (general combining ability or additivity) always retains significance although the  $b$  item denoting residual zygotic effects (specific combining ability or dominance/epistasis) is now only significant in the instances of fifth leaf width and tiller number at the sixth leaf stage. For the latter two characters the subdivided items of  $b$  may be interpreted. In neither case is  $b_1$  significant and thus no difference between the overall parental and  $F_1$  cross means is present; for tiller number at the sixth leaf  $b_2$  only is significant and this is usually taken to indicate assymetry of gene distribution; for fifth leaf width only  $b_3$  is significant—interpreted normally as “left over” non-additive genetic variation.

Thus, over all three environments, the main heritable variation is, for all four characters, attributable to average zygotic and average maternal effects of parents, and although there are some indications of residual zygotic effects for two characters, in no case is there any evidence for residual reciprocal effects. Before considering interactions of the main effects with environments it is useful to point out that we should be primarily interested in the interaction of those effects which are shown to be significant above by the more valid tests.

Two main tests for interaction of the main heritable items with environments are given in table 2. In test “A” each main effect  $\times$  environment item is compared with its corresponding main effect  $\times$  environments  $\times$  replicate interaction. Test “B” involves further tests of the zygotic  $\times$  environment items only—the  $E \times a$  item and the  $E \times b$ (?s) items are compared with the  $E \times c$  and  $E \times d$  items respectively. This second test was thought advisable in the present circumstances since it is conceivable that in the same way as, for instance, the  $a$  item may be inflated by  $c$ —so might the  $E \times a$  item be inflated by  $E \times c$ .

The most striking point to emerge from the above comparisons is that for all four characters there is significant  $a \times E$  interaction (in both tests A and B) whereas for only leaf area, is any significant interaction of the  $c$  item with environments observable and this is only barely significant at the 5 per cent. level. However, there does appear to be significant interaction of residual reciprocal effects in the instance of tiller number at the sixth leaf although  $d$  was not present as a main effect. Of less immediate interest is the observation that for the two characters (and only these) where significant residual zygotic variation was present there is also significant  $b \times E$  interaction—for fifth leaf width in both tests A and B and for tiller number at the sixth in test A only.

TABLE 2  
*Extension of Hayman (1954) analysis of variance of diallel tables—see text*

Item	Df.	Fifth leaf length		Fifth leaf width		Fifth leaf area		Tiller number at the sixth leaf stage			
		M.S.	Test A	Test B	M.S.	Test A	Test B	M.S.	Test A	Test B	
<i>a</i>	5	20010	158.80***	6.69*	144362	551.00***	148722	350.59	416850	14.28***	21.19***
<i>b</i> <sub>1</sub>	1	1	<1	<1	393	4.14	33	<1	270	2.25	<1
<i>b</i> <sub>2</sub>	5	1013	9.73***	2.68	1659	4.85***	3899	8.84***	7740	1.89	10.87***
<i>b</i> <sub>3</sub>	9	423	2.13***	1.11	2444	6.13***	2757	3.69**	1510	4.09***	2.02
<i>b</i>	15	591	3.77***	1.56	2045	5.68***	2457	5.02***	3500	9.56***	4.69***
<i>c</i>	5	2993	17.92		14707	44.98***	20666	34.29***	2210	5.56***	
<i>d</i>	10	378	1.52		793	1.33	1592	1.77	746	1.37	
<i>T</i>	35	3648			23827		25834		8794		
Environments	2	189232			619065		550134		816235		
<i>a</i> × <i>E</i>	10	3614	28.63***	13.90***	2909	10.72***	15637	36.80***	2831	8.59***	3.78*
<i>b</i> <sub>1</sub> × <i>E</i>	2	53	1.26	<1	850	8.95**	2.01	2.93	2.76		
<i>b</i> <sub>2</sub> × <i>E</i>	10	75	<1	<1	674	1.97	259	<1	1.45	<1	<1
<i>b</i> <sub>3</sub> × <i>E</i>	18	208	1.05	1.12	934	2.34	595	<1	598	<1	<1
<i>b</i> × <i>E</i>	30	154	<1	<1	842	2.33**	466	<1	928	2.52*	<1
<i>c</i> × <i>E</i>	10	260	1.55		499	1.53	1316	2.18*	778	2.13	<1
<i>d</i> × <i>E</i>	20	185	<1		422	<1	798	<1	753	1.79	<1
<i>T</i> × <i>E</i>	70	672			954		2849		1045	1.90*	
Blocks	3	1470			1120		1270		650		
<i>a</i> × <i>B</i>	15	137	1.08		183	<1	337	<1	819	2.49	
<i>b</i> <sub>1</sub> × <i>B</i>	3	333	7.92		129	1.35	587	5.10*	10	<1	
<i>b</i> <sub>2</sub> × <i>B</i>	15	109	<1		210	<1	366	<1	356	<1	
<i>b</i> <sub>3</sub> × <i>B</i>	27	122	<1		393	<1	568	<1	523	1.42	
<i>b</i> × <i>B</i>	45	132	<1		315	<1	502	<1	432	1.18	
<i>c</i> × <i>B</i>	15	205	1.22		267	<1	424	<1	333	<1	
<i>d</i> × <i>B</i>	30	208	<1		715	1.20	1012	1.13	610	1.12	
<i>T</i> × <i>B</i>	105	165			403		613		525		
<i>E</i> × <i>B</i>	6	266			1461		2368		923		
<i>a</i> × <i>E</i> × <i>B</i>	30	126			262		424		328		
<i>b</i> <sub>1</sub> × <i>E</i> × <i>B</i>	6	42			95		115		120		
<i>b</i> <sub>2</sub> × <i>E</i> × <i>B</i>	30	104			342		441		410		
<i>b</i> <sub>3</sub> × <i>E</i> × <i>B</i>	54	199			399		657		369		
<i>b</i> × <i>E</i> × <i>B</i>	90	157			360		549		366		
<i>c</i> × <i>E</i> × <i>B</i>	30	167			327		604		417		
<i>d</i> × <i>E</i> × <i>B</i>	60	249			595		895		547		
<i>T</i> × <i>E</i> × <i>B</i>	210	180			408		638		420		
Total	431	1425			5288		5615		5031		

The situation becomes somewhat confused when the interactions of the constituent items of  $b$  are examined. For fifth leaf width where  $b_3$  was evident as a main effect there is also observable interaction with environments—but in test B only, furthermore in test A, only, there is significant  $b_1 \times E$  interaction although  $b_1$  was not evident as a main effect. In the instance of Tiller number at the sixth leaf whilst  $b_2$  was apparent as a main effect it is  $b_3$  which is observed to interact with environments. This apparent confusion will be considered in the discussion.

Further tests of main effects against either their individual replicate, or Environment errors, may be effected but are not included here since table size and discussion length would be inflated, whereas these results only serve to confirm the above findings. In a similar manner the interaction of each main effect with environments may be tested against the relevant replicate interactions again with similar conclusions to those already reached.

#### 4. DISCUSSION

The results of table 1 need no elaboration only serving to confirm these of Cooper (*loc. cit.*) in revealing a high level of interaction between environments and the six parental populations for all four characters. Furthermore, inter-population diallel analysis over environments (table 2) showed that the main heritable variation was, primarily, due to average zygotic effects and average maternal effects of parents—a finding which is in general agreement with genetic analysis of each of these characters within the environments—Thomas, 1965. There are, however, minor deviations from these latter generalisations which will be dealt with below where we turn to the main topic of the investigation—to ascertain which heritable components interact with environments.

As was pointed out in the Results section the main point of interest is clearly that although both average zygotic and average maternal effects are conspicuously present as heritable components over environments it is the former which invariably interact with environments whereas the latter is usually constant over environments (except for fifth leaf area where the  $c \times E$  interaction is just significant.) The situation is nevertheless not completely straightforward—there is, for instance some evidence that residual zygotic effects also interact with environments when they are also evident as main effects (for fifth leaf width and tiller number at the sixth leaf). There is even, in one case, significant interaction of residual reciprocal effects (for fifth leaf area), although for no character was the  $d$  item significant. However, there are some inconsistencies in the test of the individual  $b$ 's  $\times E$  interactions which were alluded to previously, but these (and indeed the solitary significance of  $d \times E$ ) can probably be attributed to the “minor discrepancies” referred to above when results of analysis of main effects within and over environments were briefly compared. For instance, for fifth leaf width in environment 2 and 3,  $b_3$  was found to be highly significant, but not in environment 1 and  $b_1$  was highly significant in environment 1 only. Thus the confusion referred to in the tests for this character is in all likelihood attributable to the three constituent items of  $b$  being present differentially in the three environments—and we might conclude that this phenomenon represents inconstancy of type rather than degree of genotype environment interaction. At the risk of over-simplification, it might be preferable to

refrain from interpreting the constituent items of  $b$  (and the  $d \times E$  interaction for that matter) and only consider the interactions of the overall  $b$  item. If this is acceptable then the overall situation resolves itself somewhat. Firstly, it is apparent that it is almost exclusively the prerogative of zygotic effects to interact with environments rather than maternal effects. Secondly, it is the more powerful zygotic item-average effect of parents (*i.e.* general combining ability of additivity) which is usually responsible for these interactions and residual zygotic effects have a secondary role—as indeed they do as main effects. Thus, in general, maternal effects could be considered in the context of genotype—environment interactions as a stabilising influence and zygotic effects as acting in the direction of instability. Alternatively, it could also be said that maternal effects were a non adaptive or conservative force and zygotic effects allowed a degree of adaptation in the three environments. Both stability and adaptation are generally considered to be desirable genetic properties but they would appear superficially to be conceptually opposed—high adaptivity implying instability and vice versa. However, this apparent contradiction is a rather dialectical one—the situation being resolved in practice (albeit rather arbitrarily on occasion) in the context of particular circumstances by the author concerned who may subjectively decide, on behalf of the organism and situation, where the division between, for instance, considering a particular result as reflecting instability or high adaptivity, should lie. Although this author does not consider it particularly important or meaningful at this stage to apply such a distinction to the results presented, nevertheless it is convenient, to facilitate further discussion, to do so and maternal effects will be referred to as stabilising effects and zygotic influences as reflecting instability—bearing in mind that this is not a final decision! We may then move on to consider the implications of these findings in more detail and attempt to relate them to the known facts regarding population structure in this species.

The only published attempt at elucidating the genetic basis for stability in inter-population studies with ryegrass is by Thomas, 1968, who presents analysis of coefficients of variation (which it is argued measure the stability of the characters considered within the environment utilised) from a diallel cross series. In contrast to the present findings it is shown that maternal effects play no part at all in determining this type of stability—additive genetic variation being the main factor controlling the observed population differences in this respect. This contrast need not, however, disturb us unduly, it being neither essential nor indeed probable that the same or similar control should operate within an environment (“micro-environmental stability”) as opposed to between very dissimilar environment (“macro-environmental stability”) and thus these findings may not help us with or be relevant to an understanding of the present situation.

It is considered germane at this juncture to retrace our steps somewhat and consider the possible nature and importance of maternal effects in this species (one usually accepts the presence of zygotic influence without question). Thomas, 1965, 1967 indicated that the maternal effects observed in the same “ $F_1$ ” diallel as is here considered—in environment 1—were due neither to a gross nutritional influence of seed weight, nor unequal selfing frequencies in the parental populations. In addition reciprocal differences were shown to have been transmitted to the “ $F_2$ ” diallel generation. It was consequently suggested that maternal effects were cytoplasmic in origin and

that cytoplasmic differences between populations might well have arisen from fixation of plasmagenes which are known to exist within ryegrass populations—Breese *et al.*, 1965. This explains the present finding that maternal as well as zygotic effects are both found as overall effects over environments but it is difficult to imagine how and why reciprocal differences which are cytoplasmic in origin should remain constant over these very extreme environments since the cytoplasm is generally considered to be rather more labile and somewhat subservient to the nucleus. Nevertheless, it is interesting to postulate that this very powerful and apparently buffering influence of the cytoplasm must have a very important evolutionary significance in this species—a proposition which has been discussed by Breese, 1966, whose conclusion must be considered as still applicable. “Perhaps this dual control of variability can explain why the outbreeding *Lolium* complex has adopted itself so successfully to such a wide range of climatic and ecological conditions without any great chromosome differentiation or the changes in levels of ploidy which have been so marked in other species . . . a more detailed knowledge of genetic control is required, covering a wider range of characters and populations.”

### 5. SUMMARY

1. The presence of a high level of interaction between three greenhouse environments and six climatic populations of ryegrass for four seedling characters was demonstrated.

2. Analysis of a complete  $6 \times 6$  diallel series of intra-population (parental) and inter-population ( $F_1$ ) crosses over three environments showed that:

(a) Constant maternal and additive zygotic effects of parental populations were responsible for the heritable variation over the three environments, although for two characters—fifth leaf width and tiller number at the sixth leaf stage—residual zygotic variation was also present.

(b) The particular zygotic effects present for each character over environments were also the heritable components which interacted with environments and only for one character—fifth leaf area, was there any indication at all that maternal effects interacted with environments.

3. Some attempt was made to relate these findings to other known facts regarding the genetic architecture of *Lolium* populations.

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APPENDIX

*Diallel table of mean values over four replicates for the four characters in each of three environments*

	Fifth leaf length (in mm.) Environment 1						Fifth leaf length (in mm.) Environment 2					
	1	2	3	4	5	6	1	2	3	4	5	6
1	131	153	165	137	137	138	114	123	119	133	124	115
2	152	184	160	190	168	151	143	168	151	162	151	139
3	161	163	186	169	155	156	126	124	124	119	118	101
4	141	149	160	135	141	133	128	138	130	117	116	118
5	145	144	162	143	140	128	121	131	115	120	118	110
6	131	141	149	131	129	131	103	117	97	107	116	97

	Fifth leaf length (in mm.) Environment 3						Fifth leaf width (in mm.) Environment 1					
	1	2	3	4	5	6	1	2	3	4	5	6
1	171	203	178	189	168	174	2.67	3.24	2.79	2.70	2.61	2.70
2	232	267	202	248	224	218	3.19	4.15	3.38	3.95	3.31	3.26
3	167	179	159	186	154	150	2.67	3.08	2.71	2.73	2.63	2.55
4	185	194	189	190	196	169	2.76	3.05	2.83	2.76	2.79	2.62
5	191	210	170	181	160	155	2.65	2.95	2.86	2.81	2.67	2.52
6	172	192	157	174	170	161	2.56	2.78	2.47	2.70	2.47	2.44

	Fifth leaf width (in mm.) Environment 3						Fifth leaf width (in mm.) Environment 3					
	1	2	3	4	5	6	1	2	3	4	5	6
1	3.48	3.81	3.67	3.76	3.84	3.43	3.76	4.59	3.71	3.86	4.05	3.88
2	4.47	4.99	4.44	4.96	4.38	4.18	5.01	5.54	4.57	5.45	4.88	5.14
3	3.68	3.77	3.26	3.71	3.38	3.21	3.61	4.04	3.58	3.94	3.76	3.53
4	3.68	4.34	3.86	3.59	3.67	3.57	4.02	4.73	4.18	4.19	4.09	3.85
5	3.68	4.00	3.38	3.75	3.51	3.26	4.11	4.55	3.88	3.95	3.81	3.71
6	3.41	3.83	3.07	3.48	3.53	3.34	3.93	4.46	3.56	3.66	3.66	3.71

	Fifth leaf area (in sq. mm.) Environment 1						Fifth leaf area (in sq. mm.) Environment 2					
	1	2	3	4	5	6	1	2	3	4	5	6
1	362	509	463	371	361	375	401	480	439	488	491	398
2	498	772	553	756	564	510	646	856	729	811	633	601
3	437	492	510	463	410	399	466	475	406	449	404	328
4	389	464	460	377	393	352	476	609	511	422	432	423
5	386	438	473	404	374	329	452	529	395	453	418	366
6	334	402	374	361	373	333	355	460	307	379	414	332

[continued overleaf

	Fifth leaf area (in sq. mm.) Environment 3						Tiller number at sixth leaf Environment 1					
	1	2	3	4	5	6	1	2	3	4	5	6
1	663	957	668	742	734	682	6.13	5.83	6.75	5.45	5.58	5.35
2	1165	1496	1002	1370	1102	1156	5.98	6.28	5.90	5.75	5.75	5.05
3	612	738	577	741	592	537	5.80	5.53	4.13	5.23	5.13	4.63
4	746	948	800	802	811	664	5.78	5.53	5.70	5.85	4.95	4.75
5	782	975	667	719	614	581	5.98	5.00	6.10	5.28	6.10	4.88
6	681	872	571	644	635	605	5.20	4.68	4.30	3.95	4.95	4.15

	Tiller number at sixth leaf Environment 2						Tiller number at sixth leaf Environment 3					
	1	2	3	4	5	6	1	2	3	4	5	6
1	8.48	9.58	10.88	7.93	9.02	8.00	10.10	11.28	10.97	10.03	10.33	7.82
2	10.03	10.40	8.60	8.70	8.60	8.30	10.80	11.27	11.42	10.15	10.18	10.53
3	9.10	9.22	8.40	8.72	8.77	8.30	11.75	11.20	10.00	10.18	11.42	9.30
4	8.70	9.30	8.43	7.10	7.60	7.83	10.75	10.50	11.32	8.75	9.80	7.60
5	9.18	8.82	9.52	7.90	10.05	7.93	11.88	10.20	10.13	9.18	10.90	9.20
6	7.58	7.42	7.42	7.10	7.42	7.13	8.62	8.90	9.20	7.85	8.32	8.07