GENOTYPE-ENVIRONMENTAL INTERACTIONS IN *SCHIZOPHYLLUM COMMUNE*

II. ASSESSING THE ENVIRONMENT

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Received 1.vii.71

1. Introduction

Although the importance of genotype-environmental interactions has long been recognised, it is only in the last decade that it has been possible to describe and predict this component of an individual's phenotype. This success followed the recognition that the phenotypic character under study itself provides a quantitative assessment of an environment (Yates and Cochran, 1938), and the finding that phenotype was often linearly related to such environmental values (Finlay and Wilkinson, 1963; Perkins and Jinks, 1968). The various regression approaches that have been used have been described and compared in a previous paper (Fripp and Caten, 1971).

In most previous studies (*e.g.* Finlay and Wilkinson, 1963; Eberhart and Russell, 1966; Perkins and Jinks, 1968; Breese, 1969) the environmental values used were the mean yield in each environment of the particular set of genotypes under investigation, calculated from the actual data being analysed for its genotype-environmental interactions. This use as independent variable of values which are not independent of the phenotypic variable regressed on to them, has been criticised by Freeman and Perkins (1971). The regression technique can still be used to study genotype-environmental interactions provided the environmental values used are independent of the data being analysed.

Freeman and Perkins (1971) list and discuss a number of ways in which independent environmental values might be obtained and describe a biometrical-genetical model and a regression analysis (approach 3 in Fripp and Caten, 1971) appropriate to such values. The many possible methods of assessing the environments may be grouped into four major categories:

(1) division of the replicates available into two groups using one group to measure the genotype-environmental interactions and the second group to assess the environments (*i.e.* the trial and the assessment genotypes are replicates of the same material);
(2) inclusion of an assessment set of genotypes which are closely related to the trial genotypes;
(3) inclusion of a single, or a limited number of assessment genotypes;
(4) use of physical measures.

Only (4), the use of physical measures, does not necessitate the growing of individuals solely to provide data to assess the environments. It is not often possible, however, to use physical measures since the exact nature of the environmental variables is rarely known. Furthermore their use becomes difficult, firstly when the environment is varying for more than one factor and secondly when the scale of measurement appropriate to the biological effects of the varying factor(s) is unknown. The first of these two

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difficulties may be overcome by the use of multivariate regression analysis (Hardwick, personal communication).

Of the biological assessments (categories (1), (2) and (3) above) the environmental values given by (1) should correspond most closely to the average response of the trial individuals. Replication, however, involves a considerable increase in the size of the experiment. The same disadvantage applies to category (2), with the added possibility that the assessment genotypes may not respond in the same way as the trial genotypes.

In most practical situations where size of the experiment is often a limiting factor, the third category would be preferred provided satisfactory interpretation of the data being examined was assured. As an example of this category, Freeman and Perkins (1971) suggest the use of parental genotypes to assess the environments in studies involving either crosses between inbred lines or collections of inbred lines derived from such crosses. Previous results confirm the suitability of parental genotypes and in addition show that a single assessment genotype may be satisfactory (*e.g.* Bucio-Alanis and Hill, 1966; Bucio-Alanis, Perkins and Jinks, 1969; Fripp and Caten, 1971). It is not known, however, how precise the environmental assessments must be in practice to achieve useful predictions.

Unless different methods of assessing the environment give rise to precisely the same environmental values they will lead to different estimates of the linear (β_i) and non-linear ($\sum_j \delta_{ij}^2/(s-2)$) sensitivities of a genotype (see Fripp and Caten, 1971, for description of these measures of sensitivity). Such changes may affect the reliability of predictions made, and will have particularly serious consequences if they change the relative magnitudes of the sensitivity statistics of the genotypes under test.

In the present paper assessment methods from each of the four categories listed above are applied to single sets of experimental data, and the results obtained with each method are compared with those obtained from the use of non-independent environmental values. The latter was chosen as the standard for comparison because it can be followed for every set of data and has until now been the standard practice. The comparisons made should thus help to clarify the reliability of these previous studies where only non-independent environmental values have been used.

2. Materials and methods

The quantitative character considered is dikaryotic growth rate of the fungus *Schizophyllum commune*. The use of this character for studies of environmental and genotype-environmental variation has been discussed previously by Fripp and Caten (1971). The present data are from two experiments referred to as experiments 1 and 3. Experiment 1 has been described in detail elsewhere (Fripp and Caten, 1971). For the present purposes only the data for environments 1-2 and 5-10 of this experiment have been used.

In experiment 3 ten trial dikaryons and two assessment dikaryons were grown in each of ten environments and their linear growth over 8 days measured. The trial dikaryons consisted of parents selected for low and high growth rates (*i.e.* 3-6L and 3-6H) and eight of the "F_2" progeny of F_1 122. These eight "F_2" dikarons were selected for their low non-linear sensitivities and range of linear sensitivities in experiments 1 and 2 of Fripp

and Caten (1971). The assessment genotypes were F_1 122 and a second F_1 dikaryon, F_1 1492. Full details of the derivation of all the dikaryons except F_1 1492 can be found in the text and figure 1 of Fripp and Caten (1971). F_1 1492 was derived in the same way as F_1 122 but from a different ancestral wild isolate (Isolate No. 2 of Simchen and Jinks, 1964).

The environments in experiment 3 were produced by varying: (i) concentration of malt extract in a malt growth medium (set 3.1 in table 1), and (ii) temperature (set 3.2 in table 1). The particular concentrations

TABLE 1

Composition of the environments in experiment 3

Set*	Environment Number	Temperature °C	Medium
3·1	16	25	0·01% malt
	17	25	1·00% malt
	18	25	1·50% malt
	19	25	2·00% malt
	20	25	4·00% malt
		Average†	
3·2	21	20·8	2% malt
	22	22·6	2% malt
	23	25·2	2% malt
	24	26·5	2% malt
	25	28·9	2% malt

* Sets 1 and 2 (environments 1-15) described previously (Fripp and Caten, 1971).

† See text.

and temperatures were chosen such that these two types of environment gave rise to similar ranges of $\hat{\mu}+\hat{\epsilon}_j$, where $\hat{\mu}+\hat{\epsilon}_j$ is the mean over replicates and genotypes for the jth environment. Some difficulty was encountered in maintaining the incubators at a constant temperature, in particular environment 24, and the figures given in table 1 reflect the average temperatures experienced by the dikaryons.

Four replicates of each dikaryon were grown in a completely randomised design in each environment. For the ten trial dikaryons, two replicates were designated at random to provide the data to be analysed for its genotype-environmental interactions. The other two replicates and all four replicates of the assessment dikaryons were used to provide independent environmental vales.

In the subsequent text, the term GE-analysis denotes the complete set of analyses (*i.e.* joint regression analysis plus the individual regression analyses) carried out using a particular set of independent (z_j) or non-independent ($\hat{\epsilon}_j$) environmental values. The data whose phenotypic variation is being analysed are referred to as the trial data while those used solely to derive environmental values are referred to as assessment data. Following Freeman and Perkins (1971) b_i denotes the regression coefficient of the ith genotype, that is b denotes an estimate of a genotype's linear sensitivity, β.

3. ANALYSIS

The data analysed for their genotype-environmental interactions are the mean growth rates over replicates of each dikaryon in each environment. The

error items derived from the differences between replicates and used in the tests of significance have been adjusted accordingly.

Six different methods of assessing the environments are used in the present paper. Details of these and the symbols used subsequently to identify them are as follows:

A. Use of the actual data being analysed.
B. Replication of the trial genotypes.
C. Use of an assessment set of genotypes derived from the same population, that is a genetic replicate.
D. Use of a single assessment genotype closely related to the trial genotypes.
E. Use of a single assessment genotype not related to the trial genotype but with a similar selection history.
F. Use of a physical measure.

These involve therefore the use of a non-independent biological assessment (A), one example of each of the independent assessment categories (1), (2) and (4) (B, C and F respectively), and two examples of category (3) (D and E). Methods D and E differ in the genetic relation between the single assessment genotype and the trial genotypes. A to F are arranged in order of decreasing relationship between the trial data and the assessment data.

All six were not possible with any one set of data. The combinations of assessment method and source of data considered are given in table 2,

TABLE 2

Summary of GE-analyses

I. Comparison of various biological assessments.
II. Comparison of biological and physical assessments.

	Source of data	Assessment of the environments	$\hat{\epsilon}_j$ or z_j	Assess.* code	Analysis code
I	Experiment 1	Trial replicates of trial " F_2 " dikaryons	$\hat{\epsilon}_j$	A	1A
		Control " F_2 " dikaryons	z_j	C	1C
		F_1 122	z_j	D	1D
	Experiment 3	Trial replicates of trial dikaryons	$\hat{\epsilon}_j$	A	3A
		Assessment replicates of trial dikaryons	z_j	B	3B
		F_1 122	z_j	D	3D
		F_1 1492	z_j	E	3E
	Experiment 3 Assessment replicates	Assessment replicates of trial dikaryons	$\hat{\epsilon}_j$	A	3'A
II	Experiment 3	Trial replicates of trial dikaryons	$\hat{\epsilon}_j$	A	3.1A
		Assessment replicates of trial dikaryons	z_j	B	3.1B
		Concentration of malt as g./litre	z_j	F	3.1F
	Experiment 3	Trial replicates of trial dikaryons	$\hat{\epsilon}_j$	A	3.2A
		Assessment replicates of trial dikaryons	z_j	B	3.2B
		Average temperature as degrees Centigrade	z_j	F	3.2F

* Method of assessing environment. See text.

together with details of the observations used to derive each set of environmental values. The analysis code numbers assigned in this table indicate

both the experiment providing the data and the method of assessing the environments, and are used throughout to refer to the individual analyses.

The GE-analysis of Freeman and Perkins (1971) was used for all GE-analyses involving independent environmental values. Where non-independent values were used the approach of Finlay and Wilkinson (1963) was followed. For a discussion of these two approaches see Fripp and Caten (1971).

The statistics listed below are used to compare the assessments.

(i) r_E = the correlation value between $\hat{\epsilon}_j$ and $\mathcal{Z}_j$.
(ii) b_c—the combined regression coefficient which is, in fact, the coefficient for the regression of $\hat{\epsilon}_j$ on to $\mathcal{Z}_j$.
(iii) t_c—t value testing whether b_c differs from unity.
(iv) l%—the linear proportion (see Fripp and Caten, 1971, for definition and calculation).
(v) r_i^2—the ratio of the regression sum of squares of the ith genotype to the total of its regression and deviations from regression sums of squares. Since this total is fixed for a single set of data, this ratio is a convenient means of assessing the degree of linearity obtained.
(vi) b_i—the regression coefficient of the ith genotype.
(vii) dev. M.S.—the deviations from regression M.S. of the ith genotype.
(viii) r_s—Spearman rank correlation coefficient between the corresponding b_i's from two GE-analyses (see Snedecor and Cochran, 1967).

To allow the changes occurring with change in method of assessing the environments to be compared with those occurring in repeats of the same experiment (*i.e.* with those attributable to random variation), the data from the assessment replicates of the trial dikaryons in experiment 3 were regressed on to their own $\hat{\epsilon}_j$ values. The results of this GE-analysis are included under the analysis code number 3'A.

4. Results

The complete joint regression analysis of Freeman and Perkins (1971) is given only in table 3. In all subsequent tables the between genotypes item is omitted. In addition the various items in these subsequent analyses are denoted solely by the numbers assigned to them in table 3. The levels

Table 3

Joint regression analyses of the trial date from experiment 1

	No.*	Item	d.f.	Mean squares and significance levels† 1A	1C	1D
		Genotypes (G)	35	445·55***	445·55***	445·55***
	1.	Environments (E)	7	13,784·14***	—	—
E	2.	Combined regression	1	—	96,108·43***	95,084·38***
E	3.	E residual	6	—	63·42***	234·10***
G × E	4.	Heterogeneity of regressions	35	148·25***	146·82***	145·38***
G × E	5.	G × E residual	210	36·39***	36·62***	36·87***
	6.	Error	288	18·43	18·43	18·43

* Number used in subsequent tables to identify the items.
† See text for details of the variance ratio tests.
Significance level: *** $P < 0.005$.

of significance shown are for the following variance ratio tests: combined regression M.S. against environmental residual M.S.; heterogeneity of regressions M.S. against G × E residual M.S.; all other items against the error M.S.

The results are presented in two sections. In the first the various biological methods of assessing the environment are considered, while in the second physical assessments are compared with the most satisfactory of the biological assessments.

I. *Comparison of various biological environmental values*

To simplify the discussion only the results for experiment 3 will be considered in detail although those for experiment 1 are included in certain tables. The experiment 1 results will be referred to: (i) to support any generalisations, (ii) where a comparable analysis is not possible for experiment 3; and (iii) where the conclusions differ from those of experiment 3.

(a) *Joint regression analyses*

The joint regression analyses for experiments 1 and 3 are given in tables 3 and 4 respectively and the statistics relating to these analyses in table 5.

TABLE 4

Joint regression analyses of the trial data (3A, 3B, 3D, 3E) and assessment data (3'A) from experiment 3

Item		d.f.	Mean squares and significance levels†				
			3A	3B	3D	3E	3'A
1.		9	841·97***				784·51***
E	2.	1		7,417·06***	7,021·99***	6,539·83***	
	3.	8		20·09††	69·47***	152·24***	
G × E	4.	9	86·36***	89·13***	68·93*	73·95*	92·59***
	5.	72	29·24***	28·90***	31·42***	30·79***	22·07***
6.		100	9·85	9·85	9·85	9·85	8·81

† See text for details of variance ratio tests.

Significance levels: †† P = 0·05. * P = 0·01-0·05.
** P = 0·005-0·01. *** P = < 0·005.

In analysis 3A all items are greater ($P < 0{\cdot}005$) than the error mean square and the heterogeneity of regressions item is greater than its residual. The conclusions of importance for the present study are therefore: (i) that the regressions of individual genotypes are heterogeneous; (ii) that this heterogeneity accounts for a major part of the genotype-environmental variation; and (iii) that deviations from linear regression are also an important source of genotype-environmental interaction.

Analyses 3B, 3D and 3E involve independent environmental values and the between environments as well as the genotypes × environments variation has been partitioned. In all three analyses a large part of the between environments sum of squares is accounted for by the combined regression item. The amount of variation in $\hat{\epsilon}_j$ that is linearly related to the z_j values does, however, decrease as the assessment genotypes become less closely related to the trial genotypes. This is illustrated by both the decrease in r_e (table 5) and the increase in item 3 (tables 3 and 4) across the analyses. In fact, item 3 is barely significant in analysis 3B but highly significant in 3D. Applying the criteria of Freeman and Perkins (1971) and testing if $\bar{\delta}_j = 0{\cdot}0$ and $\bar{\beta} = 1{\cdot}0$, none of the sets of z_j values provide adequate estimates of $\hat{\epsilon}_j$.

TABLE 5

Statistics relating to the joint regression analyses in tables 3 and 4

Analysis	b_e	t_c	r_e	l%	het. b_i*
1A	1·000	—	1·000	87·9	221
1C	1·059	2·185	0·998	87·6	212
1D	0·921	1·717	0·993	87·3	186
3A	1·000	—	1·000	79·8	12
3B	1·025	0·472	0·989	80·6	11
3D	0·733	3·658**	0·963	73·3	9
3E	0·657	3·365**	0·916	75·4	8
3'A	1·000	—	1·000	86·3	18

* het. b_i = number of paris of b_i which differ significantly on a t-test.
Significance level: ** P = 0·005-0·01.

When items 4 and 5 are examined, it is obvious that the use of independent environmental values has had virtually no effect on the overall interpretation of the genotype-environmental interactions in these data and the conclusions from analyses 3B, 3D and 3E are as given for 3A. In fact the mean squares and hence the linear proportions (l% in table 5) for the four analyses are very similar. The homogeneity of l% is even more striking in experiment 1. Comparison of 3A with its replicate 3'A indicates that differences comparable to those between the assessment methods may occur between repeats of the same experiment.

(b) *Individual regression analyses—linearity of the regressions*

The joint regression analyses indicate that in the present data the use of different sets of environmental values has had little effect on how much on average of the genotype-environmental variation is explained by heterogeneity of the regressions of individual dikaryons. Despite this overall similarity, it is possible that there might be changes in the behaviour of individual genotypes which will only become apparent when the regressions for single dikaryons are considered.

The r_i^2 values are given for each dikaryon and each GE-analysis in table 6. In general r_i^2 decreases as the assessment genotypes become less

TABLE 6

Magnitude of r_i^2 *in the individual regression analyses in experiment 3. 3A, 3B, 3C and 3D involve the trial data and 3'A the assessment data*

	r_i^2				
Dikaryon*	3A	3B	3D	3E	3'A
3-6L	0·473	0·397	0·410	0·359	0·618
206	0·751	0·707	0·837	0·773	0·719
203	0·722	0·668	0·684	0·510	0·806
212	0·854	0·886	0·876	0·657	0·824
218	0·836	0·788	0·809	0·704	0·629
229	0·833	0·877	0·870	0·860	0·901
214	0·752	0·725	0·679	0·621	0·861
235	0·790	0·794	0·685	0·629	0·892
3-6H	0·770	0·809	0·659	0·636	0·809
204	0·796	0·700	0·703	0·867	0·867
Mean	0·758	0·735	0·721	0·662	0·793

* In increasing order of magnitude of b_i.

closely related to the trial genotypes, but it is clear that the method of assessing the environments has little effect compared with either the differences between the dikaryons themselves or the differences between two independent sets of data involving the same genotypes and environments (compare 3A and 3'A). Since a large part of the variation of most dikaryons is accounted for by a linear regression examination of their linear sensitivity is warranted.

(c) *Individual regression analyses—linear sensitivity*

The magnitude and ranking of the b_i's in each of the GE-analyses of the trial data in experiment 3 are given in table 7. It is apparent that although

TABLE 7

Magnitude and ranking of b_i *for the 10 dikaryons in experiment 3. 3A, 3B, 3D and 3E involve the trial data and 3'A the assessment data*

	Magnitude of b_i					Ranking of b_i				
Dikaryon†	3A	3B	3D	3E	3'A	3A	3B	3D	3E	3'A
3-6L	0·336*	0·319*	0·239*	0·210*	0·429*	1	1	1	1	1
206	0·663*	0·667*	0·533	0·483	0·514*	2	2	2	2	2
203	0·790	0·787	0·585	0·476	0·798	3	3	3	3	4
212	0·966	1·020	0·745	0·608	0·983	4	5	5	4	5
218	0·977	0·983	0·732	0·643	0·729	5	4	4	5	3
229	1·136	1·208	0·884	0·828	1·365	6	8	8	8	9
214	1·139	1·158	0·824	0·742	1·217	7	6	7	7	6
235	1·159	1·204	0·822	0·742	1·230	8	7	6	6	7
3-6H	1·326	1·408	0·934	0·864	1·390	9	9	9	9	10
204	1·507*	1·496	1·031	0·973	1·345	10	10	10	10	8
b_c	1·000	1·025	0·733	0·657	1·000					

† In increasing order of magnitude of b_i.
* Significantly different from b_c.

changing the method of assessing the environment alters the absolute magnitudes of the b_i values it has very little effect on their ranking. Comparisons with the corresponding results for GE-analysis 3'A show that the alterations that occur are no greater than those in repeats of the same experiment. In fact, the lowest rank correlation ($r_s = 0{\cdot}867$) was for GE-analyses 3A and 3'A.

(d) *Individual regression analyses—non-linear sensitivity*

The deviations from regression mean squares are given for experiment 3 in table 8. In general these increase as the control becomes less closely related to the trial genotypes. Most of this increase in non-linear sensitivity appears in the E residual items in the joint regression analyses but there are usually associated, but less striking changes in the G × E residual items.

As the deviations from regression mean squares increase, the standard deviations of the corresponding b_i's increase and any two b_i values are less likely to differ significantly on a t-test. Thus although the changes in method of assessing the environments have, as shown above, little effect on the relative magnitudes of the b_i's of a set of dikaryons they may affect the conclusions as to whether the b_i's differ from one another and from b_c. In the present sets of data the number of significant comparisons decreases as the control genotypes become less related to the trial genotypes (tables 5 and 7).

TABLE 8

Deviations from regression mean squares of the 10 dikaryons in experiment 3. 3A, 3B, 3D and 3E involve the trial data, and 3'A the assessment data

	Deviations from regression M.S.				
Dikaryon†	3A	3B	3D	3E	3'A
3-6L	11·96	13·68	13·38	14·53	10·03
206	13·83	16·28	9·02	12·58	9·11
203	22·74*	27·13*	25·90*	40·09*	13·54
212	15·16	11·85	12·88	35·51*	18·19*
218	17·78	22·92*	20·63*	31·98*	27·71*
229	24·55*	18·09	19·09	20·57*	18·03*
214	40·59*	45·04*	52·53*	61·95*	21·14*
235	33·86*	33·27*	50·74*	59·75*	16·15
3-6H	49·67*	41·26*	73·66*	78·77*	40·13*
204	33·03*	50·63*	74·42*	73·64*	24·56*
Mean	26·32	28·02	35·23	42·94	19·86

† In increasing order of magnitude of b_i.
* Significant when tested against the error M.S.

The heterogeneity chi-squares (χ^2) for Bartlett tests of the deviations from regression mean squares of the individual dikaryons are given in table 9.

TABLE 9

Heterogeneity χ^2 from Bartlett tests of the dev. M.S.'s of the individual dikaryons

Analysis	1A	1C	1D	3A	3B	3D	3E	3'A
χ^2	57·3	61·8	57·0	8·0	8·9	19·4	13·4	7·1
P	**	***	**	NS	NS	**	NS	NS

Significance levels: NS P = >0·05; ** P = 0·005-0·01; *** P = <0·005.

With the exception of GE-analysis 30 conclusions as to whether these mean squares are homogeneous are independent of the method of assessment. Whether this exception reflects a true effect of method of assessment or whether it is a chance result requires further investigation.

II. *Comparison of biological and physical environmental values*

(a) *Joint regression analyses*

The partitioning of the between environments sum of squares is of particular interest when physical measures are used to assess the environments. Knowledge of the specific factor(s) varying in a set of environments is not the sole difficulty determining the successful use of physical measures to assess environments. There is also the problem of choosing the scale on which to measure the physical variable. *A priori*, that scale which corresponds most closely to the biological scale (*i.e.* the response of the character under study to change in environment) might be expected to produce the most meaningful picture. The degree of correspondence between a particular physical scale and the biological scale is apparent from the partitioning of the between environments sum of squares in the joint regression analysis of Freeman and Perkins (1971). With complete agreement between the two scales all the between environments variation is accounted for by the

regression on to the physical scale (*i.e.* by the combined regression item). As the correspondence decreases, progressively more of the between environments S.S. will appear in the environmental residual item.

The joint regression analyses obtained when the malt (set 3.1) and temperature (set 3.2) environments in experiment 3 were considered separately and were analysed using biological and physical environmental values are given in table 10. The statistics derived from these analyses are given in table 11. From items 2 and 3 in the joint regression analyses it is obvious that when either biological or physical z_j values are used most of the between environments variation is accounted for by the combined regression

TABLE 10

Joint regression analyses for the 10 trial dikaryons in the 5 malt environments (3.1 A, B, F) and the 5 temperature environments (3.2 A, B, F), of experiment 3

			Mean squares and significance levels†					
			3.1			3.2		
Item		d.f.	A	B	F	A	B	F
	1.	4	758·86***	—	—	755·33***	—	—
E	2.	1	—	2977·72***	2890·73***	—	2966·47***	2596·57*
E	3.	3	—	19·24	48·23**	—	18·29	141·59***
G×E	4.	9	44·66	44·55	42·49	95·23***	90·76**	81·84*
G×E	5.	27	27·10**	27·13**	27·82**	24·29***	25·78***	28·76***
	6.	50	11·37	11·37	11·37	8·33	8·33	8·33

† See text for details of variance ratio tests.
Significance levels: as for table 4.

TABLE 11

Statistics relating to the joint regression analyses in table 10

Analysis	b_c	t_c	r_e	l%
3.1 A	1·000	—	1·000	67·9
3.1 B	1·026	0·31	0·990	67·8
3.1 F	5·743	6·39**	0·976	65·4
3.2 A	1·000	—	1·000	84·5
3.2 B	0·963	0·49	0·991	82·5
3.2 F	2·560	2·61	0·927	78·3

Significance level: ** P = 0·005-0·01.

item. However, the relation between $\hat{\epsilon}_j$ and z_j is less precise for the physical measures as indicated by higher environmental residual mean squares and lower r_e values (tables 10 and 11). In fact, in the present sets of data the former are only significant when physical z_j's are used. Nevertheless the r_e values are high and it is considered that the scales on which the physical variables are measured are satisfactory. Hence the partitioning of the genotypes × environments sum of squares and the examination of the regressions for the individual genotypes in analyses 3.1 F and 3.2 F should be meaningful.

Items 4 and 5 show that the three methods lead to remarkably similar partitioning of the genotypes × environments S.S. As found in section I there is a tendency for the heterogeneity of regressions item to decrease as the assessment data become less directly related to the trial data and the

physical measures give rise to the lowest heterogeneity of regressions items and hence the lowest l% values (see table 11).

(b) *Individual regression analyses*

When the r_i^2 values for the three methods (*i.e.* $\hat{\epsilon}_j$, biological z_j and physical z_j) are compared no pattern is apparent; the values for some dikaryons increase while those of others decrease with the same change in the method of assessing the environments.

TABLE 12

Magnitude and ranking of b_i *in subsets 3.1 and 3.2 of experiment 3*

	Magnitude of b_i			Ranking of b_i		
Dikaryon†	3.1 A	3.1 B	3.1 F	3.1 A	3.1 B	3.1 F
3-6L	0·200*	0·175*	1·142*	1	1	1
206	0·780	0·786	4·656	2	2	2
203	0·806	0·792	4·710	3	3	3
3-6H	0·935	1·002	4·901	4	5	4
235	0·941	0·987	5·099	5	4	5
212	0·969	1·045	5·986	6	6	6
229	1·116	1·147	6·433	7	7	7
218	1·291	1·303	7·464	8	8	8
214	1·359	1·432	8·199	9	9	9
204	1·602	1·594	8·835	10	10	10
b_c	1·000	1·026	5·743			
Dikaryon†	3.2 A	3.2 B	3.2 F	3.2 A	3.2 B	3.2 F
3-6L	0·191*	0·198*	0·608*	1	1	1
206	0·460*	0·450*	1·386	2	2	2
218	0·552*	0·550*	1·576	3	3	3
203	0·565	0·565*	1·707	4	4	4
212	0·888	0·890	2·726	5	5	7
214	1·052	0·950	1·802	6	6	5
204	1·240	1·168	2·487	7	7	6
229	1·580*	1·537*	4·284	8	8	9
235	1·665	1·554	4·013	9	9	8
3-6H	1·806*	1·770*	5·013*	10	10	10
b_c	1·000	0·963	2·560			

† In order of increasing magnitude of b_i

* Significantly different from b_c.

For most dikaryons a linear regression accounts for all or a major part of the change in phenotype with change in environment, no matter which set of environmental values is used. The values of b_i and their ranking are given in table 12. It is obvious that while the absolute magnitude of b_i changes markedly with change from a biological to a physical method of assessment, the ranking (*i.e.* relative linear sensitivity) of the dikaryons is unaffected. The changes in the absolute magnitude of b_i simply indicate that unit increase of the physical variable does not correspond to unit increase in the biological character.

The large differences in the relative linear sensitivities of these ten dikaryons in the two sets of environments will not be considered further in the present paper.

As would be expected, the deviations from regression mean squares of the individual dikaryons show the same lack of pattern as the r_i^2 values and the

values for no one assessment method are consistently higher or lower than those of the other two.

It is apparent that the three methods of assessing the environments (*i.e.* physical, independent and non-independent biological values) give the same interpretation. That is, within each set of environments, the partitioning of the between environments and genotypes × environments sums of squares, the ranking of the b_i values, and the amount of variation accounted for by the regressions, are very similar for the three methods. For the sets of environments considered herein none of the three methods can unconditionally be said to be the best. In fact it seems that for certain dikaryons in certain environments the biological values are the more useful (*i.e.* allow more of the variation to be explained by a linear regression) while for others, the physical values are.

5. Discussion

The results show that the overall interpretation of a set of data, including comparisons of the linear and non-linear sensitivities of the individual dikaryons, is remarkably little influenced by the source of the environmental values used. Indeed the magnitudes of the heterogeneity of regressions item and its residual scarcely differ from one GE-analysis to another irrespective of whether the environmental values are derived from the same source of data (1A and 3A), from a single related assessment genotype (1D and 3D), from an unrelated assessment genotype (3E) or from physical measures (3.1 F and 3.2 F). This similarity extends to the behaviour of individual dikaryons where, although the absolute values of the b_i's may change with change in the method of assessing the environment, the relative linear sensitivities of the different genotypes remain unaltered. Thus the selection of the most and least sensitive dikaryons is not influenced by the method used to assess the environments. In all GE-analyses $\hat{\epsilon}_j$ and z_j are highly correlated.

As outlined in the introduction the criticism of Freeman and Perkins (1971) raises doubts as to the validity of previous work where non-independent environmental values have been used. The present finding that the results from the independent and non-independent environmental values agree so closely suggests that the amount of bias introduced through the use of non-independent values can be small. Thus, although not statistically valid, regressions onto $\hat{\epsilon}_j$ provide biologically valid information. Hence it appears that complete confidence can be placed in the conclusions drawn by geneticists and plant breeders from the earlier experiments where non-independent values were used. Recognising, however, that independent values should be used the present results show that a single, appropriately chosen genotype can provide a satisfactory assessment of the environment.

The present conclusion that the interpretation is largely independent of the method of assessing the environment might at first seem to indicate that the choice of the assessment genotypes is of minor importance in designing an experiment. However, two features of the present experiments suggest that such a generalisation is not possible. Firstly, despite the consistency of the picture obtained there is a distinct tendency for less of the genotype-environmental interactions to be accounted for by differences in the linear regressions, and hence for the interactions to become less predictable, as the assessment data become more distantly related to the trial data. This trend

is apparent in the decreases in $l\%$, r_i^2, and the number of significant differences between regressions, and becomes particularly marked when the physical assessments are also considered. The existence of this trend suggests that the choice of the method of assessing the environment is not without limits and argues strongly for the assessment material to be as closely related to the trial material as possible. Secondly, the present data may not be typical of that encountered in many practical situations. Thus the additive environmental effects are large relative to the genotype-environmental interactions and hence a high correlation between $\hat{\epsilon}_j$ and z_j is to be expected whatever the assessment genotypes. Such a correlation need not exist where the $\hat{\epsilon}_j$ values are more uniform and genotype-environmental interactions are proportionally a more significant part of the response of a genotype to change in environment. Under the latter conditions the choice of the method and material with which to assess the environments is likely to be more critical.

From the above considerations it would appear that the decision as to what assessment material to use must be made separately for each experiment taking into account the aims of the experiment, the material and facilities available, and all previous information about the genotypes and environments under study. The following suggestions based on observations from the present and previous studies and the comments of Freeman and Perkins (1971) should lead to satisfactory results in most instances.

1. When the ancestry of the trial genotypes is known the parental genotype(s) from which they have been derived should furnish reliable assessments of the environments. Parental genotypes may not provide adequate assessments if (i) the gene frequencies in the trial genotypes are not the same as those in the parental genotypes, and/or (ii) non-allelic interactions are present.

2. When the general pattern of behaviour is known a single assessment genotype (or a limited number of assessment genotypes) known to be similar to the trial genotypes in gene frequencies and gene action should be satisfactory.

3. If little is known about the trial genotypes replication of these genotypes or inclusion of a number of potential assessment genotypes known to differ from one another in gene frequencies and gene action would seem to be necessary if independent assessments are to be used. Where the second procedure is followed the most appropriate assessment genotype would be chosen once the data were available.

4. When the number of trial genotypes and environments is reasonably large it may be worth considering the use of non-independent environmental values. Both the present results and theoretical considerations (Hardwick, personal communication) indicate that the bias introduced through their use is small, and in many situations recognition of this error may be more realistic than attempting to provide suitable independent assessments.

Once the data have been obtained it is important to determine whether the z_j values used are providing an adequate assessment of the environmental changes experienced by the trial individuals. This is particularly important where the assessment genotypes are not the same as the trial genotypes. Freeman and Perkins (1971) suggest two criteria to determine this, namely that $\beta = 1{\cdot}0$ and $\bar{\delta}_j = 0{\cdot}0$. In most of the present GE-analyses either or

both of these criteria fail, yet the regressions appear to be meaningful and consistent with those using $\hat{\epsilon}_j$. This suggests that these test criteria of Freeman and Perkins (1971) are too rigid in practice.

Failure of these two criteria indicates that the trial and assessment material differ in gene action, gene frequencies or genotype frequencies (Freeman and Perkins, 1971). Recognising that at least one of these differences exists, the problem becomes that of deciding whether the difference is sufficient to cause mis-interpretation of the trial data. Consideration of this problem suggests three principles which are important in determining the adequacy of a set of z_j's. Firstly z_j need not equal $\hat{\epsilon}_j$, rather it is only necessary that z_j and $\hat{\epsilon}_j$ respond to the different environments in the same way. Secondly z_j is itself subject to error and some allowance should be made for the error of estimation of z_j as well as that of $\hat{\epsilon}_j$. Thirdly $\hat{\epsilon}_j$ (and sometimes also z_j) is calculated by averaging the performances of a number of genotypes, and the between genotypes as well as the within genotypes variation should be recognised as a source of difference between $\hat{\epsilon}_j$ and z_j. Three approaches to determine whether a set of z_j's are adequate, based on these principles, are considered below.

The first approach is to compare the correlation between z_j and $\hat{\epsilon}_j$ (r_e) with that between replicate estimates of $\hat{\epsilon}_j$ (r_{rep}). The z_j are considered adequate if r_e is not less than r_{rep}. Thus in experiment 3, the 3B z_j values which are in fact replicate estimates of the $\hat{\epsilon}_j$'s would be considered adequate, while the 3D and 3E z_j values with their lower r_e values would be considered inadequate. With this correlation test however, while z_j values whose r_e is equal to or greater than the r_{rep} may be concluded to be adequate, no test of significance is possible as to whether r_e values lower than r_{rep} result from the method of assessment or from chance.

The second approach is to carry out a two-way analysis of variance of the z_j and $\hat{\epsilon}_j$ values and test the interaction item for significance against an error obtained by pooling the error of estimation of $\hat{\epsilon}_j$ and z_j. If the interaction is non-significant the z_j are considered adequate. In most situations, however, $\hat{\epsilon}_j$ and z_j will not be derived from the same number of genotypes and replicates and the errors of $\hat{\epsilon}_j$ and z_j will not be expected to be homogeneous. In this circumstance the analysis of variance is invalid.

In the present study the errors of $\hat{\epsilon}_j$ and z_j are expected to be homogeneous in GE-analyses 1C and 3B where $\hat{\epsilon}_j$ and z_j are derived from identical replication of the same (3B) or comparable (1C) genotypes. The analysis of variance has been carried out in these two cases (table 13). The error

TABLE 13

Analyses of variance testing the adequacy of z_j values

	1C		3B	
Item	d.f.	M.S.†	d.f.	M.S.†
1. Environments (E)	7	721·89***	9	161·73***
2. Assessments (A)	1	10·52***	1	1·20 N.S.
3. E×A	7	1·34 N.S.	9	0·92 N.S.
4. Error	1136	—	380	8·26

† All items tested against item 4.
Significance levels: N.S. $P<0·05$; *** $P<0·005$.

items in this table were obtained by summing over environments the within $\hat{\epsilon}_j$ and the within z_j sums of squares which both include a between genotypes

and within genotypes source of variation. The interaction item is non-significant in both analyses indicating that any differences that exist between $\hat{\epsilon}_j$ and z_j are consistent over environments. Hence these two sets of z_j's would be considered adequate, agreeing with the conclusion from the joint regression and individual regression analyses (see tables 7 to 9). The between assessments item is significant for GE-analysis 1C but not for 3B. This might be expected since replicates of the trial genotypes themselves were used to assess the environments in 3B whereas different (but closely related) genotypes were used in 1C.

The third approach is to carry out a regression analysis of the $\hat{\epsilon}_j$ and z_j values. The z_j are considered to be adequate if the deviations from regression mean square is non-significant. Two regressions are possible, either $\hat{\epsilon}_j$ on to z_j or z_j on to $\hat{\epsilon}_j$. Which regression is the more appropriate depends on deciding which variable should be considered as being fixed, and whether it is the error of $\hat{\epsilon}_j$ or z_j that is the more realistic in practice for testing the adequacy of the z_j's. Since $\hat{\epsilon}_j$ is the best measure available of how the environments are affecting the trial material, and since z_j can only be expected to be as good an assessment of the environmental effect as it is as an estimate of its own expected value, the regression of z_j on to $\hat{\epsilon}_j$ is considered to be the more appropriate here even though in the actual analysis of thee genotype-environmental interactions of the trial data z_j has been considered as the fixed variable.

The regression analyses testing the adequacy of z_j are shown for the present data in table 14. The deviations from regression mean square is

TABLE 14

Regression analyses of z_j on to $\hat{\epsilon}_j$, testing adequacy of z_j values

	Mean squares and significance levels†					
Item	d.f.	1C	1D	d.f.	3B	3D	3E
1. Regression	1	2372·91***	3067·68***	1	691·90***	1210·94***	1237·41***
2. Remainder	6	1·57 N.S.	7·55 N.S.	8	1·87 N.S.	11·98 N.S.	29·62***
3. Error	††	1·57(568)	14·25(8)	††	8·25(190)	7·39(30)	3·74(30)

† Item 1 tested against items 2 and 3; item 2 against item 3.
†† Degrees of freedom given in brackets after each error M.S.
Significance levels: N.S.; $P<0·05$; *** $P<0·005$.

significant only for GE-analysis 3E. Thus using this test it would be concluded that the z_j used were inadequate only in GE-analysis 3E agreeing with the results of the joint regression and individual regression analyses where the use of this unrelated assessment genotype gave rise to the greatest deviations from regression.

The z_j error item in table 14 is obtained by summing the within z_j mean squares of the individual environments and has $s(np-1)$ degrees of freedom where there are s environments and n replicates of p assessment genotypes. Where p is greater than one this error includes between genotypes as well as between replicates variation. Thus the present regression analysis differs from that contained in the test of Freeman and Perkins (1971) in two ways. Firstly z_j is regressed on to $\hat{\epsilon}_j$, and secondly the error item allows for any genetic differences as well as replicate differences present in the dependent variable.

6. Summary

1. Non-independent biological, independent biological and physical methods of assessing a single set of environments are compared to determine to what extent the regression analysis of genotype-environmental interactions is influenced by the source of the environmental values.

2. The regression analyses of individual dikaryons and hence the joint regression analyses when single sets of data are regressed on to environmental values obtained in these ways are remarkably similar. There is a tendency though for the linearity of the regressions to decrease as the assessment material becomes more distantly related to the individuals under study and when physical assessments are used.

3. The results show that a single assessment genotype can be satisfactory and that the interpretation of a set of data depends little on whether independent (z_j) or non-independent ($\hat{\epsilon}_j$) values are used. Therefore conclusions made in earlier studies where $\hat{\epsilon}_j$ has been used should be valid.

4. The criteria suggested by Freeman and Perkins (1971) to determine the adequacy of a set of z_j's are shown to be too rigid in practice. It is suggested that a set of z_j's is adequate if any differences between them and the $\hat{\epsilon}_j$'s are consistent over environments. Various means of testing for this consistency are discussed.

Acknowledgments.—I wish to thank Professor J. L. Jinks and Dr C. E. Caten for their advice and criticism throughout this investigation, and Miss F. Moffatt for her assistance with the experiments. The work was carried out while the author was in receipt of a Thomas Lawrence Pawlett Postgraduate Scholarship from the University of Sydney (Australia).

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