

ENVIRONMENTAL AND GENOTYPE-ENVIRONMENTAL COMPONENTS OF VARIABILITY

VIII. RELATIONS BETWEEN GENOTYPES GROWN IN DIFFERENT ENVIRONMENTS AND MEASURES OF THESE ENVIRONMENTS

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1. INTRODUCTION

It is commonly observed that the relative performances of different genotypes alter in different environments, *i.e.* that there exists a genotype-environment interaction. It has been further observed by various authors, *e.g.* Yates and Cochran (1938), Finlay and Wilkinson (1963), Rowe and Andrew (1964), Eberhart and Russell (1966), Perkins and Jinks (1968), Breese (1969), Baker (1969), that the relation between the performance of different genotypes in the various environments and some measure of these environments is frequently linear, or nearly so. These observations, which have all been made independently, lead to the conclusion that there may well be some genuine underlying linear relation between performance of particular genotypes and environmental conditions, even though it does not always account for all the observed interaction. Attention has thus been paid to the measurement of the environmental response and also to determination of the difference between the responses for different genotypes. For this purpose, the statistical theory of regression has been employed, but unfortunately the fundamental statistical assumptions have not been satisfied in any of the work quoted. The lines that have been drawn account for the observed results, but it is not valid to regard them as regression lines or to compare their slopes statistically.

There are two basic statistical objections that can be made to some or all of the papers cited, namely the choice of the sums of squares and degrees of freedom from which to subtract the regression components and, more important, the selection of the measurement of the environment on which to do the regression. An incorrect measure of environment was used in the first paper on this topic, that of Yates and Cochran (1938); some later authors took the procedure direct from this paper, while others independently rediscovered the same incorrect approach. The correct partitions of sums of squares are considered in the next two sections, followed by their interpretation in terms of biometrical genetics. Various possible measures of the environment are then discussed, and finally an example is given.

2. PARTITION OF SUMS OF SQUARES AND DEGREES OF FREEDOM

Suppose that the observations come from a set of t genotypes in s environments, to use the notation of Perkins and Jinks (1968), there being r replicates of each genotype in each environment. Then, by a slight extension of

Perkins and Jinks' notation, we may suppose the performance of the k th replicate of the i th genotype in the j th environment to be given by y_{ijk} , where

$$y_{ijk} = \mu + d_i + \epsilon_j + g_{ij} + e_{ijk}. \quad (1)$$

Here μ = grand mean over all replicates, genotypes and environments;
 d_i = additive genetic contribution of the i th genotype ($i = 1, \dots, t$);
 ϵ_j = additive environmental contribution of the j th environment ($j = 1, \dots, s$);
 g_{ij} = genotype-environment interaction of the i th genotype in the j th environment;
 e_{ijk} = residual variation contributed by the k th replicate ($k = 1, \dots, r$) of the i th genotype in the j th environment.

TABLE 1

Degrees of freedom and sums of squares in genotype-environment analysis of variance

Source of variation	d.f.	Sum of squares
G	$t-1$	$\sum_i Y_{i..}^2/rs - Y_{...}^2/rst$
E	$s-1$	$\sum_j Y_{.j.}^2/rt - Y_{...}^2/rst$
G \times E	$(t-1)(s-1)$	$\sum_i \sum_j Y_{ij.}^2/r - \sum_i Y_{i..}^2/rs - \sum_j Y_{.j.}^2/rt + Y_{...}^2/rst$
Error	$st(r-1)$	$\sum_i \sum_j \sum_k y_{ijk}^2 - \sum_i \sum_j Y_{ij.}^2/r$

The usual least-squares procedure requires the minimisation of the residual sum of squares S , where $S = \sum_i \sum_j \sum_k (y_{ijk} - \bar{y}_{ij})^2$, with $st(r-1)$ degrees of freedom. (The convention will be adopted here that $Y_{ij.}$ refers to a total over the suffix replaced by a dot, while \bar{y}_{ij} refers to a mean over the suffix omitted; more than one suffix can be replaced or omitted.)

Further, μ is estimated by \bar{y} , d_i by $(\bar{y}_i - \bar{y})$, ϵ_j by $(\bar{y}_j - \bar{y})$ and g_{ij} by $(\bar{y}_{ij} - \bar{y}_i - \bar{y}_j + \bar{y})$. The sums of squares and degrees of freedom in the analysis of variance for variation between genotypes (G), between environments (E), genotype-environment interaction (G \times E) and residual error are most conveniently represented as in table 1.

These expressions for the sums of squares are the easiest for calculation; for theoretical purposes the first three can be replaced by appropriate summations of the estimates of d_i , ϵ_j and g_{ij} . The total variation within genotypes is obtained by summing the E and G \times E lines in table 1, and leads to a term with $t(s-1)$ degrees of freedom, the sum of squares being expressible either as $\sum_i \sum_j Y_{ij.}^2/r - \sum_i Y_{i..}^2/rs$ or as $\sum_i \sum_j (\bar{y}_{ij} - \bar{y}_i)^2$. From these expressions it is seen that the variation within the i th genotype separately is the corresponding expression without the summation over all values of i . Each of these is then a valid sum of squares with $(s-1)$ degrees of freedom.

There is no corresponding partition of the G \times E sum of squares into contributions due to variation within each genotype separately, despite assertions to the contrary (Baker, 1969). One could envisage a sum of squares of the form $\sum_j (\bar{y}_{ij} - \bar{y}_i - \bar{y}_j + \bar{y})^2$, as Baker does, but consideration of

the alternative form of the $G \times E$ sum of squares in terms of totals shows that there is no way of dividing it into t groups. Further, the degrees of freedom are $(t-1)(s-1)$, and this number is not in general divisible by t . It is worth noting that this, superficially attractive but impossible, partition would also be expected to arise from the work of Bucio Alanis (1966). He only had two genotypes, and used the difference between them as the dependent variable in his regression equation as is perfectly legitimate. An obvious generalisation of this is to take the difference between one genotype and the mean of all, but this leads direct to the same expression as above.

Partition of the $G \times E$ sum of squares into components attributable to the various genotypes separately has not been attempted by anybody other than Baker, but in most of the published work other ways of partitioning the interaction sum of squares are considered. Rowe and Andrew (1964) and Eberhart and Russell (1966) are the only authors to work entirely with the total variation within genotypes.

3. REGRESSION SUMS OF SQUARES AND DEVIATIONS

Suppose now that some measure x_j of the environment is available, x_j being the same for all genotypes. For the moment x_j will be assumed to be measured without error. Previous workers have all taken x_j to be \bar{y}_j , or the deviation of this from the general mean \bar{y} , but it is not valid for x_j to be a linear function of \bar{y}_{ij} , because the linear regression of \bar{y}_{ij} on x_j is considered. This may be expressed as:

$$\bar{y}_{ij} - \bar{y}_i = \beta_i (x_j - \bar{x}) + \text{error term}, \quad (2)$$

where \bar{x} is the mean of the x_j ; it is convenient to write z_j for $x_j - \bar{x}$. The expression (2) is an alternative to (1) in the last section, so gives rise to a different form of the analysis of variance from that in table 1.

In equation (2) β_i is estimated by b_i , where $b_i = \frac{\sum_j \sum_k \bar{y}_{ij} z_j}{\sum_j \sum_k z_j^2} = r_{C_{xy_i}/C_{xx}}$, say. C_{xx} is the corrected sum of squares for x ; C_{xy_i} is the corrected sum of products for x and \bar{y}_i , and may also be written as $\sum_j \sum_k (\bar{y}_{ij} - \bar{y}_i) z_j$. This latter expression for C_{xy_i} confirms that b_i would be unaltered if $(\bar{y}_{ij} - \bar{y}_i)$ were regressed on x_j , but the first expression is more useful and will be used here. The sum of squares for regression, with one degree of freedom, may be variously written as $r_{C_{xy_i}}^2/C_{xx}$, $rb_i C_{xy_i}$ and $rb_i^2 C_{xx}$. It is seen to be the square of a linear function of \bar{y}_{ij} by expanding it in the form $r(\sum_j \sum_k \bar{y}_{ij} z_j)^2 / \sum_j \sum_k z_j^2$. Subtracting this expression from $\sum_j \sum_k (\bar{y}_{ij} - \bar{y}_i)^2$ gives the deviation from regression within the i th genotype as a sum of squares with $(s-2)$ degrees of freedom: summing all these expressions over i gives the total sum of squares attributable to all regressions as a component of the $(E + G \times E)$ sum of squares with t degrees of freedom.

The combined regression line over all genotypes has slope $\bar{b} = \frac{\sum_i \sum_j \sum_k \bar{y}_{ij} z_j}{\sum_i \sum_j \sum_k z_j^2} = rt_{C_{x\bar{y}}/rtC_{xx}}$, say. Since C_{xx} is common to all genotypes, this leads to the conclusion that \bar{b} is the arithmetic mean over i of all b_i . The overall regression sum of squares is $rt\bar{b}^2 C_{xx}$, i.e. $rt(\sum_j \sum_k \bar{y}_j z_j)^2 / \sum_j \sum_k z_j^2$, the square of a linear function of \bar{y}_j , and thus part of the environment sum of squares.

This is where the attempt to equate x_j to \bar{y}_j or $(y_j - \bar{y})$ breaks down. In either case $z_j = \bar{y}_j - \bar{y}$ and $\sum \bar{y}_j z_j = \sum z_j^2$; the regression sum of squares $rt\bar{b}^2 C_{xx}$ becomes the same as $rt \sum_j (\bar{y}_j - \bar{y})^2$, that is, the total sum of squares between environments, not merely part of it. Although this point has been recognised by some previous workers, its consequences have not been realised. Thus, Rowe and Andrew (1964) write this sum of squares down in two tables, assigning one degree of freedom to it in one table and $(t-1)$ in the other: Perkins and Jinks (1968) refer to "the joint regression sum of squares which in this analysis equals the environment sum of squares". This sum of squares is in fact not used much in the various developments, but nevertheless its ambiguity vitiates the subsequent analysis. Any sum of squares has a

TABLE 2

Partitioning of analysis of variance to take account of regression effects

Source of variation	d.f.	Sum of squares
Genotypes (G)	$t-1$	As table 1
Environments (E)		
Combined regression	1	$(\sum_j Y_{.j} z_j)^2 / rt \sum_j z_j^2$
Residual	$s-2$	By subtraction from E in table 1
Interaction (G \times E):		
Heterogeneity of regressions	$t-1$	$[\sum_i (\sum_j Y_{ij} z_j)^2 / r - (\sum_j Y_{.j} z_j)^2 / rt] / \sum_j z_j^2$
Residual	$(t-1)(s-2)$	By subtraction from G \times E in table 1
Error between replicates	$st(r-1)$	As table 1

unique number of degrees of freedom, and this cannot vary according to what partitioning scheme is adopted.

The sum of squares attributable to heterogeneity of regression, that is, to differences of the individual regression lines for the different genotypes from the combined regression line over all genotypes, has $(t-1)$ degrees of freedom. It can be expressed as $r \sum_i (b_i - \bar{b})^2 C_{xx}$ and expanded as either $r \sum_i [\sum_j z_j (\bar{y}_{ij} - \bar{y}_j)]^2 / \sum_j z_j^2$ or $r [\sum_i (\sum_j \bar{y}_{ij} z_j)^2 - t (\sum_j \bar{y}_j z_j)^2] / \sum_j z_j^2$. Being a component of the variation within environments it is orthogonal to the term for E in table 1. Further, from the way it is derived it is also part of the variation within genotypes, so is orthogonal to G in table 1. It is thus part of the interaction term G \times E, and a residual component of this term with $(t-1)(s-2)$ degrees of freedom can be found. The replacement of z_j by $(\bar{y}_j - \bar{y})$ leads to an invalid sum of squares here also, as this heterogeneity term would then be a term in fourth powers of y divided by a quadratic term in y , and not necessarily a quadratic term in y itself. A valid partitioning of table 1 is given in table 2; the sums of squares here, as in table 1, are given in terms of totals rather than means, as these are usually easier for calculation.

All the terms in table 2 are orthogonal, so comparisons are possible by means of F tests. It will almost always be true, if x_j is a sufficiently good measure of the environment, that the combined regression is significant by comparison with the residual within environments, so this comparison will not give much

additional information. The term for heterogeneity of regression and its residual should both be tested against the experimental error to determine whether observed differences between genotypes can be accounted for, partially or wholly, by a linear effect of environments. An approach similar to this was suggested by Perkins and Jinks (1968), but they proposed to consider separately the contributions for each genotype to the interaction, although they are not independent. Nevertheless, these terms in table 2 must be studied carefully if a full understanding of experimental results is to be obtained. If the regressions are heterogeneous, the slopes of the lines for any pair of genotypes can be compared by a t -test.

Another possible test, not derivable from table 2 as it stands, is the comparison of the individual deviations from regression for the various genotypes. This can be done by a straightforward application of Bartlett's test for comparing t variances, each with $(s-2)$ degrees of freedom.

4. BIOMETRICAL INTERPRETATIONS

Two measures of phenotypic stability of genotypes have been used by those who have calculated regressions on environmental means. The first is based on the slope of the regression line for a particular genotype (Finlay and Wilkinson 1963; Rowe and Andrew 1964). Since the mean slope is then given by the regression of the environmental mean on itself, $\bar{b} = 1$. Thus, genotypes are regarded as having a high or low degree of stability according as their values of b_i fall short of or exceed unity respectively. When x_j is a truly independent variable there is no such guarantee that the mean slope will have any particular value, but it will still be true that higher values of b_i will be associated with lower stability and conversely. Eberhart and Russell (1966), who combine the E and $G \times E$ terms in the analysis of variance, also propose a second measure of stability, derived from the deviations from regressions term described at the end of the last section. They recognise that regressing on the environmental mean will lead to inexact tests of significance but still use the deviations as a measure of phenotypic stability as does Breese (1969); this measure can be applied to any x_j .

The mean phenotype of the i th genotype in the j th environment, \bar{y}_{ij} , obtained by averaging over replicates in equation (1) is an estimate of

$$\mu + d_i + \epsilon_j + g_{ij}, \quad (3)$$

putting $\sum_k e_{ijk} = 0$.

The definitions of the environmental component ϵ_j and the genotype-environment interaction component g_{ij} can now be expanded in terms of a biometrical model corresponding to the analysis given in table 2, by comparing terms in expressions (2) and (3). Thus, $\epsilon_j = \beta z_j + \bar{\delta}_j$, where β is the combined regression slope, z_j is the independent assessment of the environment and $\bar{\delta}_j$ is the deviation of \bar{y}_j from the combined regression line. Also $g_{ij} = \beta_{ai} z_j + \delta_{aij}$, where $\beta_{ai} = \beta_i - \beta$ and δ_{aij} is the deviation of the i th line from its linear regression on z_j in the j th environment less $\bar{\delta}_j$.

The mean phenotype \bar{y}_{ij} then becomes an estimate of

$$\mu + d_i + \bar{\beta} z_j + \bar{\delta}_j + \beta_{ai} z_j + \delta_{aij}. \quad (4)$$

When a set of genotypes is grown in each of a number of environments the components of variance due to the estimates of the four parameters $\bar{\beta}$, $\bar{\delta}_j$, β_{di} and δ_{dij} correspond to the middle four items in table 2, that is the combined regression and its residual and the heterogeneity of regressions and its residual, respectively.

In the presence of unequal gene frequencies among the genes controlling the character under study and its interaction with environments the various parameters can be redefined in a similar way to that set out by Perkins and Jinks (1968). Thus:

$$\bar{y}_{ij} = \mu' + d'_i + \bar{\beta}' z_j + \bar{\delta}'_j + \beta'_{di} z_j + \delta'_{dij}.$$

As before,

$$\mu' = \mu + \sum_k (u_k - v_k) d_k,$$

$$d'_i = d_i - \sum_k (u_k - v_k) d_k,$$

where u_k is the frequency of increasing alleles at the k th locus and v_k the frequency of decreasing alleles and:

$$\bar{\beta}' = \bar{\beta} + \sum_k (u_k - v_k) \beta_{dk},$$

$$\bar{\delta}'_j = \bar{\delta}_j + \sum_k (u_k - v_k) \delta_{dkj},$$

$$\beta'_{di} = \beta_{di} - \sum_k (u_k - v_k) \beta_{dk},$$

$$\delta'_{dij} = \delta_{dij} - \sum_k (u_k - v_k) \delta_{dkj}.$$

5. CHOICE OF MEASURE OF THE ENVIRONMENT

It would be highly desirable to measure environmental effects by something quite unrelated to the organism under study, and this can occasionally be done, but it is usually very difficult in practice. Unless the environment is closely controlled, it is not very likely that only one component of the environment affects the relative growth of different genotypes. It would be possible to use controlled environment chambers to do some critical experiments, but the results would then inevitably be of more theoretical than practical interest. Further, it is always desirable to test genotypes over as wide a range of environmental conditions as possible, and the constraints of a controlled environment chamber would be somewhat limiting.

It can be concluded, therefore, that the best measure of the combined effect of all the relevant factors operating in an environment will be provided by the organism itself, although the environmental effect will no longer be measured without error. There are several ways of achieving this without using the same individuals to determine both the environmental effect and the genotype-environment interaction. The most obvious solution is simply to divide the replicates of each genotype into two groups, using one group to measure the interaction and the average of the second group over genotypes to measure the environment. In order to assess experimental error there must be some replication in at least one of these groups, so three replicates or more are needed in all. The second possibility is to use one or more genotypes that can be regarded as standards to assess the environment: the more genotypes that are used here, the more accurately the environment will

be assessed. For instance, parental genotypes could be used as standards in relation to any generation derived by crosses between them (Bucio Alanis and Hill, 1966; Breese, 1969; Bucio Alanis, Perkins and Jinks, 1969; Jinks and Perkins, 1970; Perkins, 1970), or for any lines derived from inbreeding the F_2 . Two other ways of measuring the environment biologically are to use closely related material in the same environment or replicates of the same material in a closely related and contemporary environment. A final possibility is to use one genotype of a group as a standard, but since there is usually no reason to choose one genotype rather than another this method will rarely be satisfactory.

It is possible to test the similarity of the assessments of the environment by the collection of genotypes used for this purpose and by those whose interactions with the environment are under investigation. Since x_j is not measured without error, tests, and inferences from them, are conditional. Thus, the combined regression slope \bar{b} can be tested to see if it differs significantly from unity, though variation in x will, if anything, tend to reduce \bar{b} ; the residual about this regression can also be compared with the experimental error. If neither of these tests shows significance z_j may be regarded as an estimate of ϵ_j and (4) in the last section reduces to the model proposed by Perkins and Jinks (1968), namely that \bar{y}_{ij} estimates

$$\mu + d_i + \epsilon_j + \beta_{di} \epsilon_j + \delta_{dij}.$$

The estimate of the individual regression coefficient β_i which in general is equal to $\bar{\beta} + \beta_{di}$ is then equal to $1 + \beta_{di}$ so that the significance of its deviation from unity can be tested. Since the first two tests are independent each may show significance without the other: if either is significant, the two collections of genotypes must differ in gene frequencies, gene action or genotype frequencies. For example, it can be predicted that the environmental component z_j derived from the average performance of two parents in the j th environment will be an estimate of the environmental component ϵ_j of a collection of inbred lines derived from a cross between them unless the gene frequencies in the two parents are changed during the inbreeding of their F_2 offspring or non-allelic interactions are present. Absence of non-allelic interactions is also required if the environmental component z_j derived from the average performance of two inbred parents in the j th environment is to be an adequate assessment of the additive environmental component ϵ_j of the generations derived from crosses between them (Bucio Alanis, Perkins and Jinks, 1969; Jinks and Perkins, 1970).

6. EXAMPLE

As an example of the new approach some data on the growth rate of the fungus *Schizophyllum commune* are considered. These come from a trial containing two replicates of 36 genotypes, together with a control, all grown in 8 different environments. Table 3 shows, for these data, the analysis of variance for comparing the 36 genotypes, partitioned as in table 2, the environmental effect for the 8 environments being determined from the control.

All the terms in table 3 are significant at the 0.1 per cent level when compared with the error between replicates. Further, the mean square for heterogeneity of regressions is significantly greater than its residual at the

same level. Most of the interaction is thus accounted for by the heterogeneity of regressions, a situation recognised as occurring commonly by Perkins and Jinks (1968). The values of b_i for regression of the 36 genotypes on the control range from 0.54 to 1.33, the mean value \bar{b} being 0.92 with a standard error of 0.046. Thus, this set of data does not contradict the hypothesis that $\bar{\beta} = 1$, but $\bar{\delta}_j$ is not negligible. A plot of the regression of the mean of the 36 genotypes on the control does not suggest any particular curvature, merely a high degree of scatter. The use of further controls could possibly reduce this overall scatter, but the genotypes here have different phenotypic stabilities in the sense of Eberhart and Russell (1966) and Breese (1969).

TABLE 3
Growth rate in Schizophyllum commune

Source of variation	d.f.	Mean square	F (compared with error)
Genotypes (G)	35	445.551	24.18
Environments (E)			
Combined regression	1	95084.384	5160.62
Residual	6	234.098	12.49
Interaction (G \times E)			
Heterogeneity of regressions	35	145.378	7.89
Residual	210	36.865	2.00
Error between replicates	288	18.425	

7. SUMMARY

1. Genotype-environment interactions have often been explained, wholly or in part, by linear regressions of the performance of the various genotypes on a measure of the environment. The environment has often been assessed by the mean of the genotypes grown in it, but this method leads to statistically invalid regressions in which the sum of squares for the joint regression is the same as the total sum of squares between environments, not part of it.

2. The analysis of variance is partitioned into components representing regressions on a general measure of the environment independent of the genotypes under study and deviations from regression. Interpretations of various results that could arise from this analysis of variance are given in terms of biometrical genetics.

3. The choice of an appropriate measure of the environment is considered. If, as is usually best, the environment is assessed by the responses of similar genotypes, genetically useful information can be obtained direct from the analysis of variance.

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