Among-Environment Heteroscedasticity and Genetic Autocorrelation: Implications for the Study of Phenotypic Plasticity

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ABSTRACT

The impact of among-environment heteroscedasticity and genetic autocorrelation on the analysis of phenotypic plasticity is examined. Among-environment heteroscedasticity occurs when genotypic variances differ among environments. Genetic autocorrelation arises whenever the responses of a genotype to different environments are more or less similar than expected for observations randomly associated. In a multivariate analysis-of-variance model, three transformations of genotypic profiles (reaction norms), which apply to the residuals of the model while preserving the mean responses within environments, are derived. The transformations remove either among-environment heteroscedasticity, genetic autocorrelation or both. When both nuisances are not removed, statistical tests are corrected in a modified univariate approach using the sample covariance matrix of the genotypic profiles. Methods are illustrated on a *Chlamydomonas reinhardtii* data set. When heteroscedasticity was removed, the variance component associated with the genotype-by-environment interaction increased proportionally to the genotype variance component. As a result, the genetic correlation r_g was altered. Genetic autocorrelation was responsible for statistical significance of the genotype-by-environment interaction and genotype main effects on raw data. When autocorrelation was removed, the ranking of genotypes according to their stability index dramatically changed. Evolutionary implications of our methods and results are discussed.

PHENOTYPIC plasticity is the subject of an increasing number of studies, in both the plant and the animal literature. This is well demonstrated by the wealth of papers published on plasticity in the last few years (*e.g.*, FORD and SEIGEL 1989; SCHEINER and LYMAN 1989, 1991; SCHLICHTING 1989a; SCHULTZ and WARNER 1989; PLATENKAMP 1990; TREXLER and TRAVIS 1990; TUCIC *et al.* 1990; SCHEINER *et al.* 1991; WEBER and SCHEINER 1992). Phenotypic plasticity refers to the degree to which the phenotypic expression of a genotype varies under different environmental conditions (SULTAN 1987). Its converse, called "stability," means the maintenance of a nearly constant phenotype over a wide range of conditions (BELL and LECHOWICZ 1994). When associated with high-level performance, stability is a primary goal for plant breeders and crop scientists (PLAISTED and PETERSON 1959; SHUKLA 1972; NGUYEN *et al.* 1980; KANG and MILLER 1984; WESTCOTT 1986). BRADSHAW's (1965) review provided an elegant summary of the ecological and agricultural perspectives on plastic responses. The evolutionary significance of phenotypic plasticity and its implications are also well documented (*e.g.*, SULTAN 1987; STEARNS 1989). Plasticity also applies to character correlations that may be altered by the environment, in their sign as well as in their magnitude (SCHLICHTING 1989b; STEARNS *et al.* 1991).

A standard method of carrying out a plasticity experiment is to raise various genotypes in a series of environments and to monitor the variation in responses among these environments (SCHLICHTING 1986). A nonquantitative analysis of the range of phenotypic responses or "reaction norm" (SCHMALHAUSEN 1949) for a given genotype consists of plotting its response against each growth environment (*e.g.*, GUPTA and LEWONTIN 1982; SCHEINER and LYMAN 1991). The quantitative analysis of plasticity essentially relies on the analysis-of-variance (ANOVA) approach, with the analysis of the coefficient of variation (CV) as a complement or an alternative (SCHLICHTING 1986). Individual norms of reaction are then especially useful for interpreting significant genotype-by-environment (G × E) interaction (SULTAN 1987).

In what follows, our approach to the quantification and assessment of plasticity will be based on the conventional view in quantitative genetics (VIA 1987). Using as a basis a two-way ANOVA with genotype and environment as crossed factors, the phenotypic variability among individuals that are genetically linked and sampled from a range of environments can be partitioned into genotype and environment main effects, and G × E interaction. Global measures of plasticity are based

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on an estimate of the variance component associated with the G × E interaction, combined (SCHEINER and GOODNIGHT 1984) or not (THOMPSON 1991) with the environmental one. In the ANOVA models classically defined in selection and quantitative genetics, the genotype and environment factors are usually considered random, implicitly or not, because of the associated variance components and correlation coefficients (PLAISTED and PETERSON 1959; SCHEINER and GOODNIGHT 1984; SCHEINER and TEERI 1986). Those models, however, take the genetic and environmental dependencies and variabilities into account in a rather drastic manner. They constrain the intraclass correlation coefficient among environments to be constant within each genotype, whatever the genotype and the pair of environments considered, and they impose analogous constraints on correlations among genotypes within each environment. Moreover, the genotypic and environmental variances are constrained to be constant within the environments and genotypes, respectively.

We define a multivariate ANOVA (MANOVA) model that relaxes those constraints on the intraclass correlation coefficient among environments and on the genotypic variances within environments. In this model, a norm of reaction is formally defined by the vector of mean responses of a genotype to the environments, called the genotypic profile. We quantify plasticity using the stability variance components assigned to the genotypes, which essentially result from a partition of the G × E sum of squared deviations into genotype-specific components (see APPENDIX A). Stability variance components have been available for a long time in agricultural sciences (PLAISTED and PETERSON 1959; WRICKE 1962; SHUKLA 1972). They have the advantage over global variance components that they allow assessing plasticity of each genotype separately, providing an answer to the question: Is that genotype significantly unstable or not?

From a statistical viewpoint, two classical assumptions underlying ANOVA are the homoscedasticity (equality of the variances) and independence (or to a lesser extent, the intraclass correlation structure) of the observations. These statistical assumptions can be violated when estimating and assessing global and individual variance components in plasticity analysis. On one hand, plasticity experiments are often characterized by genotypic responses that are more dispersed in some environments than in others. We refer to this heterogeneity of genotypic variances among environments as "among-environment heteroscedasticity." On the other hand, by design, plasticity experiments also involve genetic correlations among responses from a genotype to different environments, resulting in traits taking values for the genotype that are more similar (positive autocorrelation) or less similar (negative autocorrelation) than expected for pairs of observations randomly associated. This concept of a "genetic autocorrelation" extends the one used in time series and spatial data analysis. It is compatible with considering one trait expressed by a genotype in different environments as different traits with genetic correlations among them (FALCONER 1981, p. 291). To understand the evolutionary implications of the above-mentioned heteroscedasticity and autocorrelation for a plasticity analysis, we consider three transformations of the genotypic profiles defined in the MANOVA model. The first transformation removes both the among-environment heteroscedasticity and genetic autocorrelation from the genotypic profiles before the estimation of stability variance components. The other transformations account for only one of the two sources of nuisance. As an alternative, we present corrections in ANOVA F testing, inspired by the techniques developed in the analysis of repeated measures (CROWDER and HAND 1990; POTVIN *et al.* 1990), to apply in particular when removing heteroscedasticity or autocorrelation by data transformation is biologically irrelevant. Both methods are illustrated on a *Chlamydomonas reinhardtii* data set, for three stability indices used as measures of plasticity *vs.* the coefficient of variation. Guidance on how they may be carried out in SAS is provided. In the light of results, the consequences of removing among-heteroscedasticity and genetic autocorrelation before plasticity analysis are discussed, in relation with the very nature of the G × E interaction and the genetic variation in phenotypic plasticity in particular.

THE MODELS

In a standard plasticity experiment, the phenotype of individual k ($k = 1, \ldots, r_{ij}$) of genotype i ($i = 1, \ldots, g$) in environment j ($j = 1, \ldots, n$) can be expressed as

$$p_{ijk} = m + g_i + e_j + ge_{ij} + \epsilon_{ijk} \qquad (1)$$

where m is the intercept (the response expected for all the individuals or clonal replicates when the other terms in the model are random); g_i, e_j and ge_{ij} are the deviations due to genotype i and environment j, and the interaction between them, respectively; the ϵ_{ijk} are residual deviations of microenvironmental and individual nature. This model is similar in its formulation to that of SCHEINER and GOODNIGHT (1984) but in deriving our MANOVA model, we will consider the environment main effects, e_j, fixed. It follows that the expected response of an individual will depend on its location, which would not be the case if the e_j were random, and that the conclusions of the corresponding plasticity analysis will be limited to the environments under study. The other terms (g_i, ge_{ij} and ϵ_{ijk}) are all considered random, with 0 expected value. They are independently distributed for responses arising from different genotypes.

From (1), the mean response $\bar{p}_{ij}$ of genotype i in environment j (computed over the r_{ij} corresponding individuals or clonal replicates) may be expressed as

$$\bar{p}_{ij} = m + g_i + e_j + \eta_{ij} \qquad (2)$$

with $\eta_{ij} = ge_{ij} + \bar{\epsilon}_{ij}$ where $\bar{\epsilon}_{ij}$ ($i = 1, \ldots, g$; $j = 1, \ldots, n$) denotes the mean residual deviation of genotype i in environment j. In (2), the m and e_j terms are fixed, as in (1), and g_i and η_{ij} are random, with 0 expected value.

We may then define the "genotypic profile" of genotype $i = 1, \ldots, g$, as the random vector of dimension n: $\bar{\mathbf{p}}_i = (\bar{p}_{i1}, \ldots, \bar{p}_{in})$. Plotting this vector of mean responses against the environments they originate from provides the norm of reaction of genotype i. It follows from (2) that

$$\bar{\mathbf{p}}_i = \mathbf{m} + \tau_i \qquad (3)$$

with $\mathbf{m} = (m + e_1, \ldots, m + e_n)$ and $\tau_i = (g_i + \eta_{i1}, \ldots, g_i + \eta_{in})$ ($i = 1, \ldots, g$). In the resulting MANOVA model (3), the fixed part, $\mathbf{m}$, is the expected value of the genotypic profile $\bar{\mathbf{p}}_i$ for all $i = 1, \ldots, g$, and the expected value of the random part, τ_i ($i = 1, \ldots, g$), is equal to 0. More specifically, the intercept $\mathbf{m}$ is the vector of the within-environment expected values, while the multivariate error term τ_i incorporates the univariate genotype main effects, g_i, and the G × E interaction, ge_{ij}, of (1). The genotypic profiles $\bar{\mathbf{p}}_i$ are independently distributed, with expected value $\mathbf{m}$ and variance-covariance matrix $\boldsymbol{\Sigma}_i$. Their independence results, under the normality assumption, from the absence of any correlation between $\bar{p}_{ij}$ and $\bar{p}_{i'j'}$, for all $i \neq i' = 1, \ldots, g$ and $j, j' = 1, \ldots, n$ in (2). Under the hypothesis "$\boldsymbol{\Sigma}_i = \boldsymbol{\Sigma}$ for all i," the $\bar{\mathbf{p}}_i$ constitute a random sample of size g from an n-variate population with parameters $\mathbf{m}$ and $\boldsymbol{\Sigma}$. The diagonal elements of the variance-covariance matrix $\boldsymbol{\Sigma}$ are the genotypic variances that may differ among environments in (3), while the off-diagonal elements are provided by the genetic autocovariances (or nonstandardized within-genotype intraclass correlations) that are no longer constrained to be constant whatever the environments considered. The classical sample estimator of $\boldsymbol{\Sigma}$ is then given by

$$\hat{\boldsymbol{\Sigma}} = \frac{1}{g-1} \sum_{i=1}^{g} (\bar{\mathbf{p}}_i - \bar{\mathbf{p}})'(\bar{\mathbf{p}}_i - \bar{\mathbf{p}}) \qquad (4)$$

with $\bar{\mathbf{p}}$, the mean of the genotypic profiles $\bar{\mathbf{p}}_i$ ($i = 1, \ldots, g$), estimating the expected value $\mathbf{m}$, and the transpose operator ($'$). Vectors $\bar{\mathbf{p}}_i - \bar{\mathbf{p}}$ are multivariate measures of deviation, which extend the univariate ones that are squared and summed to provide the classical ANOVA sum of squared deviations. Instead of being squared, the multivariate deviations are multiplied by their transpose $(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})'$ in (4). Each element of $\hat{\boldsymbol{\Sigma}}$ is the sample estimate of the corresponding element in $\boldsymbol{\Sigma}$.

To assess the hypothesis "$\boldsymbol{\Sigma}_i = \boldsymbol{\Sigma}$ for all i," a sufficient and constant number of individuals is required for each genotype in each environment, that is, a number of clonal replicates $r_{ij} = r' > n$ for all i and j. In that case, different sample variance-covariance matrices may be used in the transformations hereafter, depending on the genotype considered. The homogeneity of the genotypic variance-covariance matrices may be assessed statistically (MORRISON 1990). However, that multivariate procedure is not very powerful, so that the equality hypothesis will often be accepted when it should not be. On the other hand, a drawback of both procedures is that correlations among individuals or clonal replicates will alter the estimation of the genotypic variance-covariance matrices; this drawback is absent from (4).

THE TRANSFORMATIONS

First of all, it must be noted that our transformations apply to the residuals, $\bar{\mathbf{p}}_i - \bar{\mathbf{p}}$, of the MANOVA model (3). Second, each transformation has a specific objective, but all preserve the within-environment means of the raw data because the mean profile $\bar{\mathbf{p}}$ is added back after manipulation of the residuals. The three transformations are formally defined below. Thereafter, a close examination of the variance-covariance matrix $\hat{\boldsymbol{\Sigma}}$ will help understand how each of them works. The first and third transformations require a number of genotypes higher than the number of environments, *i.e.*, $g > n$, to ensure the invertibility of $\hat{\boldsymbol{\Sigma}}$ in matrix algebra. The two first transformations scale all genotypic variances to a unitary value but, when justified, a scaling to a different value may be performed with slight modification. This point is elaborated at the end of the section.

Examination of the diagonal elements of $\hat{\boldsymbol{\Sigma}}$ provides a way to detect among-environment heteroscedasticity. Each of those elements is a sample genotypic variance, *i.e.*, the variance estimated among genotypes for a given environment. Under the assumption of homogeneity of the genotypic variances, the diagonal entries of $\hat{\boldsymbol{\Sigma}}$ should be approximatively equal, or at least of the same order of magnitude. When this is not the case, dividing the residuals $\bar{\mathbf{p}}_i - \bar{\mathbf{p}}$ by the square root of the genotypic variances (the genotypic standard deviations) in (6) provides transformed profiles with unitary genotypic variances. Concerning the off-diagonal elements of $\hat{\boldsymbol{\Sigma}}$, they estimate the genetic autocovariances, $\mathrm{cov}(\bar{p}_{ij}, \bar{p}_{ij'})$, and provide the genetic autocorrelations after standardization by the relevant genotypic standard deviations. The first transformation then removes both heteroscedasticity and genetic autocorrelation since by multiplying the vector of deviation $(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})'$ with the square root of the inverse of $\hat{\boldsymbol{\Sigma}}$, the genotypic variances are scaled to unitary value, and the covariances to 0. This follows from two properties of matrix algebra. First, the product of an invertible matrix by its inverse is the identity matrix (with ones on the diagonal and zeros outside). Second, premultiplying a random vector by the square root of the inverse of its variance-covariance matrix is equivalent to multiplying its variance-covariance matrix by its inverse. To remove genetic autocorrelation, the third transformation scales

up all off-diagonal entries of $\hat{\Sigma}$, while the diagonal ones remain unchanged. Guidance on how to implement the transformations using SAS is given in APPENDIX B. All the transformations may be applied to individual as well as mean genotype responses, when data are balanced. Transformation (6) is the only one that is applicable when data are unbalanced. Transformed individual responses will be analyzed in model (1), with correction (see next section) when only partially transformed.

The first transformation provides genotypic profiles $\bar{\mathbf{z}}_i = (\bar{z}_{i1}, \ldots, \bar{z}_{in})$ $(i = 1, \ldots, g)$ from which both the among-environment heteroscedasticity and the genetic autocorrelation are removed, *i.e.*, the sample variances (the genotypic variances) computed among $\bar{z}_{ij}$ $(i = 1, \ldots, g)$ are all equal to one for $j = 1, \ldots, n$, while Pearson's linear correlation coefficients (the genetic autocorrelations) computed between $\bar{z}_{ij}$ $(i = 1, \ldots, g)$ and $\bar{z}_{ij'}$ $(i = 1, \ldots, g)$ are equal to 0 for $j \neq j' = 1, \ldots, n$:

$$\bar{\mathbf{z}}_i' = \bar{\mathbf{p}}' + \hat{\Sigma}^{-0.5}(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})' \quad (i = 1, \ldots, g) \qquad (5)$$

where $\hat{\Sigma}^{-0.5}$ denotes the square root of the inverse of $\hat{\Sigma}$.

The second one provides genotypic profiles $\bar{\mathbf{z}}_i^{(1)} = (\bar{z}_{i_j}^{(1)}, \ldots, \bar{z}_{in}^{(1)})$ $(i = 1, \ldots, g)$ from which only the among-environment heterogeneity of genotypic variances is removed:

$$\bar{\mathbf{z}}_i^{(1)\prime} = \bar{\mathbf{p}}' + \{\mathrm{diag}(\hat{\Sigma})\}^{-0.5}(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})' \quad (i = 1, \ldots, g) \qquad (6)$$

where $\mathrm{diag}(\hat{\Sigma})$ denotes the matrix of same dimensions as $\hat{\Sigma}$ (*i.e.*, n rows and n columns) and whose entries are all equal to 0, except its diagonal ones which are equal to those of $\hat{\Sigma}$.

The last transformation provides genotypic profiles $\bar{\mathbf{z}}_i^{(2)} = (\bar{z}_i^{(2)}, \ldots, \bar{z}_{in}^{(2)})$ $(i = 1, \ldots, g)$ from which only the genetic autocorrelation is removed:

$$\bar{\mathbf{z}}_i^{(2)\prime} = \bar{\mathbf{p}}' + \{\mathrm{diag}(\hat{\Sigma})\}^{0.5}\,\hat{\Sigma}^{-0.5}(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})'$$
$$= \bar{\mathbf{p}}' + \{\mathrm{diag}(\hat{\Sigma})\}^{0.5}(\bar{\mathbf{z}}_i - \bar{\mathbf{p}})' \quad (i = 1, \ldots, g) \qquad (7)$$

As mentioned above, scaling the genotypic variances to a common value other than the unitary one in (6) and (7) may be performed with slight modification. Such values are provided for instance by the geometric mean of the genotypic variances for the raw data or by the generalized variance defined as the determinant of $\hat{\Sigma}$ (ANDERSON 1984). In the example, we used a unitary variance as genotypic variance common to all environments. This choice of a standard value allows comparisons among traits observed at different scales. Also in the example (and this feature is not restricted to the Chlamydomonas data set; results not reported here), using a different common genotypic variance does *not* change the significance testing of the genotype main effects and G × E interaction, and as a result, of the stability indices; only the significance of the environment main effects appears to be dependent on it. More puzzling is the fact that, depending on the choice of the formula and model used for estimating the genetic correlation r_g, FRY's (1992, p. 543) formula (5) for the SAS model or formula (6) for the SCHEFFÉ mixed model, the value chosen as common genotypic variance has an effect, or does not. This needs further investigation.

THE CORRECTIONS

Circularity is the most general necessary and sufficient condition allowing unmodified F testing in ANOVA models in the presence of limited forms of heteroscedasticity and autocorrelation (CROWDER and HAND 1990; POTVIN *et al.* 1990). A particular case is provided by compound symmetry that combines homoscedasticity and intraclass correlation structure. In plasticity experiments, circularity means that the variances of all pairwise differences of the trait between two environments are equal for a given genotype whatever the environments, while compound symmetry refers to homogeneity of the genotypic variances and constancy of the correlations among all environments for any given genotype. The corrections in testing outlined below are only required when the circularity condition is not satisfied.

To evaluate the departure from circularity due to among-environment heteroscedasticity and genetic autocorrelation in (1), we have used BOX's epsilon (BOX 1954a,b; see also CROWDER and HAND 1990; POTVIN *et al.* 1990). The statistic is based on the trace of a contrast transformation of the estimated variance-covariance matrix $\hat{\Sigma}$; for the transformed genotypic profiles, that matrix is computed as in (4) by substituting $\bar{\mathbf{p}}_i$ by $\bar{\mathbf{z}}_i$, $\bar{\mathbf{z}}_i^{(1)}$ or $\bar{\mathbf{z}}_i^{(2)}$ $(i = 1, \ldots, g)$, respectively. For the untransformed genotypic profiles for instance, BOX's epsilon is estimated by

$$\hat{\epsilon} = \frac{[\mathrm{tr}(\mathbf{C}'\hat{\Sigma}\,\mathbf{C})]^2}{(n-1)\,\mathrm{tr}[(\mathbf{C}'\hat{\Sigma}\,\mathbf{C})^2]} \qquad (8)$$

where $\mathbf{C}$ denotes a matrix of $n - 1$ orthonormal contrasts of dimension n.

BOX's epsilon value ranges from $1/(n-1)$ to 1.0. The smaller it is, the less the circularity condition is satisfied and, as a result, the more genotypic variances are heterogeneous among environments and the more genetic autocorrelations are unequal and different from 0. Note that $\hat{\epsilon} = 1.0$ for the transformed genotypic profiles $\bar{\mathbf{z}}_i$ $(i = 1, \ldots, g)$ because their sample variance-covariance matrix is reduced to the identity matrix (with ones on the diagonal and zeros outside), as a direct result of the definition of transformation (5). BOX's epsilon must be used in F testing when required, to take into account the genetic autocorrelations

among individual responses from the same genotype and the heterogeneity of their dispersion from one environment to the other.

Under the normality assumption on random effects in (1), where only the intercept and the environment main effects are fixed, the resulting corrected F tests are for the genotype main effects:

$$F[g-1, \hat{\epsilon} \sum_{i=1}^{g} \sum_{j=1}^{n} (r_{ij}-1)] = \text{Genotype MS/Error MS};$$

for the environment main effects:

$$F[\hat{\epsilon}(n-1), \hat{\epsilon}(g-1)(n-1)] = \text{Environment MS/G} \times \text{E Interaction MS};$$

and for the G × E interaction:

$$F[\hat{\epsilon}(g-1)(n-1), \hat{\epsilon} \sum_{i=1}^{g} \sum_{j=1}^{n} (r_{ij}-1)] = \text{G} \times \text{E Interaction MS/Error MS}.$$

THE EXAMPLE

C. reinhardtii has been used in a series of experiments (BELL 1990a,b, 1991) focusing on the G × E interaction as a way to maintain genetic variation in heterogeneous environments. We chose a subset of the data published by BELL (1991) to assess the impact of among-environment heteroscedasticity and genetic autocorrelation on the results of plasticity experiments. Strain CC-410 (mt-) of *C. reinhardtii* was grown in eight different environments where nitrate, phosphate and bicarbonate were manipulated. Twelve different genotypes were grown in each environment; the design was replicated twice. Growth of each culture, based on transmittance at 665 nm, was measured 16 times during the course of the experiment. A logistic equation was fitted to the 16 scores to obtain a relative growth rate, r. Details on the experimental design procedures can be found in BELL (1991). In this paper, we retained log r as the variable of interest to analyze the plasticity of the 12 genotypes of Chlamydomonas. Three stability indices were used to quantify phenotypic plasticity and illustrate the effects of removing heteroscedasticity and autocorrelation, by working on raw versus transformed phenotypic responses; the indices are defined in APPENDIX A. Thereafter, a global testing procedure was carried out, with and without corrections, for the G × E interaction and the genotype and environment main effects in (1), and the stability of each genotype was assessed statistically using SHUKLA's index I_3. Finally, variance component estimation was performed before genetic correlation analysis.

RESULTS

For the raw data, BOX's $\hat{\epsilon}$ is equal to 0.460, a value much smaller than 1.0, indicating severe violation of the circularity condition as a result of the high heteroscedasticity and the autocorrelation of the Chlamydomonas data set (Table 1). Appropriate statistical analysis should take this heterogeneity and dependency into account. When data are transformed following (6) to remove among-environment heteroscedasticity, $\hat{\epsilon} = 0.536$. Similarly, $\hat{\epsilon}$ rises to 0.604 when genetic autocorrelation is corrected for after (7). As mentioned above, when transformation (5) is applied, $\hat{\epsilon} = 1.0$, showing that the corresponding transformed responses successfully meet the minimum criterion of circularity. Furthermore, these transformed responses are even homoscedastic and uncorrelated among the environments.

Norms of reaction were defined for log r and drawn across the eight environments for the 12 Chlamydomonas genotypes. The pattern of among-environment heteroscedasticity is illustrated in Figure 1A for the raw data, where different environments are characterized by different genotypic variances and high (low) average performance is associated with large (small) variability among genotypes. Norms of reaction for genotypic profiles transformed after (6), which removes heteroscedasticity, are displayed in Figure 1B. They show a pattern strikingly different from the one observed with raw data, and this, even though the mean of each environment is preserved by transformation; as mentioned above, the property of preserving the within-environment means holds true, by definition, for the other two transformations. Differences in performance existing among genotypes in each environment are still noticeable in Figure 1B because the ranking of the 12 genotypes according to their performance is unchanged, but the genotypic variances are now equal. This is particularly true for the environments 5, 7 and 8 that originally generated low genotypic variances. Likewise, transformation (7), which removes genetic autocorrelations among observations collected for a given genotype in different environments, seriously alters the norms of reaction (Figure 1C). Both effects are combined in Figure 1D, according to transformation (5). All those alterations illustrate how important the violation of the circularity condition is, as uncovered by BOX's $\hat{\epsilon}$. This preliminary examination of the data, before and after transformation, will help understand the effect that transformations have on the values taken by the indices.

Index I_1 (9), the ecovalence I_2 (10) of WRICKE (1962), and index I_3 (11) of SHUKLA (1972) were analyzed for raw and transformed data. These stability indices point out to what extent various genotypes are stable across different environments. The larger the value of the index, the less stable (or the more plastic) the corresponding genotype. Results are presented in Tables 2–4. Though indices I_2 and I_3 differ in their numerical value, the genotype rankings they provide

TABLE 1

Genotypic variances and genetic autocorrelations of the log relative growth rate of *Chlamydomonas reinhardtii*

Environment	1	2	3	4	5	6	7	8
1	0.1110	0.0054	0.0475	0.0556	−0.0025	0.0176	−0.0037	−0.0029
2	0.0681	0.0567	0.0301	0.0043	−0.0026	0.0126	0.0044	0.0044
	(0.8335)							
3	0.4842	0.4294	0.0865	0.0377	0.0082	0.0125	0.0005	−0.0086
	(0.1107)	(0.1636)						
4	2.7829	0.0848	0.6018	0.0455	0.0055	0.0049	−0.0032	−0.0055
	(0.0260)	(0.7934)	(0.0384)					
5	−0.0980	−0.1394	0.3593	0.3342	0.0060	−0.0062	−0.0007	−0.0049
	(0.7620)	(0.6657)	(0.2514)	(0.2884)				
6	0.3562	0.3578	0.2877	0.1547	−0.5424	0.0220	−0.0011	0.0072
	(0.2557)	(0.2535)	(0.3646)	(0.6312)	(0.0685)			
7	−0.1887	0.3199	0.0297	−0.2555	−0.1611	−0.1272	0.0034	0.0026
	(0.5571)	(0.3107)	(0.9271)	(0.4228)	(0.6170)	(0.6936)		
8	−0.0838	0.1803	−0.2819	−0.2481	−0.6111	0.4707	0.4308	0.0106
	(0.7957)	(0.5749)	(0.3747)	(0.4369)	(0.0348)	(0.1225)	(0.1620)	

Environments 1–8 are ordered from best to worst performance and an average high performance appears to be associated with high variance among genotypes (see also Figure 1). The upper triangular part (with the diagonal) and the lower triangular part of the matrix above respectively concern the genetic autocovariances and genotypic variances, and the genetic autocorrelations. Genetic autocorrelations (autocovariances) are computed as PEARSON's correlation coefficients (sample covariances) among environments, across the 12 genotypes, and numbers in parentheses are the corresponding probabilities. Genetic autocorrelation and among-environment heteroscedasticity (heterogeneity of genotypic variances) are responsible for a reduction in the number of degrees of freedom in corrected analyses of variance, as quantified by BOX's epsilon estimate $\hat{\epsilon}$. Geometric mean of the genotypic variances: 0.0242. Generalized variance (ANDERSON 1984): $7.575\ 10^{-16}$.

are identical. On that basis, and because I_1 is but a first step in the development of the other two indices, we restricted significance testing to SHUKLA's unbiased I_3. Figure 2 illustrates the shifts in ranking the genotypes according to their stability once genotypic profiles are transformed to remove among-environment heteroscedasticity and genetic autocorrelation, jointly or separately. It is informative to follow the effect of each transformation on each individual genotype. For example, genotype 1, which is the most stable for raw data, becomes relatively unstable once among-environment heteroscedasticity is removed. Graphical examination suggests that the shifts in genotype ranking are less important when responses are transformed only for among-environment heteroscedasticity; that visual observation is strengthened by the significance of KENDALL's tau coefficient of concordance. Genotype ranking is very sensitive to the removal of genetic autocorrelation, with or without adjustment for among-environment heteroscedasticity. Genotype 10, for instance, changes from extremely stable to highly unstable when its profile is transformed according to (7). Results based on the coefficient of variation markedly differ from those using any of the three stability indices, and data transformations before CV computation also alter genotype ranking (Table 5). When testing for stability, as measured by I_3, the importance of among-environment heteroscedasticity and genetic autocorrelation is once again obvious (Table 6). Whereas SHUKLA's index for raw data indicates significant instability for six of the 12 genotypes, only four genotypes are found unstable once heteroscedasticity has been removed, and none, if data are transformed to avoid autocorrelation.

Raw data were analyzed using the mixed ANOVA model (1); three missing data were estimated by the method given in SCHEINER and GOODNIGHT (1984). Analyses of variance were also performed on transformed data. As they did for SHUKLA's index, transformations alter the observed significance levels of the global testing procedures (Table 7). When performing ANOVA on raw data, the G × E interaction and the genotype and environment main effects are all very highly significant. Removing among-environment heteroscedasticity by using a unitary genotypic variance common to all environments results in nonsignificant environment main effects, while transformation for genetic autocorrelation acts similarly on the G × E interaction and genotype main effects. When data are transformed for both heteroscedasticity and autocorrelation, ANOVA fails to detect any significant effect or interaction.

Corrections in the number of degrees of freedom associated with mean squares involving the environment factor were applied in order to take into account the violation of the circularity condition. In our example, although those corrections change the P values, they do not alter the conclusions drawn from the analysis (Table 7).

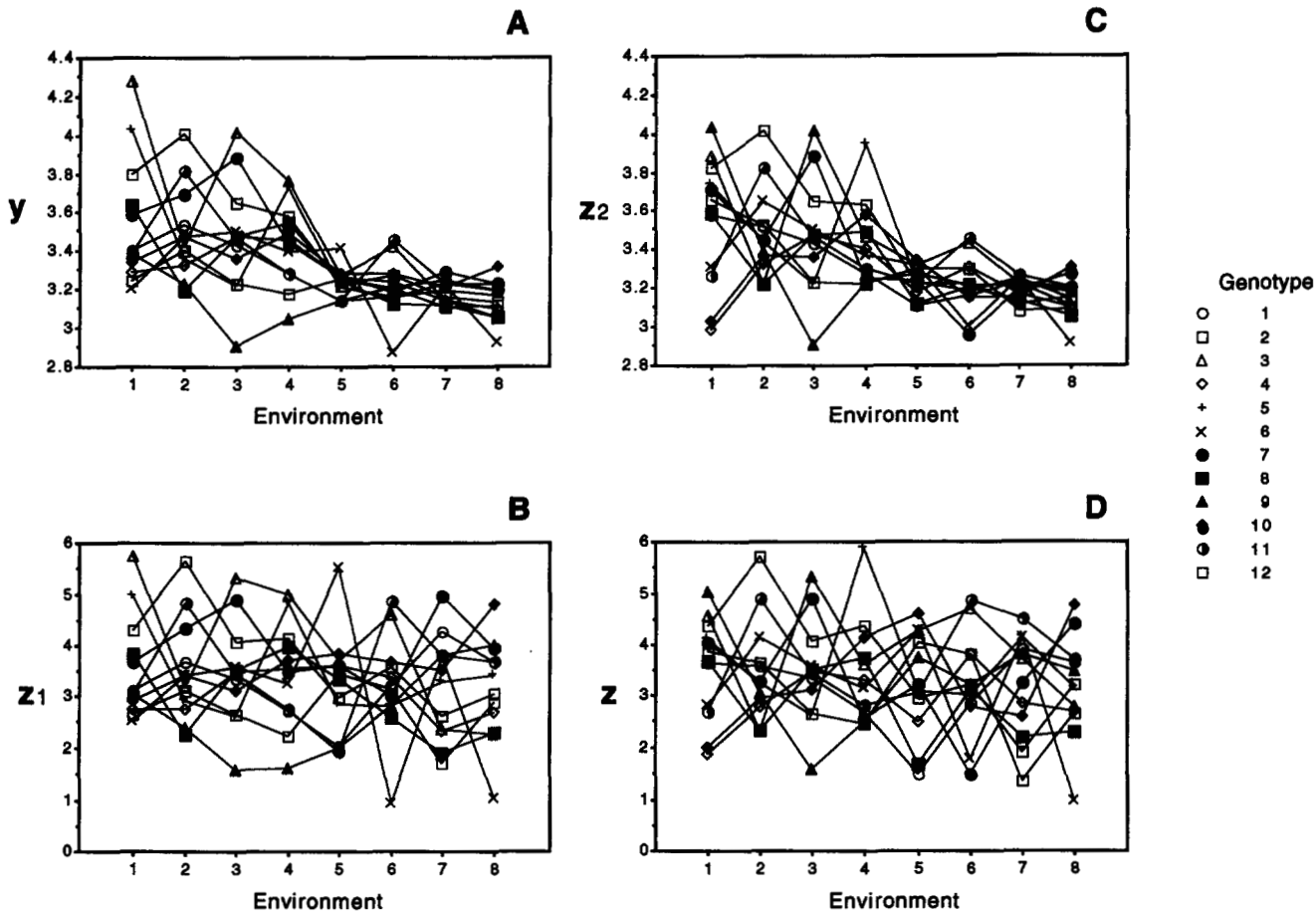

FIGURE 1.—Norms of reaction for the log relative growth rate (r) of 12 genotypes of *Chlamydomonas reinhardtii* in eight environments. Norms of reaction are presented (A) for raw log r data (**y**) and for transformed data from which (B) among-environment heteroscedasticity has been removed ($\mathbf{z}_1$), (C) genetic autocorrelation has been removed ($\mathbf{z}_2$), and (D) both sources have been removed (**z**).

Finally, variance components attributable to the random terms in (1), *i.e.*, the genotype main effects, the G × E interaction and the error term, were computed using the expected values of mean squares given by WINER *et al.* (1991, p. 304) for the two-way mixed ANOVA model. Their relative proportions differ substantially whether computed on raw or transformed data. In particular, removing among-environment heteroscedasticity increases the proportion of variance due to the G × E interaction with respect to the genotype variance component (Table 8). On the other hand, the sum of squares associated with the environment main effects that are the only fixed effects in (1) with the intercept remains constant because all transformations preserve the within-environment means (Table 7).

DISCUSSION

Stability indices as alternative measures of plasticity: Phenotypic plasticity is considered increasingly important to evolutionary theory as it plays a central role in buffering the selection acting on genotypes (GRANT 1985). Plasticity is believed to evolve through natural selection in heterogeneous environments (BELL and LECHOWICZ 1994). However, there is no consensus whether selection acts directly on plasticity (SCHEINER and LYMAN 1989) or whether reaction norms evolve indirectly through selection on phenotypes in distinct environments (VIA and LANDE 1985). The understanding of phenotypic plasticity is impaired by the lack of reliable measures to quantify it (SCHLICHTING 1986; WESCOTT 1986). A critical issue is to determine to what extent genotypic response varies across environments (BELL 1991). To quantify the plasticity of individual genotypes, population biologists often relied on individual CV and global variance components (SCHEINER and GOODNIGHT 1984; SCHLICHTING 1986; SULTAN 1987). For their part, and in an approach that may seem to be at first sight diametrically opposite, breeders generally considered that high G × E interaction reduces selection rates and makes it difficult to determine the superiority of a genotype over others (EBERHART and RUSSELL 1966). Consequently, they fostered the development of stability analyses aimed at determining which genotypes are most stable across different environments (COMSTOCK and MOLL 1963). We believe, and we hope to

TABLE 2

Index of stability (I_1) for each of 12 genotypes of *Chlamydomonas reinhardtii*

Genotype	y	z_1	z_2	z
1	0.130 (4)	3.16 (4)	0.215 (3)	4.33 (2)
2	0.062 (1)	2.18 (2)	0.281 (4)	6.10 (5)
3	1.317 (12)	12.56 (11)	0.749 (11)	7.06 (6)
4	0.125 (3)	1.67 (1)	0.188 (1)	2.66 (1)
5	0.718 (10)	5.95 (7)	0.611 (8)	7.46 (9)
6	0.407 (8)	15.95 (12)	0.404 (7)	10.14 (12)
7	0.451 (9)	3.34 (5)	0.633 (9)	8.08 (10)
8	0.356 (7)	4.35 (6)	0.288 (5)	4.34 (3)
9	0.143 (5)	6.12 (8)	0.733 (10)	7.13 (8)
10	0.064 (2)	2.26 (3)	0.204 (2)	7.08 (7)
11	0.325 (6)	6.92 (10)	0.300 (6)	5.61 (4)
12	0.751 (11)	6.46 (9)	0.836 (12)	9.17 (11)

Original data are log relative growth rates (r) over day (BELL 1991). Results are presented for raw log r data (**y**) and for data transformed for among-environment heteroscedasticity ($\mathbf{z}_1$), genetic autocorrelation ($\mathbf{z}_2$), and both sources (**z**). Numbers in parentheses refer to the genotype stability ranking, from the most (1) to the least (12) stable.

have provided a convincing example, that the tools used in stability analysis can be helpful to evolutionary biologists.

Early attempts to characterize genotype stability across environments were based on a regression approach in which the genotypic values were regressed on environmental means (YATES and COCHRAN 1938; FINLAY and WILKINSON 1963). Since FREEMAN and PERKINS (1971) have shown the statistical invalidity of testing the homogeneity among regressions, stability indices were developed (WRICKE 1962; SHUKLA 1972). Those indices essentially partition the G × E interaction sum of squared deviations into the components associated with different genotypes (see APPENDIX A);

TABLE 3

Ecovalence (I_2) for each of 12 genotypes of *Chlamydomonas reinhardtii*

Genotype	y	z_1	z_2	z
1	0.053 (1)	3.53 (6)	0.045 (1)	4.21 (3)
2	0.106 (4)	2.38 (2)	0.125 (3)	5.68 (4)
3	0.665 (12)	10.65 (11)	0.436 (11)	6.91 (6)
4	0.079 (2)	1.68 (1)	0.281 (7)	3.11 (1)
5	0.384 (11)	5.27 (8)	0.350 (10)	7.11 (7)
6	0.252 (9)	15.46 (12)	0.171 (4)	9.35 (12)
7	0.147 (6)	3.35 (3)	0.316 (8)	7.64 (10)
8	0.118 (5)	3.48 (5)	0.084 (2)	3.42 (2)
9	0.334 (10)	7.47 (10)	0.595 (12)	7.55 (9)
10	0.095 (3)	3.41 (4)	0.340 (9)	8.30 (11)
11	0.217 (7)	7.20 (9)	0.267 (5)	6.46 (5)
12	0.236 (8)	4.87 (7)	0.279 (6)	7.25 (8)

Results are presented for raw log r data (**y**) and for data transformed for among-environment heteroscedasticity ($\mathbf{z}_1$), genetic autocorrelation ($\mathbf{z}_2$), and both sources (**z**). Ranks in genotype stability are indicated in parentheses.

TABLE 4

SHUKLA's stability index (I_3) for each of 12 genotypes of *Chlamydomonas reinhardtii*

Genotype	y	z_1	z_2	z
1	0.0056 (1)	0.516 (6)	0.0035 (1)	0.622 (3)
2	0.0147 (4)	0.319 (2)	0.0172 (3)	0.874 (4)
3	0.1106 (12)	1.737 (11)	0.0705 (11)	1.085 (6)
4	0.0101 (2)	0.199 (1)	0.0439 (7)	0.433 (1)
5	0.0622 (11)	0.813 (8)	0.0557 (10)	1.119 (7)
6	0.0397 (9)	2.560 (12)	0.0250 (4)	1.503 (12)
7	0.0218 (6)	0.486 (3)	0.0499 (8)	1.210 (10)
8	0.0167 (5)	0.508 (5)	0.0102 (2)	0.487 (2)
9	0.0538 (10)	1.191 (10)	0.0977 (12)	1.194 (9)
10	0.0128 (3)	0.495 (4)	0.0540 (9)	1.323 (11)
11	0.0336 (7)	1.145 (90)	0.0415 (5)	1.007 (5)
12	0.0370 (8)	0.746 (7)	0.0435 (6)	1.143 (8)

Results are presented for raw log r data (**y**) and for data transformed for among-environment heteroscedasticity ($\mathbf{z}_1$), genetic autocorrelation ($\mathbf{z}_2$), and both sources (**z**). Ranks in genotype stability are indicated in parentheses.

they are commonly referred to as stability variance components. A genotype will be considered stable if its stability variance component is equal to the within-environment variance. LIN *et al.* (1986) and WESCOTT

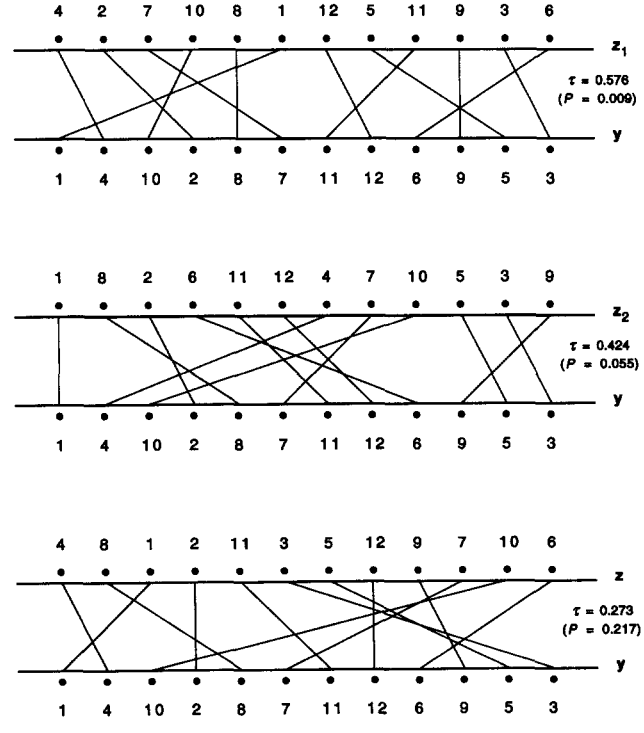

FIGURE 2.—Comparison in ranking the 12 genotypes of *C. reinhardtii* on the basis of SHULKA's index (see Table 4), from the most to the least stable, depending on whether indices are computed on raw log r data (**y**) or on transformed data from which among-environment heteroscedasticity has been removed ($\mathbf{z}_1$), genetic autocorrelation has been removed ($\mathbf{z}_2$), or both sources have been removed (**z**). KENDALL's tau (τ) coefficient of concordance between rankings for raw and transformed data is reported with the associated probability.

TABLE 5

Coefficients of variation for each of 12 genotypes of *Chlamydomonas reinhardtii*

Genotype	y	z_1	z_2	z
1	4.12 (4)	87.56 (12)	5.29 (3)	25.00 (3)
2	2.93 (2)	20.42 (4)	6.07 (4)	30.52 (8)
3	12.24 (12)	33.34 (9)	9.46 (10)	25.73 (4)
4	4.07 (3)	15.91 (2)	5.05 (1)	21.18 (1)
5	9.47 (11)	26.64 (7)	8.71 (8)	28.27 (6)
6	7.42 (9)	50.47 (11)	7.31 (7)	38.93 (12)
7	7.37 (8)	17.34 (3)	2.93 (9)	31.68 (11)
8	6.73 (7)	26.61 (6)	6.18 (6)	27.92 (5)
9	4.52 (5)	35.20 (10)	9.79 (11)	30.68 (9)
10	2.85 (1)	15.71 (1)	5.20 (2)	29.92 (7)
11	6.40 (6)	28.03 (8)	6.14 (5)	23.95 (2)
12	9.42 (10)	25.29 (5)	9.94 (12)	30.68 (10)

Results are presented for raw log r data (y) and for data transformed for among-environment heteroscedasticity (z_1), genetic autocorrelation (z_2), and both sources (z_1). Ranks in genotype stability are indicated in parentheses.

TABLE 6

Observed F values in assessing the significance of log r instability of 12 genotypes of *Chlamydomonas reinhardtii*, as measured by SHUKLA's index

Genotype	y	z_1	z_2	z
1	0.524^{ns}	1.340^{ns}	0.088^{ns}	0.494^{ns}
2	1.376^{ns}	0.828^{ns}	0.429^{ns}	0.694^{ns}
3	10.345***	4.507**	1.762^{ns}	0.861^{ns}
4	0.943^{ns}	0.518^{ns}	1.096^{ns}	0.344^{ns}
5	5.822**	2.110^{ns}	1.392^{ns}	0.889^{ns}
6	3.710*	6.644***	0.625^{ns}	1.193^{ns}
7	2.036^{ns}	1.260^{ns}	1.248^{ns}	0.961^{ns}
8	1.559^{ns}	1.317^{ns}	0.254^{ns}	0.386^{ns}
9	5.032**	3.089*	2.440^{ns}	0.948^{ns}
10	1.197^{ns}	1.285^{ns}	1.349^{ns}	1.050^{ns}
11	3.146+	2.971+	1.036^{ns}	0.800^{ns}
12	3.459+	1.935^{ns}	1.086^{ns}	0.907^{ns}

With P the probability of observing a higher F value, ns, $P \geq 0.05$; *$0.01 \leq P < 0.05$; **$0.001 \leq P < 0.01$; ***$P < 0.001$. Results are presented for raw data (y) and for data transformed for among-environment heteroscedasticity (z_1), genetic autocorrelation (z_2), and both sources (z).

(1986) provide an overview of the various statistical methods available in stability analysis. Although stability indices and the CV both provide information at the level of individual genotypes, the realities they concern can be very different. For example, the ecovalence I_2 (10) may be defined as the sum of squared deviations associated with a genotype after centering on the within-environment means, that is, once the environment main effects in (1) have been removed (see APPENDIX A). It follows that I_2 directly relates to the genotype-specific portions of the genetic variance of phenotypic plasticity and provides a measure of the heritable portion of the total environmental variance relative to each genotype if plasticity is heritable. On the other hand, the CV combines the environment main effects with the G × E interaction in its computation, because it is based on the total variance of a given genotype among the environments; this can be shown by applying the properties of the general linear model and its quadratic forms (see, *e.g.*, WINER *et al.* 1991). Therefore, we contend that stability indices might, often, be more relevant to evolutionary theory than the CV. They also allow statistical assessment of which genotypes are significantly unstable and which are not (Table 6), but they do *not* allow testing if a given genotype is more or less stable than another, in accordance with the random nature of the genotype factor in (1). Stability indices however provide a ranking of the genotypes on the basis of the observed values of the statistic (Tables 2–4).

Appropriate quantification and assessment of stability can have important consequences. Among others, it has been suggested that stable genotypes tend to have lower yield (HARDWICK 1981). This however might be a mere artifact of the methods used to measure stability (SCHLICHTING 1986; WESCOTT 1986). Our analyses tend to support this suspicion. When the stability of Chlamydomonas genotypes is assessed by the CV on raw data, genotypes 3, 5 and 12, which are the most unstable, are fast growing. Across genotypes, a highly significant correlation coefficient of 0.757 ($P = 0.004$) confirms that highest relative growth rate is associated with highest variability. However, when stability is measured by SHUKLA's index I_3, the correlation decreases to 0.477, which is not significant ($P = 0.116$). On the other hand, when the data are corrected for heteroscedasticity, the unstable genotypes are not among the fastest growing and the correlation is not statistically significant, either computed from the CV or the index I_3. Therefore, stability is apparently not necessarily negatively correlated with the grand mean, which is coherent with the evidence that independent genetic systems control a character and its plasticity (PERKINS and JINKS 1973; KHAN *et al.* 1976; see also SCHEINER and LYMAN 1991).

The impact of among-environment heteroscedasticity on the analysis of phenotypic plasticity: In plasticity experiments, the responses of various genotypes are compared under different environmental conditions. It has been known for a long time that in certain environments phenotypic expression will diverge, while in others it will converge. Accordingly, environments may produce more, or less, variation in genotypic response (CLAUSEN *et al.* 1948; HARBERD 1957; FELDMAN and LEWONTIN 1976; BELL 1990a,b). Experimental results in insects (SCHARLOO 1989), fishes (TREXLER and TRAVIS 1990) and plants (TUCIC *et al.* 1990; BELL 1991), suggest that differences in variance among environments are a frequent phenomenon. For instance, when they were looking at the phenotypic plasticity of *Iris*

TABLE 7

Summary of univariate analyses of variance for the log relative growth rate of 12 *Chlamydomonas reinhardtii* genotypes

Source	SS	*df*	*F*	*P*	Corrected *P*
Raw log *r*					
Genotype	2.147	11	9.12	<0.0001	<0.0001
Environment	4.304	7	8.81	<0.0001	<0.0001
Genotype × Environment	5.373	77	3.26	<0.0001	<0.0001
Residual	2.053	96			
Without among-environment heteroscedasticity					
Genotype	38.475	11	4.54	<0.0001	<0.0001
Environment	4.304	7	0.34	0.931	0.838
Genotype × Environment	137.526	77	2.32	<0.0001	0.002
Residual	74.003	96			
Without genetic autocorrelation					
Genotype	0.940	11	1.07	0.396	0.401
Environment	4.304	7	7.20	<0.0001	<0.0001
Genotype × Environment	6.579	77	1.07	0.379	0.400
Residual	7.687	96			
Without both sources					
Genotype	22.001	11	0.79	0.646	
Environment	4.304	7	0.31	0.949	
Genotype × Environment	154.004	77	0.78	0.853	
Residual	241.871	96			

Genotypes were cultured in each of eight environments, with two replicates. The analyses used raw log *r* data and transformed data after removing among-environment heteroscedasticity, genetic autocorrelation, and both sources. Probabilities were corrected for the assumptions underlying ANOVA, which are violated by raw and partially transformed data.

pumila, TUCIC *et al.* (1990) quantified that heterogeneity, and reported that environments were significantly heteroscedastic for 22 out of the 26 characters observed. Among-environment heteroscedasticity has thus been reported in the past, but was not fully taken into account statistically. However, early in the statistical litterature, heteroscedasticity has been recognized as a nuisance that cannot be ignored when performing ANOVA or related techniques (COCHRAN 1947; see DUTILLEUL and LEGENDRE 1993 for a review joining the statistical and biological perspectives). In particular, when some treatment comparisons give rise to much smaller error variances than others, significance levels are highly distorted. If that heteroscedasticity is an inherent characteristic of the treatment, usual data transformation will not be able to remove it (COCHRAN 1947).

While preserving within-environment means, transformation (6) provides reaction norms from which among-environment heteroscedasticity has been removed. As shown by KENDALL's tau coefficient of concordance computed for SHUKLA's index (Figure 2), primary evolutionary information is retained after filtering of the heteroscedastic noise. Nevertheless, among-environment heteroscedasticity altered all the measures of phenotypic plasticity that we considered. This is particularly true for the variance component associated with the G × E interaction, which represents the variance available for selection to act upon if plasticity is heritable (SCHEINER and LYMAN 1989, 1991) and to which SCHLICHTING (1986) and THOMPSON (1991) refer as the genetic component of phenotypic plasticity (see also BELL and LECHOWICZ 1994). In the Chlamydomonas example, the variance component ascribed to the G × E interaction after (6) is

TABLE 8

Variance component estimation in the univariate ANOVA model for the log relative growth rate of *Chlamydomonas reinhardtii*

Source	Variance component estimate
Raw log *r*	
Genotype	0.0109
Genotype × Environment (genetic correlation: $r_g = -0.054$)	0.0242
Error	0.0214
Without among-environment heteroscedasticity	
Genotype	0.1704
Genotype × Environment (genetic correlation: $r_g = -0.196$)	0.5076
Error	0.7709

Variance component estimation is restricted to random terms in the ANOVA model; it has been carried out under WINER *et al.*'s (1991, p. 304) mixed model, called the "Scheffé model" by FRY (1992) in reference to SCHEFFÉ (1959). Accordingly, genetic correlation computation was based on FRY's (1992, p. 543) formula (6). Data refer to raw log *r* data and transformed data after removing among-environment heteroscedasticity, respectively.

much higher than for raw data, proportionally to the variance component associated with the genotype main effects in the ANOVA model (Table 8), while the environment main effects are no longer significant (Table 7). That proportional increase suggests that the genetic component of plasticity might be more important than first thought, as a proof that among-environment heteroscedasticity can affect our understanding of the selective forces acting on the genotypes.

Response to selection is not only attributable to the magnitude of the G × E variance component, but also to the magnitude and direction of the genetic correlation, r_g (VIA 1984). The fact is that heteroscedasticity is responsible for a bias in the estimation of r_g in the two-way mixed ANOVA model (FRY 1992). In particular, an underestimation of r_g results from differences in the among-family variance among environments (YAMADA 1962). It follows that the ability of (6) to remove among-environment heteroscedasticity allowed us to examine its impact on the estimation of the genetic correlation. Using the formula of YAMADA (1962) for SCHEFFÉ's mixed model [formula (6) of FRY (1992), p. 543], we estimated r_g for raw data and for data transformed after (6). Strikingly, heteroscedasticity substantially altered the magnitude of the genetic correlation because the observed values are −0.05 and −0.20, respectively (Table 8). This is in accordance with the proportional increase reported above for the G × E variance component after (6). Genetic correlation r_g can be used to predict how phenotypic plasticity will evolve (ANDERSSON and SHAW 1994). There will be little opportunity for adaptative plasticity to evolve if characters are strongly positively correlated across environments (VIA and LANDE 1985; PLATENKAMP and SHAW 1992). Our results point out that plasticity of the log relative growth rate of Chlamydomonas, log r, would be more likely to evolve in an homoscedastic than in an heteroscedastic world.

Among-environment heteroscedasticity also has a substantial impact on the assessment of genotype stability through stability variance components. This is not surprising because stability analysis is related to ANOVA and thus shares the same basic assumptions. Accordingly, transformation (6) altered the ranking of the 12 Chlamydomonas genotypes concerning their stability, whatever the index used. In particular, the shift in stability of genotype 1 suggests that identification of stable genotypes may be seriously confused by heteroscedasticity. When present and not taken into account, among-environment heteroscedasticity might invalidate the results of stability analysis and lead to selection of less than optimal genotypes. Equally important is that unstable genotypes are less abundant after transformation (6). For example, the instability of genotypes 5 and 12 observed on raw data appears to be essentially a response to the heteroscedasticity of the environments used in the experiment (Table 6).

Proof is thus given that among-environment heteroscedasticity can have a strong influence on the output of plasticity analysis: in the Chlamydomonas example, it radically changed some results, their interpretation, and the conclusions drawn. Consequently, the greatest care must be taken to assess the circularity condition prior to any statistical analysis. The norms of reaction themselves can clearly illustrate the differences in genotypic variance among the environments (Figure 1A). The likelihood that data violate circularity due to some environmentally-induced heterogeneity of genotypic variances can be evaluated by computing BOX's $\hat{\epsilon}$ for raw data and for data transformed after (6). More generally, the importance of heteroscedasticity in a plasticity experiment should be assessed by comparing the results based on raw data with those based on transformed data. If there is a large discrepancy in the results, it should be decided whether among-environment heteroscedasticity is biologically important. Because it preserves both the genotypic mean within each environment and the ranking of genotypes according to the magnitude of their response, transformation (6) retains the inherent characteristics of the genotypes and offers a solution that is statistically correct while retaining the evolutionary potential. Furthermore, results are more general since they depend less on specific environments that might induce heteroscedasticity. However, in some cases, crucial information may be contained in the differences in the genotypic variance among environments. For example, when novel environments or environmental extremes increase the variance of the response (*e.g.*, COLEMAN *et al.* 1989; BAZZAZ *et al.* 1990), it may be inappropriate to apply (6). Statistical analysis should then be based on corrected tests using (8), as a statistically sound alternative.

The importance of genetic autocorrelation for the study of plasticity: According to FALCONER (1952, 1981), the expression of a trait in two different environments can be viewed as two distinct characters, allowing the computation of a genetic correlation between them (see also SCHEINER and GOODNIGHT 1984; VIA and LANDE 1985). In the univariate ANOVA models used in plasticity experiments (*e.g.*, SCHEINER and GOODNIGHT 1984; SCHEINER and TEERI 1986), the G × E interaction is considered to be a measure of that genetic correlation. The drawback in this approach is that those models constrain the intraclass correlation coefficient within each genotype to be constant among all the environments. This assumption is violated by our example, as shown by BOX's epsilon. In the MANOVA model (3) considered here, genetic autocorrelations (within a genotype, among environments) are incorporated into the variance-covariance matrix of the multivariate error term, τ_i, and the assumption

about the intraclass correlation structure is relaxed. Our approach allows us to account for genetic autocorrelation in two ways: it can be removed specifically by applying transformation (7) to the raw data or the significance levels observed in hypothesis testing can be corrected by using BOX's epsilon in modifying the numbers of degrees of freedom of the F statistics. The importance of genetic autocorrelation in plasticity experiments is illustrated by the Chlamydomonas example: when not removed, genetic autocorrelation is responsible for the significance of the G × E interaction and genotype main effects; once autocorrelation is removed, the ranking of genotypes according to their stability is dramatically altered.

Even if genetic autocorrelation is a statistical nuisance, it is essential to decide if it must be looked upon as such, and therefore eliminated from the data, or if the process it represents is biologically meaningful, and should be retained. In the latter case, statistical analysis should be modified accordingly, by relaxing the assumptions of the univariate ANOVA model (1) and providing corrected tests as a proper solution. Removing genetic autocorrelation could only be advised when the action of sets of genes is independent from one environment to the next. Within a species, this is most likely untenable. Moreover, detecting genetic correlations among environments is often the very focus of plasticity experiments (ANDERSSON and SHAW 1994). We believe that genetic autocorrelation is likely always present in plasticity experiments, and that removing it by data transformation is biologically inappropriate. Furthermore, AYERS and THOMAS (1990) and FRY (1992) emphasize that the circularity assumption is often biologically untenable. As an alternative, when the circularity assumption is violated, we recommend hypothesis testing based on corrected P values in (1), using BOX's $\hat{\epsilon}$ derived from (8). This procedure allows correct statistical analysis while retaining crucial biological information contained in the autocorrelated data. Our MANOVA model (3) also offers a statistically and biologically valid solution to the problem.

Our results clearly established that among-environment heteroscedasticity affects the results of plasticity experiments. Correcting for it in the Chlamydomonas example changed, among other things, the proportion of the genetic component of phenotypic plasticity and the magnitude of the genetic correlation; in some other examples, the direction of the genetic correlation could even be reversed. It is suggested in the quantitative genetics literature (*e.g.*, FALCONER 1981) that selection partitions the genetic and environmental variances in ways similar to an analysis of variance. If transformation (6) provides an accurate estimate of reality, this will mean that phenotypic plasticity does not always respond to selection as little, or as much, as thought using raw data. Given the controversy regarding the heritability of plasticity (SCHEINER and LYMAN 1989, 1991; VIA 1987, 1991), the implications of among-environment heteroscedasticity for the study of phenotypic plasticity are potentially important. When heteroscedasticity violates a basic assumption of ANOVA, we believe that the predictions derived from analyses performed on raw data, without transformation or correction, are highly suspect. Admittedly, we came to this problem with a statistical background. We very much hope that evolutionary biologists will examine the evolutionary significance of heteroscedasticity. A most important question to ask is: Does natural selection act on a phenotype as in an homoscedastic or in an heteroscedastic world?

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APPENDIX A: STABILITY INDICES

The three indices below provide as many ways of quantifying phenotypic plasticity. Each of them is based on the G × E interaction sum of squared deviations, combined or not with the one associated with the environment main effects, and a given decomposition into genotype-specific components. As a result, these indices can differ in the stability variance components they ascribe to genotypes, both in their value and in their meaning.

For a raw genotypic profile $\bar{\mathbf{p}}_i$, the first primary index considered here is defined as

$$I_{1i} = \sum_{j=1}^{n} (\bar{p}_{ij} - \bar{p}_i)^2. \qquad (9)$$

It measures the variation of genotype i (= 1, . . . , g) among the n environments, around its mean response $\bar{p}_i = (1/n)\ \Sigma_{j=1}^{n}\ \bar{p}_{ij}$ computed over those environments; this is in accordance with BRADSHAW's (1965) qualitative definition of phenotypic plasticity (see also SCHEINER and GOODNIGHT 1984).

With $\bar{p}$ the global mean response computed over all individuals whatever the genotype and the environment, and $\bar{p}_j$ the mean response in environment j (= 1, . . . , n),

$$I_{2i} = \sum_{j=1}^{n} (\bar{p}_{ij} - \bar{p}_i - \bar{p}_j - \bar{p})^2 \qquad (10)$$

This second index or ecovalence (WRICKE 1962; see also KANG and MILLER 1984), as rewritten here, is but one of the g terms defining the G × E interaction sum of squared deviations in (1). In other words, and while the environment main effects are incorporated into I_{1i} in (9), the ecovalence I_{2i} measures the among-environment variation of genotype i (= 1, . . . , g) after adjustment for those environmental effects. It follows that index I_2 provides a measure of the portion of the genetic component of phenotypic plasticity attributable to each genotype; this holds true for I_3 defined hereafter.

The third index due to SHUKLA (1972) provides an unbiased estimate of the stability variance component assignable to genotype i, as an alternative to I_{2i}:

$$I_{3i} = \frac{1}{(n-1)(g-1)(g-2)} \times \left[g(g-1)I_{2i} - \sum_{i=1}^{g} I_{2i}\right]. \qquad (11)$$

The stability of each genotype can be assessed statistically on the basis of SHUKLA's index, according to the criterion suggested by JOHNSON (1962) as reported in SHUKLA (1972). We retained I_3 instead of I_2 in significance testing because of I_3's unbiasedness property, even if its computation is more complex. When dealing with transformed data, raw mean responses $\bar{p}_{ij}$, $\bar{p}_i$, $\bar{p}_j$, and $\bar{p}$ in (9) – (11) are substituted by similar quantities expressed in terms of $\bar{z}^{(1)}$, $\bar{z}^{(2)}$ or $\bar{z}$, depending on the transformation.

APPENDIX B: PRACTICAL ASPECTS IN SAS

Computation of (9): Use PROC SORT for sorting observations BY genotype and environment and thereafter, PROC MEANS for computing mean responses $\bar{p}_{ij}$ BY genotype i (= 1, . . . , g) and environment j (= 1, . . . , n); when calling PROC MEANS, specify an OUTPUT DATASET into which mean responses will be saved. Then, call PROC UNIVARIATE BY genotype with these mean responses, and the CSS (corrected sum of squares) statistic computed will provide the observed value of (9) for each genotype.

Computation of (10): Call PROC STANDARD for centering (without modifying the variance) the mean responses $\bar{p}_{ij}$ with respect to $\bar{p}_i$ BY genotype i and specify an OUTPUT DATASET into which the mean responses once centered $\bar{p}_{ij} - \bar{p}_i$ are saved. Call a second time (after using PROC SORT BY environment) PROC STANDARD BY environment with the mean responses once centered $\bar{p}_{ij} - \bar{p}_i$ and specify a second OUTPUT DATASET into which the mean responses twice centered $\bar{p}_{ij} - \bar{p}_i - (\bar{p}_j - \bar{p})$ will be saved. Finally, call PROC UNIVARIATE BY genotype (after using PROC SORT BY genotype) where the CSS statistic computed provides the value of (10) for each genotype.

Computation of $\hat{\Sigma}$ by (4): Using a matrix algebra procedure (*e.g.*, in SAS: PROC IML in Version 6), a matrix algebra software (*e.g.*, MATLAB) or a spreadsheet program (*e.g.*, EXCEL, LOTUS123), range the centered mean responses $\bar{p}_{ij} - \bar{p}_j$ of genotype i (= 1, . . . , g) in environment j (= 1, . . . , n) into a two-dimensional table whose n columns correspond to the environments and the g lines to the genotypes. The matrix operations are then reduced to the sum of products of the transpose of each line $(\bar{\mathbf{p}}_i - \bar{\mathbf{p}})$ of the data table by the same line nontransposed. Finally, divide the sum by $g - 1$. In SAS for instance, the centered mean responses, saved in an OUTPUT DATASET after calling PROC STANDARD BY environment, can be read and reshaped in PROC IML.

Transformation (5): Multiply matrix $\hat{\Sigma}^{-0.5}$ by the transpose of each genotypic profile once centered (i = 1, . . . , g). This may be done in a single operation, by the product of matrix $\hat{\Sigma}^{-0.5}$ by the transpose of the data table whose entries are the centered mean responses $\bar{p}_{ij} - \bar{p}_j$ (genotypes = rows, environments = columns); the result is a data table of same dimensions and whose entries are the centered transformed mean responses

$\bar{z}_{ij} - \bar{p}_j$. Matrix $\hat{\Sigma}^{-0.5}$ is the inverse of the square root of $\hat{\Sigma}$; in SAS, it may be computed by PROC IML and the HALF function which provides the Cholesky decomposition $\hat{\Sigma} = \mathbf{U}'\,\mathbf{U}$ where $\mathbf{U} = \hat{\Sigma}^{0.5}$ is an upper triangular matrix.

Transformation (6): For genotype i ($= 1, \ldots, g$) and environment j ($= 1, \ldots, n$), multiply the centered mean response $\bar{p}_{ij} - \bar{p}_j$ by the inverse of the square root of the genotypic variance estimated for environment j, *i.e.*, of the jth diagonal element of $\hat{\Sigma}$; the result is the centered transformed mean response $\bar{z}_{ij}^{(1)} - \bar{p}_j$.

Transformation (7): For genotype i ($= 1, \ldots, g$) and environment j ($= 1, \ldots, n$), multiply the centered transformed mean response $\bar{z}_{ij} - \bar{p}_j$ by the square root of the genotypic variance estimated for environment j; the result is the centered transformed mean response $\bar{z}_{ij}^{(2)} - \bar{p}_j$.

Transformations are completed by adding $\bar{\mathbf{p}}$ to the vectors resulting from the above-described operations.