

ALTERNATIVE FORMULATIONS OF THE MIXED-MODEL ANOVA  
APPLIED TO QUANTITATIVE GENETICS

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Many recent studies in the field of quantitative genetics draw on results from mixed-model factorial experiments (Ayres et al., 1987; Futuyma and Philippi, 1987; Grosberg, 1987; Hare and Kennedy, 1986; James et al., 1988; Mazer, 1987; Pashley, 1988a; Petranka and Sih, 1987; Shaw, 1986; Stephenson and Winsor, 1986; Via, 1984). But the analysis of such experimental data is confounded by discrepancies in the recommendations of various authors (Table 1). Some widely used computer packages (e.g., SAS [SAS Institute, 1985] and BMDP [Dixon, 1985]) employ different algorithms, which are derived from different statistical models and are based on different sets of assumptions (Hocking, 1973). These alternative models lead to different expected mean squares. Because it is the expected mean squares that determine the appropriate denominator for conducting  $F$  tests, different expected mean squares lead to different  $F$  tests and can alter important biological conclusions; estimates of genetic variance and heritability are especially sensitive to the choice of model. Here, we identify features that distinguish the models, evaluate the effect of model choice on two recent studies, and offer some recommendations for future research.

*The Mixed-Model ANOVA in  
Quantitative Genetics*

Mixed-model designs are characterized by the presence of both “fixed” and “random” effects as sources of variation (Scheffé, 1959 pp. 4–6, 261–289). For example, Pashley (1988a) examined the growth performance (larval mass at day 10, larval duration, pupal weight, and survival) of eight full-sib families of fall armyworm, *Spodoptera frugiperda*, on two alternative host plants (rice and corn). “Family” was taken to be a random effect because Pashley wished to draw inferences about variance among fall armyworm families in general and was not specifically interested in differences among the eight families used in the experiment. Conversely, “host” was taken to be a fixed effect, as Pashley was specifically interested in differences between rice and corn, not in the variance among host plants in general. Differential mortality resulted in unequal sample sizes.

This powerful factorial experiment allowed Pashley to test simultaneously for 1) genetic variance among families, 2) differences between rice and corn, and 3) genotype  $\times$  environment interactions, where the rel-

TABLE 1. Some common statistical references dealing with mixed-model ANOVAs categorized by the model type(s) they discuss.

Reference	Discusses model that excludes mixed interactions from expected mean squares of random effects	Discusses model that includes mixed interactions in expected mean squares of random effects
Anderson and McLean (1974)	yes	no
Dixon (1985) (BMDP)	yes	no
Hicks (1982)	yes	no
Hocking (1985)	yes	yes
Kempthorne (1957, 1979)	yes	no
Milliken and Johnson (1984)	no	yes
Neter et al. (1985)	yes	no
SAS Institute (1985)	no	yes
Scheffé (1959)	yes	yes
Searle (1971)	yes	yes
Snedecor and Cochran (1967)	yes	no
Sokal and Rohlf (1981)	yes	no
Steel and Torrie (1980)	yes	no
Zar (1984)	yes	no

ative performance of different families varies depending upon the particular host. The basic experimental design, in which different families or genotypes (a random effect) are tested across some ecologically relevant fixed effect, such as host quality, temperature, salinity, or nutrient availability, provides an extremely useful framework for testing evolutionary hypotheses. We have chosen Pashley's (1988a) study for reanalysis because it was a well-designed and carefully conducted experiment that exemplifies a larger body of work. Our intent is not to challenge her particular conclusions, but to constructively illustrate hazards that are likely to be encountered in the analysis and interpretation of future studies. The problems became apparent to us while studying Pashley's paper partly because she did a good job of describing her analyses. Such a reanalysis would have been impossible for some of the papers cited above.

#### *Variants of the Model Can Lead to Different Conclusions*

The expected mean squares appropriate for analyzing the three-way mixed model of Pashley (1988a) differ depending upon the statistical model that is invoked. Scheffé (1959 pp. 261–289) developed a very general model for the analysis of mixed-effect experiments, and this is the model presented in many familiar references (Table 1). However, a different model is occasionally discussed as the standard for analyzing mixed-effect experiments. In particular, this model is implemented by the SAS statistical package in its "General Linear Models" (GLM) procedure. It is one of the few statistical routines designed to accommodate unequal sample sizes in its mixed-model analyses of variance and was used by Pashley in her study. Compared to the Scheffé model, the SAS model includes one additional term ( $k_2\sigma_{FH}^2$ ) in the expected mean square

for the random effect of family (Table 2). As a consequence, the appropriate denominator for constructing the  $F$  statistic becomes  $MS_{FH}$  rather than  $MS_E$ , and the main effect of family becomes insignificant ( $F = 1.14$ ), rather than highly significant ( $F = 8.29$ ), as it would be in the Scheffé model (Table 3). In the SAS analysis, the estimated variance among families ( $\hat{\sigma}_F^2$ ), which is an approximation of the genetic variance, is very small and does not differ significantly from zero, while in an analysis based on the Scheffé model, the genetic variance is quite large (coefficient of variation =  $\hat{\sigma}_F/\hat{\mu} \approx 0.17$ ) and highly significant. Both analyses indicate that the growth performance varies depending upon the particular combination of family and host plant (significant family  $\times$  host interaction; Table 3). In five of eight ANOVAs reported by Pashley (1988a) the main effect of family was nonsignificant but would have been significant had she followed the Scheffé model rather than the SAS model.

These alternative analyses of the same experimental results can lead to rather different interpretations. The SAS analysis suggests a biological system with strong genotype  $\times$  environment interactions, where the relative growth rate of different families depends on the host plant, even though family growth rates do not differ when averaged across host plants (case B in Fig. 1). In such a system, one may expect selection to favor traits that limit oviposition to the optimal host and that restrict interbreeding between genotypes with different optimal hosts; speciation is a possible consequence (Diehl and Bush, 1984). This contrasts with the Scheffé analysis, which includes a significant family effect, indicating that some families grow faster than other families regardless of the host plant (case A in Fig. 1). Here, the relative advantage enjoyed by fast-growing families may differ with host plant (family  $\times$  host interaction), but nonetheless, fast-growing families on rice are also fast-growing families on corn. Thus, because the same families are favored on each host plant (though by different margins), there is no reason to predict the development or reinforcement of reproductive isolation. Lewontin (1974) and Rausher (1983) have previously stressed the importance of distinguishing between the multiple biological phenomena that can lead to significant interaction terms in mixed-model ANOVAs. Figure 1 and our discussion only describe a subset of the possibilities.

As a second example of the discrepancy that can result from applying different models to the same data set, Table 4 contrasts the results obtained using SAS GLM and BMDP 8V (Dixon, 1985) to analyze another published study. BMDP 8V is consistent with Scheffé (1959) in its formulation of expected mean squares, but carefully restricts its usage to perfectly balanced data sets. The design specifications and data are taken from Ayres et al. (1987). The growth rates of six larvae from each of eight full-sib families of *Epirrita autumnata* (Geometridae) were measured on eight different genets of mountain birch (*Betula pubescens tortuosa*) on each of two different dates. The total possible sample size was 768 ( $6 \times 8 \times 8 \times 2$ ), but 15 of 768 measurements were lost due to larval mortality. Here, for purposes of illustration, we replaced the 15 missing values with random numbers (drawn from normal populations with the same mean and variance as the cells into which they were inserted) to generate a bal-

TABLE 2. A comparison of the expected mean squares generated by SAS GLM with those suggested by Scheffé (1959 pp. 261–289) for a three-way mixed-model ANOVA. The design specifications are taken from Pashley (1988a). Random effects are denoted as  $\sigma$  and fixed effects as  $Q$ ;  $k_i$ 's are coefficients. Note that  $\sigma_F^2$  and  $\sigma_f^2$  are not equivalent but are defined differently in the two models.

Source	Effect type	Expected mean squares generated by SAS GLM	Expected mean squares suggested by Scheffé (1959)
Sex (S/s)	fixed	$\sigma_E^2 + k_1 Q_S$	$\sigma_e^2 + k_1 Q_s$
Family (F/f)	random	$\sigma_E^2 + k_2 \sigma_{FH}^2 + k_3 \sigma_F^2$	$\sigma_e^2 + k_3 \sigma_f^2$
Host (H/h)	fixed	$\sigma_E^2 + k_4 \sigma_{FH}^2 + k_5 Q_H$	$\sigma_e^2 + k_4 \sigma_{fh}^2 + k_5 Q_h$
Family $\times$ host (FH/fh)	random	$\sigma_E^2 + k_6 \sigma_{FH}^2$	$\sigma_e^2 + k_6 \sigma_{fh}^2$
Error (E/e)		$\sigma_E^2$	$\sigma_e^2$

anced data set. Three terms (“family,” “tree,” and family  $\times$  tree) differ in their expected mean squares, depending on the computer algorithm that was used. Consequently, the denominator of the  $F$  test, and the  $F$  test results differed in all three terms (Table 4). The variance among families was nonsignificant according to SAS, but significant according to BMDP. Variance among trees was substantially larger in the BMDP analysis. The family  $\times$  tree interaction was small and nonsignificant in both analyses. Because the SAS and BMDP computer packages analyze different models, they produced different results, even with analogous computer instructions and identical data. This discrepancy was not appreciated by Ayres et al. (1987) when they published.

*Distinctions Among the Models*

The choice of models for experiments involving both fixed and random effects has been a matter of considerable discussion and debate among statisticians (e.g., Scheffé, 1956, 1959 pp. 261–271; Kempthorne, 1957 pp. 250–264; Hartley and Searle, 1969; Searle, 1971 pp. 400–404; Hocking, 1985 pp. 330–334). Hocking (1973) described three such models. We adopt his notation in labeling these models I, II, and III. The two most prominent models are fully specified in the Appendix: Model I follows Scheffé (1959) and is implemented by BMDP (Dixon, 1985), while model II is implemented by SAS (SAS Institute, 1985). Model III is a special case of model I (Hocking, 1973) which assumes a particular covariance structure by requiring that interaction effects are uncorrelated (compare state-

ments 2 in models I and II of the Appendix). For example, if the growth rate of ten larvae (replicates) from each of eight families (where “family” is a random effect) is measured on each of three hosts (where “host” is a fixed effect), model II assumes that the covariance of growth rates for individuals from the same family but on different hosts is the same for each pair of hosts. Scheffé (1956, 1959 pp. 264–265) argued that this restriction on the covariance structure had to be specifically justified and could not be assumed without scrutiny. For the case of biological systems, we agree that this is a dangerous assumption. In fact, it seems to be an interesting hypothesis in its own right, because it addresses the nature of genotype  $\times$  environment interactions. In our example, the assumption becomes particularly suspect if two hosts are quite similar but differ from a third host. The necessary symmetry is guaranteed when there are only two levels of the fixed factor ( $a = 2$ ; as in Pashley [1988a] and Ayres et al. [1987]), but the conditions required to satisfy the assumption become increasingly numerous as  $a$  becomes larger.

The models further differ in that the variance components are defined differently (see Appendix: statement 4 in model I; statements 3, D, and E in model II). Specifically,

$$\sigma_a^2 = \sigma_B^2 - \frac{\sigma_{AB}^2}{a}$$

where  $\sigma_a^2$  denotes the variance as defined by SAS,  $\sigma_B^2$  denotes the variance as defined by Scheffé; and  $a$  is the number of levels in the fixed factor. Compared to the

TABLE 3. The error terms and ANOVA results following SAS GLM compared to those obtained following Scheffé (1959). The dependent variable is larval mass at day 10 (grand mean = 0.173 g). The degrees of freedom and mean squares are from Pashley (1988a table 2). See Table 2 for model specifications and expected mean squares.

Source	d.f.	MS	Analysis following SAS GLM		Analysis following Scheffé (1959)	
			Error term	F	Error term	F
Sex	1	0.0013	MS <sub>E</sub>	0.66	MS <sub>E</sub>	0.66
Family	7	0.0164	MS <sub>FH</sub>	1.14	MS <sub>E</sub>	8.29***
Host	1	0.1810	MS <sub>FH</sub> <sup>a</sup>	12.54**	MS <sub>FH</sub>	12.54**
Family $\times$ host	7	0.0144	MS <sub>E</sub>	7.26***	MS <sub>E</sub>	7.26***
Error	125	0.0020				

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

<sup>a</sup> Pashley inadvertently reported tests using MS<sub>E</sub> as the error term for host; MS<sub>FH</sub> is the correct  $F$  test denominator following SAS GLM or Scheffé (1959). The error was corrected in Pashley (1988b; see also EVOLUTION 42(6) p. 1363).

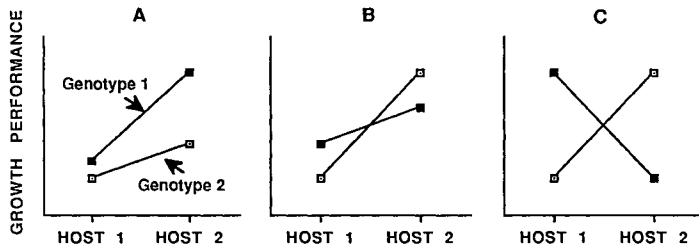


FIG. 1. A depiction of three plant-herbivore systems with genotype  $\times$  host interactions: A) with main effects of both genotype and host; B) with a main effect of host but not genotype; C) with no main effects.

Scheffé model, the SAS model tends to attribute less variance to the random main effect. The larger the interaction variance, the larger the difference. We know of no explicit consideration of the biological merits of the competing variance definitions. The genetic models that relate variance terms in mixed-model ANOVA to additive genetic variance seem to have been initially based on variance terms as defined by Scheffé (e.g., Kempthorne, 1957 pp. 250-264; Griffing and Langridge, 1963). We also note that for the experiment analyzed in Table 4, variance estimates derived from the Scheffé model seem to be more congruent with results obtained by partitioning the analysis into two two-way random models (i.e., analyzing each date separately). Based on this simpler model, on which SAS and Scheffé agree, variance between families was significant on the first date (fourth-instar larvae) but not on the second date (fifth-instar larvae); variance estimates were  $830 \times 10^{-6}$  and  $42 \times 10^{-6}$ , respectively. For the full three-way mixed model, the estimated variance between families was  $249 \times 10^{-6}$  following Scheffé, or  $39 \times 10^{-6}$  following SAS. Hence, the Scheffé estimate approximates the arithmetic mean of the two variances calculated separately, while the SAS estimate falls slightly outside the range of the two variances. The SAS VARCOMP procedure implements the same models as GLM and produced equivalent variance estimates using all methods available in SAS (TYPE I, MIVQUE0, ML, and REML; see SAS Institute, 1985).

As a consequence of these differences between the models, the expected mean squares of any random effect in model II includes variance components due to interactions between the random effect and other fixed effects in the model. These interaction terms do not contribute to the expected mean squares under model I (compare the expected mean squares of family in Table 2). Hence, model II leads to a ratio of mean squares to test for random effects that differs from many references (Table 1) and results in a different analysis, even when simplified to the case of equal sample sizes (Table 4; Hartley and Searle, 1969; Hocking, 1973; SAS Institute, 1986 pp. 129-139). Unlike models II and III, model I does not generalize to the unbalanced case. Hence, a very practical advantage of model II is that it allows the exact computation of expected mean squares with unbalanced data, which is mathematically intractable under the Scheffé model (Goodnight and Speed, 1978). Although this is an appealing argument for general application of the SAS model (Searle, 1971 p. 403), we stress, as have Hocking (1973) and Scheffé (1956), that the simplicity of the result cannot be used as justification for the assumptions required to obtain it.

Yet a third model has been discussed (Graybill, 1961 pp. 396-403; model III in Hocking [1973]) which shares the variance definitions and expected mean squares of model I but which requires assumptions about the covariance structure similar to those of model II. Hocking

TABLE 4. The error terms and ANOVA results obtained using SAS GLM and BMDP 8V (mean squares and degrees of freedom were identical). The dependent variable is relative growth rate of insects (grand mean =  $0.570 \text{ mg} \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ ). "Family" and "tree" were specified as random effects and "date" was considered to be a fixed effect. The data are from Ayres et al. (1987). The expressions given for error terms in footnotes a-c follow Neter et al. (1985 pp. 833-835).

Source	df.	MS	SAS GLM		BMDP 8V	
			Error term	F	Error term	F
Date	1	7.4204	0.0397 <sup>a</sup>	186.91***	0.0397 <sup>a</sup>	186.91***
Family	7	0.0318	0.0281 <sup>b</sup>	1.13	MS <sub>FT</sub>	4.05**
Tree	7	0.3593	0.0218 <sup>c</sup>	16.48**	MS <sub>FT</sub>	45.73***
Date $\times$ family	7	0.0258 (MS <sub>DF</sub> )	MS <sub>DFT</sub>	4.61***	MS <sub>DFT</sub>	4.61***
Date $\times$ tree	7	0.0195 (MS <sub>DT</sub> )	MS <sub>DFT</sub>	3.48*	MS <sub>DFT</sub>	3.48*
Family $\times$ tree	49	0.0079 (MS <sub>FT</sub> )	MS <sub>DFT</sub>	1.41	MS <sub>E</sub>	1.32
Date $\times$ family $\times$ tree	49	0.0056 (MS <sub>DFT</sub> )	MS <sub>E</sub>	0.94	MS <sub>E</sub>	0.94
Error	640	0.0060 (MS <sub>E</sub> )				

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .  
<sup>a</sup>  $MS_{DF} + MS_{DT} - MS_{DFT}$  (df. = 10).  
<sup>b</sup>  $MS_{DF} + MS_{FT} - MS_{DFT}$  (df. = 8).  
<sup>c</sup>  $MS_{DT} + MS_{FT} - MS_{DFT}$  (df. = 5).

(1985 sect. 10.4) further compares models II and III. Additionally, Searle (1971 pp. 401–404) discussed two variants of model II which differ in that one requires the additional assumption that, for any level of the random component, the interaction parameters sum to zero across levels of the fixed component; Hocking (1973) argued that this additional assumption cannot be justified.

#### Recommendations

Researchers should determine whether model I (Scheffé) or model II (SAS) is appropriate for their experimental situation, regardless of the balance of the data. When there are only two levels of the fixed component, the choice of model depends only on the definition of the variance components; the covariance assumption required by model II is automatically satisfied. When there are more than two levels of the fixed component(s), model II should not be used unless the covariance assumption and choice of variance components can be justified (Hocking, 1973; Scheffé, 1956, 1959 pp. 264–265). Consultation with a statistician is recommended. Once the model is chosen, the expected mean squares and testing procedure can be identified.

When data are mildly unbalanced and model II cannot be justified, one can use the mean squares and degrees of freedom generated by SAS, but test hypotheses using the expected mean squares and mean-square ratios recommended by Scheffé (1959) and others (see middle column of Table 1). SAS allows the construction of such tests. Resultant  $F$  tests are approximate, but the consequences of the approximation are expected to be less serious than those associated with a different  $F$ -test denominator. For example, the approximate  $F$  statistics calculated by Ayres et al. (1987), all differed by less than 4% from the exact  $F$  tests calculated using a similar data set (with 15 of 768 values randomly generated; BMDP 8V in Table 4), while altering the denominators changed three  $F$  tests by 72%, 64%, and 7% (SAS GLM vs. BMDP 8V in Table 4). The reliability of such  $F$ -test approximations must be a function of how unbalanced the data are, but their sensitivity to unequal sample size remains poorly known.

When data are unbalanced and model II is justifiable, then SAS can be used for analysis. However, we urge caution in the choice of method to estimate variance components (e.g., see Milliken and Johnson, 1984 p. 290). When data are highly unbalanced and model II cannot be justified, then more complex alternatives that are presently unavailable in most statistical packages are required (e.g., Hocking, 1985 pp. 334–350; Milliken and Johnson, 1984 pp. 289–295, 247–262).

We regret that we are unable to provide a simpler resolution, but our needs as evolutionary biologists have outstripped the refinement of appropriate statistical techniques. This makes it important that we carefully consider and carefully describe the analysis of mixed-model experiments. If an ANOVA is reported, then the model that was used should be explicitly defined and carefully justified. The model and associated assumptions can be simply and efficiently specified by indicating which of the Hocking (1973) models (I, II, or III) is being used. ANOVA tables should indicate how the  $F$  tests were constructed (e.g., table 2 in Pashley [1988] and tables 2 and 3 in Ayres et al. [1987]). If the

mean squares and degrees of freedom are also given, it is easy for readers who may not agree with the model to construct alternative  $F$  tests. Researchers should be alert for cases in which the interpretations are potentially affected by selection of the model. The greatest discrepancies will occur when the interaction variance (e.g.,  $\sigma_{FH}$  in Table 2) is large and significant; when the interaction variance is zero, the results become identical. Furthermore, ANOVA tables should be used to supplement, rather than replace, tabular or graphical presentation of the data, especially when important interactions exist. Figure 2 in Ayres et al. (1987) and figure 3 in Via (1984) are two attempts at allowing visual evaluation of the results. Careful graphical analysis may be the best safeguard against erroneous biological conclusions.

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APPENDIX

Alternative formulations of the two-way mixed-model ANOVA as contrasted by Hocking (1973) are listed below. Note that Hocking's (1973) table 2 is incorrectly labeled as comparing the expected mean squares of models I and III; it actually compares models I and II as below.

Model I (after Scheffé, 1959)	Model II (Searle, 1971; Hocking, 1985; SAS Institute, 1985)
$y_{ijk} = \mu + A_i + B_j + [AB]_{ij} + e_{ijk}$	$y_{ijk} = \mu + \alpha_i + \beta_j + [\alpha\beta]_{ij} + e_{ijk}$
<p>where:</p> <ol style="list-style-type: none"> <li>1) <math>\mu</math> and <math>A_i</math>, (<math>i = 1, \dots, a</math>) are parameters with <math>\sum_i A_i = 0</math>;</li> <li>2) <math>B_j</math>, <math>[AB]_{ij}</math>, and <math>e_{ijk}</math> are random variables with means = 0 and covariance structure described in terms of the <math>a \times a</math> positive symmetric matrix <math>\Sigma = [\sigma_{ir}]</math>, as follows  <math>\text{Var}(e_{ijk}) = \sigma^2</math>  <math>\text{Var}(B_j) = \bar{\sigma}_{..}</math>  <math>\text{Cov}([AB]_{ij}, [AB]_{i'j'}) = \sigma_{i'j} - \bar{\sigma}_{i'..} - \bar{\sigma}_{.j'..} + \bar{\sigma}_{..}</math>  <math>\text{Cov}(B_j, [AB]_{ij}) = \bar{\sigma}_{.j'..} - \bar{\sigma}_{..}</math>                      and with all other covariances = 0;</li> <li>3) <math>[AB]_{.j} = 0</math> (<math>j = 1, \dots, b</math>);</li> <li>4) the variance components are defined as  <math>\sigma_B^2 = \bar{\sigma}_{..}</math>  <math>\sigma_{AB}^2 = \sum_i (\sigma_{ii} - \bar{\sigma}_{..}) / (a - 1)</math>.</li> </ol>	<p>where:</p> <ol style="list-style-type: none"> <li>1) <math>\mu</math> and <math>\alpha_i</math> (<math>i = 1, \dots, a</math>) are parameters;</li> <li>2) <math>\beta_j</math>, <math>[\alpha\beta]_{ij}</math>, and <math>e_{ijk}</math> are uncorrelated random variables with zero means and  <math>\text{Var}(e_{ijk}) = \sigma^2</math>  <math>\text{Var}(\beta_j) = \sigma_\beta^2</math>  <math>\text{Var}([\alpha\beta]_{ij}) = \sigma_{\alpha\beta}^2</math>;</li> <li>3) the variance components are defined to be <math>\sigma_\beta^2</math> and <math>\sigma_{\alpha\beta}^2</math>.</li> </ol> <p>Model II is a special case of model I with these relations:</p> <ol style="list-style-type: none"> <li>A) <math>\mu + A_i = \mu + \alpha_i</math>;</li> <li>B) <math>\sigma_{ii} = \sigma_\beta^2 + \sigma_{\alpha\beta}^2</math>;</li> <li>C) <math>\sigma_{i'j} = \sigma_\beta^2</math> (<math>i \neq i'</math>);</li> <li>D) <math>\sigma_B^2 = \sigma_\beta^2 + \sigma_{\alpha\beta}^2 / a</math>;</li> <li>E) <math>\sigma_{AB}^2 = \sigma_{\alpha\beta}^2</math>;</li> <li>F) <math>B_j = \beta_j + [\alpha\beta]_{.j}</math>;</li> <li>G) <math>[AB]_{ij} = [\alpha\beta]_{ij} - [\alpha\beta]_{.j}</math>.</li> </ol>