Analysis of two factor experiments

Suppose we have two treatment factors, A and B, with a and b levels respectively, and we have r replications of a completely randomized design in these treatments. The linear model can be written as

$$Y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk} \tag{1}$$

where τ_i is the effect of level i of treatment A, β_j is the effect of level j of treatment B and $(\tau\beta)_{ij}$ is the interaction.

The analysis of variance table is constructed from the identity

$$Y_{ijk} = \bar{Y}_{\ldots} + (\bar{Y}_{i..} - \bar{Y}_{\ldots}) + (\bar{Y}_{.j.} - \bar{Y}_{\ldots}) + (\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{\ldots}) + (Y_{ijk} - \bar{Y}_{ij.})$$

leading to

Source	df	Sum of Squares	Mean Square
A	$a-1$	$\sum_{ijk}(\bar{Y}_{i..} - \bar{Y}_{\ldots})^2$	MS_A
B	$b-1$	$\sum_{ijk}(\bar{Y}_{.j.} - \bar{Y}_{\ldots})^2$	MS_B
$A \times B$	$(a-1)(b-1)$	$\sum_{ijk}(\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{\ldots})^2$	MS_{AB}
residual	$ab(r-1)$	$\sum_{ijk}(\bar{Y}_{ijk} - \bar{Y}_{ij.})^2$	MS_{resid}

This analysis of variance uses the variation between units within each treatment combination as an estimate of error. This would underestimate the true error if, for example, the r observations at each treatment combination were simply r measurements on the same experimental unit. It would be appropriate if there were available rab units, and the experiment was a completely randomized design.

In many cases the measurement of error will be associated instead with replications of the whole experiment, for example on different days, or in different locations, and so on. Many writers refer to this as "true replication", to distinguish it from repeated observations within a treatment combination. In this case the analysis of variance table will have a separate line for the main effect of replicates, and the residual will be the replicate-treatment interaction. This is the case in Example K of [CS]. The use of interaction as an estimate of error is discussed in Section 4.13 of [CS]. The model analogous to (1) but with a replicate effect is

$$Y_{ijk} = \mu + \tau_i + \beta_j + \gamma_k + (\tau\beta)_{ij} + \epsilon_{ijk}. \tag{2}$$

Some authors make a distinction between "crossed" and "nested" factors: in (1) replicates are nested within treatment and in (2) replicates are crossed with treatment.

Some insight into the distinction between true replicates and repeat observations can also be obtained by considering three possible sets of assumptions for the treatment effects in (1). In the usual analysis, summarized in the analysis of variance table above, we have

Source	df	Mean Square	Expected Mean Square
A	$a-1$	MS_A	$\sigma^2 + rb\sum\tau_i^2/(a-1)$
B	$b-1$	MS_B	$\sigma^2 + ra\sum\beta_j^2/(b-1)$
AB	$(a-1)(b-1)$	MS_{AB}	$\sigma^2 + r\sum(\tau\beta)_{ij}^2/\{(a-1)(b-1)\}$
residual	$ab(r-1)$	MS_{resid}	σ^2

where the expected mean squares are calculated from (1) under the assumption $E\epsilon_{ijk} = 0$, $var(\epsilon_{ijk}) = \sigma^2$, and the errors are independent. (As usual an equivalent conclusion can be reached using a randomization analysis.)

One way to compute the expected mean squares from first principles is to impose the summation restrictions $\sum\tau_i = 0$, $\sum\beta_j = 0$, $\sum_i(\tau\beta)_{ij} = 0$, and $\sum_j(\tau\beta)_{ij} = 0$. Then, for example,

$$\begin{aligned}
E(MS_{AB}) &= E\{r\sum_{ij}(\bar{Y}_{ij.} - \bar{Y}_{i..} - \bar{Y}_{.j.} + \bar{Y}_{...})^2\}/\{(a-1)(b-1)\} \\
&= rE\sum_{ij}\{(\tau\beta)_{ij} + (\bar{\epsilon}_{ij.} - \bar{\epsilon}_{i..} - \bar{\epsilon}_{.j.} + \bar{\epsilon}_{...})\}^2/\{(a-1)(b-1)\} \\
&= \{r\sum_{ij}(\tau\beta)_{ij}^2 + r\sum_{ij}E(\bar{\epsilon}_{ij.} - \bar{\epsilon}_{i..} - \bar{\epsilon}_{.j.} + \bar{\epsilon}_{...})^2\}/\{(a-1)(b-1)\}.
\end{aligned}$$

The last expectation is that of a quadratic form in $\bar{\epsilon}_{ij.}$ of rank $(a-1)(b-1)$ and hence equal to $\sigma^2(a-1)(b-1)/r$, leading to the result above.

Under the assumption that the ϵ's are normally distributed, we have the results that SS_{AB}/σ^2 follows a noncentral χ^2 distribution on $(a-1)(b-1)$ degrees of freedom with noncentrality parameter $r\sum_{ij}(\tau\beta)_{ij}^2/(a-1)(b-1)$, and SS_{resid}/σ^2 follows a central χ^2 distribution on $ab(r-1)$ degrees of freedom, and hence that MS_{AB}/MS_{resid} follows a non-central F-distribution, which can be used for power or sample size calculations. The central $F_{(a-1)(b-1),ab(r-1)}$ distribution is used for testing the null hypothesis of no interaction.

A different model for the replicated factorial is the socalled mixed effects model where we assume the τ_i are fixed effects and we impose the restriction $\sum \tau_i = 0$, but we assume $(\tau\beta)_{ij} \sim (0, \sigma^2_{ab})$ is a random effect. As usual we assume that $\epsilon_{ijk} \sim (0, \sigma^2)$, and the ϵ's and $\tau\beta$'s are all mutually uncorrelated. The table of expected mean squares is

Source	df	Mean Square	Expected Mean Square
A	$a-1$	MS_A	$\sigma^2 + r\sigma^2_{ab} + rb\sum \tau_i^2/(a-1)$
B	$b-1$	MS_B	$\sigma^2 + ra\sum \beta_j^2/(b-1)$
AB	$(a-1)(b-1)$	MS_{AB}	$\sigma^2 + r\sigma^2_{ab}$
residual	$ab(r-1)$	MS_{resid}	σ^2

Note that the correct F test for main effects of factor A is now computed from the ratio of MS_A to MS_{AB}, the interaction mean square. This is one way of seeing that the AB interaction really is the correct way to measure 'error' in the replicated factorial design discussed above.

This model would usually only be appropriate when the factors A and B are not on the same footing: in Section 6.5 we illustrate this by assuming that A is a treatment factor of the usual sort, but that the levels of B correspond to different centers, or laboratories, or times; B is a "nonspecific" factor.

It is often assumed in the mixed model that β_j are also random effects, but we argue in our book (p.148) that this doesn't really make sense. In some treatments of the mixed model a further assumption is added re the interaction terms: $\sum_j (\tau\beta)_{ij} = 0$, which induces a correlation among the random interaction terms. Arguments against this are also presented in our book (p.148), and in DV, 17.8.2. DV give a nice explanation of how this relates to using interaction mean square for error in a randomized block design in 17.9.

A third possibility in (1) is to model the effects of both A and B by independent random variables, supposing that $\tau_i \sim (0, \sigma^2_a)$, $\beta_j \sim (0, \sigma^2_b)$, and $(\tau\beta)_{ij} \sim (0, \sigma^2_{ab})$. Note that then $var Y_{ijk} = \sigma^2_a + \sigma^2_b + \sigma^2_{ab} + \sigma^2$. The table of expected mean squares is now

Source	df	Mean Square	Expected Mean Square
A	$a-1$	MS_A	$\sigma^2 + rb\sigma^2_a + r\sigma^2_{ab}$
B	$b-1$	MS_B	$\sigma^2 + ra\sigma^2_b + r\sigma^2_{ab}$
AB	$(a-1)(b-1)$	MS_{AB}	$\sigma^2 + r\sigma^2_{ab}$
residual	$ab(r-1)$	MS_{resid}	σ^2

In this case both A and B are assessed by comparing their mean squares to MS_{AB}, and AB is assessed relative to MS_{resid}. The fully crossed factorial model with all effects assumed to be random is not very usual in applications; more common is a model in which random effects are nested within each other. Suppose for example that the levels of B at one level of A have no relation to the levels of B at another level of A. For example B could index repeated samples of some experimental material. Then a nested model with random effects would take the form

$$Y_{ijk} = \mu + \tau_i + \beta_{j(i)} + \epsilon_{k(ij)}$$

say, where we might assume $\tau_i \sim N(0, \sigma_a^2)$, $\beta_{j(i)} \sim N(0, \sigma_b^2)$, and $\epsilon_{k(ij)} \sim N(0, \sigma^2)$. The analysis of variance is formed from the decomposition

$$Y_{ijk} = \bar{Y}_{...} + (\bar{Y}_{i..} - \bar{Y}_{...}) + (\bar{Y}_{ij.} - \bar{Y}_{i..}) + (Y_{ijk} - \bar{Y}_{ij.})$$

leading to the analysis of variance

Source	df	Mean Square	Expected Mean Square
A	$a-1$	MS_A	$\sigma^2 + r\sigma_b^2 + rb\sigma_a^2$
B(in A)	$a(b-1)$	MS_B	$\sigma^2 + r\sigma_b^2$
residual	$ab(r-1)$	MS_{resid}	σ^2

where each effect is tested using the mean squares in the line just below it.

In R mixed and random effects models can be analysed using `lme`, in the library `nlme`. Some of the features are described in Venables and Ripley (2004). Split plot designs, having two (or more) error mean squares, can also be analysed using `lme`. The book by Pinheiro and Bates (2000) *Mixed-Effects Models in S and S-PLUS* discusses nonlinear mixed effects models as well.