Variations on Split Plot and Split Block Experiment Designs

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To Edna, my lovely wife and helpmate

To Fidela, my loved wife and our sons
Emmanuel, Willy, Fabrice and Yves.

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Preface

When it comes to designed experiments, researchers often end up creating complex designs without having sufficient analytical expertise to handle. Researchers in plant breeding, animal science, health sciences and so forth, come to statistical consulting with data from rather very complex designs from time to time. Unfortunately, statistical courses taken by these researchers may not have covered these sophisticated designs. To make matters even more severe, there is an alarming shortage of textbooks covering complex designs. To help alleviate the analytical challenges of researchers dealing with complex designs, we have decided to write this book and we do hope that it will be helpful to a lot of researchers. Understanding and mastery of the designs covered here, assume a prior exposure to the basic experimental designs such as: one-way completely randomized design, completely randomized factorial experiment designs, randomized complete blocks with one or more factors, incomplete blocks, row-column designs, Latin-square designs and so forth. These basic designs are easy to analyze since one is dealing with one experimental error given one has a single level of randomization of the treatment combinations between the levels of various factors to the experimental units. Nonetheless, this type of randomization might be rather simplistic and inappropriate depending on the existing experimental conditions along with the constraints imposed by limited resources. As a result, the experimenter might be forced to have different randomizations and therefore experimental units of unequal sizes at different levels of randomization, to overcome logistical and/or technological constraints of an experiment. This opens up a class of more complex designs called split plot designs or split block designs with at least two types of experimental errors. In either case, several variations can occur with a possibility of a further partitioning of the experimental units, leading to smaller and smaller experimental units paralleled with more error terms used to test the significance of various factors' effects. Furthermore, an experiment design might consist of a combination of these two types of designs, along with treatments arranged following the basic designs for some of the factors under investigation. A textbook on variations of split plot and split block designs points in the right direction by addressing the urgent need of researchers dealing with complex designs for which no reference is available to the

best of our knowledge. We have encountered a few researchers in this type of situation through our statistical consulting activities. We are therefore convinced that this book will be a valuable resource not only to researchers but also to instructors teaching experiment designs courses. It is also important to adequately equip graduate students with the important skills in complex designs for a better readiness to real life situation challenges as far as designed experiments are concerned. Another important innovation of this textbook consists of tackling the issue of error reduction through blocking, analysis of covariance, or both. While blocking relatively homogeneous experimental units into groups might help reduce substantially the experimental error, there are situations where it is neither sufficient by itself nor feasible at all. Thus, use of available auxiliary information on the experimental units has proven to significantly reduce the experimental error through analysis of covariance. Analysis of covariance enables one to better control the experimental error when covariates are judiciously chosen. We have added a chapter on analysis of covariance to specifically provide researchers with helpful analytical tools needed when dealing with covariates in complex designs.

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May 2006

CHAPTER 1

The Standard Split Plot Experiment Design

1.1. INTRODUCTION

Prior to starting the topic of this book, it was deemed advisable to present some design concepts, definitions, and principles. Comparative experiments involve a number, v, treatments (factors) where a *treatment* is an item of interest to the experimenter. A treatment could be a medical treatment, a drug application, a level of a factor (amount of a drug, fertilizer, insecticide, etc.), a genotype, an agricultural practice, a marketing method, a teaching method, or any other item of interest. The selection of the v treatments for an experiment is known as the *treatment design*. The selection of an appropriate treatment design is a major element for the success of an experiment. It may include checks (standards, placebos) or other *points of reference*. The treatments may be all combinations of two or more factors and this is known as a *factorial arrangement* or *factorial treatment design*. A subset of a factorial is denoted as a *fractional replicate* of a factorial.

The arrangement of the treatments in an experiment is known as the *experiment design* or the *design of the experiment*. The term experimental design is of frequent use in statistical literature but is not used here. There are many types of experiment designs including: unblocked designs, blocked designs (complete blocks and incomplete blocks), row-column experiment designs, row-column designs within complete blocks, and others. Tables of designs are available in several statistical publications. However, many more experiment designs are available from a software package such as GENDEX (2005). This package obtains a randomized form of an experiment design and the design in variance optimal or near optimal.

There are three types of units to be considered when conducting an experiment. These are the observational unit, the sample or sampling unit, and the experimental

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unit (Federer, 1991, Chapter 7). The *observational unit* is the smallest unit for which a response or measurement is obtained. A population or distribution is composed of *sample units or sampling units*. The *experimental unit* is the smallest amount of experimental material to which one treatment is applied. In many experiments, these three types of units are one and the same. In other cases, they may all be different. For example, suppose a treatment is a teaching method taught to a group of thirty students. The experimental unit is the group of thirty students for the period of time used to evaluate a teaching method. The sampling unit is the student, from a population of all students, for which inferences are to be made about this teaching method. Suppose that several examinations are given during the period of time the method is applied, the result from each examination is an observation or response and the observational unit is one examination from one student. In some investigations like sampling for water quality, obtaining a measurement on produce for a genotype from a plot of land measuring 1 m by 10 m (an experimental unit), etc., the sampling units are undefined.

Fisher (1966) presented three principles of experiment design. These are *local control (blocking, stratification), replication,* and *randomization*. Owing to random fluctuations of responses in any experiment or investigation, there is variation. The variation controlled should not be associated or interacting with treatment responses. For example, if an animal dies during the course of conducting an experiment and the death is not caused by the treatment, it should be considered as a missing observation and not as a zero response. Blocking (stratification) or local control is used to exclude extraneous variation in an experiment not associated with treatment effects. The blocking should be such as to have maximum variation among blocks and minimum variation within blocks. This makes for efficient experimentation and reduces the number of replicates (replications) needed for a specified degree of precision for treatment effects.

To reduce the effect of the variation in an experiment on measuring a treatment effect, the sample size or the number of replicates needs to be increased. Replication allows for an estimate of the random variation. Replication refers to the number of experimental units allocated to a particular treatment. The variation among the experimental units, eliminating treatment and blocking effects, is a measure of experimental variation or error. The number of replications should not be confused with the number of observations. For example, in a nutrition study of several regimes with an experimental unit consisting of one animal, weekly measurements (observations) may be taken on the weight of the animal over a 6-month period. These week-by-week measurements do not constitute replications. The number of replications is determined by the number of experimental units allocated to one treatment and not by the number of observations obtained.

Randomization is necessary in order to have a valid estimate of an error variance for comparing differences among treatments in an experiment. Fisher (1966) has defined a *valid estimate of an error variance or mean square* as one which contains all sources of variation affecting treatment effects except those due to the treatments themselves. This means that the estimated variance should be among experimental units treated alike and not necessarily among observations.

An appropriate response model needs to be determined for each experiment. It is essential to determine the pattern of variation in an experiment or investigation and not assume that one response model fits all experiments for a given design. With the availability of computers, *exploratory model selection* may be utilized to determine variation patterns in an experiment (Federer, 2003). The nature of the experiment design selected and the variation imposed during the conduct of an experiment determine the variation pattern. The conduct of an experiment or investigation is a part of the design of the experiment or investigation. This fact may be overlooked when selecting a response model equation for an experiment. For example, a randomized complete block design may be selected as the design of the experiment. Then, during the course of conducting the experiment, a part of the replicate of the experiment is flooded with water. This needs to be considered as a part of the design of the experiment and may be handled by setting up another block, using a covariate, or missing experimental units. This would not be the response model envisioned when the experiment design was selected. Or, it may be that the experimenter observed an unanticipated gradient in some or all of the blocks. A response model taking the gradients within blocks into consideration should be used in place of the model presumed to hold when the experiment was started. More detail on exploratory model selection may be found in Federer (2003).

For further discussion of the above, the reader is referred to Fisher (1966) and Federer (1984). The latter reference discusses a number of other principles and axioms to consider when conducting experiments.

An analysis of variance is considered to be a partitioning of the total variation into the variation for each of the sources of variation listed in a response model. An F-test is not considered to be a part of the analysis of variance as originally developed by Sir Ronald A. Fisher. Statistical publications often consider an F-test as part of the analysis of variance. We do not, as variance component estimation, multiple range tests, or other analyses may be used in connection with an analysis of variance. Some experimenters do consider the term analysis of variance to be a misnomer. A better term may be a partitioning of the total variation into its component parts or simply variation or variance partitioning.

1.2. STATISTICAL DESIGN

The standard split plot experiment design (SPED) discussed in several statistics textbooks has a two-factor factorial arrangement as the treatment design. One factor, say A with a levels, is designed as a randomized complete block design with r complete blocks or replicates. The experimental unit, the smallest unit to which one treatment is applied, for the levels of factor A treatments is called a *whole plot experimental unit* (wpeu). Then each wpeu is divided into b *split plot experimental units* (speus) for the b levels of the second factor, say B. Note that either or both factors A and B could be in a factorial arrangement or other treatment design rather than a single factor. A schematic layout of the standard SPED is shown below.

Standard split plot design with r replicates, a levels of factor A, and b levels of factor B

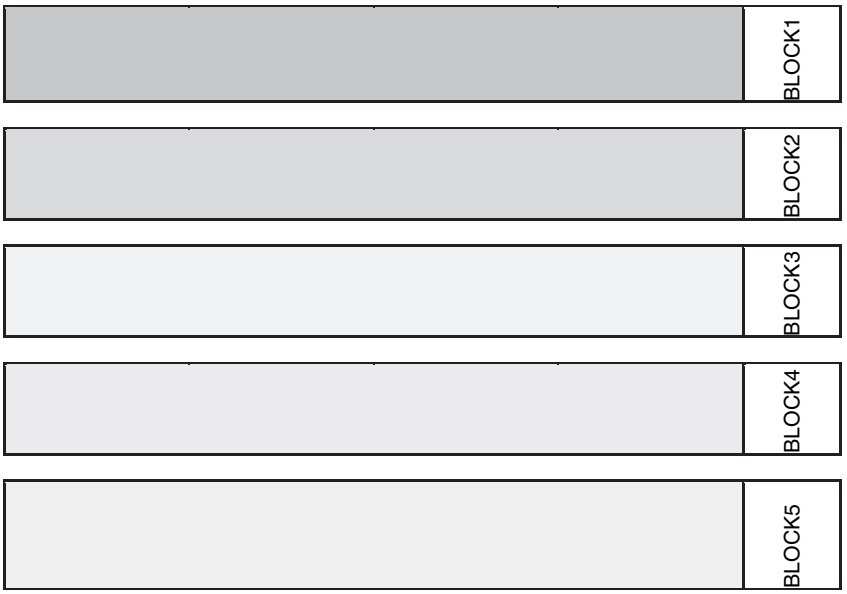
Replicate	1	2	3	...	r
Whole plot factor A	1 2 ... a	1 2 ... a	1 2 ... a	...	1 2 ... a
Split plot factor B	1 1 ... 1	1 1 ... 1	1 1 ... 1		1 1 ... 1
	2 2 ... 2	2 2 ... 2	2 2 ... 2		2 2 ... 2
	.....	.....	.....		.....
	b b ... b	b b ... b	b b ... b		b b ... b

The a levels of factor A are randomly and independently allocated to the a wpeus within *each* of the r complete blocks or replicates. Then within *each* wpeu, the b levels of factor B are independently randomized. There are r independent randomizations for the a levels of factor A and ra independently assigned randomizations for b levels of factor B. The fact that the *number of randomizations and the experimental units* are different for the two factors implies that each factor will have a separate error term for comparing effects of factor A and effects of factor B.

Even though the standard SPED has the whole plot factor A treatments in a randomized complete block design, any experiment design may be used for the factor A. For example, a completely randomized experiment design, a Latin square experiment design, an incomplete block experiment design, or any other experiment design may be used for the whole plot treatments. These variations are illustrated in Chapter 3.

The three steps in randomizing a plan for a standard or basic split plot experiment design consisting of $r = 5$ blocks (replicates), $a = 4$ levels of whole plot factor A, and $b = 8$ levels of split plot factor B are shown below:

Step 1: Divison of the experimental area or material into five blocks



Step 2: Randomizaton of four levels of whole plot factor *A* to each of five blocks

				BLOCK1
A3	A2	A1	A4	
				BLOCK2
A4	A1	A3	A2	
				BLOCK3
A2	A3	A4	A1	
				BLOCK4
A4	A2	A3	A1	
				BLOCK5
A3	A4	A1	A2	

Step 3: Randomization of eight levels of split plot factor B within each level of whole plot factor A

B1	B2	B7	B2	
B4	B3	B8	B4	
B5	B4	B4	B7	
B3	B5	B2	B5	
B6	B1	B5	B8	
B8	B6	B3	B1	
B7	B8	B6	B6	
B2	B7	B1	B3	BLOCK1
A3	A2	A1	A4	
B7	B6	B2	B5	
B2	B1	B3	B4	
B4	B4	B5	B2	
B6	B3	B7	B8	
B3	B7	B8	B3	
B8	B2	B1	B6	
B1	B5	B6	B7	
B5	B8	B4	B1	BLOCK2
A4	A1	A3	A2	
B4	B7	B1	B6	
B6	B8	B2	B1	
B1	B2	B4	B3	
B8	B6	B3	B5	
B5	B3	B7	B4	
B7	B1	B8	B8	
B3	B5	B6	B7	
B2	B4	B5	B2	BLOCK3
A2	A3	A4	A1	

B3	B7	B5	B8	
B8	B6	B2	B5	
B5	B2	B3	B4	
B6	B8	B4	B1	
B1	B4	B7	B3	
B7	B5	B6	B6	
B4	B1	B8	B7	
B2	B3	B1	B2	BLOCK4
A4	A2	A3	A1	
B3	B1	B7	B1	
B1	B6	B2	B7	
B5	B2	B3	B4	
B6	B7	B4	B8	
B8	B4	B5	B3	
B7	B5	B6	B6	
B4	B8	B1	B5	
B2	B3	B8	B2	BLOCK5
A3	A4	A1	A2	

If an experiment design involving blocking is used for the b split plot treatments, factor B, should be *within each* whole-plot-treatment wpeu, as this facilitates the statistical analysis for an experiment as orthogonality of effects is maintained. If the experiment design for the split plot factor B treatments is over levels of the whole plot treatments within one complete block, confounding of effects is introduced and the statistical analysis becomes more complex (Federer, 1975). This may not be a computational problem as available statistical software packages can be written to handle this situation. However, the confounding of effects reduces the precision of contrasts and estimates of effects.

1.3. EXAMPLES OF SPLIT-PLOT-DESIGNED EXPERIMENTS

Example 1—A seed germination test was conducted in a greenhouse on $a = 49$ genotypes of guayule, the whole plots (factor A), with four seed treatments (factor B) applied to each genotype as split plot treatments (Federer, 1946). The wpeu was a greenhouse flat for one genotype and 100 seeds of each of the four seed treatments (factor B) were planted in a flat, as more information on seed treatment than on genotype was desired and this fitted into the layout more easily than any other arrangement. The speu consisted of 1/4 of a greenhouse flat in which 100 seeds were planted. The 49 genotypes were arranged in a triple lattice incomplete block experiment design with $r = 6$ complete blocks and with an incomplete block size of $k = 7$ wpeus. The four seed treatments were randomly allocated to the four speus in a flat, that is, within each genotype wpeu. The data for eight of the 49 genotypes in three of the six replicates are given as Example X-1 of Federer (1955) and as Example 1.2. The whole plot treatments, 49 genotypes, are considered to be a random sample of genotypes from a population of genotypes, that is, they are

considered to be random effects whereas the seed treatments are fixed effects as these are the only ones of interest.

Example 2—Example X-2 of Federer (1955) contains the yield data for $b = 6$ genotypes which are corn double crosses. The data are from two of the twelve districts set up for testing corn hybrids in Iowa. The $a = 2$ districts are the whole plots, and the six corn double crosses, the split plot treatments, are arranged in a randomized complete block design within each district. The yield data (pounds of ear corn) arranged systematically are given below:

District 1, *A*					
Double-cross, factor *B*	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total
1-1	34.6	33.4	36.5	33.0	137.5
2-2	34.5	39.1	35.4	35.6	144.6
4-3	30.1	30.8	35.0	33.3	129.2
15-45	31.3	29.3	29.7	33.2	123.5
8-38	32.8	35.7	36.0	34.0	138.5
7-39	30.7	35.5	35.3	30.6	132.1
Total	194.0	203.8	207.9	199.7	805.4

District 2, *A*					
Double-cross, factor *B*	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Total
1-1	33.1	24.6	33.8	34.6	126.1
2-2	46.4	36.9	36.3	45.3	164.9
4-3	32.3	38.7	37.5	37.6	146.1
15-43	37.5	39.2	39.1	34.1	149.9
8-38	31.2	40.8	46.1	44.1	162.2
7-39	35.8	38.2	38.8	39.6	152.4
Total	216.3	218.4	231.6	235.3	901.6

Example 3—Cochran and Cox (1957), page 300, present the data for an SPED with $a = 3$ recipes, the whole plots (factor A), for chocolate cakes baked at $b = 6$ temperatures, the split plots (factor B). The response was the breaking angle of the cake. Enough batter for one recipe was prepared for the six cakes to be baked at the six temperatures. That is, the wpeu was one batter for six cakes. The three recipes were arranged in a randomized complete block design with $r = 15$ replicates.

Example 4—Federer (1955), page 26 of the Problem Section, presents the data for an SPED with $a = 2$ whole plot treatments (factor A) of alfalfa or no alfalfa and $b = 5$ split plot treatments of bromegrass strains. The bromegrass strains were intercropped (mixed together) with the alfalfa and no alfalfa (See Federer,

1993, 1999). The whole plot treatments were arranged in a randomized complete block design with $r = 4$ replicates. The dry weights (grams) of hay arranged systematically are:

Bromegrass strain, factor B	Replicate 1 Factor A		Replicate 2 Factor A		Replicate 3 Factor A		Replicate 4 Factor A	
	alfalfa	alone	alfalfa	alone	alfalfa	alone	alfalfa	alone
a	730	786	1004	838	871	1033	844	867
b	601	1038	978	1111	1059	1380	1053	1229
c	840	1047	1099	1393	938	1208	1170	1433
d	844	993	990	970	965	1.308	1111	1311
e	768	883	1029	1130	909	1247	1124	1289

Example 5—Das and Giri (1979), page 150, present an example of three varieties forming the whole plots and $b = 4$ manurial treatments forming the split plots in an SPED with $r = 4$ replications.

Example 6—Gomez and Gomez (1984), page 102, give a numerical example of six levels of nitrogen applications forming the whole plots and $b = 4$ rice varieties forming the split plots in an SPED with $r = 3$ replications.

Example 7—Raghavarao (1983), page 255, presents a numerical example where the whole plots were $a = 3$ nitrogen levels and the $b = 4$ split plot treatments were insecticides in an SPED with $r = 4$ replications.

Example 8—Leonard and Clark (1938), Chapter 21, give a numerical example of a split plot experiment design with $a = 10$ maize hybrids as the whole plots of 36 hills (3 plants per hill). The wpeus were divided into thirds with 12 hills making up the speu. The $b = 3$ split plot treatments were seeds from the three generations F1, F2, and F3. Two replicates were used and the response was the yield of ear corn.

Example 9—In a setting other than agriculture, three types of schools (public, religious, and private) were the whole plots. Four types of teaching methods formed the split plots. This arrangement was replicated over r school districts. The response was the average score on standardized tests.

Example 10—Two types of shelters (barn and outdoor) were the whole plot factor A treatments and two types of shoes for horses were used as the factor B split plot treatments. There were to be $r = 5$ sets (replicates) of four horses used. Two horses, wpeu, of each set would be kept in a barn and two would be kept outdoors. One horse, speu, had one type of shoe and the second horse received the other type of shoe. The response was length of time required before reshoeing a horse was required.

Example 11—In a micro-array experiment, the two whole plot treatments were methods one and two. The two split plot treatments were red color-label 1 and green

color-label 2 for method 1 and were green color-label 1 and red color-label 2 for method 2. There were $r = 10$ sets of whole plots. The color by label interaction is completely confounded with method in the SPED experiment performed.

Example 12—Three types of managements (factor A) constituted the whole plots that consisted of a litter of six male rats. The $b = 6$ medical treatments (factor B) were the split plot treatments with one rat constituting the speu. Three litters, wpeus, were obtained from each of $r = 6$ laboratories.

Example 13—A randomized complete block experiment design with $a = 5$ treatments (factor A) and $r = 5$ replicates was conducted to determine the effect of the treatments on the yield and the quality of strawberries. The experiment was laid out in the field in five columns, the blocks or replicates, and five rows. Hence, this is a row-column design as far as spatial variation is concerned. A 5×5 Latin square experiment design should have been used but was not. The strawberries in each of the 25 wpeus were graded into $b = 4$ quality grades (factor B) that were the split plot treatments. Responses were the weight and the number of strawberries in each of the grades within a wpeu.

Example 14—Jarmasz et al. (2005) used several forms of a split plot experiment design to study human subject perceptions to various stimuli. The factor sex was not taken into account when analyzing the data presented in the paper. Taking the factor sex into account adds to the splitting of units and the complexity of the analysis. Several variations of the SPED were used. The split-plot-designed experiment is of frequent occurrence in this type of research investigation.

Numerous literature citations of split plot designs are given by Federer (1955) in the Problem Section at the end of the book. This type of design appears in many fields of inquiry and is of frequent occurrence. Kirk (1968) lists ten references as representative applications of split plot designs in literature involving learning and other psychological research. The Annual Reports of the Rothamsted Experiment Station, the International Rice Research Institute (IRRI), and other research organizations give data sets for split-plot-designed experiments.

1.4. ANALYSIS OF VARIANCE

A partitioning of the degrees of freedom in an analysis of variance table for the various sources of variation is one method for writing a linear model for a set of experimental data. Alternatively, writing a linear model in equation form is another way of presenting the sources of variation for an experiment. A linear response model for the SPED for fixed effects factors A and B is usually given as

$$Y_{hij} = \mu + \rho_h + \alpha_i + \delta_{hi} + \beta_j + \alpha\beta_{ij} + \varepsilon_{hij}, \tag{1.1}$$

where Y_{hij} is the response of the hijth speu,
μ is a general mean effect,

ρ_h is the hth replicate effect which is identically and independently distributed with mean zero and variance σ_ρ^2,

α_i is the effect of the ith whole plot factor A treatment,

δ_{hi} is a whole plot random error term which is identically and independently distributed with mean zero and variance σ_δ^2,

β_j is the effect of the jth split plot factor B treatment,

$\alpha\beta_{ij}$ is the interaction effect of the ith whole plot treatment with the jth split plot treatment, and

ε_{hij} is a split plot random error effect identically and independently distributed with mean zero and variance σ_ε^2.

The ρ_h, ε_{hi}, and δ_{hij} in Equation (1.1) are considered to be mutually independent variables.

Prior to calculating an analysis of variance, ANOVA table for the above response model, it is often instructive and enlightening to construct an ANOVA table for *each* whole plot as follows:

Whole plot level	A1		A2		...	Aa	
Source of variation	DF	SS	DF	SS	...	DF	SS
Total	rb	T1	rb	T2	...	rb	Ta
Correction for mean	1	C1	1	C2	...	1	Ca
Replicate	$r-1$	R1	$r-1$	R2	...	$r-1$	Ra
Split plot factor B	$b-1$	B1	$b-1$	B2	...	$b-1$	Ba
$R \times B$ = Error	$(r-1)(b-1)$	E1	$(r-1)(b-1)$	E2	...	$(r-1)(b-1)$	Ea

DF is degrees of freedom and SS is sum of squares. The dot notation is used which indicates that this is a sum over the subscripts replaced by a dot. The sums of squares for the ith whole plot treatment, $i = 1, 2, \ldots, a$, are:

$$\mathrm{Ti} = \sum_{h=1}^{r}\sum_{j=1}^{b} Y_{hij}^2$$

$$\mathrm{Ci} = \mathrm{Y}_{.i.}^2/\mathrm{br}$$

$$\mathrm{Ri} = \sum_{h=1}^{r} Y_{hi.}^2/b - Y_{.i.}^2/br = b\sum_{i=1}^{r}(\bar{y}_{hi.} - \bar{y}_{.i.})^2$$

$$\mathrm{Bi} = \sum_{j=1}^{b} Y_{.ij}^2/r - Y_{.i.}^2/br = r\sum_{j=1}^{b}(\bar{y}_{.ij} - \bar{y}_{.i.})^2.$$

These are the usual equations for computing sums of squares for data from a randomized complete block designed experiment. Ei is obtained by subtraction.

Data from a split-plot-designed experiment should not be analyzed as a three-factor factorial of the three factors A, B, and R. This is *not correct* as can be seen from the above and noting that the b $R \times B$ interactions are *nested* within whole plot treatments. This means that this interaction is completely confounded with the $R \times A \times B$ interaction. The replicates for different wpeus are not the same even though they may have the same numbering. They are from different parts of the experiment. The calculations can be performed but this does not validate the partition for these two interactions.

A combined ANOVA is easily obtained from the above analyses as indicated in the table that follows.

Source of variation	Degrees of freedom	Sum of squares
Total	rab	$\mathrm{T1} + \mathrm{T2} + \ldots + \mathrm{T}a$
Correction for mean	1	CFM Compute as usual
Whole plot treatment A	$a - 1$	$\mathrm{C1} + \mathrm{C2} + \ldots + \mathrm{C}a - \mathrm{CFM}$
Replicate within A	$a(r - 1)$	$\mathrm{R1} + \mathrm{R2} + \ldots + \mathrm{R}a$
Replicate	$r - 1$	Compute as usual
Error $A = R \times A$	$(a - 1)(r - 1)$	Subtraction
Split plot treatment B within A	$a(b - 1)$	$\mathrm{B1} + \mathrm{B2} + \ldots + \mathrm{B}a$
Split plot treatment B	$a - 1$	Compute as usual
$A \times B$	$(a - 1)(b - 1)$	Subtraction
Error $B = R \times B$ within A	$a(b - 1)(r - 1)$	$\mathrm{E1} + \mathrm{E2} + \ldots + \mathrm{E}a$

The Replicate within A sum of squares with $a(r - 1)$ degrees of freedom is the sum $\mathrm{R1} + \mathrm{R2} + \ldots + \mathrm{R}a$. This is the Replicate sum of squares + the Error A sum of squares. The additional sums of squares required for the above table are obtained from the following equations:

$$\mathrm{CFM} = Y_{\ldots}^2/abr$$

$$\text{Replicate} = \sum_{i=1}^{r} Y_{h..}^2/ab - Y_{\ldots}^2/abr$$

$$\text{Split plot treatment } B = \sum_{i=1}^{b} Y_{.j}^2/ar - Y_{\ldots}^2/abr.$$

Using this format for obtaining an ANOVA for an SPED can be enlightening for information on the nature of the factor B responses at each level of factor A and for observing the homogeneity of the error mean squares $\mathrm{Ei}/(rb - r - b - 1)$ at each level of factor A.

In the above form, it may be instructive in some situations to partition each of the Ei sum of squares into Tukey's one-degree-of-freedom for nonadditivity (see e.g., Snedecor and Cochran, 1980, Section 15.8) and a residual sum of squares with $rb - r - b$ degrees of freedom. Likewise, the $R \times A$ sum of squares may be partitioned

to check for nonadditivity. The formula for computing Tukey's one-degree-of-freedom sum of squares for a two-way layout is

$$\text{TNA} = \frac{\left[\sum_{h=1}^{r}\sum_{j=1}^{b} Y_{hij}(\bar{y}_{hi.} - \bar{y}_{.i.})(\bar{y}_{.ij} - \bar{y}_{.i.})\right]^2}{\sum_{h=1}^{r}(\bar{y}_{hi.} - \bar{y}_{.i.})^2 \sum_{j=1}^{b}(\bar{y}_{.ij} - \bar{y}_{.i.})^2}. \tag{1.2}$$

The mean of combination *hi* is $\bar{y}_{hi.}$. $\bar{y}_{.i.}$ is the *i*th whole plot mean. $\bar{y}_{..j}$ is the mean of the *j*th split plot treatment, and $\bar{y}_{.ij}$ is the mean of treatment combination *ij*. For the numerical example, Example 1.2, in Section 1.7 and $i = 0$, the differences of replicate means from the overall mean are $-5/12$, $-2/12$, and 7/12. The differences of seed treatment means from the overall mean are 500/12, $-156/12$, $-148/12$, and $-196/12$. The replicates by seed treatment responses for genotype 0 are:

	Seed treatment					
Replicate	0	1	2	3	Total	$\bar{y}_{h0.} - \bar{y}_{.0.}$
1	66	12	13	6	97	$-5/12$
2	63	10	13	12	98	$-2/12$
3	70	13	11	7	101	7/12
Total	199	35	37	25	296	—
$\bar{y}_{.0j} - \bar{y}_{.0.}$	500/12	$-156/12$	$-148/12$	$-196/12$		

Using Equation (1.2) for the above data, TNA is computed as:

$$\begin{aligned}&[66(500/12)(-5/12) + 63(500/12)(-2/12) + 70(500/12)(7/12)\\ &\quad + \ldots + 7(-196/12)(7/12)]^2/[\{(-5/12)^2 + \ldots + (-196/12)^2\}]\\ &\quad = [-1,145 + 65 + \ldots - 79 - 67]^2/(2.167/4)(6,972/3) = 2.80.\end{aligned}$$

1.5. F-TESTS

The replicate effects should always be considered as random effects. Considering them as fixed effects makes no sense as an experimenter is concerned with inferences beyond these particular replicates. This means that the Error *A* mean square is the appropriate error term for testing significance of whole plot treatment main effects, that is, factor *A* effects. Depending on the validity of the assumption that the Error *A* effects, δ_{hi}, are normally, identically, and independently distributed with zero mean and common variance σ_δ^2, that is, NIID(0,σ_δ^2), an F-test of the Factor *A* mean square divided by the Error *A* mean square is appropriate for testing the null hypothesis that the *A* effects are zero.

When the whole plot treatment effects are fixed effects and the assumption of normality of the random error effects is correct, an F-test of the null hypothesis of zero split plot treatment effects is performed using the Error B mean square. Likewise, an F-test to test the null hypothesis of zero $A \times B$ interaction effects is obtained using the Error B mean square. Note that the normality assumption is not crucial in most cases as an F-test is quite robust, especially when the number of degrees of freedom associated with the denominator mean square is not small.

When the whole plot treatments are random effects, the appropriate error mean square for testing the null hypothesis of zero split plot treatment effects is the $A \times B$ interaction mean square. The appropriate error term for testing the null hypothesis of zero $A \times B$ interaction effects is the Error B mean square.

When the split plot treatments are random effects and whole plot treatments are fixed effects, the appropriate error mean square for testing the null hypothesis for zero split plot treatment effects is the Error B mean square. For the interaction variance component for factors A and B defined as $\sigma^2_{\alpha\beta}$, the error mean square

$$\sigma^2_\varepsilon + b\sigma^2_\delta + \frac{ra\sigma^2_{\alpha\beta}}{a-1}$$

is the appropriate mean square for testing for zero factor A effects. The degrees of freedom associated with the above mean square are unknown and will need to be approximated (see, e.g., Snedecor and Cochran, 1980, Section 6.11). The expected value of the interaction mean square is

$$\sigma^2_\varepsilon + \frac{ar\sigma^2_{\alpha\beta}}{a-1}.$$

The following table presents the expected values of the mean squares in an analysis of variance table for factors A and B as fixed effects and as random effects:

Source of variation	Degrees of freedom	Expected value of mean square	
		Fixed A and B	Random A and B
Replicate	$r-1$	$\sigma^2_\varepsilon + b\sigma^2_\delta + ab\sigma^2_\rho$	$\sigma^2_\varepsilon + b\sigma^2_\delta + ab\sigma^2_\rho$
Factor A	$a-1$	$\sigma^2_\varepsilon + b\sigma^2_\delta + f(\alpha_i)$	$\sigma^2_\varepsilon + b\sigma^2_\delta + r\sigma^2_{\alpha\beta} + rb\sigma^2_\alpha$
Error A	$(a-1)(r-1)$	$\sigma^2_\varepsilon + b\sigma^2_\delta$	$\sigma^2_\varepsilon + b\sigma^2_\delta$
Factor B	$b-1$	$\sigma^2_\varepsilon + f(\beta_j)$	$\sigma^2_\varepsilon + r\sigma^2_{\alpha\beta} + ar\sigma^2_\beta$
$A \times B$	$(a-1)(b-1)$	$\sigma^2_\varepsilon + f(\alpha\beta_{ij})$	$\sigma^2_\varepsilon + r\sigma^2_{\alpha\beta}$
Error B	$a(b-1)(r-1)$	σ^2_ε	σ^2_ε

The variance components for factor A effects and factor B effects are σ^2_α *and* σ^2_β, respectively. The other variance components have been defined previously. The term $f(x)$ refers to a function of the sum of squares of the parameter x inside the parentheses.