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# Principles of the experiment design

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## Abstract

Reference books usually present experiment designs as recipes, one of which should be chosen for each experiment. This approach extends to teaching and leads the researcher to understand that he is limited to the use of these experiment designs. The consequences are the adaptation of research plans to this restrict set of designs and the adoption of inappropriate designs to achieve research objectives. This approach arose from the relatively simple calculations required to analyze the results of experiments with those designs at a time when computing resources were precarious. The evolution of computing resources no longer justifies the restriction to experiment designs that require easy calculations. These resources made possible the elaboration of designs with properties appropriate for efficient experiments. This article considers the properties that constitute principles of the experiment design that must be considered when planning the experiment. Compliance with these principles allows the researcher to elaborate the most appropriate experiment design for each experiment.

**Keywords:** Experiment structure; Repetition; Local Control; Randomization; Orthogonality; Balance; Confounding; Efficiency.

## 1. Introduction

Based on the foundations laid out by Fisher [1,2] and the contributions of Yates [3,4,5], many experiment designs have been developed. The ease of the calculations for analyzing data from experiments with these designs has made them widely used. This facility gained importance at a time when resources for processing statistical analyzes were precarious. Consequently, it has become common for researchers to adapt experimental plans to these designs. This approach has led to the frequent use of inappropriate designs for the purposes and conditions of experiments. Federer [6] commented that often the researcher and statistician think that the choice of an experiment design is limited to those that appear in the literature; however, the experiment should be considered as it will be conducted, rather than being changed to fit an available design.

Computational resources turned possible the use of the most appropriate experiment design for each situation and, consequently, planning more efficient experiments. However, adopting this approach requires knowledge of the conceptual and methodological bases of experimental research and an understanding of important properties that constitute principles of experimental design. Silva [7,8] proposed a conceptual and methodological basis for experimental research, rational and coherent with the logical sequence of the experimental research process. Silva [9, 10] reviewed and updated these contributions and Silva [11, 12] presented a synthesis of them.

This article discusses the properties considered as principles of the experiment design: repetition, local control, randomization, orthogonality, balance, confounding and efficiency. The correct adoption of these principles allows the researcher to elaborate the most proper design for each experiment. This article is based on the contributions of Fisher [1,2], Yates [3,4,5], Federer [6], Silva [7,8,9,10,11,12], Cochran & Cox [13], Steel & Torrie [14], Mead [15], Kuehl [16],

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Preece [17], Bailey [18] and Fisher & Yates [19], which are explicitly referred in the text, and Federer [20,21], Finney [22], Giesbrecht [23], Hinkelmann & Kempthorne [24], Kempthorne [25,26], Ostle & Malone [27], Pearce [28,29], Petersen [30], Selwyn [31], Shadish, Cook & Campbell [32].

## 2. Experiment Design

The design of the experiment is defined by the structure of the conditions, the structure of the units and the association of these two structures, which makes up the structure of the experiment. The *condition structure* comprises the relationships between the levels of the experimental factors, and the *unit structure*, the relationships between the levels of the unit factors. The *unit factors* comprise the classifications of the observation units into themselves, and the groupings of these units originated from the local control and the formations of experimental units of experimental factors. Unit factors are random factors, i.e., factors with random effects. [Silva, 10.11,12].

The observed values of the response variable express the effects from experimental factors and unit factors. Experimental factor effects are inevitably confounded with unit factor effects, which constitute *experimental error*. Valid inferences about effects of experimental factors require that this confounding is unbiased and that those effects are not confounded with the effects of other experimental factors. This requirement is achieved by treatment factors through proper experimental control, particularly randomization. However, intrinsic experimental factors are not subject to this level of control, which implies that their effects are biasedly confounded with unit factor effects. Unit factors stratify the experimental error and originate strata of the experiment. Each of these strata may or may not include an experimental factor. In each stratum where an experimental factor is located, there is a correspondence between the levels of this factor and the levels of the corresponding unit factor. The levels of a unit factor associated with an experimental factor are the *experimental units* of this experimental factor. The *experimental error that affects the effect of an experimental factor* is the variation of the extraneous characteristics between the experimental units of this factor [Silva, 9,10].

In general, the unit factor is coarser or equivalent to the experimental factor, that is, if B is a unit factor and A is an experimental factor, to each level of factor A correspond one or more levels of B, which is symbolized by B⩾A. Thus, the correspondence between A and B can be of two forms:

a) To each level of A corresponds more than one level of B, which is symbolized by B≻A. In this case, there is more than one experimental unit for levels of A, which means that there are repetitions for levels of experimental factor A. Experimental units with distinct levels of A are different, but for a same level of A there is more than one experimental unit. This implies that effects of the experimental factor A are completely confounded with experimental error (effect of unit factor B), but this experimental error is partially confounded with effects of A. In this situation, with proper experimental control, particularly randomization, A is a treatment factor.

The experimental error that affects the effects of treatment factor A depends on the unit structure: i) If B is the only unit factor, it comprises the variation between experimental units with same level of A, which means that the experimental error that affects the effects of treatment factor A comes exclusively from stratum B. ii) If B is nested in a unit factor D, there are two possibilities: if the size of the unit factor B equals the number of treatments and this unit factor includes all treatments, the experimental error that affects effects of A comprises the variation among the levels of B within the levels of D; if the size of B is less than the number of treatments, that experimental error includes also variation between levels of D.

b) To each level of the experimental factor A corresponds a level of unit factor B, that is, there is a one-to-one correspondence between the levels of these two factors, which means that A and B are equivalent factors and is symbolized by B≡A. Therefore, the effects of A and B are completely confounded. In this situation, the stratum corresponding to unit factor B does not have pure experimental error as one of its components. The equivalence of an experimental factor and a unit factor occurs in the following circumstances: i) The experimental factor A is an intrinsic factor. Then, valid inferences about the effects of this experimental factor cannot be derived. ii) The experimental factor A is a treatment factor with a single repetition of each of its levels. In this situation, inferences about the treatment factor cannot be derived, except in experiments with factorial condition structures in which high order interactions can be assumed non-existent so that their components can be attributed to experimental error. This assumption can be tenable under stable environmental and management conditions, as can occur in industry.

## 3. Principles of the Experiment Design

The desirable properties of inferences about the effects of treatment factors require that the experimental plan ensures an experiment design that is consistent with the research objectives and properly satisfies important properties. These properties or *principles of the experiment design* are repetition, local control, randomization, orthogonality, balance, confounding and efficiency. The first three principles – repetition, randomization and local control, usually called *basic principles of the experiment design*, were laid down by Fisher [1], in his first formal exposition of experiment design. Repetition provides estimates of the experimental errors that affects effects of treatment factors, local control allows the reduction of these estimates and randomization, their unbiasedness. Orthogonality, balance and confounding were discussed by Yates [3,4,5]. With orthogonality, the effects of an experimental factor are restricted to a stratum of the experiment. Orthogonality is a property satisfied by the treatment factor A and unit factor B in situation a-i, and can be applied in a-ii when the number of treatments equals the number of levels of the unit factor B within each level of unit factor D. In the absence of orthogonality, the effect of an experimental factor can be expanded to a higher-level stratum. For experiments in which orthogonality is not convenient or proper, the properties of balance and confounding are useful. Balance and confounding are used in situation a-ii when the number of levels of treatment factor A is larger than the number of levels of the unit factor B, respectively when A is a simple or complex factor. Efficiency is a property that refers to the precision of a design compared to alternative designs in achieving the same objective with the same experimental material.

### 3.1. Repetition

Inferences about an effect of an experimental factor require an estimate of the error affecting it. This estimate comes from experimental units with the same level of this factor. Thus, obtaining this estimate requires at least two experimental units with the same level of this factor.

*- The experimental unit with a level of an experimental factor is a **repetition** of this level. The number of experimental units with a level of an experimental factor is the **number of repetitions** of this factor level.*

Repetition is a property that characterizes the treatment factor. In experiments with many treatment factors, in some circumstances where higher order interactions are known to be non-existent, the absence of repetition can be circumvented. However, the absence of repetition makes inferences about effects of intrinsic factor impossible.

The experimental unit is the unit of information of the experimental error that affects the effects of a treatment factor. Thus, the correct identification of the experimental units of treatment factors and, therefore, of the repetitions of their levels is essential in experiments.

Sometimes values of a response variable observed in two or more fractions or successive instants in an experimental unit are incorrectly considered to come from several repetitions of the experimental condition in this experimental unit. These observed values are *multiple observations* or *repeated observations* in the experimental unit; they are not repetitions of the experimental condition. The following examples are illustrative.

**Example 1**. In an experiment to investigate the effect of diet supplementation after weaning male lambs of a breed with two treatments: with supplementation and without supplementation, 32 animals are allocated to each of two fields. Supplementation is assigned to the animals in one of the fields, and the animals in the other field remain without supplementation. The measurements of response variables related to the performance of the animals (body weight at slaughter, for example) are performed individually in each of the 64 animals. Thus, the 32 animals in one field are conditioned to receive the same treatment. Therefore, the experimental unit for the experimental factor supplementation is a field with 32 animals; each animal is a unit of observation. Therefore, this design includes only one repetition for each of the two treatments.

The variation between animals within fields underestimates the variance of the error that affects differences between treatments assigned to different fields and implies biased inferences. In fact, in the example, the difference between the effects of treatments with supplementation and without supplementation comes from the means observed in the two fields, which expresses effects of animals, fields and treatments, if any, while the variation between animals within fields expresses only animal effects. A valid (unbiased) error for inferences about the effects of these two treatments must have the same composition as the error that affects effects of treatments, excluding the effect of treatments, that is, effects of animals and fields. This error must come from the variation between fields with the same treatment, which requires more than one repetition of treatments.

**Example 2**. Suppose the plan of the experiment considered in Example 1 is reformulated so that the 64 animals are allocated in 16 pens, each with four animals, and each of the two treatments is randomly assigned to eight pens. As the treatments are assigned to the pens so that each pen receives a treatment independently of the other pens, now the experimental unit is the pen with four animals. Therefore, this design provides eight repetitions for each of the two treatments. With this unit structure, the error that affects the effects of treatments is provided by the variation between pens with the same treatment.

These examples highlight the importance of distinguishing between experimental unit and observation unit, or between repetition and multiple observation, since the estimation of the variance of the observation error, obtained from multiple observations in the experimental units, generally underestimates the variance of the error that affects effects of treatments, which is provided by experimental units or repetitions.

**Example 3**. An experiment is planned to verify the effectiveness of the control of giberela in wheat crops with fungicide. As the fungicide effect may depend on the cultivar's susceptibility to giberela, alternative cultivars are considered. Then, two treatment factors are defined: fungicide and cultivar, respectively with four and three levels in the target population and the sample. The experiment is carried out in one place, in one year, on a ground that will be divided into 48 plots. These plots will be randomly assigned to the 12 combinations of the fungicide and cultivar levels so that each plot is associated with one of these combinations and each combination results in four plots. At the maturation stage, each plot will be harvested to determine the grain yield, the hectoliter weight and the 1000-grain weight. Therefore, plot is the experimental unit of both treatment factors fungicide and cultivar, and the observation unit of the important response variables. Thus, the experiment comprises two treatment factors: fungicide and cultivar with crossed relation, and one unit factor: plot. As the levels of the treatment factor fungicide∧cultivar will be assigned to four plots, each fungicide will be present in 12 plots and each cultivar, in 16 plots. Therefore, this design defines 12, 16 and 4 repetitions for the levels of the fungicide, cultivar and fungicide∧cultivar factors, respectively.

Besides to provide an estimate of the experimental error that affects treatment effects, repetition has other important functions: a) control the error variance and improve the precision of inferences about treatment effects; b) provide a better representation of the target population and improve the scope of the inferences from the experiment, c) reduce the bias that may result from discrepant observations due to unforeseen accidents, and d) allow verification of the reproducibility of results.

Repetition is particularly important for increasing the precision of estimates of treatment means and comparisons of these means. This property can be observed by the expression of the estimate of the variance of the estimate of the mean of a treatment: $\widehat{Var}(\bar{y}_i) = s^2/r$, where r is the number of repetitions of the treatment i and $s^2$ is the estimate of the variance from experimental error.

In many experiments, repetition is a means of obtaining a better representation of the target population by the experimental material. This is the case, for example, of agricultural experiments with the purpose of recommending technologies to farmers in a region. In these experiments, the treatments should be evaluated under the variation of environmental conditions present in the region, which is provided by repeating the experiment in several places that represent the amplitude of the variation of soil and climate conditions of the region, and for several years so that the annual variations of the climate can be manifested. This means repetitions in space and time. The same principle should be used in some experiments under apparently controlled conditions. For example, it may be convenient for a laboratory or greenhouse experiment to be repeated in several laboratories or greenhouses to determine whether the effects of treatments are repeated under the various conditions that may occur in different facilities and over time. Repetition in space and time also implies an increase in the number of treatment repetitions, which contributes to increasing the precision of estimates of treatment means.

## 3.2. Local control

*- Local control consists of classifying observation units into groups according to the levels of one or more extraneous characteristics and considering this classification in the assignment of the levels of treatment factors to the experimental units so that the variation between these groups due to these extraneous characteristics does not affect relevant effects of these treatment factors and is separated from the variation due to experimental error that affects these effects.*

If the experimental material is heterogeneous, the researcher can leave among the groups of units constituted by the local control a considerable proportion of the extraneous variation. Efficient local control allows greater precision of inferences about effects of treatment factors; so, more sensitivity of the experiment to detect differences in treatment effects. This control allows that, even with appropriately heterogeneous experimental material to achieve the

representation of the target population, the experiment can be sufficiently sensitive to detect important effects of treatment factors. Thus, the skillful exercise of local control is crucial for the construction of an efficient experiment design, that is, a design whose variance of the experimental error that affects important effects of treatment factors is small.

In the simplest situation of local control, observation units are the elementary experimental units and are classified into blocks of units according to the levels of a relevant extraneous characteristic. Then, the complete set of the treatments is randomly assigned to the experimental units of each block. With this experimental design, the relevant treatment effects are not confounded with block effects. Illustrations of this block design are given by the following two examples.

**Example 4**. If the experiment to investigate the effect of supplementation of the lamb diet considered in Example 2 is carried out on flat ground, pastures in nearby pens can be expected to be more homogeneous than pastures in distant pens, because of the usual tendency of variability of soil characteristics to increase with distance. In these circumstances, it may be convenient to perform local control of these characteristics with the classification of the 16 pens into eight blocks of two contiguous pens, each pen with four animals. Then, the two pens of each block are randomly assigned to the two treatments, separately and independently for each block.

With local control performed by this unit structure, the variation between the blocks due to extraneous characteristics is separated from the experimental error that affects the effects of the treatments. If this extraneous variation is greater than the number of independent units of information (degrees of freedom) that correspond to it, the precision of inferences about treatment effects is increased in relation to the precision that would be achieved by a structure without local control for the same experimental material.

This experiment design comprises eight replications of each treatment, provided by the eight blocks. The expected heterogeneity of pastures might also be controlled by the formation of four blocks of four experimental units (pens), with two replicates of the treatments per block. This design would provide four repetitions of each treatment by the four blocks and two replications by each block, also comprising eight repetitions of each treatment. This alternative design could be more convenient because it involves less loss of degrees of freedom to estimate the experimental error variance, which would possibly be considerably smaller than the fraction of the experimental error separated to constitute the stratum of the experimental error corresponding to blocks.

**Example 5**. If the experiment on the effectiveness of the control of giberela in wheat crops (Example 3) is carried out in a place of heterogeneous soil characteristics, it is recommended local control with the formation of four blocks of 12 homogeneous plots. Then, the 12 plots of each block are randomly assigned to the 12 combinations of the four levels of fungicide and the three cultivars.

Local control can be used in experiments with more than one formation of experimental units.

**Example 6**. Suppose in the experiment considered in Example 5 the treatment factor fungicide is more important than the treatment factor cultivar. Then, to allocate more precision for the fungicide factor, in each block, the twelve plots are classified into three sets of four nearby plots, and these three sets of plots are randomly assigned to the three cultivars and the four plots of each of these sets to the four fungicides. Therefore, the experimental unit of the treatment factor fungicide is the plot and of the treatment factor cultivar, the set of three plots. Thus, the unit structure comprises two formations of experimental units, one formation for each of the two treatment factors. This design assigns four repetitions for each cultivar and 12 repetitions for each fungicide.

Local control with two or more classifications of the experimental units may be necessary for efficient control of the heterogeneity of the extraneous characteristics of the experimental material. Example 7 provides an illustration.

**Example 7**. In a nutrition experiment with Holstein dairy cows, four different food supplements are considered. Four animals are available at the same lactation stage and the effects of the supplements are short-term, so that it is expected that no residual effect of the supplement administered in one short interval manifest in the next interval. Then, the four supplements will be administered to the four animals at each of four successive intervals of three weeks. The relevant extraneous characteristics are the individual characteristics of the four animals and the characteristics of the environment over the four intervals. In these circumstances, it can be expected that efficient local control will be achieved by assigning the supplements to the animals so that each of the animals receives all supplements and each supplement appears in each interval. Thus, the experimental unit of the treatment factor supplement is an animal in an interval. This unit structure with double local control implies separating from the experimental error that affects the effects of treatments the variation due to extraneous characteristics among animals and among lactation intervals.

The most effective local control encompasses the important extraneous characteristics with the fewest classifications of the experimental units. This can be achieved by the association of the classifications of the experimental units according to two or more relevant extraneous characteristics for the constitution of a single classification for local control and adopting this same classification in the order of execution of experimental techniques. Suppose that in the experiment of Example 4 in which local control of the characteristics of the environment is carried out by classifying the 16 pens in eight blocks of two contiguous pens of four animals, the variation of weaning dates of the 64 animals is considerable. In these circumstances, it may also be appropriate to classify the 64 animals into eight blocks of eight animals with weaning at close dates and then associate these blocks of animals with the blocks of pens, constituting eight blocks of two contiguous pens, each pen with four animals. Then, the two pens of each block are randomly assigned to the two treatments, separately and independently for each block. This same classification of the experimental units can be considered in the implementation of management techniques that may imply heterogeneity of the experimental material, executing them block by block. In the experiment of Example 5, the same classification of the plots according to proximity constituted for local control of soil characteristics can be used to control relevant extraneous characteristics related to cultivation techniques, such as planting, applications of fungicide, insecticide and herbicide, and harvesting.

### 3.3. Randomization

- ***Randomization*** *is the random association of extraneous characteristics with treatment factors.*

Randomization is performed by random association of experimental units to treatments.

Repetition provides an estimate of the variance of the experimental error affecting the effects of treatment factors; local control allows the reduction of this estimate. However, these two principles do not ensure its validity.

**Example 8**. In the experiment on the supplementation of the diet of male lambs with a block design with one or two replications per block (Example 4), the local control controls the variation of the extraneous characteristics related to soil between the blocks. However, other sources of extraneous variation remain uncontrolled. Particularly, if the treatments are allocated to the pens in the same positions within each block, the difference of the average weights of the pens with the two treatments will be an estimate of the difference in the effects of the treatments plus the difference attributable to the position of the treatments with and without supplementation within the blocks. To illustrate the difficulty that this design may imply for inferences about the effects of supplementation, suppose that the treatment with supplementation is allocated in the pens to the right of each block and the treatment without supplementation on the left and that the pasture in the pens to the right of the blocks is favored by soil characteristics. In these circumstances, if the experiment reveals that the average weight of animals with supplementation is higher than that of animals without supplementation, the researcher will not know what to conclude.

The flaw of this design is the bias that can result from the gradient of soil characteristics along the blocks. Suppose that, instead of systematically allocating the two treatments to pens in the same positions, the researcher determines the allocation of the two treatments to the pens of each block by separate and independent randomization for each block. Thus, in each block the two treatments have the same chance of being allocated to any of the pens and, consequently, the same chance of being favored or harmed by position within the block.

This example illustrates trends in experimental material that can be expected when designing the experiment. In experiments where the researcher has less knowledge of the variability of the experimental material, unexpected trends can be revealed. To avoid bias of these origin, the researcher needs some means or resource to ensure that treatments are not systematically favored or harmed by some source of extraneous variation, known or unknown. The resource is randomization. Of course, the result of any specific randomization can favor or disfavor treatments. The basis of the principle of randomization is that over the course of repetitions of experiments the confounding of effects of extraneous characteristics with effects of treatments becomes equally influential for all treatments.

Randomization is restricted by local control and formations of experimental units. In experiments with one formation of experimental units and without local control, randomization is performed without any restrictions. Thus, in the experiment in Example 2, randomization must be performed to assign the eight pens to each of the two treatments, with equal chances of association of pens and treatments. In experiments with simple local control and a unique formation of experimental units, randomization must be performed within each block, separately and independently for each block. Thus, in the experiment considered in Example 4, the pens of each block must be randomly assigned to the two treatments; in the experiment of Example 5, the plots of each block must be assigned to the 12 treatments. In experiments with more than one formation of experimental units, as in Example 6, randomization must be performed

separately for each factor in the corresponding formation of experimental units. In experiments with double local control (Example 7), randomization must be carried out so that the allocation of treatments to the experimental units is in accordance with the defined experiment design. To achieve this purpose, randomization can be performed from standard Latin square plans, available in reference books (Cochran & Cox [13], Steel & Torrie [14], Mead [15], Kuhel [16]).

In a formation for two or more treatment factors, randomization should be performed for the complex factor generated by the largest number of these simple factors. This randomization implies randomization of the simple and complex factors that generate it. On the other hand, the separate randomization of factors in different formations of experimental units implies randomization of the complex factors they form. In the experiment in Example 5, randomization is performed for the factor fungicide^cultivar, which implies the randomization of each of the fungicide and cultivar factors; with the design in Example 6, the separate randomizations of fungicide and cultivar factors implies randomization of factor fungicide^cultivar.

Randomization of a treatment factor must be carried out through a draw that guarantees that all experimental units of this factor have the same chance of being assigned to any of its levels, according to the defined experiment design. Thus, it depends on the structure of the conditions, the structure of the units and the defined structure of the experiment. Randomization will be considered in more detail in the Sections 3.4-3.6 for designs that adopt the principle of orthogonality, balance, or confounding.

Randomization must also be considered in the execution of experimental techniques that can give rise to extraneous characteristics whose effects may be considerable confounded with effects of treatment factors. This randomization consists in performing the technique in the experimental units in a random order, with the same restrictions indicated for randomization of treatments.

## 3.4. Orthogonality

Orthogonality is an important property of the experiment design, as it allows inferences about the effects of treatment factors separately and independently from the effects of unit factors and other experimental factors. The term "orthogonal design" has been used in distinct circumstances and with several meanings (Preece [17], Federer [6]). Here, the abbreviated version of the definition by Bailey [18] suggested by Silva [10] is adopted, with the approach that considers the design of the experiment as the association between the condition structure, defined by the objectives of the experiment, and the unit structure, which expresses relevant classifications of the observation units.

Orthogonality of experiment design requires orthogonality of the condition structure and the unit structure, which demands orthogonality of the experimental and unit factors.

**Orthogonality of factors**

*- Factors A and B are **orthogonal** if:*

*• A and B have nested (hierarchical) relation; or*

*• A and B have crossed relation and the parts (sets of conditions or observation units) corresponding to the levels of $A \wedge B$ have sizes proportional to the product of the sizes of the correspondent parts of A and B; that is, $n_{ab} = (n_{a.} \times n_{.b})/n_{..}$, for all levels of factor $A \wedge B$, where $n_{a.}$, $n_{.b}$ and $n_{..}$ are the sizes of the a-th part of A, the b-th part of B and of the total of the parts of $A \wedge B$, respectively.* This condition is satisfied particularly for the A^B part sizes illustrated in Figure 1.

| A | B | | | |
|---|---|---|---|---|
| | $b_1$ | $b_2$ | $b_3$ | $b_4$ |
| $a_1$ | 4 | 4 | 4 | 4 |
| $a_2$ | 4 | 4 | 4 | 4 |

a)

| A | B | | | |
|---|---|---|---|---|
| | $b_1$ | $b_2$ | $b_3$ | $b_4$ |
| $a_1$ | 4 | 4 | 4 | 4 |
| $a_2$ | 2 | 2 | 2 | 2 |

b)

| A | B | | | |
|---|---|---|---|---|
| | $b_1$ | $b_2$ | $b_3$ | $b_4$ |
| $a_1$ | 4 | 5 | 3 | 4 |
| $a_2$ | 4 | 5 | 3 | 4 |

c)

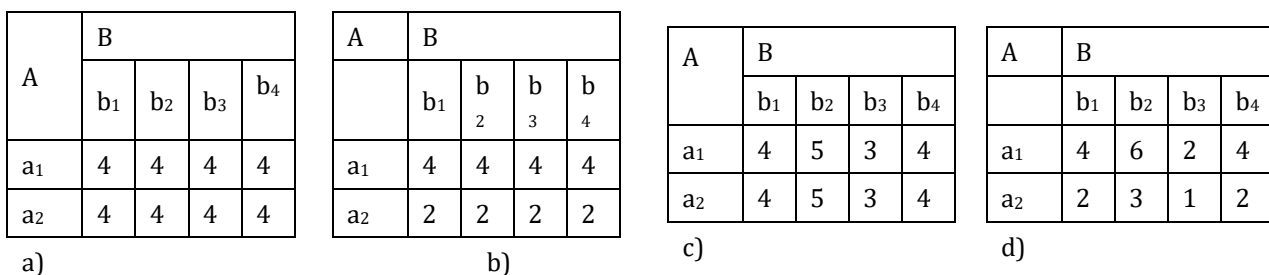| A | B | | | |
|---|---|---|---|---|
| | $b_1$ | $b_2$ | $b_3$ | $b_4$ |
| $a_1$ | 4 | 6 | 2 | 4 |
| $a_2$ | 2 | 3 | 1 | 2 |

d)

**Figure 1** Size of parts of A^B that satisfy the orthogonality condition of factors A and B.

### Orthogonality of the structure of conditions and the structure of units

*- The structure of conditions is* **orthogonal** *if:*

*a) The special factor $M_C$ that aggregates the experimental conditions belongs to the structure,*

*b) experimental factors are orthogonal.*

*- The structure of units is* **orthogonal** *if:*

*a) The special factor $M_U$ that aggregates the observation units belongs to the structure,*

*b) unit factors are uniform,*

*c) unit factors are orthogonal.*

### Orthogonality of the experiment design

*- The experiment structure is* **orthogonal** *if:*

*a) The M factor, which results from the association of special factors $M_C$ and $M_U$, belongs to the structure,*

*b) the condition structure is orthogonal,*

*c) the unit structure is orthogonal,*

*d) the experimental factors remain orthogonal,*

*e) the experimental factors are orthogonal to the unit factors.*

Experimental factors which are orthogonal in the condition structure remain orthogonal in the experiment structure when they have equal replications or a nested structure.

Examples 2 to 8 in the previous Sections illustrate experiments with orthogonal design. Example 9 considers again the experiment in Example 4 to illustrate the procedure for checking orthogonality.

**Example 9** – In the experiment with lambs considered in Example 4, the two levels of treatment factor A - supplementation: $a_1$ - without and $a_2$ - with, are assigned to 64 pens grouped into four blocks of four pens each with four animals, as represented by the sketch in Figure 2, where $b_1$, $b_2$, $b_3$ and $b_4$ are the levels of the block unit factor.

| $b_1$ | |
|---|---|
| $a_2$ ○○○○ | $a_1$ ○○○○ |
| $a_1$ ○○○○ | $a_2$ ○○○○ |

| $b_2$ | |
|---|---|
| $a_2$ ○○○○ | $a_2$ ○○○○ |
| $a_1$ ○○○○ | $a_1$ ○○○○ |

| $b_3$ | |
|---|---|
| $a_1$ ○○○○ | $a_2$ ○○○○ |
| $a_1$ ○○○○ | $a_2$ ○○○○ |

| $b_4$ | |
|---|---|
| $a_1$ ○○○○ | $a_1$ ○○○○ |
| $a_2$ ○○○○ | $a_2$ ○○○○ |

**Figure 2** Sketch of the experiment in Example 9 with experimental factor A with two levels $a_1$ and $a_2$ in four blocks of four pens, each with four animals (○).

This experiment considers one treatment factor: A - supplementation and comprises three unit factors: B – block, P – pen and E - animal with nested relation. Therefore, the condition structure and the unit structure are both orthogonal. The structure of the experiment, generated by the association of treatment factor A with unit factor P, constitutes the stratum of the experiment P≡A⋏B and cross structure A∗B. It is verified next that the treatment factor A and the unit factor B are orthogonal.

The repetition numbers of factor A levels in each of the four blocks are shown in Table 1.

**Table 1** Number of repetitions of the two levels of experimental factor A in the three blocks of the experiment in Example 9.

| A: Supplementation | B: Block | | | | Sum |
|---|---|---|---|---|---|
| | $b_1$ | $b_2$ | $b_3$ | $b_4$ | |
| $a_1$ | 8 | 8 | 8 | 8 | 32 |
| $a_2$ | 8 | 8 | 8 | 8 | 32 |
| Sum | 16 | 16 | 16 | 16 | 64 |

For the combination of levels $a_1b_1$: $n_{11}=8$, $n_{1.}=32$, $n_{.1}=16$, $n_{..}=64$. Then, $(n_{1.} \times n_{.1})/n_{..} = (32 \times 16)/64 = 8$. This same result is obtained for the other combinations of levels of A and B. Thus, the orthogonality condition is satisfied, which implies that treatment factor A and unit factor B are orthogonal.

As the condition structure and the unit structure are both orthogonal, the treatment factor A and unit factor B are orthogonal, and the experiment comprises only one treatment factor: A, the structure of the experiment is orthogonal.

**Example 10**. Nitrogen soil fertilization experiment for irrigated rice cultivation with two treatment factors: nitrogen, with three levels - 0, 60 and 120 kg/ha, and time of application, with two levels - planting and coverage at 30 days. The experiment is carried out in four blocks of six plots, and the plots of each block are randomly assigned to six combinations of the levels of nitrogen and coverage factors, separated and independently. The response variables are measures of characteristics of the grain collected in each plot. Therefore, plot is the experimental unit and the observation unit.

The combinations of the zero level of the nitrogen factor with the planting and coverage levels of the time factor are not distinguished; therefore, they constitute the same treatment - without nitrogen. Thus, the 3x2 = 6 combinations of the levels of nitrogen and time constitute, in fact, five treatments of a crossed structure of two factors - nitrogen with two levels: 60 and 120 kg/ha and time with two levels: planting and coverage, extended by an additional treatment: no nitrogen (Figure 3). Thus, the six treatments can be expressed as a mixed factorial structure of three factors: fertilization (F) with two levels - without and with, nitrogen (N) with two levels - 60 and 120 kg/ha, and time (T) with two levels - planting and coverage. Factors N and T are crossed, and both are nested in factor F, constituting a mixed factorial structure symbolized by $F/(N*T)$. The crossed factors N and T are orthogonal, and these two factors are both nested in factor F, which mean that are orthogonal to F. Therefore, the condition structure is orthogonal. On the other hand, the unit structure comprises two unit factors: plot and block with nested relation. Thus, the unit structure is also orthogonal. Since the experimental factors N and T have levels with equal repetitions, they remain orthogonal in the experiment structure. Therefore, this structure is orthogonal.

| T | N | | |
|---|---|---|---|
| | 0 | 60 | 120 |
| Planting | 8 | 4 | 4 |
| Coverage | | 4 | 4 |

**Figure 3** Tabular representation of the crossed structure of treatment factors nitrogen (N) and time (T), extended by the additional treatment no nitrogen.

Randomization for orthogonal designs was already illustrated by Examples in Sections 3.3. In general, with orthogonal designs, randomization is performed in formations of experimental units, within each stratum of the experiment. In each stratum, randomization assigns the experimental units to the levels of the corresponding treatment factor, simple or complex. In case of a complex treatment factor, randomization should be performed for the complex factor generated by the largest number of simple factors. Thus, in the experiment in Example 5, randomization is performed for the factor fungicide^cultivar; in the design in Example 6, randomization is carried out separately for the fungicide and cultivar factors, located in two distinct strata.

A consequence of orthogonality is the simplicity of the calculations involved in statistical analysis procedures and the ease of interpretation of its results. The simplicity of calculations was very important before the advent of electronic

computing, but it has become greatly reduced with the availability of equipment, programs and systems that automate the execution of data analysis. Consequently, this convenience of using orthogonal designs decreased.

Orthogonality is a highly desirable property. However, non-orthogonality is not a serious defect of a design, provided that relevant effects of treatment factors are not affected by experimental error and effects of other experimental factors. This property is provided by the principles of balance and confounding.

## 3.5. Balance

Balanced designs may be suitable for experiments with unifactorial treatment structures in situations where using orthogonal designs is impractical or inconvenient. For example, it may not be possible to achieve local control that classifies experimental units into blocks of sufficiently homogeneous units each of which including the complete set of treatments. This situation is usual in experiments with a high number of treatments and can occur even in experiments with a small number of treatments when there are restrictions on the experimental material to form complete blocks homogeneous regarding to extraneous characteristics. In these circumstances, it may be necessary to resort to design with incomplete blocks, that is, blocks smaller than the number of treatments. With these designs, differences in the effects of treatments in different blocks are more affected by extraneous variation than differences in the same block. This would imply these differences to be estimated with different precisions. This inconvenience can be avoided by constructing designs in which each treatment is present with each of the other treatments in the same number of blocks. Thus, the effects of differences between blocks on simple comparisons of treatments are balanced, which guarantees equal precision for inferences about all differences between two treatments.

This concept of design balance is expressed as follows:

*- An incomplete block design is* **balanced** *if each two treatments appear together in a block the same number of times. This property implies equal precision for all simple comparisons of treatments, that is, for all comparisons between two treatments.*

Balanced designs were introduced by Yates [4] for experiments in plant breeding and selection where it is desired to make all comparisons of pairs of treatments with the same precision. The design can be arranged in a layout of incomplete blocks or latin squares and can be balanced or partially balanced.

Example 11 illustrates the use of balanced incomplete block design in an experiment with one experimental factor.

**Example 11**. An experiment on the effect of temperature on tomato seed germination with the consideration of four temperatures: 1 - 40°C, 2 - 45°C, 3 - 30°C e 4 - 35°C must be conducted in three chambers A, B and C with controlled temperature that will have to be used repeatedly to build up the desired number of repetitions. As the variation between stages of chamber use is a source of relevant extraneous variation, appropriate local control is exercised to constitute stage as a block of three experimental units. Therefore, only three of the four temperatures can be assigned to each block. This characterizes a balanced incomplete block design with four treatments (temperatures) in four blocks (stages) of three experiments units (chambers), in which each pair of temperatures appears twice in the same block. Figure 4 presents a sketch of this design.

| Block | Chamber | | |
|---|---|---|---|
| | A | B | C |
| (1) | 1 | 4 | 3 |
| (2) | 4 | 3 | 2 |
| (3) | 3 | 2 | 1 |
| (4) | 2 | 1 | 4 |

**Figure 4** Balanced incomplete block design for an experiment with four treatments (1, 2, 3 and 4) in four blocks of three experimental units.

In this design, blocks cannot be grouped into separate repetitions. In general, the blocks of an incomplete block design can be grouped into separate repetitions when the number of blocks is divisible by the number of repetitions. This

property is important in experiments with large number of treatments as it provides additional local control through larger complete blocks made up by the repetitions.

Example 12 shows a design with nine treatments in 12 incomplete blocks grouped into four separate repetitions in which each pair of treatments appears once in some block. In this design the number of blocks (12) is divisible by the number of repetitions (4).

**Example 12**. An experiment to compare nine wheat cultivars will be conducted in a design with four repetitions each with three incomplete blocks of three experimental units, the sketch of which is shown in Figure 5.

| Block | Rep. 1 | | | Block | Rep. 2 | | | Block | Rep. 3 | | | Block | Rep. 4 | | |
|-------|--------|---|---|-------|--------|---|---|-------|--------|---|---|-------|--------|---|---|
| (1) | 1 | 2 | 3 | (4) | 1 | 4 | 7 | (7) | 1 | 5 | 9 | (10) | 1 | 8 | 6 |
| (2) | 4 | 5 | 6 | (5) | 2 | 5 | 8 | (8) | 7 | 2 | 6 | (11) | 4 | 2 | 9 |
| (3) | 7 | 8 | 9 | (6) | 3 | 6 | 9 | (9) | 4 | 8 | 3 | (12) | 7 | 5 | 3 |

**Figure 5** Balanced incomplete block design of an experiment with nine treatments (1, 2, …, 9) in 12 blocks of three experimental units grouped into four repetitions.

In this design, each pair of treatments appears once within some block. The blocks can be grouped into separate repetitions, since 12 is divisible by 4. This design belongs to the class of *lattice designs*, since each repetition has the form of a square lattice with the number of treatments being an exact square and the number of units per block its square root.

For some numbers of treatments and experimental units per block, the two previous forms of design can be laid out in a form of latin square layout to allow the elimination of variation arising from two sources.

**Example 13**. Design for an experiment with nine treatments and four repetitions with the 36 experimental units laid out with double grouping in each repetition, forming blocks in two directions, named rows and columns. The layout is shown in Figure 6.

| Rep I | | | | Rep II | | | | Rep III | | | | Rep IV | | | |
|-------|--------|---|---|--------|--------|---|---|---------|--------|---|---|--------|--------|---|---|
| Row | Column | | | Row | Column | | | Row | Column | | | Row | Column | | |
| (1) | 1 | 2 | 3 | (4) | 1 | 4 | 7 | (7) | 1 | 6 | 8 | (10) | 1 | 9 | 5 |
| (2) | 4 | 5 | 6 | (5) | 2 | 5 | 8 | (8) | 9 | 2 | 4 | (11) | 6 | 2 | 7 |
| (3) | 7 | 8 | 9 | (6) | 3 | 6 | 9 | (9) | 5 | 7 | 3 | (12) | 8 | 4 | 3 |

**Figure 6** Balanced design for an experiment with nine treatments (1, 2, …, 9) in four lattice squares.

It may be verified that each pair of treatment appear once in the same row and once in the same column. This implies equal precision for all comparisons between two treatments. This design is known as *lattice square*.

A balanced incomplete block design can be constructed for any number of treatments and any number of units per block. These two definitions fix the minimum number of repetitions and, consequently, the size of the experiment. Usually, this size is too large. Plans for small numbers of treatments are available in Cochran & Cox [13], Fisher & Yates [19] and Kuhel [16].

In many circumstances, the researcher must resort to a design that lack the complete symmetry of a balanced design. Examples of these *partially balanced designs* are parts of lattice designs, illustrated in Example 12.

Designs with the two first replications of a balanced lattice are called *simple lattice* and with the first three replications *triple lattice*. These designs require the number of treatments to be a perfect square.

Another set of designs useful for very large number of treatments are the *cubic lattices*. In these designs the number of treatments is a cube of the number of units per block, which implies a drastic reduction in the size of the blocks. The number of repetitions is three or a multiple of three.

Partially balance designs lack the important property of equal precision for all simple comparisons of treatments. This means that some treatment pairs are compared more precisely than others. These differences in precision are more pronounced the further the design deviates from the symmetry of the balanced design.

A detailed description and an extensive catalogue of plans of incomplete balanced and partially balanced designs are given in Cochran and Cox [13].

Randomization for balanced and partially balanced designs may be performed from the plans available in Cochran & Cox [13], Fisher & Yates [19] and Kuhel [16]. In general, it consists of two steps: 1) random assignment of the experimental units of each incomplete block to the treatments included in the block, and 2) randomization of the incomplete blocks within replications, if the incomplete blocks form larger complete blocks.

### 3.6. Confounding

Confounding of characteristics is common in research, particularly in experimental research. A concern of the researcher is to make inferences about the effects of treatment factors free from confounding with effects of relevant extraneous characteristics. The principles of local control and randomization are intended to reduce this confounding and avoid bias that may arise for inferences.

In some experiments, however, the confounding of effects of treatment factors with effects of unit factors may be inevitable and become a resource for achieving greater precision for inferences about the most relevant effects of treatment factors at the cost of sacrificing information about less important effects. Thus, in experiments with two or more treatment factors with a high number of combinations of levels, or with constrains or considerably heterogeneity of experimental material, it may be convenient to sacrifice inferences about effects of some irrelevant interactions and to adopt a design with incomplete blocks. With this design, the effects of these interactions are confounded with effects of incomplete blocks, in favor of greater precision for the inferences about the main effects and the relevant interactions.

**Example 2**. Consider an experiment with three treatment factors A, B and C each with two levels, with three repetitions of each of the eight combinations of levels, in which constraints of the experimental material only allow the formation of blocks of four plots. In these circumstances, the researcher is willing to sacrifice inferences about the interaction of the three factors: AxBxC.

To ease understanding, the notation of a combination of the levels of the factors is adopted that symbolizes the levels of the factors present in the combination by the lowercase forms of the letters denoting the factors, and the levels of the factors absent by the absence of the letters that denote them. So, a combination of levels is denoted by juxtaposing the notations of the levels present in the combination. Thus, for example, ab denotes the combination of levels in which the levels of factors A and B are present, and the level of factor C is absent. The combination in which the levels of the three factors are absent is denoted by (1).

With this notation, the contrasts between the eight combinations of the levels of factors A, B and C that express the main effects and interactions of these factors are presented in Table 2. The main effect of a factor is the contrast between the combinations with the presence of the factor and the combinations with its absence, indicated by the + and - signs, respectively. The factor interactions are contrasts between combinations with the + and - signs defined by the signs of the corresponding factors, determined by the rule of signs in multiplication, that is, combination of levels with an even number of signs: +; with odd number of signs: –.

**Table 2** Main effects and interactions of A, B and C factors expressed as contrasts between the eight combinations of the levels of these factors.

| Effect | Combination | | | | | | | |
|--------|-----|---|---|---|----|----|----|-----|
|        | (1) | a | b | c | ab | ac | bc | abc |
| A      | -   | + | - | - | +  | +  | -  | +   |
| B      | -   | - | + | - | +  | -  | +  | +   |
| C      | -   | - | - | + | -  | +  | +  | +   |
| AxB    | +   | - | - | + | +  | -  | -  | +   |

| AxC | + | - | + | - | - | + | - | + |
|------|---|---|---|---|---|---|---|---|
| BxC | + | + | - | - | - | - | + | + |
| AxBxC | - | + | + | + | - | - | - | + |

Confounding of the AxBxC interaction is achieved by assigning the eight combinations of the levels of factors A, B and C to two incomplete blocks of four experimental units, combinations with + sign to one block and those with – sign to the other block, as shown in the sketch of Figure 7.

Repetition:  1  2  3

Block:  1  2  3  4  5  6

| a | (1) |
|---|-----|
| b | ab |
| c | ac |
| abc | bc |

| a | (1) |
|---|-----|
| b | ab |
| c | ac |
| abc | bc |

| a | (1) |
|---|-----|
| b | ab |
| c | ac |
| abc | bc |

**Figure 7** Sketch (before randomization) of a design with three treatment factors A, B and C with three replications each, consisting of two blocks of four experimental units with confounding of the effect of the AxBxC interaction with block effects (Example 14).

Example 14 illustrates confounding of the interaction of the three factors A, B, and C in all three repetitions. In these circumstances the effect of the AxBxC interaction is completely confounded. In some situations, it may be convenient to obtain partial information about an interaction effect by confounding it with block effects in some of the repetitions and confounding the effects of other interactions in other repetitions.

**Example 3**. Suppose the researcher prefers to obtain partial information regarding the AxBxC interaction by confounding it only in one of the three repetitions, namely the first, at the expense of the partial confounding of the interactions of two factors AxB and AxC respectively in repetitions 2 and 3. So, the appropriate sketch (before randomization) is the one presented in Figure 8.

Repetition:  1  2  3

Block:  1  2  3  4  5  6

| a | bc |
|---|-----|
| b | ac |
| c | ab |
| abc | (1) |

| (1) | a |
|-----|---|
| c | b |
| ab | ac |
| abc | bc |

| (1) | a |
|-----|---|
| b | c |
| ac | ab |
| abc | bc |

Confounded interactions:  A×B×C  A×B  A×C

**Figure 8** Sketch (before randomization) of an experiment with three experimental factors A, B and C with three replications each consisting of two incomplete blocks of four experimental units with partial confounding of the interactions AxBxC, AxB and AxC (Example 15).

Examples 14 and 15 illustrate confounding of interactions in incomplete block designs. The principle of confounding also applies to confounding of main effects in experiments with two or more treatment factors with different importance to attribute greater precision for inferences about the most important factors. The experiment on control of giberela in wheat crops in Example 6 illustrates a design with two treatment factors: fungicide and cultivar in which cultivar is assigned to large plots and fungicide to divisions of these plots (subplots). In this design, plots are incomplete blocks for cultivar factor, which imply that the effects of this treatment factor are confounded with the effects of the plot unit factor. Therefore, this design attributes larger precision for the main effect of the more important treatment factor: fungicide, which is applied to divisions of the plot (subplots).

In some experiments the confounding principle is adopted for practical convenience. As an illustration, suppose that the experiment in Example 11, which considers the effect of temperature on tomato seed storage with four temperatures, includes three cultivars, and must be conducted in four chambers with controlled temperature. For practical convenience, the levels of the temperature factor will be assigned to the chambers that will be used repeatedly over four successive periods to build up the proper number of repetitions. In each period, the four temperatures will be randomly assigned to the four chambers and the three cultivars to three positions within each chamber. So, this experiment comprises two treatment factors: temperature and cultivar, with 4 and 3 levels, respectively, and three unit factors: chamber (plot), position in the chamber (subplot) and period (block). This design attributes greater precision for inferences about the effects of treatment factor cultivar than for temperature. Thus, it would be proper if cultivar factor were more important for the purpose of the experiment than temperature. It could also be justified, on practical grounds, if both factors were equally important. However, in this experiment, the design with two formations of experimental units, corresponding to the unit factors plot and subplot, is questionable for assigning less precision to temperature, the most important factor.

## 3.7. Efficiency

The principles previously considered are properties of individual experiment designs. Efficiency is a property of a design compared to an alternative simpler design for the same objective of experiment and experimental material:

- *An experiment design is more **efficient** than another design if the precision it provides is greater than that provided by this alternative design.*

This is an important property that must be considered in many situations, particularly: a) for the decision between alternative designs in *plan*ning experiments based on the results of earlier experiments, and b) to verify the desirability of having adopted a particular design in an experiment already carried out in relation to a simpler alternative design. This second use is important in the case of experiments that are repeated over time, such as plant breeding experiments. For example, if a design with incomplete blocks is not more efficient than a design with complete blocks, there is no reason to continue using that design in the coming years.

The **relative efficiency** of a design $D_1$ in relation to an alternative $D_2$ design is expressed by the quotient of the precisions provided by *designs $D_1$ and $D_2$*, that is: $\frac{1/\sigma_1^2}{1/\sigma_2^2} = \frac{\sigma_2^2}{\sigma_1^2}$, where $\sigma_1^2$ and $\sigma_2^2$ are the population variances of the experimental errors for $D_1$ and $D_2$ designs. As these variances are unknown, one should resort to estimates and express the relative efficiency of design $D_1$ compared to design $D_2$ by $RE(D_1|D_2) = \frac{1/s_1^2}{1/s_2^2} = \frac{s_2^2}{s_1^2}$, where $s_1^2$ and $s_2^2$ are the estimates of the variances of the errors of the designs $D_1$ and $D_2$, respectively, and ?$_1$ and ?$_2$ are the degrees of freedom of these estimates. Design $D_1$ will be more efficient than design $D_2$ if $RE(D_1|D_2) > 1$.

If the degrees of freedom for the estimate of error variance $s_2^2$ is under 20, it is important to consider the loss of precision resulting from fewer degrees of freedom associated with this estimate, by multiplying the precision factor obtained by $\frac{(v_1+1)(v_2+3)}{(v_2+1)(v_1+3)}$, as suggested by Fisher [1,2], where $n_1$ and $n_2$ are the degrees of freedom associated with error for the designs $D_1$ and $D_2$, respectively (Cochran & Cox [13], Steel & Torrie [14]).

**Example 16**. The simplest situation is the relative efficiency of an experiment with a randomized complete block design $D_1$ compared to a completely randomized design $D_2$. Denoting the estimates of the variances of the error and block for design $D_1$ by $s_e^2$ and $s_b^2$, the estimate of the variance of the error for design $D_2$ with the same experimental material would be $s_2^2 = \frac{v_b s_b^2 + (v_t+v_e)s_e^2}{v_b+v_t+v_e}$, where $n_b$, $n_t$ and $n_e$ are the degrees of freedom of block, treatment and error. Therefore, the relative efficiency of design $D_1$ relative to design $D_2$ is:

$$RE\ (D_1|D_2) = \frac{1/s_1^2}{1/s_2^2} = \frac{s_2^2}{s_1^2} = \frac{v_b s_b^2 + (v_t+v_e)s_e^2}{(v_b+v_t+v_e)s_e^2} = \frac{v_b s_b^2 + (v_t+v_e)s_e^2}{v_b s_e^2 + (v_t+v_e)s_e^2}.$$

Thus, design $D_1$ will be more efficient than design $D_2$ if $s_b^2 > s_e^2$, i.e., the variance of the error between blocks is larger than the variance of the error among experimental units within blocks. This condition requires that the variation among blocks which is separated from the experimental error by the local control be larger than the corresponding degrees of freedom.

**Example 17**. Consider, now, the efficiency of a Latin square design (LSD) compared to a randomized complete block design (RCBD). Denote the estimates of the row, column and error of the LSD by $s_R^2$, $s_C^2$ and $s_e^2$. Considering row as the only block factor, the estimate of the error variance in the RCBD would be $s_2^2 = \frac{v_C s_C^2 + (v_t + v_e)s_e^2}{v_C + v_t + v_e}$, where $n_C$, $n_t$ and $n_e$ are the degrees of freedom for column, treatment and error in the latin square. Then, the relative efficiency of the Latin square design compared to the complete block design, considering the rows as the blocks, is expressed by:

$$\text{RE(LSD} - \text{column|RCBD)} = \frac{v_C s_C^2 + (v_t + v_e)s_e^2}{(v_C + v_t + v_e)s_e^2} = \frac{v_C s_C^2 + (v_t + v_e)s_e^2}{v_C s_e^2 + (v_t + v_e)s_e^2}.$$

Then, the Latin square design will be more efficient than the randomized complete block design, considering row of the Latin square as block, if the column variance estimate is greater than error variance estimate of Latin square design.

Similar results are obtained considering column of the Latin square as the only block factor of the complete block design.

**Example 17**. In a design in randomized block with two treatment factors A and B, factor A is in large plots and factor B in small plots inside the large plots. In this split plot *design*, the experimental error in the stratum of factor A is the extraneous variation that is confounded with the interaction A.C: $s_{C.A}^2$, *where C denotes the unit factor block,* and the experimental error in the stratum of factor B is a composition of the interactions $C$.B and $C$.A.B: $\frac{v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{v_{C.B} + v_{C.A.B}}$. With a randomized complete block with the same experimental material, with both treatment factors in a unique stratum, the experimental error would be a composition of the interactions $C$.A, $C$.B and $C$.A.B: $\frac{v_{C.A} s_{C.A}^2 + v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{v_{C.A} + v_{C.B} + v_{C.A.B}}$. Then, the relative efficiency of the design in randomized blocks with two formations of experimental units in the stratum of factor A compared to design in randomized complete blocks is expressed by:

$$\text{RE(SPD|RCBD in stratum of factor A)} = \frac{v_{C.A} s_{C.A}^2 + v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{(v_{C.A} + v_{C.B} + v_{C.A.B})s_{C.A}^2} = \frac{v_{C.A} s_{C.A}^2 + v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{v_{C.A} s_{C.A}^2 + (v_{C.B} + v_{C.A.B})s_{C.A}^2}.$$

In general, the error variance among experimental units of factor A within blocks ($s_{C.A}^2$) is larger than the variance among experimental units of factor B within experimental units of factor A: $\frac{v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{v_{C.B} + v_{C.A.B}}$, This implies:

$$v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2 = \frac{(v_{C.B} + v_{C.A.B})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)}{(v_{C.B} + v_{C.A.B})} < (v_{C.B} + v_{C.A.B})s_{C.A}^2. \text{ Therefore:}$$

$$\text{(SPD|RCBD in stratum of factor A)} < 1.$$

The relative efficiency of the design in randomized blocks with two formations of experimental units in the stratum of factor B compared to design in randomized complete blocks is expressed by:

$$\text{RE(SPD|RCBD in stratum of factor B)} = \frac{(v_{C.B} + v_{C.A.B})(v_{C.A} s_{C.A}^2 + v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)}{(v_{C.A} + v_{C.B} + v_{C.A.B})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)} =$$

$$= \frac{(v_{C.B} + v_{C.A.B})(v_{C.A} s_{C.A}^2) + (v_{C.B} + v_{C.A.B})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)}{(v_{C.A})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2) + (v_{C.B} + v_{C.A.B})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)} =$$

$$= \frac{(v_{C.B} + v_{C.A.B})(v_{C.A} s_{C.A}^2) + K}{v_{C.A}(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2) + K}, K = (v_{C.B} + v_{C.A.B})(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2).$$

Dividing numerator and denominator by $(v_{C.B} + v_{C.A.B})v_{C.A}$, follows:

$$\text{RE(SPD|RCBD in stratum of factor B)} = \frac{s_{C.A}^2 + K_1}{\frac{v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2}{v_{C.B} + v_{C.A.B}} + K_1}, K_1 = \frac{(v_{C.B} s_{C.B}^2 + v_{C.A.B} s_{C.A.B}^2)}{v_{C.A}}$$

$$\text{RE(SPD|RCBD in stratum of factor B)} = \frac{\text{Error variance between experimental units of factor A} + K_1}{\text{Error variance between experimental units of factor B} + K_1} > 1.$$

These results show that for an experiment with two treatment factors, the design with two formations of experimental units, one of large units and the other of subdivisions of those units, provides gain in precision for the factor allocated

in the small units compared to the design with a unique formation of experimental units, but a loss of precision for the factor allocated in the large units. Therefore, although sometimes the design with two sizes of experimental units has practical convenience, it should be used only when this convenience implies that the most important factor is allocated to the small units.

The previous examples highlight that, like precision, the expression of relative efficiency depends on the design and can vary with the treatment factor. They consider experiments with orthogonal designs, in which effects of a treatment factor manifest in a unique stratum. In experiments with more complex designs, as balanced incomplete block designs and factorial designs with confounding, effects of a treatment factor can be situated in more than one stratum. Consequently, estimates of error variances with these designs are compositions of error variance estimates in these strata. Considerations about these designs are available in Bailey [18], Cochran & Cox [13], Fisher & Yates [19] and Kuhel [16].

## 4. Conclusions

The experiment should provide inferences about the relevant effects of treatment factors. To this end, the experiment plan must be drawn up in line with the research objectives, considering the available experimental material, the required properties of the experiment and the appropriate principles of experiment design: repetition, local control, randomization, orthogonality, balance, confounding and efficiency. Compliance with these principles allows the elaboration of the most appropriate experiment design for the experiment.

## Compliance with ethical standard

*Disclosure of conflict of interest*

The author declare that no conflict of interest exist.

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