DESIGNS FOR RESEARCH AND OBSERVATIONAL EXPERIMENTS

Chapter I.

INTRODUCTION

by

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Facts concerning the design of experiments are scattered throughout statistical literature. No single text suffices entirely for a course in experimental design. Leonard and Clark (1939), Goulden (1939), and Love (1936, 1943), have written texts which are suitable for students of agronomy. The above three references are excellent for their discussions on experimental techniques and procedures. Fisher's (1942) "The Design of Experiments" is suitable as a reference for research workers who have mastered the elementary statistical methods and is more advanced than desired for students learning the elements of experimental design. Cochran and Cox (unpublished) have planned to write a book on experimental designs but as far as known little or nothing will be included on the estimation and use of variance components. Therefore, in an attempt to fill this need and to give the elementary mathematical theory on experimental designs, the present manuscript has been written.

The student will need to understand basic statistical methods as illustrated and explained by Professors G.W. Snedecor, "Statistical Methods", 1946, and R.A. Fisher, "Statistical Methods for Research Workers", 1944. The latter reference is somewhat more advanced in some sections than is required for an understanding of the present dissertation on experimental designs. For the full comprehension of the mathematical theory underlying the analysis of various designs, the student would need to have mastered the principles of calculus and matrix algebra. However, an attempt was made to write the text in such a fashion that the student may be able to grasp the essential ideas concerning the mathematical theory with only a good basic understanding of college algebra. Occasionally, some concepts of calculus and matrix algebra may creep in but in these cases the explanation will be given. The less advanced students should concentrate on the first part of the chapters, confining themselves to learning the "how" rather than the "why" of designing experiments for research or observational work.

At this point an explanation should be given concerning the terms "Research Experiments" and "Observational Experiments". But first of all it is necessary to define what is meant by the term "research". In the broad sense, research is the collection and analysis of data. As is well-known, there are many degrees of research. The scale runs all the way from doing something absolutely new down to the "refinding" of wellknown and well established facts. The upper part of the scale might be termed research while the lower end, the refinding of wellknown facts, might be termed "re-search". The scale in between has no clear-cut division but perhaps some experimental facts are desired and may be obtained by people who have not had the training required for carrying out the more technical phases of research problems. "Reflective thinking" and historical studies should not (unfortunately they are by some groups) be termed research <u>unless</u> the results are used to bring out new ideas and facts. In addition, to be of most value the results of a research study need to be put in a form that is available to others. Experimentation for personal satisfaction alone is useless to the advance of science and as one able administrator once put it: "One could not imagine a greater fools' paradise than to merely experiment in whatever direction the mind might wander, with little heed paid to and no deductions made concerning the results obtained."

The term "research experiment" may be applied to a study investigating new ideas or facts. The exercisable degree of control on experimental conditions by the experimenter is quite adequate and is almost complete in some cases. The external factors affecting the character under observation may either be controlled or eliminated, i.e. they are held constant while the character under observation is allowed to vary. In some more complex research experiments two or more characters are allowed to vary while other factors that may affect the experimental results are kept constant. Of course, it is impossible to control all external factors and in practice the main ones are controlled and the factors with lesser effects are allowed to vary.

On the other hand, the experimenter may have little or no control over the influential external factors and he may have to choose a range of the character that appears in the population under surveillance. Also he may know, in general, the expected results of the experiment but wishes to obtain some measure of the character under observation. In this case the experimenter may choose some experimental layout or design from the following chapters or some other source which may be the same design as that for the research experiment. Since the degree of control over experimental conditions is limited and since the range of the character may be fixed in the population, it is suggested that this type of study be called an observational experiment in contradistinction to the research experiment which may include a range of the character larger than present in the population and is subject to a high degree of control. Of course the argument over the division line between the two types of experiments boils down to an argument similar to the pros and cons over "What is truth?" Regardless of the terms used to designate the various experiments, the experimental designs or the field or laboratory layout will be the same. Therefore, this manuscript will be confined to a discussion of the various experimental designs with regard to the choice of the experimental material; size, shape, and number of the individual units; the number of repetitions; construction, randomization, field or laboratory lay-out, and statistical analysis; the choice of appropriate experimental errors; the choice of an experimental design; and the elementary theory involved in tests of significance and in estimation of variance components.

In all cases except for the chapter on systematic designs, the discussion will be limited to designs which are subject to statistical analysis. The allotment of a particular treatment (variety, feeding ration, size of farm, level of fertilizer, baking condition, etc.) to a specified plot or "area" in the experimental area (the site at which an experiment may be conducted, e.g. a greenhouse bench, a set of ovens, a period of time, etc.) must be at random. The element of chance in the random allotment of the treatment to the experimental plot permits the experimenter to obtain unbiased estimates of the means and variance and to make probability statements concerning them. Fisher, "The Design of Experiments," 1942, gives an excellent discussion on validity and randomization and tests of hypotheses.

The following is a general classification of experimental designs.

Systematic
Completely Randomized
Randomized Complete Blocks

Latin Squares and Variations
- Latin Square
- Graeco-latin Squares
- Other Latin Squares (plaid, half-plaid, and quasi-)
- Cross-over
- Switch-back or Reversal

Incomplete Blocks
- Split-plot
- Lattice
- k^n or n-dimensional Lattices
- p x q Lattices
- Incomplete Lattice Squares
- Youden Squares

As with all statistical manuscripts, the present one is subject to the criticism that the author did not use "standard" statistical symbolism. Since the last three words of the preceding sentence imply something different to writers in the statistical field, it is perhaps best to list and define the symbols used in the majority of places in the text.

X_i or Y_i, $i = 1,2,\ldots, n$ = a specified number, is the individual measurement or count on the observed character. X_1 = measurement on the first individual, X_2=measurement on the second individual, and

X_{ij} or Y_{ij}, $i=1,2,\ldots,n$ and $j=1,2,\ldots,m$, is the record of the i th individual of the j th classification.

$\frac{\sum_{i=1}^{n} X_i}{n} = \frac{X.}{n} = \bar{x}$, i.e., the mean of n observations $X_1, X_2, \ldots, X_n$, is the sum of the observations, $X_1 + X_2 + \ldots X_n$, divided by the number n. $\bar{y}$ is obtained similarly. Some texts use m for a mean with m_x denoting the mean of the X_i and m_y the mean of the Y_i. $\bar{x}$ in normal populations is an unbiased estimate of the population mean μ, a known or unknown parameter.

$\frac{1}{nm} \sum_{i=1}^{n} \sum_{j=1}^{m} X_{ij} = \frac{X_{..}}{mn} = \bar{x} =$ overall mean of a 2-way classification.

$\frac{1}{m} \sum_{j=1}^{m} X_{ij} = \frac{X_{i.}}{m} = \bar{x}_{i.} =$ the mean over all $j = 1,2,\ldots, m$ for the i th classification. Likewise,

$\frac{1}{n} \sum_{i=1}^{n} X_{ij} = \frac{X_{.j}}{n} = \bar{x}_{.j}$.

$(X_i - \bar{x}) = x_i =$ deviation from the mean.

$\sum_{i=1}^{n} (X_i - \bar{x})^2 = \sum_{i=1}^{n} x_i^2 =$ sum of squares of deviations from the mean.

$\frac{\sum_{i=1}^{n} x_i^2}{n-1} = s^2 =$ the variance or sometimes mean square of a single observation and is an unbiased estimate of the population parameter σ^2 in normal populations.

$\frac{\sum_{i=1}^{n} x_i^2}{n(n-1)} = s^2_{\bar{x}} =$ the variance of a mean of n individuals and is an unbiased estimate of the population parameter $\sigma^2_{\bar{x}}$ in normal populations.

$\sqrt{s^2} = s =$ the standard error of a single observation or the standard deviation.

$\sqrt{s^2_{\bar{x}}} = s_{\bar{x}} =$ the standard error of a mean.

$\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}} = s_{\bar{x}_1 - \bar{x}_2} = s_d =$ the standard error of a difference between 2 means based on n_1 and n_2 individuals, respectively. In the event that $n_1 = n_2$, $s\sqrt{\frac{2}{n}} = \sqrt{2}\, s_{\bar{x}} = s_d$.

b_{12} = regression coefficient of the variate X_{1i} on the variate X_{2i} and is an unbiased estimate of the population parameter β_{12}. Other symbols will be used to designate regression coefficients also but these will be explained when used.

$b_{12.3}$ = partial regression coefficient of X_{1i} on X_{2i} independent of X_{3i}.

r_{12} = total or zero order (Snedecor, 1946) correlation coefficient of X_{1i} with X_{2i} and is an estimate (though biased) of the population parameter, ρ.

v = coefficient of variation or variation coefficient, which has often been designated as c.v. or just c.

d.f. = degrees of freedom.

s.s. = sums of squares.

m.s. = mean square.

A. of V. = analysis of variance.

EMS = error mean square.

Eff = efficiency expressed as a percentage.

$F = \dfrac{\text{greater m.s.}}{\text{lesser m.s.}}$ = Snedecor's F = a test of significance.

t = "Student's" t = a test of significance.

χ^2 = chi-square = a test of significance.

$z = \frac{1}{2} \log_e F$ = Fisher's z = a test of significance.

A significance level is a predetermined probability of obtaining deviations as large as or larger than a specified number. Values from tests of significance of approximately the specified constant or larger are deemed significant.

A variable is the characteristic or trait under observation and of interest at the moment. The variable may be continuous (measurement data) or discrete (enumeration data). The individual measurements or counts of the variable are called the variates. Thus heights of people represent a variable, say X, and the height 67 inches represents the variate.

A population represents all the individuals of interest or of a given characteristic (or characteristics). The number of individuals in the population may be finite or infinite. For example a discrete population might represent the plants on a greenhouse bench. Usually the population about which inferences are made is infinitely large and a fraction is all that is ever observed.

A sample represents a fraction of the population. A representative sample is as its name specifies, representative of the population from which drawn. A random sample is a sample drawn in such manner that every possible sample will have an equal chance of being drawn. For example there are 20 possible samples of 3 taken from 6 objects and a random sample of 3 items would be one that was drawn in such a manner that any of the other 19 samples have equal chances of being drawn.

A parameter is a fixed value, e.g. 2, and is the value of the variable which specifies the population. The population parameter may be known or unknown.

A statistic is an estimate of the population parameter. If the average of a large number of estimates does not approach the population parameter, then the statistic is said to be biased, and conversely for unbiased statistics.

A <u>distribution</u> of the variates for the population may be represented by some frequency curve or by a mathematical formula, for example the formula for a random sample of n individuals from the normal, binomial, and Poisson distributions, respectively are of the form

$$\int_{-\infty}^{\infty}\cdots\int_{-\infty}^{\infty}\left(\frac{1}{2\pi\sigma^2}\right)^{n/2} e^{-\frac{\sum_{i=1}^{n}(X_i-\mu)^2}{2\sigma^2}}\,dX_i \quad ,$$

$$\sum_{X=0}^{n} {}_nC_X p^X q^{n-X} \quad ,$$

and

$$\sum_{X=0}^{n} e^{-m}\frac{m^X}{X!} \quad .$$

An <u>individual unit</u> or <u>unit of observation</u> represents the smallest unit for which an observation is made.

The <u>treatments</u> in an experiment represent items which are being tested. The term "treatments" is very general and is often used in place of varieties, fertilizers, baking treatments, feeding treatments, method of dying fabrics, dosages of drugs, etc.

The <u>experimental site</u> or <u>area</u> represents the place or time over which an experiment is conducted. In field trials, it may represent the land which the experiment occupies; in home economics experiments, it may represent the time and pieces of equipment used, etc.

If all treatments have been included a proportional (usually equal) number of times and are together in one part of the experimental area, this fraction of the total site is called the <u>replicate</u>.

If the replicate is repeated, this is known as a replication or repetition of the treatments. The treatments may be replicated without having replicates (see Chapter III).

Uniformity or blank trial data represent the measurements taken for a variable on several (usually many) units of observation of the same treatment. Various experimental designs may be laid out on the uniformity trial data and the "treatments" may be assigned to the plot yields at random. The comparisons among these "treatments" would represent comparisons for the same treatment or "dummy" comparisons.

The term deviation has been explained previously to denote the discrepancy between the variate, X_i, and the estimate of the parameter, say $\hat{\mu}$. The term difference refers to the discrepancy between two variates, say X_1 and X_2, or between two treatment means (Snedecor, 1946, Examples 2.6 and 2.7).

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SOME PRINCIPLES OF EXPERIMENTAL DESIGN

by

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Our courses in statistical methods and experimental design tend to emphasize the mechanical features of the subject--the lay-out of the experiment and the calculation of statistics. It is my purpose to discuss and illustrate two of the more fundamental principles that govern design. These are simple and obvious; yet they seem to include the important practical requirements of good experimentation. The philosophical aspects of the subject I do not intend to mention.

The two principles of design which I have in mind are as follows:

First: To provide unambiguous answers to the questions asked, and

Second: To get the answers with a minimum expenditure of resources.

As a preliminary, let us consider for a moment the questions that may be asked. From a long experience in reading project outlines, I conclude that these questions are often not very definite. Frequently I have found the proposed experiment wholly inadequate to realize the stated objectives. For instance, the objective may read, "To determine the adaptability of some varieties to Iowa conditions," followed by a proposal to try the varieties during a single season on some one set of plots. Of course, I realize that Iowa is supposed to exhibit a uniform climate and soil, but no Iowa agronomist would admit this.

At times, it would appear that the experimenter is concerned merely in demonstrating his own superior knowledge. I have in mind the case of a man who had designed a split-plot experiment to answer this question: When is the best time in the fall to make the last cutting of a certain perennial forage crop? While I was examining the first year's results, he explained that one average was unusual because variety A should have been superior to variety B in the earlier cuttings. He even described the physiological characteristics that made this so. The other averages he considered normal. When I asked for the results in succeeding years in order to get an answer to the question presumably asked--which treatment maximized the yield over the effective life of the plots?--I was blandly informed that other experiments demanded his attention after one season so that this one was abandoned. I gathered that he already knew all the answers to the experiment as conducted and that he really wasn't much interested in the specified objective of his project. The experiment seemed to be a filler which warranted the payment of his salary and expenses until something more promising turned up.

If you say that my examples are unusual, I fear you are wrong. My experience leads me to suspect that some large fraction of our so-called experimentation, perhaps even a majority of it, is conducted in an equally vague and unproductive fashion. Experimenters often remind me of small boys investigating the mechanism of a clock. They are eager to see what makes it tick, but not much interested in devising a superior time-piece, much less in writing a scientific report of their investigation.

Some good experimenters do not require very definite answers to questions. This is notably true in breeding tests with large numbers of varieties. The answer desired is given by an array of the varieties according to their performance in one or more respects. An over-all test of significance is available for one character--tests of other characters would not be independent of the first, I suppose. No way is provided for combing the various measurements into a single one determining excellence. No test is furnished for distinguishing between individual varieties. The only question asked, usually, is about the yield of those varieties that prove to have acceptable stalk strength, for example; or ear height. Even nutritive value is not often used as a criterion of selection. Yet the notable achievements of the breeders is convincing evidence that this simple answer is the only one needed.

Contrasted with these experiments in which vague or few questions are asked are those in which many individual comparisons are planned, each with an appropriate test of significance. The factorial experiment is a shining example of this type. But I wish to avoid any implication that an experimenter should choose a design merely because it answers many questions. The efficient design is the one that answers the questions which he is asking. If he has a single question, then answers to other questions may be superfluous and the more complicated designs may be inefficient.

Assuming then that the experimenter has one or more definite questions, he should choose a design that will furnish unambiguous answers to them. This is the first of the principles which I wish to discuss. A single, clear-cut answer from an experiment is not easy to attain. Some type of replication is always necessary. Appropriate controls must be set up to segregate the desired effects from those that confuse the issue. Correlated variables difficult to regulate may be measured and controlled by covariance. Tests of significance must be provided to distinguish between treatment effects and sampling variation. These are some of the familiar devices for achieving unambiguous answers.

Ambiguous answers mean little or no information from an experiment. Let me illustrate. Not long ago I was asked by an experimenter to give an opinion as to which of four methods was superior for testing significance. Six protein supplements had been tried on chicks and all the 15 pairs of treatments were to be tested, ignoring the fact that only five degrees of freedom were available. This aroused my suspicion, so I made detailed inquiries about the design. I found that all the chicks receiving Treatment A were kept in a single separate enclosure, as were the lots getting the other treatments. I found that the sexes were mixed in the lots in unknown ratios. I found that one supplement had been substituted for another in equal weights irrespective of protein content. Of course, no record was kept of individual food consumption.

Practically any question asked of this experiment would have half a dozen different answers. If the lot having Treatment A gained significantly more than that with Treatment B the answer might be (i) that the concentration of protein in A was greater than that in B; or (ii) that the biological value of the first protein was superior to that of the second; or (iii) that there was a larger proportion of males in lot A than in lot B; or (iv) that environmental conditions (including incidence of disease) were more favorable in pen A than in pen B; or (v) that there was correlation among the gains of the birds within the lots resulting in a downward bias in the estimate of error; or (vi) that ration A was more appetizing than B. So far as the gain is concerned, the last answer could be eliminated by using some ratio such as gain per unit of food eaten; but the ambiguity would not be removed because there is no test of significance provided for the ratio.

Remember, now, that this man was asking about the merits of various tests of significance, seemingly unaware that the differences he was testing were completely meaningless. Such is the effect of the manner in which we teach statistics!

I have cited an extreme example, but not one that is unique. I remember some data that were sent around to several statisticians only a few years ago. The data were from a ten-year experiment on cultural treatments of pecan trees. All the statisticians agreed that the investigator had been particularly ingenious in arranging the experiment so that every comparison was ambiguous. But the U.S.D.A. paid money to this man for 10 years, money from your pocket and mine, with never a competent check on the design or progress of the experiment. I suspect that there are many of this type of experiment now in progress.

Another common kind of ambiguity is one already mentioned, the trial of three or more treatments with no specified degrees of freedom to be tested. The means are estimated in an unbiased manner and can be arrayed according to the order in which they turned up in the particular experiment. But there is no way to differentiate the populations. Even though the F-test indicates population differences, the answer as to which population differs from which has no single answer.

For illustration, I return to the chick-feeding experiment. There were five protein supplements tried in addition to the standard. Of course, each of the five could be compared to the standard, but this is not an efficient set of comparisons. It turned out that there were three vegetables and two animal sources, so that four of the possible five independent comparisons could be specified. The only remaining ambiguity would be in the two degrees of freedom for vegetable sources.

I believe that careful forethought would eliminate this type of ambiguity in a great many experiments. Among the experimental treatments, there are nearly always relations which would lead to at least a partial set of orthogonal comparisons. Questions based on these comparisons would be answered unambiguously, with less definite information about the remaining ones.

A somewhat more subtle type of ambiguity is that which springs from samples which are too small. An experimenter may know with reasonable certainty that a difference cannot be larger than 20 percent, yet he may use only enough replications to detect a difference of 50 percent. At the end, he is unable to distinguish between a real difference and sampling error.

In reviewing project outlines, I have often had to call attention to the fact that the proposed sample size was too small to detect any but the most obvious differences. Experimenters often take a peculiar view of this situation. They say that they don't expect significant differences, but they just wish to observe how the treatments behave under trial. They are the small boys of whom

I spoke before--they like to play around with the experimental material, hoping, no doubt, that something interesting may turn up.

It is hard for many experimenters to realize that, before the experiment is performed, definite statements can be made about the prospect of an unambiguous answer. Foreknowledge of the probability that an experiment will detect a specified difference is confused with fore-knowledge that there is such a difference. If you tell these people that, under specified conditions, the probability is 0.8 that their experimental questions will be answered, they scornfully say, "Well, why do the experiment? You can tell us now what the answer is."

I think this is one of the most spectacular feats of statistics. Experimenters have always begged us to tell them how large a sample to take, but when we tell them, the language seems unrealistic. Also, the necessary number of replications is ofttimes so great that it will not be believed. The new tables of Mood, et al., now in press, enable one to make decent estimates of the number of replications necessary to yield unambiguous answers to specified questions. The experimenter himself must decide on the size of the population difference he wishes to detect and must help to estimate the standard deviation of the population. The latter is the weakest link in the chain, but it can often be made quite strong. Under the specified assumptions, a determinate sample size will, with stated probability, yield a significant difference. If it does not, the answer is that any difference there may be in the population is less than the one which the experiment was designed to detect.

The experimenter who insists on using too small a sample often feels justified by his results. Even if the population difference is zero, he has a five-in-a-hundred chance of significance; while if there is a real difference, his chance of detecting it is greater. But the canny investigator will not easily be misled by such an outcome. He will be wary of a large sample difference, because he knows that a large population difference would have been discovered long ago. He will be equally wary of a small experimental error--he knows the usual sizes in such experimentation. But with an inadequate sample size, about the only chance of attaining significance, in case the population difference is as small as suspected, is an unusually large sample difference or an unusually small sample error. So the competent experimenter, knowing the size of sample necessary to detect a reasonable population difference if it exists, is not deceived by significance in an experiment of inadequate size. But, of course, this competent investigator would not have wasted his time and my money in carrying out such a too-small experiment. It is the incompetent who braves the inadequate sample and proudly publishes his accidental findings.

I had quite a lengthy correspondence, a couple of years ago, with a botanist in one of our greater universities who asked me about the adequacy of the size of his experiment. He was seeking to detect a small difference with large variation. When I told him the required size he was frankly scornful. I cited formulas and suggested that he have them checked by a girl in his employ who had completed a course in statistics given by an uncontaminated, pure mathematician. I never heard from him again. I suspect his work has been fruitful of publications and that he is held in great esteem by his brethren.

Having assured ourselves of an experiment that will yield unambiguous results, we set up the second principle that it shall be done at a reasonable cost. "At minimum cost" is the phrase which I used in the beginning, but that is an unattainable ideal. I think we shall not be censured even if we habitually waste a dollar or two on our experiments. In general, economy means low experimental error.

For a selected set of answers, to be attained with specified accuracy, economy is achieved in three familiar ways. First, there is the selection of homogeneous experimental material. When this is possible, the experimenter must beware lest he make the base of his structure too narrow. He might get answers about a small segment of his population, and these might not hold in other related parts. If this is a reasonable conjecture, then he should first investigate such possible interactions. If he finds them absent, he can proceed with his more detailed experiment with the selected portion of the population. Otherwise, he must enlarge his plans so as to answer the questions, at least ultimately, for each of the homogeneous parts of the population concerned. I suspect that the existence of these interactions is unusual in many fields of investigation. Nonetheless, their possible presence must not be ignored.

The second method of achieving economy is by choice of an appropriate plan for the experiment. Randomized blocks, the latin square, and various incomplete block devices are familiar to you. This is the method of economizing which most taxes the ingenuity of the investigator. In this conference, you will hear one of the speakers describe his device of splitting plants in order to have more homogeneous material for his experiments. He also had to decide how to use the pairs of plants to test three fertilizers--calcium, phosphorous and potassium. He could have chosen a balanced incomplete block plan with two combinations per block; this would have given him equal amounts of information on main effects and interactions. But he decided that the interactions were not pertinent to his inquiry, so he chose a combination of paired experiments in which the three main effects were evaluated with maximum efficiency, leaving the interactions to be tested with the large error due to the variation among half-plants from different plants.

This is a nice decision which experimenters tend to shirk. Over and over again I have tried to get men to decide which set of treatments to put on the split plots and which on the main. They are avaricious of information and are reluctant to sacrifice any from either set. The same kind of decision often has to be made in order to get an experiment large enough to promise unambiguous answers--some treatments must be sacrificed, and giving them up is like having eyeteeth pulled. The ideal experimenter is he who can choose that plan which will give unambiguous answers to necessary questions without undue expenditure.

The third method of achieving economy, or reducing experimental error, is by statistical control of extraneous variables. This is the appropriate method if homogeneous material is either undesirable or not available. It has the virtue of broadening the basis of inference. If related variables can be measured rather than experimentally controlled, their effects can be eliminated from the estimate of error; and in the end one has not only the answers desired about the experimental variable but also measures of its relations to the others. I think this method of covariance is growing in popularity. It is effective not only in reducing experimental error but also in broadening the scope of information at small cost.

In conclusion, I wish to discuss one of the causes of ambiguity, with consequent high cost, in much of our experiment station work. A friend of mine, shrewd but inclined to be caustic, placed the fraction of useful expenditure of station funds at 3 percent. My own estimate is somewhere between 20 percent and 40 percent. Why is this?

One reason is that experimenters, eager for information, are too impatient to allow the necessary time to get it. They want it all the first year. The result is that even a big experiment is far too small to yield unambiguous answers. So, the inadequate experiment must be tried again next year. This goes on indefinitely. After ten or twenty years of this kind of thing, the fundamental questions are still unanswered, but the leader is ever hopeful that next year he will have the longed-for results. If he is told that the experiment is too small to yield the answers he wants, he complacently replies that the director will not provide him with sufficient funds; so, he continues to fritter away the resources he has.

Every experimenter should have some kind of twenty-year plan. The early years should be spent in getting reasonably certain answers to subsidiary questions about techniques, the effects of environment, etc. Each experiment should be large enough to get an unambiguous answer to at least one question. As the climax approaches, the main question emerges with increasing clarity, and at the end is answered without ambiguity.

I am not so naive as to suppose that this ideal program will move along smoothly and without interruption. At the end of twenty years the question may seem as far from solution as it was at the start. But every preliminary answer is available information and denotes progress toward ultimate success.

I hope I have succeeded in convincing you that my main thesis is true: that the investigator may, with specifiable certainty, know in advance that his experiment will, without excessive cost, yield unambiguous answers to the questions asked.

Chapter II.

SYSTEMATIC DESIGNS

by

W.T. Federer.

Prior to the development of modern experimental designs, experimenters had tried various arrangements which are not subject to the laws of chance. Various systematic schemes of ordering or arranging the treatments in the various repetitions have been devised. One such scheme might be to arrange all duplicates, triplicates or etc. of the treatment together. Suppose the experimenter wished to test three treatments A, B, and C and that he decided to have 4 repetitions of each treatment. With the above scheme the arrangement of the 3 treatments over the experimental area could be one of the following:

A A A A	B B B B	C C C C

,

A A A A
B B B B
C C C C

,

A	B	C
A	B	C
A	B	C
A	B	C

,

A A	B B	C C
A A	B B	C C

From fertilizer, yield, and other trials or experiments it was evident that it might be better to test treatments A, B, and C together in a compact block and then to repeat these blocks with a systematic ordering of the treatments in each block or repetition. One of the more common types of systematic arrangements in which the treatments are repeated several times is the following:

Replicate I	Replicate II	Replicate III
A B C	A B C	A B C

or

Replicate I	A	B	C
" II	A	B	C
" III	A	B	C

In this case the ordering of the treatments is exactly the same in every replicate (a unit which contains all the treatments). Another systematic arrangement is the following:

Replicate I	Replicate II	Replicate III
A B C	C A B	B C A

In this case each treatment occupies each order in the replicate.

From the last systematic arrangement experimenters may have felt it necessary to place the treatments so as to eliminate soil heterogeneity in two directions and proposed the "diagonal square" (see Fisher, 1942); for three treatments the design would be:

A	B	C
C	A	B
B	C	A

and for 5 treatments the design would be:

A	B	C	D	E
E	A	B	C	D
D	E	A	B	C
C	D	E	A	B
B	C	D	E	A

In order to eliminate the effect of A appearing on one diagonal, a systematic arrangement involving the Knight's Move was used, i.e. one down and two over. This arrangement for 5 treatments in 3 replicates gives the following design:

Replicate I	A	B	C	D	E
" II	D	E	A	B	C
" III	B	C	D	E	A

and involving 5 replicates the arrangement is:

A	B	C	D	E
D	E	A	B	C
B	C	D	E	A
E	A	B	C	D
C	D	E	A	B

Fisher (1942, The Design of Experiments) states that the above design has been known in Denmark since about 1872 but that it is usually ascribed to the Norwegian, Knut Vik, and called the Knut Vik Square. A fairly good account of this design is given by Fisher (1942), but the statistical analysis for it has only recently been worked out (Övind Nissen, 1949, unpublished paper.)

Numerous other systematic arrangements have been devised, the various experimenters attempting to outguess natural variation. Regardless of the type of systematic design they all have relatively the same advantages and disadvantages. The advantages are often given as (the quotation marks are those of the author):

(i) Simplicity. Many experimenters feel that planting, note-taking, and harvesting in agronomic trials are facilitated by using systematic arrangements. In judging or scoring experiments it is sometimes felt that the judge will be better able to "discriminate" between the treatments if he (the judge) knows the order in which the treatments occur in the different repetitions.

(ii) The systematic design provides "adequate" sampling of the experimental area. That is, it allows for "intelligent placement" of the various treatments.

(iii) Varieties may be arranged in order of maturity or fertilizer treatments in order of increasing fertility.

(iv) It may be desirable to alternate dissimilar varieties (say, bearded versus beardless barley) so that natural crossing or mechanical mixtures can be detected in subsequent years.

(v) There is no need to randomize since the heterogeneity of the experimental site is such as to randomize the effects on the treatments. (This does not lessen the effect of one treatment on another or of a single arrangement; these facts should not be ignored.)

The disadvantages of the systematic designs are that there is no correct measure of the variation and the correlation between adjacent plots may lead to systematic errors in assessing treatment differences. The latter point is easily illustrated by the following systematic arrangement:

Replicate I	Replicate II	Replicate III
A B C	A B C	A B C

,

where the yield gradient is assumed to exist from left to right. Even though treatments A, B, and C may be the same thing, the experiment would show A to be better than B, and B better than C. In the event that the treatments were different, their differences may be exaggerated or underestimated depending upon the arrangement of the treatments.

Fisher (1942, The Design of Experiments, sections 27 and 34) discusses the effect of systematic arrangements on tests of significance and in the estimation of an error variance. Suppose that systematic arrangements are tried on uniformity trial data, (Plot data on the same treatment over the whole of the experimental site or area).[1] Then, the treatments in the experimental area would all be the same thing. The total sum of squares would be a constant regardless of what arrangement was chosen. If the experimenter was able to "intelligently" place the treatments so that all were subjected to about the same heterogeneity, then the sum of squares due to the differences between dummy or pseudo-varieties would be decreased. The decrease must be counterbalanced by an increase in the error or remainder sum of squares since the total is a constant, or

Total s.s. = s.s. among dummy var.+ s.s.within var.

If,on the other hand, the experimenter does a lousy job of "intelligently" placing the dummy varieties, the estimate of the error sum of squares will be smaller than it really should be and the differences between the varieties will be exaggerated. Some arrangements may consistently underestimate the error variance. The amount of underestimation is unknown and any attempt to obtain an estimate of the error variance from systematic arrangements is pretty much a matter of guesswork.

It is suggested that students in experimental design read this chapter for its historical value. They should never design an experiment in a systematic manner but rather should choose an experimental design that is subject to statistical analysis. The remainder of the manuscript will be confined to a discussion of such designs.

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1. For work on use of systematic designs on uniformity trial data see Odland and Garber, 1928, Journ. Amer.Soc.Agron.,20:93-108; Tedin,1931, Jour.Agr.Sci.21:191-208; and Pan,1935, Jour.Amer. Soc.Agron. 27:279-285. The first reference states that the standard deviations obtained from systematic arrangements were somewhat lower in all cases,than those obtained from random arrangements on soybean uniformity trial data. Tedin(1931) found that the variation within 6x5 blocks was uninfluenced by arrangement, diagonal or random,for estimating the error. He suggested random arrangements for the highest degree of scientific accuracy. On rice uniformity yield data Pan (1935) found that the deviations among varieties (in reality they are dummy or pseudo-varieties) were much larger than might be explained on the basis of random sampling.

Chapter III.

COMPLETELY RANDOMIZED DESIGNS.

by

W. T. Federer.

The simplest of all designs having a random arrangement is the completely randomized design. The design may be defined as one in which the treatments are randomly arranged over the whole of the experimental site. No effort is made to confine treatments to any portion of the whole area. The number of repetitions of any one treatment may vary. The completely randomized design is usually chosen when the variation over the whole experimental unit is relatively small. An example of the lay-out of a completely randomized design would be the following, in which the five treatments A, B, C, D, and E are repeated four times each on the twenty units representing the whole of the experimental area. -

(E) (1)	(E) (8)	(C) (9)	(B) (16)	(E) (17)
(A) (2)	(D) (7)	(D) (10)	(B) (15)	(A) (18)
(B) (3)	(C) (6)	(A) (11)	(C) (14)	(B) (19)
(E) (4)	(D) (5)	(A) (12)	(D) (13)	(C) (20)

Such an experiment might have been designed for 20 pots on a greenhouse bench, a series of 20 soil analyses, the 20 animals in a feeding trial, 20 cake-pans in an oven or the 20 successive bakings of single cakes in an oven, records on five litters of four pigs each, or some other type of experimental material.

Example III-1

In order to best illustrate the statistical analysis a numerical example was chosen from a guayule experiment on the dry weight of the shrub (leaves not included) on plants that had completed one year's growth in the field. The plants of variety 109 (a 54 $\pm$ chromsome strain of guayule) were classified with regard to trueness of type. The characteristic plants of 109 were listed as normals = N. The remainder of plants differed considerably in appearance from the normals and were divided into two categories, offtypes = O and aberrants = A. It was desired to know if the three types of plants varied with regard to dry weight of shrub. A random selection was made of 5 plants of each type and the dry weights obtained. The 15 selected plants were scattered over the experimental area in the following manner (the type of plant, A, N, or O, is listed first followed by number of plant and dry weight of shrub in grams):

A-1-34

O-3-84 N-2-87

A-4-12

A-5-20 N-6-167

N-7-112

N-9-104 O-8-134

A-10-5

O-11-86

N-12-106

O-13-120

A-14-48 O-15-108

The table of yields and sums of squares is given below:

	Normals =N	Offtypes =O	Aberrants =A	Total
	87	84	34	
	167	134	12	
	112	86	20	
	104	120	5	
	106	108	48	
Totals = ΣX	576	532	119	1227
Means	115.2	106.4	23.8	81.8
ΣX_i^2	70054	58472	4029	132555
$(\Sigma X)^2/n$	66355.2	56604.8	2832.2	100368.6
Σx_i^2	3698.8	1867.2	1196.8	32186.4

Since such comparisons as normals versus offtypes and aberrant versus the mean of the normals and offtypes are logical comparisons to make and since they formed a part of the hypothesis in designing the experiment, these contrasts are given in the following analysis of variance table:

Source of variation.	Degrees of freedom.		Sum of squares.		Mean squares.	
Among types	2		25,423.6		12,712.8	
Nvs O		1				193.6
N+Ovs2A		1				25,230.0
Within types	12		6,762.8		563.6	
Within N		4		3,698.8		924.7
" O		4		1,867.4		466.8
" A		4		1,196.8		299.2
Total	14		32,186.4			

The total sum of squares is

$$\sum_{i=1}^{n}\sum_{j=1}^{k} X_{ij}^2 - \frac{X^2_{..}}{nk}$$

$$= 87^2 + 167^2 + \ldots + 5^2 + 48^2 - \frac{(1227)^2}{15}$$

$$= 132555.0 - 100,368.6 = 32,186.4 \text{ with } 14 \text{ d.f.}$$

The sum of squares among types is

$$\sum_{i=1}^{n} \frac{X_{i.}^2}{k} - \frac{X^2_{..}}{nk}$$

$$= 125{,}792.2 - 100{,}368.6 = 25{,}423.6 \text{ with 2 d.f.}$$

The sum of squares among plants within normals is

$$\sum_{j=1}^{k} X_{1j}^2 - \frac{X_{1.}^2}{k} = 87^2 + \ldots + 106^2 - \frac{576^2}{5}$$

$$= 70{,}054 - 66{,}355.2 = 3{,}698.8 \text{ with 4 d.f.}$$

The sums of squares among plants within offtypes and aberrants is 1,867.2 and 1,196.8 respectively. The pooled within type sum of squares is 3,698.8 + 1,867.2 + 1,196.8 = 32,186.4 with 12 degrees of freedom.

An orthogonal set of comparisons among the three types would be:

Comparison	N	O	A
NvsO	+	-	0
N+O vs A	+	+	-2

The sum of squares for the comparison normals versus aberrants, is

$$\frac{(576 - 532)^2}{5(1 + 1)} = 193.6$$

and for the comparison, normals and offtypes versus aberrants, is

$$\frac{\{576 + 532 - 2(119)\}^2}{5\ (1 + 1 + 4)} = 25{,}230.0$$

The pooled error mean square, 563.6, with 12 degrees of freedom is used to test the comparisnns of the normals with offtypes and the normals and offtypes with the aberrants, with the respective values of

$$F = \frac{193.6}{563.6} = 0.34$$ and

$$F = \frac{25230.0}{563.6} = 44.77$$

There is a strong hint that the within type variances for the three types of plants are different. However, one should not expect to detect differences in variances unless the degrees of freedom are fairly numerous or unless the variances are extremely divergent. Bartlett's test for homogeneity of variances as illustrated by Snedecor (1946,p.250) for equal numbers of individuals per lot follows:

$$\chi^2 = 2.3026\ (5-1)\ \left(3 \log_{10} 563.6 - \log_{10} 924.7 - \log_{10} 466.8 - \log_{10} 299.2 \right)$$

$$= 1.306 \text{ with 2 degrees of freedom.}$$

The corrected χ^2 value is

$$\chi^2_e = \frac{1.306}{1.111} = 1.18$$

A chi-square value of 1.18 or larger with 2 degrees may be expected to be exceeded in random sampling from a homogeneous population in about 75% of the times. There is little evidence that within type variances are different, hence the pooled error mean square 563.6 was used to test the comparisons made.

The conclusion would be reached that the difference between the means of normal and offtype plants is less than ordinarily expected in random sampling from the same population. On the other hand the difference between the means of the offtype and normal plants and of the aberrants is much larger than can be attributed to chance sampling fluctuations. There is little doubt that the aberrants are much lower in dry weight of shrub than are the other types of plants.

Various other statistics may be computed from the data given. For example the standard error of a mean is

$$s_{\bar{x}} = \sqrt{\frac{563.6}{5}} = 10.62,$$

the standard error of a mean difference is,

$$s_{\bar{d}} = \sqrt{2}\ s_{\bar{x}} = 1.414(10.62) = 15.02,$$

the coefficient of variation is

$$v = \frac{s}{\bar{x}} = \frac{\sqrt{563.6}}{81.8} = 29 \text{ percent},$$

the intraclass correlation is (see Snedecor, P.245)

$$\frac{12712.8 - 563.6}{12712.8 + 4(563.6)} = .81,$$

and so forth.

In addition the experimenter may wish to compute such statistics as the t values for various comparisons. The contrast of the means of normals and offtypes is tested by

$$t = \frac{115.2 - 106.4}{\sqrt{\frac{2}{5}\left(\frac{3698.8+1867.4}{4+4}\right)}} = \frac{8.8}{16.7} = 0.53$$

The standard error of a mean difference, 16.7 is the appropriate one (see Fisher, 1942, The Design of Experiments) for comparing these means. The pooled error variance, 695.8, has 8 degrees of freedom. Therefore, a t value of 0.53 or larger with 8 degrees of freedom has a probability of occurrence greater than 50 per cent in sampling from homogeneous populations. In the event that a pooled error variance with 8 d.f. was not considered appropriate, but that each within type variance was an estimate of a different parameter the resulting t value would correspond to the tabled t values for four degrees of freedom (see Snedecor, P.83).

The remainder of the contrasts may be made in a similar manner. A single least or minimum significant different (lsd or msd) would be appropriate only if the variances were considered to be from the same population. The experimental evidences against heterogeneity is insignificant ($.7 < p < .8$ for [illegible] value) and one may be justified in computing a single lsd or msd equal to

$$t_{.05}s_{\bar{d}} = 2.179\ (15.02) = 32.7,$$

since the comparisons of interest do not represent a grouping of the data after the results have been scrutinized but were made prior to the selection of the plants for dry weights. Cochran (Emp.Jour.Agric, 6:157,1938) gives an excellent discussion of the various tests of significance among a group of treatments. Fisher, (1942. Section 24, The Design of Experiments) Love (1943,P.34), and Leonard and Clark (1939,Chapter 11) are among other writers who have discussed this problem and there should be no need for repetition except that experimenters have consistently misused least significant differences, especially with regard to the highest versus the lowest. For the comparisons of fortuitous groupings of the data, the present tables of probability values have little value.

The above illustration was given to illustrate the computational procedure for completely randomized design with equal numbers of repetitions of each of the treatments. For the case of n classes with k individuals per class the following breakdown of the degrees of freedom are appropriate:

Source of variation.	Degrees of freedom.
Among n classes	n - 1
Among individuals within classes	n(k-1)
Total	nk -1

Some experimenters are not so lucky as to always obtain equal numbers for each class. If the experimenter is working with animals, some of the experimental animals may become sick or die, leaving the experimenter with unequal numbers. Likewise, in the laboratory, an assistant may unwittingly bulk items, may forget to record the data, or may inadvertently lose some results in one way or another and the experimenter is left with unequal numbers of individuals.

The analysis for unequal numbers in a completely randomized design is little affected; the only real effect is that comparisons among treatments with fewer numbers is less precise than among treatments with larger numbers.

Example III-2.

Example III-1 represents only a part of the plants of 109 for which dry weight of shrub was obtained. From the entire area planted to variety 109, 54 plants were selected at random. Of these plants 27 were normals, 15 offtypes and 12 aberrants. The dry weight of shrub for the plants of the three types are given in Table III.1 along with the means, sums of squares and standard errors of a mean.

The analysis of variance for example III.2 is

Source of variation.	Degrees of freedom.		Sums of squares.		Mean squares.	
Among types	2		67,566.7		33,783.4	
N vs.O		1		2,436.8		
N +O vs 2A		1		65,129.9		
Within types	51		45,750.1		897.1	
Within N		26		29,348.3		1,128.8
" O		14		13,430.9		959.4
" A		11		2,970.9		270.1
Total	53		113,316.8			

Thetotal sum of squares is obtained by squaring all individual weights and subtracting the overall sum squared divided by the total number

$$\sum_{i=1}^{n} \sum_{j=1}^{k_i} X_{ij}^2 - \frac{X^2..}{\sum_{i=1}^{n} k_i}$$

$$= 58^2 + 109^2 + \ldots + 17^2 + 65^2 - \frac{4935^2}{54}$$

$$= 564,321.0 - 451,004.2 = 113,316.8 \text{ with } 53 \text{ d.f.}$$

TABLE III-1. Dry weight of shrub (without leaves) in grams.

Number	Normals	Offtypes	Aberrants	Total
1	58	103	34	
2	109	84	12	
3	87	88	20	
4	101	109	5	
5	105	134	48	
6	94	106	32	
7	167	86	21	
8	141	149	19	
9	112	64	24	
10	104	120	20	
11	58	108	17	
12	98	82	65	
13	106	112	-	
14	120	129	-	
15	65	22	-	
16	100	-	-	
17	117	-	-	
18	82	-	-	
19	133	-	-	
20	172	-	-	
21	133	-	-	
22	165	-	-	
23	150	-	-	
24	116	-	-	
25	120	-	-	
26	192	-	-	
27	117	-	-	
Total	3122	1496	317	4935
Mean	115.6	99.7	26.4	91.4
s.s.$=\Sigma X_i^2$	390344	162632	11345	564321
$(\Sigma X_i)^2/k$	360995.7	149201.1	8374.1	451004.2
Σx_i^2	29348.3	13430.9	2970.9	113316.8
$s_{\bar{x}}$	6.5	8.0	4.7	

The sum of squares among types with 2 d.f. is

$$\sum_{i=1}^{3} \frac{X_{i.}^2}{k_i} - \frac{X_{..}^2}{\sum_{i=1}^{3} k_i} = \frac{3122^2}{27} + \frac{1496^2}{15} + \frac{317^2}{12} - \frac{4935^2}{54}$$

$$= 518{,}570.9 - 451{,}004.2 = 67{,}566.7$$

The sums of squares among plant weights within normals, offtypes and aberrants are obtained from the formula

$$\sum_{j=1}^{k_i} X_{ij} - \frac{X_{i.}^2}{k_i}$$

and are given in Table III-1.

The sums of squares for the 2 orthogonal comparisons given in the analysis of variance table are

$$\frac{3122^2}{27} + \frac{1496^2}{15} - \frac{4618^2}{42} = 2436.8 \qquad \text{and}$$

$$\frac{4618^2}{42} + \frac{317^2}{12} - \frac{4935^2}{54} = 65{,}129.9$$

The standards errors of a mean (Table III-1) are computed from the formula

$$s_{\bar{x}} = \sqrt{\frac{\sum_{j=1}^{k_i} X_{ij}^2 - \frac{X_{i.}^2}{k_i}}{k_i\,(k_i - 1)}}\ .$$

The individual within type variances appear to be quite different. Bartlett's test for homogeneity results in the following chi-square value,

$$X^2 = 2.3026\ (51 \log 897.1 - 26 \log 1128.8 - 14 \log 959.4 - 11 \log 270.1)$$
$$= 6.29$$

$$X^2_c = \frac{6.29}{1 + \frac{1}{6}\left(\frac{1}{26} + \frac{1}{14} + \frac{1}{11} - \frac{1}{51}\right)} = \frac{6.29}{1.028} = 6.12,$$

with 2 d.f. The probability of obtaining a chi-square value as large or larger than 6.12 would occur about 2-5 per cent of the times in random sampling. Hence it is concluded that the variances differ. The X^2_c value = 6.12 with 2 d.f. may be partitioned into two single d.f. with corrected chi-square values of 0.12 and 6.00 for comparisons among plant variances for normals versus offtypes and of the pooled among plant variances for normals and offtypes versus aberrants. Apparently the variation in individual plant weights is much smaller for aberrants than for the other plant types, but the variation among plant weights for normals and offtypes is approximately equal. One could then use the pooled within plant variance, 1069.5 with 26 + 14 = 40 degrees of freedom for testing the difference between the means of the normals and offtypes thus,

$$F = \frac{2436.8}{1069.5} = 2.28$$

The corresponding F value for 1 and 40 degrees of freedom at the 5 per cent level is 4.08 and we would conclude that the difference in means of normals and offtypes could be obtained fairly frequently in random sampling. The experimenter may wish to be more conservative and also may not wish to assume that the error variances in normals and offtypes are estimates of the same parameter. He may have observed that the last weight for the offtype plants, 22, was unusually low and that for the last aberrant plant, 65, was unusually high. These values tend to increase the variances of both types. A copying error was suspected but a check showed this not to be the case. A misclassification was suspected but could not be verified. In view of this then, the experimenter may wish to make a more conservative test. Such a test would be to use an F with less than 40 degrees of freedom, say 14 or mid-way between 14 and 26 degrees of freedom. An approximate significance level of t may be computed from the formula given by Cochran and Cox (1944,Experimental Designs, mimeo), and illustrated by Snedecor (P.84, 1946),

$$t_{o5} = \frac{t_{o5}(k_1\text{-1d.f.})\,\frac{s_1^2}{k_1} + t_{o5}\,(k_2\text{ -1d.f})\,\frac{s_2^2}{k_2}}{\frac{s_1^2}{k_1} + \frac{s_2^2}{k_2}}$$

$$= \frac{2.056\left(\frac{1128.8}{27}\right) + 2.145\left(\frac{959.4}{15}\right)}{\frac{1128.8}{27} + \frac{959.4}{15}}$$

$= 2.110$, which is equivalent to about 17 degrees of freedom. The experimental t value is

$$t = \frac{115.6 - 99.7}{\sqrt{\frac{1128.8}{27} + \frac{959.4}{15}}} = \frac{15.9}{10.28} = 1.55,$$

which is somewhat smaller than the calculated five per cent value, 2.110. In this instance the means agree sufficiently well so that the same conclusion is reached regardless of the test used. An illustration of the opposite situation is given by Snedecor (section 4.6, 1946)

Similarly the mean difference of aberrants and offtypes may be tested by the statistic

$$t = \frac{99.7 - 26.4}{\sqrt{\frac{959.4}{15} + \frac{270.1}{12}}} = \frac{73.3}{9.30} = 7.88$$

The 5 per cent level of t for this comparison is

$$t_{o5} = \frac{2.145\left(\frac{959.4}{15}\right) + 2.201\left(\frac{270.1}{12}\right)}{\frac{959.4}{15} + \frac{270.1}{12}} = 2.160$$

and the 1 per cent level of t is

$$t_{o1} = \frac{2.977\left(\frac{959.4}{15}\right) + 3.106\left(\frac{270.1}{12}\right)}{\frac{959.4}{15} + \frac{270.1}{12}} = 3.011$$

The mean weight difference for the 2 types of plants, offtypes and aberrants, is much larger than could be logically attributed to chance sampling fluctuations.

Other comparisons forming a part of the hypothesis may be tested similarly.

The coefficient of variation for the whole experiment has little meaning, but for illustrative purposes it is,

$$v = \frac{s}{\bar{x}} = \frac{\sqrt{397.1}}{91.4} = 33 \text{ per cent.}$$

The coefficients of variations for the three types of plants, normals, off-types, and aberrants, are

$$\frac{\sqrt{1128.8}}{115.6} = 29 \text{ per cent,}$$

$$\frac{\sqrt{959.4}}{99.7} = 31 \text{ per cent,} \quad \text{and}$$

$$\frac{\sqrt{270.1}}{26.4} = 62 \text{ per cent.}$$

One might suspect that the means and standard deviations were related in a linear manner and then the coefficients of variations should have been approximately equal (this still may be true if there was a misclassification of the last individual, for both offtypes and aberrants, i.e. the values 22 and 65). Despite this, there appears to be a relationship between the means and variances and in order to use the generalized error with 51 degrees of freedom some transformation of the data is necessary (M.S. Bartlett has discussed this subject to some extent, see Biometrics 3: 39,1947)

The above examples illustrate the procedures and complexities that may be encountered in experimental work. Rubber percentage data were taken for the 3 types of plants (see problem III-1) on the same 54 plants. The error variances or within type variances for normals, offtypes, and aberrants are the reverse in order of magnitude. The normals seemed to be less variable than are the other types with the aberrants being the most variable. On the other hand, the variation in grams of rubber per plant (see

Problem III-2) and resin percentage is approximately equal for the three plant types.

The chief advantages of the completely randomized design are:

a) The ease of laying out the design.

b) The design allows for the maximum number of degrees of freedom for the error sum of squares.

c) Ease of analysis. A completely randomized design has the simplest analysis of all experimental designs subject to statistical analysis.

d) Unequal numbers of repetitions for the various treatments may be included without complicating the analysis in most cases.

The chief disadvantage of the design is that it is usually suited only for small numbers of treatments. When large numbers of treatments are included the material must necessarily be spread over a relatively large experimental area. This generally increases the variation among the treatment responses. For the case in which the variation over the whole of the experimental area is relatively large, it is possible to select more efficient designs than the completely randomized one. Quite frequently the treatment means are measured more precisely in the more efficient designs with fewer replicates. Completely randomized blocks are seldom, if ever, used for field lay-out of experiments, the reason being that experience has shown that other designs are much more suitable.

Problem III-1.

Fifty-four plants were selected at random from the area planted to variety 109. These are the same plants on which dry weight of shrub was obtained in Table III-1. The character rubber percentage was obtained on the individual plants. The data are:

Plant No.	Normals.	Offtypes.	Aberrants.	Total.
1	6.97	6.21	4.28	
2	7.11	5.70	7.71	
3	7.26	6.04	6.48	
4	6.80	4.47	7.71	
5	7.01	5.22	7.37	
6	7.00	5.55	7.20	
7	6.35	4.45	7.06	
8	6.37	4.84	6.40	
9	7.29	5.88	8.93	
10	7.31	5.82	5.91	
11	6.86	6.09	5.51	
12	6.81	5.59	6.36	
13	6.43	6.06		
14	7.43	5.59		
15	6.68	6.74		
16	7.29			
17	7.12			
18	6.68			
19	7.34			
20	5.15			
21	6.41			
22	6.45			
23	6.32			
24	6.82			
25	6.86			
26	6.48			
27	7.28			
Total				
Mean				

(i) Test the mean differences of normals and offtypes and of offtypes and aberrants by t-test.

(ii) Are the variances homogeneous?

(iii) Run covariance analysis of rubber-percentage (Y) on dry weight of shrub.

(iv) Does the regression of the means differ from the average within regression?

(v) Do the within-type regressions differ from the average within regression?

(vi) Is the variation among dry weight of shrubs significantly greater than that among rubber percentages for aberrants?

Problem III-2.

The following data on estimated grams of rubber per plant were obtained on the same plants of variety 109 as given in problem III-1.

Plant No.	Normals.	Offtypes.	Aberrants.	Total.
1	4.07	6.39	1.46	
2	7.73	4.77	0.89	
3	6.29	5.33	1.30	
4	6.84	4.88	0.41	
5	7.35	7.00	3.57	
6	6.57	5.90	2.34	
7	10.60	3.82	1.51	
8	8.99	7.23	1.20	
9	8.16	3.77	2.19	
10	7.58	7.00	1.17	
11	4.00	6.61	0.91	
12	6.67	4.61	4.11	
13	6.78	6.77		
14	8.91	7.23		
15	4.32	1.51		
16	7.30			
17	8.30			
18	5.47			
19	9.74			
20	8.86			
21	8.52			
22	10.62			
23	9.46			
24	7.93			
25	8.20			
26	12.47			
27	8.52			
Total				

(i) Are the within type variances homogeneous?

(ii) Under the assumption of homogeneity of variances test the mean of highest versus lowest and give level of significance at 5 and 1 per cent levels.

(iii) Compute coefficients of variation for each type and for the experiment.

Problem III-3.

The 54 plants variety 109 were analyzed for resin percentages. The data follow:

Plant No.	Normals.	Offtypes.	Aberrants.	Totals.
1	5.71	6.17	3.97	
2	6.15	6.04	6.65	
3	6.05	5.89	5.44	
4	5.64	5.91	7.20	
5	3.85	5.22	6.52	
6	5.62	5.75	6.51	
7	5.60	5.38	5.92	
8	5.00	5.99	6.81	
9	6.06	5.44	7.34	
10	6.05	5.88	5.55	
11	5.24	6.13	5.22	
12	5.66	5.83	5.95	
13	5.53	5.88		
14	6.25	6.34		
15	6.06	5.83		
16	6.10			
17	6.07			
18	7.13			
19	6.53			
20	5.83			
21	5.85			
22	5.67			
23	6.01			
24	5.64			
25	5.88			
26	5.63			
27	6.35			
Total				

(i) Do the types differ with regard to resin percentages at the end of one year's growth?

(ii) Compute a least or minimum significant difference. Does it have any meaning for these data?

(iii) Compute the coefficient of variation. Do you believe that the variation among plants was so great as to obscure differences among the types for resin percentages?

Problem III-4.

For the students' interest it is suggested that the following list of problems or examples be scrutinized for whatever value they might have in connection with the design and analysis for a completely randomized design.

G.W. Snedecor, Statistical Methods, 1946

Page	Example	
226	Example	10.5
227	"	10.8
232	Table	10.12
235	Example	10.15
235	"	10.16
236	Table	10.15
242	Example	10.19
244	Table	10.19
247	"	10.20
318	"	12.1
341	"	13.1

C.H. Goulden, Methods of Statistical Analysis, 1939

Page 125 - Example 29

W.H.Leonard and A. Clark, Field Plot Technique, 1939

Chapter 12, Table 1.

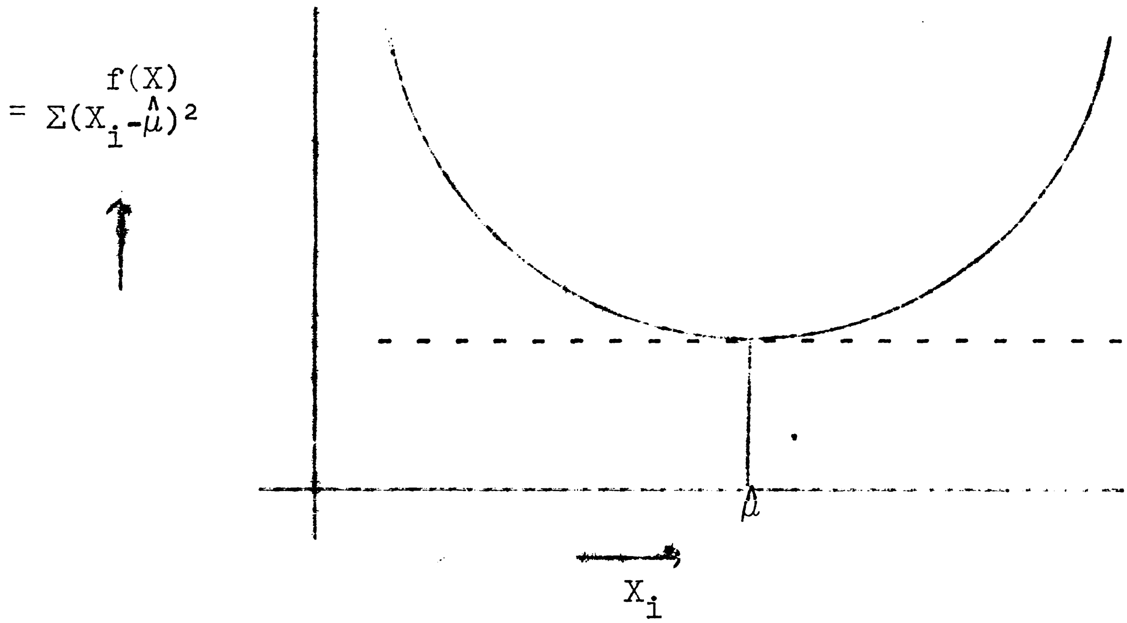
LEAST SQUARES SOLUTIONS.

It is possible to use a variety of estimates of the population parameters in summarizing the data from a sample. The estimates will have various properties, such as minimum variance, biased, unbiased, etc. Of the several estimation procedures, the Least Squares estimate of a population parameter is "best" in the sense (i) that the sum of squares of the observed values from the Least Squares estimate is a minimum, that is, the sum of squares of residuals or deviations is a minimum and (ii) that among all unbiased estimates which are linear functions of the sample data, the Least Squares estimate has the smallest sampling variance. A number of other methods of estimation, Maximum Likelihood, Moment, Minimum Chi-square, etc., may give the same estimate of a parameter as the method of Least Squares. The latter method is not unique in the sense that it alone gives a particular form of the estimate of a population parameter.

Before obtaining the Least Squares estimates and their variances for a completely randomized design, the Least Squares estimate of the mean, $\bar{x}$, and of the linear regression coefficient are obtained.

Least Squares estimate of the Mean.

Suppose that the population mean is designated as the parameter μ and the Least Squares estimate as $\hat{\mu}$. It is desired to choose $\hat{\mu}$ such that the sum of squares of the deviations from $\hat{\mu}$ is a minimum. Graphically, this may be represented as the point on the curve at which the tangent to the curve has a slope of zero. Borrowing a method from differential calculus, the point



at which f(X) has a minimum is easily found. Since $\hat{\mu}$ is the variable, the differentiation is with respect to $\hat{\mu}$, thus

$$\frac{\partial f(X)}{\partial \hat{\mu}} = \text{slope of curve at point } X = \hat{\mu}.$$

The resulting function, after partial differentiation, is set equal to zero and the solution for $\hat{\mu}$ is obtained, thus

$$\frac{\partial \left(\Sigma(X_i-\hat{\mu})^2\right)}{\partial \hat{\mu}} = -2 \sum_{i=1}^{n} (X_i - \hat{\mu}) = 0$$

and $\hat{\mu} = \dfrac{\Sigma X_i}{n} = \bar{x} =$ arithmetic mean.

(The symbol $\hat{\mu}$ replaces μ whenever the n individuals observed represent a random sample of the total population. If all individuals in the population were observed, μ could be calculated exactly and there would be no need for estimating the population mean). It turns out that the ordinary arithmetic mean is the Least Square estimate of the population parameter μ and the sum of squares of the observed values X_i from $\bar{x}$ is minimum. This also means that the sum of the sample deviations, $\Sigma(X_i - \bar{x})$, equals zero.

The mean, $\bar{x}$, is subject to sampling variation, i.e., if another random sample were drawn from the population and an estimate of μ obtained, it is highly improbable that these values will be identical. The redeeming feature is that the estimates fall, in a specifiable proportion of the cases, with a calculable interval. To calculate the interval, an estimate of the variance of the population is needed. By the method of Moments it is possible to calculate the sampling variance.

The first moment about the origin is defined as $E(X_i) = v_1 = \mu$ the expected value or average value of any randomly drawn element from the population under observation. The above agrees with the Least Squares solution for μ, i.e., if all items in the population were averaged the result would be μ. The second moment about the origin is defined as $E\,X^2 = v_2$ and the second moment about the mean as $E\,X^2 - (EX)^2 = v_2 - v_1{}^2 = \sigma^2$ where σ^2 is defined as the variance. In the normal distribution $\mu \pm \sigma$ represents the points of inflection on the normal curve. Now the following is true:

$$E(X-\mu)^2 = E(X^2 - 2X\mu + \mu^2) = EX^2 - 2\mu EX + \mu^2$$

$$= EX^2 - \mu^2 = EX^2 - (EX)^2,$$

by use of the theorems (i) that the expected value of a sum equals the sum of the expected values, and (ii) that the expected value of a constant is the constant. The variance in the population of N individuals, when μ is known, is

$$\frac{E\left[\sum_{i=1}^{N}(X_i-\mu)^2\right]}{N} = \frac{1}{N} E\left[\sum_{i=1}^{N} X_i^2 - \frac{(\Sigma X)^2}{N}\right]$$

$$= \frac{N}{N} E[X^2] - \frac{1}{N^2} E[\Sigma X_i]^2 = E[X^2] - \frac{1}{N^2}[N\mu]^2$$

$$= EX^2 - \mu^2 = \sigma^2.$$

In most instances only a random sample of n individuals is obtainable. In this case $\bar{x} = \hat{\mu}$ is the estimate of μ and a mean unbiased estimate of the sampling variance is obtained as follows:

The observation X_i may be represented as the mean of the population plus some random deviation (plus or minus) in the form of the <u>linear</u> <u>model</u>,

$$X_i = \mu + e_i .$$

The mean of n observations may be represented by the linear model

$$\bar{x} = \mu + \frac{\Sigma e_i}{n} .$$

Now,
$$E[X_i-\mu]^2 = E[\mu + e_i - \mu]^2 = E[e_i^2] = \sigma_e^2 = \sigma^2$$

and
$$E\sum_{i=1}^{n}(X_i-\bar{x})^2 = E\left[\sum_{i=1}^{n} X_i^2 - \frac{(\Sigma X)^2}{n}\right]$$

$$= \sum_{i=1}^{n} E[\mu+e_i]^2 - \frac{1}{n}E[n\mu + \Sigma e_i]^2$$

$$= n\mu^2 + n\sigma_e^2 - n\mu^2 - \sigma_e^2 = (n-1)\sigma_e^2,$$

since $E[\mu e_i] = 0 = E[e_i e_j, j \neq i]$ and $E[e_i^2] = \sigma_e^2$.

In other words the deviations are random and the correlation of any two is, on the average, equal to zero.

From the above expectation of the sum of squares of the deviations from the sample mean, it is obvious that division by n-1 instead of n results in a mean unbiased estimate, $\hat{\sigma}_e^2$, of the population variance $\sigma_e^2 = \sigma^2$.

The sampling variance of the mean has the expectation

$$E(\bar{x}-\mu)^2 = E(\mu + \frac{\Sigma e_i}{n} - \mu)^2 = E\left[\frac{\Sigma e_i}{n}\right]^2$$

$$= \frac{1}{n^2} E\left[e_1 + \ldots + e_n\right]^2 = \frac{\sigma_e^2}{n}.$$

Therefore the variance of the sample mean is estimated by the formula

$$\hat{\sigma}_{\bar{x}}^2 = \frac{\Sigma(X_i - \bar{x})^2}{n(n-1)}.$$

The sampling variance of the sample may be viewed in another manner, which is very useful for later developments. Suppose that it is desired to calculate the <u>reduction in the total sum of squares</u> ΣX_i^2 due to fitting the constant, $\bar{x}$ = an estimate of μ. The expected reduction in the sum of squares is

$$E(\bar{x}\Sigma X_i) = \frac{E(\Sigma X_i)^2}{n} = \frac{E[\Sigma(\mu + e_i)]^2}{n} = \frac{n^2\mu^2 + n\sigma_e^2}{n} = n\mu^2 + \sigma_e^2.$$

The total sum of squares has the expectation

$$E\Sigma X_i^2 = \sum_{i=1}^{n} E(\mu + e_i)^2 = n\mu^2 + n\sigma_e^2$$

Therefore the residual sum of squares after fitting the "regression coefficient" for the mean is

$$n\mu^2 + n\sigma_e^2 - n\mu^2 - \sigma_e^2 = (n-1)\sigma_e^2,$$

with n-1 degrees of freedom.

Least Squares Estimate of the Linear Regression Coefficient.

The method of Least Squares for estimating the regression of one variable, say Y_i, on another, say X_i, and the intercepts of the line is the equation

$$\hat{Y}_i = a + bX_i$$

is discussed by Wilks, Elementary Statistical Analyses, 1949, sections 13:21 to 13:24, and by several other authors.

Suppose that some variable is observed on n individuals randomly drawn from a population and that the measurements or counts are recorded as $Y_1, Y_2, \ldots, Y_n$. Also suppose than an additional observation is made on each of the n individuals, i.e., $X_1, X_2, \ldots, X_n$. The X_i are called the independent variates and are known without error. This means that on each individual item a pair of observations, Y_i and X_i, are made. If the paired data are plotted in the XY plane, the n pairs of observations form n points or coordinates. The resulting plot is a dot or scatter diagram. Now it is desired to determine the best fitting straight line to the n points or coordinates in the sense that the sum of squares of the deviations of the observed values, Y_i, from the corresponding value, $\hat{Y}_i$, on the line will be a minimum. In order to do this, it is necessary to determine the intercept a and the slope of the line, b. Since these values are determined to make the sum of squares of residuals a minimum in the sample, the partial differentiation is with respect to a and then to b. The value for any randomly drawn value Y_i may be expressed in the linear form in terms of the population parameters α and β,

$$Y_i = \alpha + \beta X_i + e_i ,$$

where e_i is the deviation between the observed Y_i and the calculated $\hat{Y}_i$ from the equation $\hat{Y}_i = a + b\,X_i$, where a and b are the Least Squares estimates of α and β. respectively. The sum of squares

$$\sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2 = \sum_{i=1}^{n} (Y_i - a - b\,X_i)^2$$

is to be made a minimum by the proper choice of a and b. Now,

$$\frac{\partial[\Sigma(Y_i - a - b\,X_i)^2]}{\partial\, a} = -2\Sigma(Y_i - a - b\,X_i) = 0$$

and

$$na = \Sigma Y_i - b\Sigma X_i$$

or

$$a = \bar{y} - b\bar{x} .$$

$$\frac{\partial[\Sigma(Y_i - a - bX_i)^2]}{\partial\, b} = -2\Sigma\, X_i(Y_i - a - bX_i) = 0 .$$

$$b\Sigma X_i^2 = \Sigma X_i Y_i - a\Sigma X_i .$$

The 2 equations and 2 unknowns may be solved as follows: Multiply the first normal equation by n and the second by ΣX_i.

$$[b\Sigma X_i^2 + a\Sigma X_i = \Sigma X_i Y_i]\; n$$

$[b\Sigma X_i \quad na = \Sigma Y_i]\; \Sigma X_i$ and subtract to obtain

$$b[n\Sigma X_i^2 - (\Sigma X_i)^2] = n\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i$$

or the Least Squares estimate of the slope is

$$b = \frac{n\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i}{n\Sigma X_i^2 - (\Sigma X_i)^2} = \frac{\Sigma(X_i - \bar{x})(Y_i - \bar{y})}{\Sigma(X_i - \bar{x})^2}$$

and of the intercept is

$$a = \bar{y} - b\bar{x} = \bar{y} - \bar{x}\,\frac{\Sigma(X_i - \bar{x})(Y_i - \bar{y})}{\Sigma(X_i - \bar{x})^2} .$$

a and b define the "best" fitting straight line to the n pairs of points in the scatter diagram.

In order to introduce the reader to the solution of simultaneous equations by the method of determinants, the following solutions of a and b from the 2 normal equations

$$\beta\Sigma X_i{}^2 + \alpha\Sigma X_i = \Sigma X_iY_i$$

$$\beta\Sigma X_i + n\alpha = \Sigma Y.$$

are:

$$b = \frac{\begin{vmatrix} \Sigma X_iY_i & \Sigma X_i \\ \Sigma Y_i & n \end{vmatrix}}{\begin{vmatrix} \Sigma X_i{}^2 & \Sigma X_i \\ \Sigma X_i & n \end{vmatrix}} = \frac{n\Sigma X_iY_i - \Sigma X_i\Sigma Y_i}{n\Sigma X_i{}^2 - (\Sigma X_i)^2}$$

$$= \frac{\Sigma(X_i - \bar{x})(Y_i - \bar{y})}{\Sigma(X_i - \bar{x})^2},$$

and

$$a = \frac{\begin{vmatrix} \Sigma X_i{}^2 & \Sigma X_iY_i \\ \Sigma X_i & \Sigma Y_i \end{vmatrix}}{\begin{vmatrix} \Sigma Y_i{}^2 & \Sigma X_i \\ \Sigma X_i & n \end{vmatrix}} = \frac{\Sigma Y_i\Sigma X_i{}^2 - \Sigma X_i\Sigma X_iY_i}{n\Sigma X_i{}^2 - (\Sigma X_i)^2}$$

$$= \frac{\Sigma Y_i}{n}\left(\frac{n\Sigma X_i{}^2 - (\Sigma X_i)^2}{n\Sigma X_i{}^2 - (\Sigma X_i)^2}\right) + \frac{\Sigma Y_i(\Sigma X_i)^2 - n\Sigma X_i\Sigma X_iY_i}{n(n\Sigma X_i{}^2 - (\Sigma X_i)^2]}$$

$$= \bar{y} - \bar{x}\left(\frac{n\Sigma X_iY_i - \Sigma Y_i\Sigma X_i}{n\Sigma X_i - (\Sigma X_i)^2}\right)$$

$$= \bar{y} - \bar{x}\,\frac{\Sigma(X_i - \bar{x})(Y_i - \bar{y})}{\Sigma(X_i - \bar{x})^2}.$$

For more complicated situations the notion of a matrix and an inverse matrix (see Ferrar, Algebra, 1941) is of considerable importance for obtaining the variances of Least Squares estimates.

A matrix is an array of numbers and may be represented symbolically as

$$\left\| X_{ij} \right\| = \left\| \begin{matrix} X_{11} & X_{12} & X_{13} & \cdots & X_{1n} \\ X_{21} & X_{12} & X_{23} & \cdots & X_{2n} \\ \cdot & & & & \\ \cdot & & & & \\ \cdot & & & & \\ X_{k1} & X_{k2} & X_{k3} & \cdots & X_{kn} \end{matrix} \right\|$$

If n = k, then the above matrix is known as a square matrix, and there is a determinant of the matrix. The matrix in itself has no numerical value but the determinant of this array of numbers may be calculated. The determinant is symbolized by single bars, thus

$$\left| X_{ij} \right| = \left| \begin{matrix} X_{11} & X_{12} & \cdots & X_{1k} \\ \cdot & & & \\ \cdot & & & \\ \cdot & & & \\ X_{k1} & X_{k2} & \cdots & X_{kk} \end{matrix} \right|$$

For the preceding example the matrix of coefficients is

$$\left\| \begin{matrix} \Sigma X_i^2 & \Sigma X_i \\ \Sigma X_i & n \end{matrix} \right\|$$

and the determinant of the matrix is

$$\left| \begin{matrix} \Sigma X_i^2 & \Sigma X_i \\ \Sigma X_i & n \end{matrix} \right| = n\Sigma X_i^2 - (\Sigma X_i)^2 = D.$$

The inverse or reciprocal matrix is denoted by the symbol

$$\left\| X_{ij} \right\|^{-1}$$

and is such that

$$\left\| X_{ij} \right\|^{-1} \left\| X_{ij} \right\| = \text{unit matrix,}$$

which is a matrix with ones in the leading diagonal and zeros elsewhere,

$$\begin{Vmatrix} 1 & 0 & 0 & \cdots & 0 \\ 0 & 1 & 0 & \cdots & 0 \\ 0 & 0 & 1 & \cdots & 0 \\ \vdots & & & \ddots & \\ 0 & 0 & 0 & \cdots & 1 \end{Vmatrix} = \text{unit matrix.}$$

The inverse of the matrix of coefficients is

$$\begin{Vmatrix} \frac{n}{D} & -\frac{\Sigma X_i}{D} \\ \frac{-\Sigma X_i}{D} & \frac{\Sigma X_i^2}{D} \end{Vmatrix} = \frac{1}{D} \begin{Vmatrix} n & -\Sigma X_i \\ -\Sigma X_i & \Sigma X_i^2 \end{Vmatrix}$$

$$= \begin{Vmatrix} \Sigma X_i^2 & \Sigma X_i \\ \Sigma X_i & n \end{Vmatrix}^{-1} .$$

The inverse of a 3x3 matrix is

$$\begin{Vmatrix} X_{11} & X_{12} & X_{13} \\ X_{21} & X_{22} & X_{23} \\ X_{31} & X_{32} & X_{33} \end{Vmatrix}^{-1} =$$

$$\frac{1}{D} \begin{Vmatrix} (X_{22}X_{33}-X_{23}X_{32}) & -(X_{21}X_{33}-X_{23}X_{31}) & (X_{21}X_{32}-X_{22}X_{31}) \\ -(X_{12}X_{33}-X_{13}X_{32}) & (X_{11}X_{33}-X_{31}X_{13}) & -(X_{11}X_{32}-X_{12}X_{31}) \\ (X_{12}X_{23}-X_{13}X_{22}) & -(X_{11}X_{23}-X_{13}X_{21}) & (X_{11}X_{22}-X_{12}X_{21}) \end{Vmatrix} ,$$

where

$$D = \begin{vmatrix} X_{11} & X_{12} & X_{13} \\ X_{21} & X_{22} & X_{23} \\ X_{31} & X_{32} & X_{33} \end{vmatrix} = X_{11}X_{22}X_{33} + X_{12}X_{23}X_{31} + X_{13}X_{21}X_{32} - X_{13}X_{22}X_{31} - X_{12}X_{21}X_{33} - X_{11}X_{32}X_{23} .$$

Also,

$$\frac{1}{D} \begin{Vmatrix} n & -\Sigma X_i \\ \Sigma X_i & \Sigma X_i^2 \end{Vmatrix} \cdot \begin{Vmatrix} \Sigma X_i^2 & \Sigma X_i \\ \Sigma X_i & n \end{Vmatrix} = \frac{1}{D} \begin{Vmatrix} n\Sigma X_i^2-(\Sigma X_i)^2 & n\Sigma X_i-n\Sigma X_i \\ -\Sigma X_i^2\Sigma X_i+\Sigma X_i\Sigma X_i^2 & n\Sigma X_i^2-(\Sigma X_i)^2 \end{Vmatrix}$$

$$= \begin{Vmatrix} 1 & 0 \\ 0 & 1 \end{Vmatrix} = \text{unit matrix.}$$

The predicted values in terms of the Least Squares estimates are

$$\hat{Y}_i = a + bX_i = \bar{y} + b(X_i - \bar{x}) .$$

The variance of the Least Squares estimate of the population intercept α is $Ea^2 - \alpha^2$. Now,

$$Ea^2 = E\left[\frac{\Sigma Y_i}{n} - \frac{\Sigma X_i}{n}\frac{(n\Sigma X_i Y_i - \Sigma X_i \Sigma Y_i)}{(n\Sigma X_i^2 - (\Sigma X_i)^2)}\right]^2$$

$$= E\left[\alpha + \frac{\beta\Sigma X_i}{n} + \frac{\Sigma e_i}{n} - \frac{\Sigma X_i}{n}\left(\frac{n\alpha\Sigma X_i + n\beta\Sigma X_i^2 + n\Sigma X_i e_i - \Sigma X_i(n\alpha + \beta\Sigma X_i + \Sigma e_i)}{n\Sigma(X - \bar{x})^2}\right)\right]^2$$

$$= E\left[\alpha + \beta\bar{x} + \frac{\Sigma e_i}{n} - \bar{x}\left(\frac{n\beta\Sigma X_i^2 - \beta(\Sigma X_i)^2 + n\Sigma X_i e_i - \Sigma X_i \Sigma e_i}{n\Sigma(X - \bar{x})^2}\right)\right]^2$$

$$= E\left[\alpha + \frac{\Sigma e_i}{n} - \bar{x}\left(\frac{n\Sigma X_i e_i - \Sigma X_i \Sigma e_i}{n\Sigma(X - \bar{x})^2}\right)\right]^2$$

$$= E\left[\alpha + \frac{\Sigma e_i}{n} - \bar{x}\,\frac{\Sigma(X - \bar{x})(e_i - \bar{e})}{\Sigma(X - \bar{x})^2}\right]^2$$

$$= \alpha^2 + E\left[\left(\frac{\Sigma e_i}{n}\right)^2 + \bar{x}^2\left(\frac{\Sigma(X - \bar{x})(e_i - \bar{e})}{\Sigma(X - \bar{x})^2}\right)^2 + \text{cross products}\right]$$

$$= \alpha^2 + \frac{\sigma_e^2}{n} + \frac{\bar{x}^2\sigma_e^2\Sigma(X - \bar{x})^2}{[\Sigma(X - \bar{x})^2]^2} = \alpha^2 + \sigma_e^2\left[\frac{1}{n} + \frac{\bar{x}^2}{\Sigma(X - \bar{x})^2}\right]$$

$$= \alpha^2 + \frac{\sigma_e^2\Sigma X_i^2}{n\Sigma(X - \bar{x})^2} .$$

Therefore the variance of the Least Squares estimate a is

$$Ea^2 - \alpha^2 = \frac{\hat{\sigma}_e^2\Sigma X_i^2}{n\Sigma(X - \bar{x})^2} ,$$ where $\hat{\sigma}_e^2$ is the sample estimate of the population parameter $\sigma_e^2 = \sigma^2$.

$$E(b-\beta)^2 = Eb^2 - \beta^2 .$$

$$Eb^2 = E\left[\frac{\Sigma(X-\bar{x})(Y-\bar{y})}{\Sigma(X-\bar{x})^2}\right]^2 = E\left[\frac{\Sigma XY - \Sigma X\Sigma Y/n}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= E\left[\frac{\Sigma(X_i\alpha + \beta X_i^2 + e_iX_i) - \frac{\Sigma X(n\alpha+\beta\Sigma X_i+\Sigma e_i)}{n}}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= E\left[\frac{\alpha\Sigma X_i + \beta\Sigma X_i^2 + \Sigma e_iX_i - \alpha\Sigma X_i - \beta\frac{(\Sigma X_i)^2}{n} - \frac{\Sigma X\Sigma e_i}{n}}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= E\left[\frac{\beta\Sigma(X-\bar{x})^2 + \Sigma(X_i-\bar{x})(e_i-\bar{e})}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= E\left[\beta + \frac{\Sigma(X_i-\bar{x})(e_i-\bar{e})}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= \beta^2 + 2\beta E\,\frac{\Sigma(X_i-\bar{x})(e_i-\bar{e})}{\Sigma(X-\bar{x})^2} + E\left[\frac{\Sigma(X-\bar{x})(e_i-\bar{e})}{\Sigma(X-\bar{x})^2}\right]^2$$

$$= \beta^2 + 0 + E\left[\frac{\Sigma(X-\bar{x})(e_i-\bar{e})}{[\Sigma(X-\bar{x})^2]^2}\right]^2 = \beta^2 + \frac{\sigma_e^2}{\Sigma(X-\bar{x})^2} .$$

Therefore,

$$E(b)^2 - \beta^2 = \frac{\sigma_e^2}{\Sigma(X-\bar{x})^2} , \quad \text{and}$$

$$\sigma_b^2 = \hat{\sigma}_\beta^2 = \frac{\hat{\sigma}_e^2}{\Sigma(X-\bar{x})^2} .$$

The covariance of a and b is

$$E(ab) = \sigma_{ab} = \text{cov}(ab) = \frac{-\sigma_e^2\Sigma X_i}{n\Sigma(X_i-\bar{x})^2} \quad \text{and}$$

is estimated by $-\dfrac{\hat{\sigma}_e^2\Sigma X_i}{n\Sigma(X_i-\bar{x})^2}$.

It might be pointed out that the elements of the inverse matrix times $\hat{\sigma}_e^2$ gives the variance of the Least Squares estimates a and b, thus

$$\sigma_b^2 = \hat{\sigma}_\beta^2 = \frac{n}{D}\hat{\sigma}_e^2 = \frac{\hat{\sigma}_e^2}{\Sigma(X-\bar{x})^2} ,$$

$$\sigma_a^2 = \hat{\sigma}_\alpha^2 = \frac{\Sigma X_i^2}{D}\hat{\sigma}_e^2 = \frac{\Sigma X_i^2\hat{\sigma}_e^2}{n\Sigma X_i - (\Sigma X_i)^2}$$

$$= \frac{\Sigma X_i^2 \ \hat{\sigma}_e^2}{n\Sigma(X-x)^2} \text{ , and}$$

$$\sigma_{ab}^2 = \hat{\sigma}_{\alpha\beta} = \frac{-\Sigma X_i}{D}\hat{\sigma}_e^2 = \frac{-\hat{\sigma}_e^2 \ \Sigma X_i}{n\Sigma(X_i-\bar{x})^2} .$$

The above use of the inverse matrix is extremely helpful in obtaining the variance of Least Squares estimates in the more complex cases.

With these results in mind it is possible to obtain the reduction in sum of squares due to fitting the intercept a (in reality a regression coefficient) and to fitting the linear regression coefficient or the slope, b. The reduction is the value of the quantity

$$a\Sigma Y_i + b\Sigma X_i Y_i.$$

It will be noted that the totals ΣY_i and $\Sigma X_i Y_i$ are the right hand sides of the normal equations

$$\beta\Sigma X_i^2 + \alpha\Sigma X_i = \Sigma X_i Y_i$$
$$\beta\Sigma X_i + n\alpha = \Sigma Y_i$$

The total sum of squares, ΣY_i^2, minus the reduction due to fitting the regression coefficients a and b has the expectation

$$E\left[\sum_{i=1}^{n} Y_i^2 - a\Sigma Y_i - b\Sigma X_i Y_i\right] = E\left[\sum_{i=1}^{n} Y_i^2 - \frac{(\Sigma Y)^2}{n} - b^2\Sigma(X-\bar{x})^2\right]$$

$$= \sum_{i=1}^{n} E(\alpha+\beta X_i+e_i)^2 - \frac{1}{n}E(n\alpha+\beta\Sigma X_i+\Sigma e_i)^2 - E\Sigma(X-\bar{x})^2 b^2 = n\alpha^2+\beta^2\Sigma X_i^2$$

$$+n\sigma_e^2 - n\alpha^2 - \frac{\beta^2(\Sigma X)^2}{n} - \sigma_e^2 - \Sigma(X-\bar{x})^2(\sigma_b^2+\beta^2) = (n-1)\sigma_e^2 - \sigma_b^2\Sigma(X-\bar{x})^2$$

$$= (n-2)\sigma_e^2 .$$

Hence, division of the residual sum of squares by n-2 results in an unbiased estimate of the variance in the population after taking account of the variation caused by the mean and of the related variation in the X_i.

The expectation of the residual sum of squares may be obtained similarly for multiple regression, that is, for each observation, Y_i, some related values X_{1i}, X_{2i}, etc. may be observed. Their effect on the variation in the Y_i may be removed and the residual sum of squares obtained. For the case of 2 independent variables, X_1 and X_2, the sum of squares to be minimized is

$$\Sigma(Y_i - a - bX_{1i} - cX_{2i})^2 ,$$

the 3 normal equations are

$$an + b\Sigma X_{1i} + c\Sigma X_{2i} = \Sigma Y_i$$
$$a\Sigma X_{1i} + b\Sigma X_{1i}^2 + c\Sigma X_{1i}X_{2i} = \Sigma X_{1i}Y_i$$
$$a\Sigma X_{2i} + b\Sigma X_{1i}X_{2i} + c\Sigma X_{2i}^2 = \Sigma X_{2i}Y_i$$

and the reduction in the total sum of squares due to fitting the 3 estimated regression coefficients a, b, and c, is

$$a\Sigma Y_i + b\Sigma X_{1i}Y_i + c\Sigma X_{2i}Y_i \quad .$$

The residual sum of squares has the expectation

$$(n-3)\,\sigma_e^2 \quad .$$

<u>Least Squares Estimates and Variances for a Completely Randomized Design.</u>

For a completely randomized design the yield of any treatment may be represented by the linear model

$X_{ij} = \mu + \tau_i + e_{ij}$ = treatment mean + random deviation, where μ represents the population mean, τ_i = the effect peculiar to the ith treatment or = the partial regression coefficient for the ith treatment, e_{ij} represents the random deviation of the jth member of the ith treatment, and i = 1,2,..., k = number of treatments. The subscript j(= 1,2,... n_i= number of repetitions or individuals in the ith treatment) denotes the particular individual in a treatment. For the first example consider an equal number n = n_i of repetitions per treatment.

The partial regression coefficients to be estimated are

$$\mu, \tau_1, \tau_2, \ldots, \tau_k,$$

or k+1 constants. However, in order to obtain a unique solution the restriction that the sum of the t_i, where the t_i are the Least Squares estimates of τ_i, must equal zero,

$$\sum_{i=1}^{k} t_i = 0$$

is imposed.

The sum of squares to be minimized is

$$\sum_i \sum_j (X_{ij} - \hat{\mu} - t_i)^2 = \text{Res.}^2$$

and the partial differentials are set equal to zero,

$$\frac{\partial \text{Res}^2}{\partial \hat{\mu}} = -2 \sum_{i,j} (X_{ij} - \hat{\mu} - t_i) = 0$$

$$\frac{\partial \text{Res}^2}{\partial t_1} = -2 \sum_j (X_{1j} - \hat{\mu} - t_1) = 0$$

$$\frac{\partial \text{Res}^2}{\partial t_2} = -2 \sum_j (X_{2j} - \hat{\mu} - t_2) = 0$$

$$\vdots$$

$$\frac{\partial \text{Res}^2}{\partial t_k} = -2 \sum_j (X_{kj} - \hat{\mu} - t_k) = 0$$

From the above, the k+1 normal equations are obtained:

$$X_{\cdot\cdot} = \sum_{i=1}^{k} \sum_{j=1}^{n} X_{ij} = n(t_1 + t_2 + t_3 + \dots + t_k) + nk\hat{\mu}$$

$$X_{1\cdot} = \sum_{j=1}^{k} X_{1j} = nt_1 + n\hat{\mu}$$

$$X_{2\cdot} = \Sigma X_{2j} = nt_2 + n\hat{\mu}$$

$$\vdots$$

$$X_{k\cdot} = \Sigma X_{kj} = nt_k + n\hat{\mu}$$

Remembering that $\Sigma t_i = 0$, the Least Squares estimate of the mean is $\hat{\mu} = \bar{x} = \dfrac{\Sigma\Sigma X_{ij}}{nk} = \dfrac{X_{\cdot\cdot}}{nk}$.

Using this estimate of μ, the remaining constants may be estimated,

$$t_1 = \frac{X_{1\cdot}}{n} - \hat{\mu} = \bar{x}_1 - \bar{x}$$

$$t_2 = \frac{X_{2\cdot}}{n} - \hat{\mu} = \bar{x}_2 - \bar{x}$$

$$\vdots$$

$$t_k = \frac{X_{k\cdot}}{n} - \hat{\mu} = \bar{x}_k - \bar{x}$$

Now $E(t_i^2) = \sigma_\tau^2$, $Ee_{ij}^2 = \sigma_e^2$, $E\mu^2 = \mu^2$, and the expected value of all cross products is zero.

The reduction in the sum of squares due to fitting $\hat{\mu} = \bar{x}$ has the expectation

$$E[\hat{\mu}X_{\cdot\cdot}] = \frac{E(\Sigma\Sigma X_{ij})^2}{nk} = \frac{1}{nk} E[nk\mu + n(\Sigma\tau_i + \Sigma\Sigma e_{ij}]^2$$

$$= nk\mu^2 + n\sigma_\tau^2 + \sigma_e^2.$$

The reduction in the sum of squares due to fitting the t_i has the average value

$$E[t_1X_{1.} + t_2X_{2.} + t_3X_{3.} + \ldots + t_kX_{k.}]$$

$$= E[\frac{\Sigma X_{i.}^2}{n} - \hat{\mu}\,\Sigma X_{i.}]$$

$$= E[\frac{\Sigma X_{i.}^2}{n} - \frac{X_{..}^2}{nk}]$$

$$= \sum_{i=1}^{k} \frac{E[n\mu + nt_i + (e_{i1} + e_{i2} + \ldots + e_{in})]^2}{n} - \frac{E\,X_{..}^2}{nk}$$

$$= nk\mu^2 + nk\sigma_\tau^2 + k\sigma_e^2 - nk\mu^2 - n\sigma_\tau^2 - \sigma_e^2$$

$$= (k-1)(\sigma_e^2 + n\sigma_\tau^2).$$

The residual sum of squares after fitting the constants, μ and t_i has the expectation:

$$E[\sum_{ij}\Sigma X_{ij}^2 - \hat{\mu}\,X_{..} - \sum_{i=1}^{k} t_iX_i]$$

$$= \sum_{i=1}^{k}\sum_{j=1}^{n}(\mu + \tau_i + e_{ij})^2 - E[\frac{\Sigma X_{i.}^2}{n} - \frac{X_{..}^2}{nk} + \frac{X_{..}^2}{nk}]$$

$$= nk\mu^2 + nk\sigma_\tau^2 + nk\sigma_e^2 - (nk\mu^2 + nk\sigma_\tau^2 + k\sigma_e^2)$$

$$= k(n-1)\sigma_e^2.$$

The total sum of squares corrected for the mean has the expectation

$$E[\sum_{ij}\Sigma X_{ij}^2 - \frac{X_{..}^2}{nk}] = nk\mu^2 + nk\sigma_\tau^2 + nk\sigma_e^2 - nk\mu^2 - n\sigma_\tau^2 - \sigma_e^2$$

$$= (k-1)n\sigma_\tau^2 + (nk-1)\sigma_e^2 = (k-1)(\sigma_e^2 + n\sigma_\tau^2) + k(n-1)\sigma_e^2$$

= treatment sum of squares + within sum of squares.

For the case where the $n_i \neq n$, the normal equations become

$$X_{..} = k\Sigma n_i\hat{\mu} + \Sigma n_i\tau_i$$

$$X_{1.} = n_1\tau_1 + n_1\hat{\mu}$$

$$X_{2.} = n_2\tau_2 + n_2\,\hat{\mu}$$

$$\vdots$$

$$X_{k.} = n_k\tau_k + n_k\,\hat{\mu}$$

Here, the Least Squares estimates are not so easily obtained. Applying the preceding method for estimating μ,

$$\hat{\mu} = \frac{X_{..}}{k\Sigma n_i} - \frac{n_1 t_1 + n_2 t_2 + \ldots + n_k t_k}{k\Sigma n_i}$$

which is not free of the t_i. Now any class mean minus the general mean results in an estimate of the t_i

$$t_1 = \frac{X_{1.}}{n_1} - \hat{\mu}$$

$$t_2 = \frac{X_{2.}}{n_2} - \hat{\mu}$$

$$t_3 = \frac{X_{3.}}{n_3} - \hat{\mu}$$

$$\vdots$$

$$t_k = \frac{X_{k.}}{n_k} - \hat{\mu}$$

Summing the above k equations, an estimate of μ is obtained,

$$\sum_i t_i = 0 = \sum_i \frac{X_{i.}}{n_i} - k\hat{\mu}$$

$$\therefore\ \hat{\mu} = \frac{1}{k}\,\Sigma\,\frac{X_{i.}}{n_i} = \frac{\Sigma\bar{x}_i}{k}$$

= arithmetic average of the treatment means.

The expectation of the total sum of squares is:

$$E\left[\sum_{i=1}^{k}\sum_{j=1}^{n_i} X_{ij}^2\right] = \sum_{i=1}^{k} n_i\ (\mu^2 + \sigma_\tau^2 + \sigma_e^2)$$

for the correction term for the mean;

$$E\left[\frac{X..^2}{\sum_{i=1}^{k} n_i}\right] = (\sum_{i=1}^{k} n_i)\mu^2 + \frac{\sum_{i=1}^{k} n_i^2\sigma_\tau^2}{\Sigma n_i} + \sigma_e^2$$

for the treatment sum of squares:

$$E\left[\sum_{i=1}^{k} \frac{X_{i.}^2}{n_i} - \frac{X..^2}{\Sigma n_i}\right] = (k-1)\sigma_e^2 + \left(\Sigma n_i - \frac{\Sigma n_i^2}{\Sigma n_i}\right)\sigma_\tau^2$$

and for the error sum of squares:

$$E\left[\sum_{i=1}^{k}\sum_{j=1}^{n_i} X_{ij}^2 - \sum_{i=1}^{k} \frac{X_{i.}^2}{n_i}\right]$$

$$= \sum_{i=1}^{k} n_i\ (\mu^2 + \sigma_\tau^2 + \sigma_e^2) - (k\sigma_e^2 + \Sigma n_i(\sigma_\tau^2 + \mu^2))$$

$$= \sigma_e^2\ \left(\sum_{i=1}^{k} n_i - k\right)\ .$$

If the $n_i = n$, the above coefficients are the same as those obtained above for equal numbers of individuals per treatment.

Chapter IV.

RANDOMIZED COMPLETE BLOCKS DESIGN.

by

W.T. Federer.

A randomized complete blocks design is one in which the site of the experiment is divided into a number of compact blocks, each block containing as many plots as there are treatments. The treatments are assigned at random to the plots in each block. There are slight variations in the various randomized blocks designs. In some instances, a check variety or treatment may be included more than once in each block.

Using the same example as in the design above, the five treatments A, B, C, D, and E may be included in each of the four blocks once and only once. The following diagram illustrates the experimental lay-out for the field, laboratory, or greenhouse.

Block I	(E) (1)	(A) (2)	(C) (3)	(B) (4)	(D) (5)
" II	(A) (10)	(D) (9)	(B) (8)	(C) (7)	(E) (6)
" III	(B) (11)	(C) (12)	(A) (13)	(E) (14)	(D) (15)
" IV	(E) (20)	(D) (19)	(A) (18)	(B) (17)	(C) (16)

The breakdown of the total degrees of freedom is:

Source of variation	Degrees of freedom	Mean square
Among 5 treatments	4	T
" 4 blocks	3	R
Remainder or error	12	E
Total	19	

As is apparent from the analysis, three of the degrees of freedom are segregated from the error degrees of freedom, for a completely randomized design. These three degrees of freedom are associated with the sum of squares attributable to the differences among the means of the four blocks.

If an experiment had been conducted as a randomized complete blocks design, it is possible to determine what the efficiency would have been had the experiment been conducted as a completely randomized design. The calculated variance for the latter design is obtained from the sum of squares for blocks plus the sum of squares obtained by multiplying the error mean square by the treatment plus error degrees of freedom and dividing by the total degrees of freedom. Symbollically, this is:

$$E' = \frac{(\text{treatment plus error degrees of freedom})E + (\text{Block degrees of freedom})R}{\text{treatment + error + block degrees of freedom}}$$

The efficiency of the randomized complete blocks design relative to the completely randomized design is the ratio of the amount of informations on the designs. The amount of information (Fisher, Design of Experiments 1942, and Snedecor, Statistical Methods, 1946) is defined as the reciprocal of the error variance. The efficiency of the randomized complete blocks design relative to what it would have been had a completely randomized design been used is

$$\frac{1}{E} \Big/ \frac{1}{E'} = \frac{E'}{E} \quad \text{in percent.}$$

The increase in efficiency due to the use of randomized complete blocks is equal to

$$1 - \frac{E'}{E} \quad \text{in percent.}$$

The shape of the complete block is usually as nearly square as possible, the reason for this being that the experimenter has little or no knowledge regarding the variation in perpendicular directions. Therefore, he usually selects a square or nearly square complete block and hopes that the variation is about equal in both directions. In some instances, it may be extremely undesirable to use square blocks. An example of this would be the lay-out of a randomized complete blocks experiment on contours. A single block should probably be confined to one contour, which would result in a long narrow block. The variation down one contour would probably be more nearly equal to the variation along the oontour than if the complete block were designed to include several contours. Another example of this would be in the design of a greenhouse experiment for the case where the heat source might be at one side of the experiment. Here again, the experimenter might profitably choose a long narrow block rather than a square one. Although it had been commonly advocated that only square blocks be used, the experimenter may be better off on the average if he follows the general rule to select a replicate shape that would make the variation in both directions approximately equal, and, consequently, making the variation within the whole block as small as possible. The variation among the replicates possible for the experimental site should be maximized.

The size and shape of the plots within a complete block have been discussed by a number of workers. (Love, Hutchinson and Panse, Cochran, etc.) In general it may be advocated that long narrow plots are preferable to square ones. The object in this case is to select plot sizes and shapes so that the variation among them is as small as possible.

The amount of replication required will depend upon the precision with which the experimenter wishes to measure the treatment m means. He usually has some idea regarding the co-efficient of variation in the material under observation. Also he has some idea of the size of the difference between two treatments which is of practical significance. With these facts then, he may decide the approximate number of replicates to use, by choosing the number of replicates giving the desired degree of precision.

The chief advantages of the randomized complete blocks design are:

(i) Accuracy. This design has been shown to be more accurate than the previous design for most types of experimental work. The elimination of the blocks sum of squares from the error sum of squares usually results in a decrease in the error mean square.

(ii) Flexibility. The design places no restrictions on the number of treatments or on the number of replicates. In general, however, at least two replicates are required to obtain tests of significance,(see later chapters for exceptions). In addition, the standard or check treatments may be included more than once with little complication to the analysis.

(iii) Ease of analysis. The statistical analysis is simple and rapid. Moreover, the error of any treatment comparison can be isolated and any number of treatments may be omitted from the analysis without complicating it. These facilities may be useful when certain treatment differences turn out to be very large, when some treatments produce crop failures or when the experimental material is heterogeneous.

The chief disadvantage of the randomized blocks design is that it is not too suitable for large numbers of treatments, or for cases in which the complete block contains considerable variability.

Because of its advantages regarding accuracy, flexibility, and ease of analysis, the randomized complete blocks design is probably the most widely used of any design.

The advantages, computational procedure, and efficiency of randomized complete block designs are illustrated in the following examples.

Example IV-1.

Uniformity trial data on corn (Zuber, 1940) were used to construct the first numerical example illustrating statistical computations involved for data obtained from randomized complete blocks experiments. The 12 plot yields in Table IV-1 represent the yield in pounds of ear corn per 2x10 hill plot. The spacing between hills was 3.5 feet. Thus, the dimensions of an individual plot are 7x35 feet and of the complete block, 35x21 feet. The plot and block shape agree fairly well with the principles enunciated in the foregoing section. Before proceeding with the computations, it should be remembered that the 3 so-called varieties, A, B, and C, are the same thing. The comparisons among the "varieties" may be called dummy comparisons, but these are made only to illustrate the numerical procedure and some interpretations involved in the course of experimentation.

All computations necessary to obtain the analysis of variance table are given in Tables IV-1 and 2. The F values were obtained as

TABLE IV-1. Field arrangement of 3 varieties of corn in 4 randomized complete blocks. Plot sizes of 2x10 hills of corn with 3.5 feet between hills.

	I			II	
1 - C 30.6	2 - B 32.0	3 - A 30.3	4 - A 33.0	5 - B 34.4	6 - C 33.1
12 - C 29.9	11 - A 31.6	10 - B 32.5	9 - A 31.0	8 - C 29.2	7 - B 29.7

70 ft.

42 feet

	Totals	Means
Replicate I	92.9	-
" II	100.5	-
" III	89.9	-
" IV	94.0	-
Variety A	125.9	31.48
" B	128.6	32.15
" C	122.8	30.70
Total	377.3	31.44

TABLE IV-2. Sums of squares and analysis of variance table for data in Table IV-1.

Correction term $= \frac{(377.3)^2}{12} = 11{,}862.94$

Total sum of squares =

$$30.6^2 + \ldots + 29.9^2 - \frac{(377.3)^2}{12} = 11{,}890.97 - 11{,}862.94$$
$$= 28.03 \text{ with 11 d.f.}$$

Replicate sum of squares =

$$\frac{92.9^2 + \ldots + 94.0^2}{3} - \frac{(377.3)^2}{12} = 11{,}882.89 - 11{,}862.94$$
$$= 19.95 \text{ with 3 d.f.}$$

Variety sum of squares =

$$\frac{125.9^2 + 128.6^2 + 122.8^2}{4} - \frac{(377.3)^2}{12} = 11{,}867.15 - 11{,}862.94$$
$$= 4.21 \text{ with 2 d.f.}$$

Interaction sum of squares by subtraction =

$28.03 - 19.95 - 4.21 = 3.87$ with 6 d.f.

Analysis of variance of yields:

Source of variation	d.f.	s.s.	m.s.	F
Replicates	3	19.95	6.650	10.31
Varieties	2	4.21	2.105	3.26
Error	6	3.87	0.645	
Total	11			

$$F = \frac{2.105}{0.645} = 3.26 \quad (F_{05} = 5.14)$$

and

$$F = \frac{6.650}{0.645} = 10.31 \quad (F_{01} = 9.78)$$

The latter F value exceeds the tabulated F value at the one percent level of probability, for 3 degrees of freedom in the greater mean square and 6 in the lesser mean square. A more appropriate F test of the differences among whole blocks (for the case of uniformity trial data <u>only</u>) would be the replicate mean square divided by the within replicate mean square,

$$F = \frac{6.650}{\frac{4.21 + 3.87}{8}} = \frac{6.650}{1.01} = 6.58$$

where F_{05} (3 and 8 d.f.) = 4.07 and F_{01} = 7.59.
In either case, the particular layout was effective in removing variation.

The F test of the "variety" differences indicates that the variation among the 3 means are not to be considered unusually large. The probability of obtaining an F of 3.26 or larger may be <u>approximated</u> from the formula given by E. Paulson (Annals.Math. Stat. 1942). This formula is applicable for 3 or more d.f. in the error variance. Using Paulson's formula an F of 3.26 or larger occurs in 20 to 30 percent of the cases, thus

$$U = \frac{\left(1 - \frac{2}{9n_e}\right) F^{\frac{1}{3}} - \left(1 - \frac{2}{9n_g}\right)}{\sqrt{\frac{2}{9n_e} F^{2/3} + \frac{2}{9n_g}}}$$

(where n_e = error degrees of freedom and n_g = d.f. for mean square in the numerator of F)

$$= \frac{\left(1 - \frac{2}{9(6)}\right)(3.26)^{1/3} - \left(1 - 2/9(2)\right)}{\sqrt{\frac{2}{9(6)}(3.26)^{2/3} + \frac{2}{9(2)}}}$$

= 1.23, which may be compared with the tabulated values of t for 6 d.f. (see Fisher, Statistical Methods, Table IV, p.169, 1944).

A more accurate probability value for an experimental F may be obtained from the formula set forward by Bancroft (Annals of Math.Stat. 194?)

In general practice, non-significance among the 3 means would be the end of computations. However, the experimenter may still want to know the coefficient of variability and the magnitude of differences necessary for significance. The coefficient of variation is

$$v = \frac{s}{\bar{x}} = \frac{\sqrt{0.645}}{31.44} = \frac{0.803}{31.44} = 2.6 \text{ percent},$$

which is low for most experimental work on corn yields with 2x10 hill plots (the average coefficient of variation for corn yield trials in Iowa is about 9-12 percent for randomized complete blocks designs. Federer, 1948).

The average least significant difference between 2 variety means is

$$\text{lsd} = t_{05}(6 \text{ d.f.})s_d = 2.447 \sqrt{\frac{2(0.645)}{4}} = 1.39.$$

The least significant difference for the comparison of the highest with the lowest yielding variety in a sample of 3 (Snedecor, 1946 Table 5.5) is

$$\text{lsd} = 3.34(.568) = 1.90.$$

The standard error of a mean is

$$s_{\bar{x}} = \frac{s}{\sqrt{n}} = \frac{0.803}{\sqrt{4}} = 0.40 \ .$$

The efficiency of this design compared to what it would have been had a completely randomized design been used is the ratio of the two variances,

$$\frac{\dfrac{(0.645)(2 + 6) + 6.650(3)}{2 + 6 + 3}}{0.645} = \frac{2.283}{0.645} = 354 \text{ percent.}$$

This means that (3.5)(4) = 14 replicates of a completely randomized design would have been required to estimate the means with the same precision as the present design, with only 4 replicates. It might be pointed out that such large gains in efficiency, 254 percent, are not generally expected.

The z test (Fisher, Statistical Methods) may be used in lieu of the F test if desired. For this example

$$Z = \frac{1}{2}\left(\log_e 2.105 - \log_e 0.645\right)$$
$$= \frac{1}{2}\left(.7444 - 9.561 + 10\right) = .592 ,$$

and the z value (Table VI, Fisher, Statistical Methods) at the 5 percent point for $n_2 = 6$ and $n_1 = 2$ degrees of freedom is 0.8188. The z test agrees with the F as it should since

$$z = \frac{1}{2} \log_e F.$$

Example IV-2.

The preceding example illustrates the analysis for one unit per plot. In some instances several units per plot may be measured. In a feeding trial of weight groups and rations several steers could be included in each cell or lot of the two-way classification or for a randomized blocks field experiment several observations of the same variable could be taken on each plot.

The data presented in Table IV-3 represent the grams of rubber obtained from 2 randomly selected plants in a plot for each of the 7 varieties of guayule planted in the 5 replicates. The allocation of the varieties to the seven plots in each replicate was random. The plot size was 28 plants long by 12 rows wide with 20" between plants within a row and 24" between rows resulting in a plot of $\frac{1}{12}$ [(28x20) x (12x24)] = $46^{2}/3$' x 24'. The replicate size was 7x24' by $46^{2}/3$' = 168' x $46^{2}/3$'. The shape of the replicate may not have been the most desirable except for the fact that the irrigation was perpendicular to the length of the replicate. In such an experiment, the best guess was to have a rectangular shaped replicate in preference to a square one, since the plots in each replicate would be treated similarly with regard to time and amount of irrigation. However, plots 6 rows wide by 56 plants long may have been the best shape in relation to replicate shape for this experiment. In instances where the plot shape is fixed the rectangular shaped replicate may prove to be the most efficient for irrigation experiments.

The sums, means, and sums of squares for the data in Table IV-3 are presented in Table IV-4. The results are summarized in Table IV-5. The mean squares are obtained from the division of the sums of squares by the appropriate degrees of freedom.

TABLE IV-3. Field arrangement of 7 varieties of guayule in 5 randomized complete blocks and weight (grams) of rubber for 2 randomly selected plants.

5x28= 140 ft.

≠ 7-130-4.06 -3.75	6-406-6.65 -6.17	5-593-6.85 -4.94	4-109-1.46 -6.39	3-416-2.96 -2.71	2-405-2.53 -6.93	1-407-2.06 -6.12
8-109-4.07 -7.73	9-593-5.92 -5.00	10-405-1.85 -6.44	11-406-4.06 -6.65	12-416-4.35 -5.85	13-130-9.27 -6.64	14-407-5.00 -5.12
21-593-3.88 -6.22	20-407-2.59 -4.79	19-406-7.77 -6.91	18-416-2.03 -5.03	17-130-6.42 4.72	16-405-5.20 0.90	15-109-6.29 -4.77
22-130-4.43 7.31	23-109-6.84 -0.39	24-405-6.49 3.55	25-416-5.41 -0.87	26-593-6.71 6.67	27-407-6.46 -10.66	28-406-6.12 -8.21
35-593-5.32 -5.08	34-130-6.64 -5.92	33-416-0.48 1.97	32-405-7.30 -4.19	31-406-8.11 -5.95	30-109-7.35 -5.33	29-407-7.66 -5.00

12x7 =84 ft.

≠ First no.= plot no.; second no.= varietal designation,and last two numbers equal weight of rubber in grams from the two plants.

TABLE IV-4. Totals of plots yields and sums of squares.

Variety	I	II	III	IV	V	Total.	Mean.
			Replicate Number				
109	7.85	11.80	11.06	7.73	12.68	51.12	5.112
130	7.81	15.91	11.14	11.74	12.56	59.16	5.916
405	9.46	8.29	6.10	15.04	11.49	50.38	5.038
406	12.82	10.71	14.68	14.33	14.06	66.60	6.660
407	8.18	10.12	7.38	17.12	12.66	55.46	5.546
416	5.67	10.20	7.11	6.28	2.45	31.71	3.171
593	11.79	10.92	10.10	13.38	10.90	57.09	5.709
Total	63.58	77.95	67.57	85.62	76.80	371.52	5.307

Total sum of squares:

$$2.06^2 + 6.12^2 + 2.53^2 + \ldots + 5.82^2 + 5.08^2 - \frac{(371.52)^2}{70} = 2287.489 - 1971.816$$
$$= 315.673$$

Sum of squares for replicates

$$\frac{63.58^2 + \ldots + 76.80^2}{14} - \frac{(371.52)^2}{70} = 1993.811 - 1971.816 = 21.995$$

Sum of squares for varieties:

$$\frac{51.12^2 + \ldots + 57.09^2}{10} - \frac{(371.52)^2}{70} = 2042.747 - 1971.816 = 70.931$$

Sum of squares of plot totals

$$\frac{7.85^2 + 11.80^2 + \ldots + 10.90^2}{2} - \frac{(371.52)^2}{70} = 2148.102 - 1971.816 = 176.286$$

Sum of squares for interaction of replicates and varieties by subtraction =

$$176.286 - 70.931 - 21.995 = 83.360 \text{ with } 24 \text{ d.f.}$$

Within plot sum of squares either by subtraction or by

$$\frac{(6.12 - 2.06)^2}{2} + \frac{(6.93 - 2.53)^2}{2} + \ldots + \frac{(5.82 - 5.08)^2}{2}$$

$$= 6.12^2 + 2.06^2 - \frac{(6.12 + 2.06)^2}{2} + 6.93^2 + 2.53^2 - \frac{(6.93 + 2.53)^2}{2}$$

$$+ \ldots + 5.82^2 + 5.08^2 - \frac{(10.90)^2}{2}$$

$$= 2287.489 - 2148.102 = 139.387.$$

Table IV-5. Analysis of Variance for the Data of Table IV-3.

Source of variation	d.f.	s.s.	m.s.	F
Replicates	4	21.995	5.4988	1.58
Varieties	6	70.931	11.8218	3.40
Experimental error	24	83.360	3.4733	
Sampling error	35	139.387	3.9825	
Total	69	315.673	-	

In the preceding table, two errors are listed, experimental and sampling. The experimenter may often be in a quandary as to which one to use. The answer depends upon which hypothesis he desires to test. If the worker wishes to confine his remarks to the particular 5 replicates used above, then the sampling error should be used for testing the variation among variety means. If on the other hand, the experimenter is not so conservative and wishes to make an inference about the true differences among the 7 varieties from the sample of 5 replicates, then the experimental error should be used. The last cited instance is the one of practical importance in most cases.

The sampling error for the data in Table IV-3 is larger than the experimental error but not significantly so. If the variation of plot means from plot to plot after removing replicate and variety effect is zero in the population then it would be expected that the experimental error would be smaller in about 50 percent and larger in 50 percent of the samples. If the latter error is significantly smaller than the sampling error, it would be concluded that a significant negative intraclass correlation (Snedecor and Fisher) existed. The explanation would depend upon the particular type of biological material involved.

Even though the experimental error is smaller, it is the best estimate of the error term for testing the significance of the difference among treatment means. The experimenter may wish to be more conservative and use the sampling error and the degrees of freedom associated with the experimental error. Other schemes could be followed, but the most logical one is to use the experimental error as the estimate of error variation in making various tests of hypotheses.

The F test of the difference among the 7 treatment means is

$$F = \frac{11.8218}{3.4733} = 3.40.$$

For 24 and 6 degrees of freedom the F values at the 5 and 1 percent points are 2.51 and 3.67, respectively. An F value as large as or larger than the experimental F value, has a probability of occurrence equal 2 to 3 percent.

The next question of importance would be to determine which, if any, of the 7 presumably unrelated varieties are significantly different with respect to yield of rubber at the end of one growing season. The answer to this question has only recently been supplied. Duncan (1947, Ph.D.Thesis, Iowa State College) obtained the test of significance for any pair among the 3 or 4 items in the test. Tukey (Biometrics, In press), using a different approach, considered the problem by segregating the k means into homogeneous subgroups. This test probably is more conservative than the significance level indicates, but it is easy to use and makes uses of published probability tables.

In making use of Tukey's ingenious method to determine which varieties differ significantly, the various steps are illustrated with the data of the present example.

Step 1. Choose a significance level.

The 5 percent level is chosen for this example.

Step 2. Calculate the difference which would have been significant if there were but two varieties.

This is equivalent to computing a least or minimum significant difference. The standard error of a variety mean is

$$s_{\bar{x}} = \sqrt{\frac{3.4733}{10}} = 0.589.$$

Therefore,

$$\text{lsd} = t_{05}\ (24\text{d.f.})\ \sqrt{2}\ s_{\bar{x}} = 2.064(1.414)(0.589)$$
$$= 1.719.$$

Step 3. Arrange the means in order and if any two adjacent means deviate by more than the lsd, consider them as subgroup endpoints.

The seven means arranged in order and the difference between 2 adjacent means are:

Variety	Mean Yield	Difference
406	6.660	
		0.744
130	5.916	
		0.207
593	5.709	
		0.163
407	5.546	
		0.434
109	5.112	
		0.074
405	5.038	
		1.867
416	3.171	
Average	5.307	

The mean of variety 416 is more than one lsd lower than the next adjacent mean, variety 405. Therefore at the end of step 3, the 7 varieties are divided into 2 groups, one containing 6 varieties and the other one variety. If no group contains more than 2 means, the process terminates.

Step 4. In each group of 3 or more means find the grand mean, the most divergent mean, and the difference of these 2 divided by $s_{\bar{x}}$. Convert these ratios into approximate unit normal deviates by finding

$$\frac{\dfrac{\bar{x}_t-\bar{x}_d}{s_{\bar{x}}} - \dfrac{6}{5}\log_{10} k}{3\left(\dfrac{1}{4}+\dfrac{1}{n_e}\right)} \quad (k > 3 \text{ means in a group}).$$

or

$$\frac{\dfrac{\bar{x}_t-\bar{x}_d}{s_{\bar{x}}} - \dfrac{1}{2}}{3\left(\dfrac{1}{4}+\dfrac{1}{n_e}\right)} \quad (3 \text{ means in a group}).$$

Separate off any straggling or divergent mean for which this is significant at the chosen two-sided significance level for the normal.

For the group of 6=k means the average is $\bar{x}_t=5.664$ and the most divergent mean from the general mean is that for variety 406, $\bar{x}_d= 6.660$. The error degrees of freedom (Table IV-5) is $n_e=24$. Substituting these values in the formula,

$$\frac{\dfrac{|\bar{x}_t-\bar{x}_d|}{s_{\bar{x}}} - \dfrac{6}{5}\log k}{3\left(\dfrac{1}{4}+\dfrac{1}{n_e}\right)} = \frac{\dfrac{6.660-5.664}{0.589} - \dfrac{6}{5}\log 6}{3\left(\dfrac{1}{4}+\dfrac{1}{24}\right)}$$

$$= \frac{8}{7}(1.691 - 0.934) = 0.865.$$

A normal deviate of 0.86 or larger is expected to occur about 39 percent of the time in random sampling (Table I, Fisher, Statistical Methods).

Thus this step has produced no further subdivision of the group of 6 means. If such had been the case the above process would have been continued until no further subdivision into subgroups was possible.

Step 5. Calculate the sum of squares of deviations from the group mean, and the corresponding mean square for each group or subgroup of 3 or more resulting from step 4. Using $s_{\bar{x}}^2$ as the denominator, calculate the variance ratio and apply the F test.

The sum of squares of the deviations of the 6 means from their average mean is

$6.660^2 + 5.916^2 + \ldots + 5.038^2 - 6(5.6635)^2$

$= 194.2194 - 192.4514 = 1.7680.$

The mean square is 0.3536 and the F is

$$F = \frac{0.3536}{3.4733/10} = 1.02.$$

The nonsignificant F indicates no overall evidence of difference in yield for the 6 varieties.

Thus, the method of Tukey indicates that variety 416 was significantly lower than the others in yield of rubber and that the variation in yield among the remaining 6 varieties was no larger than might logically be ascribed to chance.

The above method is applicable to a group of unrelated varieties or treatments and was carried through to illustrate the procedure. However, considerable information concerning the relationships of the 7 varieties was available. Variety 109 was the only $54^{\pm}$ chromosome variety in the group, the remaining were in the $72^{\pm}$ category. A logical comparison would be the mean of the 72's versus the mean of the 54 chromosome variety,

$$\frac{[6(51.12) - 59.16 - 50.38 - \ldots - 57.09]^2}{10[36 + 1 + 1 + 1 + 1 + 1 + 1]} =$$

$$\frac{51.12^2}{10} + \frac{[59.16 + \ldots + 57.09]^2}{60} - \frac{371.52^2}{70} = 0.4456.$$

Also, it was known that varieties 406 and 130 were selections from 593. The 2 degrees of freedom among these three means could logically be partitioned into 2 single degrees of freedom representing the comparison of the two selections with the parent variety and the comparison between the selections.

Among 130, 406, and 593:

$$\frac{59.16^2 + 66.60^2 + 57.09^2}{10} - \frac{182.85^2}{30} = 5.0026.$$

130 + 406 versus 593:

$$\frac{[59.16 + 66.60 - 2(57.09)]^2}{10[1+1+4]} = \frac{57.09^2}{10} + \frac{(59.16 + 66.60)^2}{20}$$

$$- \frac{[59.16 + 66.60 + 57.09]^2}{30} = 2.2349.$$

130 versus 406:

$$\frac{(59.16 - 66.60)^2}{10(1+1)} = 2.7677.$$

Furthermore varieties 130, 406, and 593 are phenotypically different from the remaining 3 varieties, 405, 407, and 416. The former have round greenish leaves and short branching habit while the latter group have long serrated grayish green leaves and longer branches. A logical comparison would be between the 2 groups of means,

$$\frac{[59.16 + 66.60 + 57.09 - 50.38 - 55.46 - 31.71]^2}{10(1 + 1 + 1 + 1 + 1 + 1)} = 34.2015.$$

The remaining 2 degrees of freedom make up the comparisons among the 3 varieties 405, 407, and 416, with the following sum of squares,

$$\frac{50.38^2 + 55.46^2 + 31.71^2}{10} - \frac{137.55^2}{30} = 31.2813.$$

It was not known what relationship existed among the 3 varieties and without further information, the partitioning of the variety sum of squares is finished. Tukey's test may be applied to these 3 means and it was found that 2 subgroups are formed consisting of 405 and 407 in one group and 416 in the other.

The sums of squares are summarized in Table IV-6 and as a partial check they should add up to the total, 70.931.

TABLE IV-6. Partitioning of the treatment sum of squares from Table IV-5.

Source of variation.	d.f.		Sum of squares.	Mean square.	F
Varieties	6		70.931	11.8218	3.40
109 vs. others		1	0.4456	0.4456	-
130+406 vs. 593		1	2.2349	2.2349	-
130 vs. 406		1	2.7677	2.7677	-
130, 406, 593 vs. 405, 407, 416		1	34.2015	34.2015	9.85
Among 405, 407, 416		2	31.2813	15.6406	4.50
Exp. error	24		83.360	3.4733	

F= 9.85 exceeds the tabulated F at the one percent point and F=4.50 exceeds the F value at the 5 percent point. The mean of the 3 varieties, 130, 406, and 593 and of the 3, 405, 407, and 416 cannot be considered as coming from the same general population. However, upon examination of the latter 3 varieties, it was found that they did not represent a homogeneous group and that the very low yield of variety 416 accounts for the large F values in both instances.

The amount of variability relative to the mean in this experiment was much higher than desired. The coefficient of variation is

$$\frac{\sqrt{3.4733/2}}{5.307} = \frac{1.318}{5.307} = 25 \text{ percent.}$$

The standard deviation <u>per</u> <u>plant</u> <u>mean</u> <u>yield</u> is $\sqrt{3.4733/2}$ or it is the standard deviation resulting from an analysis of the plot means. The verification that division of the error mean square by 2 (equals number of items from each plot) results in the same value as that obtained from using the <u>plot</u> <u>means</u> in the analysis, is left as an exercise for the student.

The efficiency of this design relative to what it would have been had a completely randomized design been used is

$$\text{Eff.} = \frac{\dfrac{21.995 + 3.4733(6+24)}{4 + 6 + 24}}{3.4733} = \frac{3.7116}{3.4733} = 107 \text{ percent,}$$

or a gain in efficiency of 7 percent.

Problem IV-1.

For example IV-1, make the assumption that varieties B and C are two entries of the same variety, say D. Complete the following analysis of variance table:

Source of variation	d.f.
Replicates	3
D vs. A	1
B vs. C	1)
Remainder	6)
Total	11

Under what conditions would the pooled error with 7 d.f. be used? Compute the standard error for testing the difference between the means of varieties A and D and the least or minimum significant difference.

Problem IV-2.

Compute the coefficient of variation, the standard error of a mean, and standard error of a difference for a mean on a plot total basis for example IV-2. If the means were on a basis of the plot total of 2 plants, would the efficiency of the design be changed? Explain.

Problem IV-3.

Use Tukey's method for testing the significance of the means in Tables 10.3 and 11.9 and example 11.28, Snedecor, Statistical Methods, 1946. Assume no relationship among the entries in the test. Why?

Problem IV-4.

Obtain the expected values for the example IV-1 from the formula

$$\hat{X}_{ij} = \text{experimental mean + variety effect + replicate effect}$$

$$= \bar{x} + v_i + r_j$$

$$= \text{variety mean + replicate mean - experiment mean.}$$

and compute the following sums of squares

$$\sum_{j=1}^{4} (X_{1j}-\hat{X}_{1j})^2 = \Sigma e_{1j}^2$$

$$\sum_{j=1}^{4} (X_{2j}-\hat{X}_{2j})^2 = \Sigma e_{2j}^2$$

and

$$\sum_{j=1}^{4} (X_{3j}-\hat{X}_{3j})^2 = \Sigma e_{3j}^2 .$$

What conclusions would you draw from the computations made?

Problem IV-4.

The following data on resin percentage and shrub weight (grams) of plants were obtained for the varieties and replicates of example IV-2. Do the varieties differ with regard to resin percentage after being corrected to a constant shrub weight? (See De Lury, Biometrics, Sept. 1948). Would you suggest a transformation for these percentages? Why or why not?

Problem IV-2 (contd.) Plot number, variety number, resin percentage, and shrub weight of 2 randomly selected plants from each of 7 guayule varieties grown in five randomized complete blocks.

5x28= 140 ft.

≠ 7-130 5.24-61 5.84-50	6-406 4.85-84 5.64-91	5-593 5.99-86 5.22-83	4-109 3.97-34 6.17-103	3-416 5.50-65 4.78-53	2-405 4.49-88 3.36-143	1-407 5.74-39 5.71-102
8-109 5.71-58 6.15-109	9-593 5.15-89 5.69-76	10-405 5.15 -28 5.10-146	11-406 4.86 -67 5.65-110	12-416 6.00 -96 5.43-104	13-130 5.49-152 5.54-106	14-407 6.15-88 5.76-92
21-593 5.88- 58 5.59-101	20-407 4.88-61 5.97-93	19-406 5.82-125 5.20-112	18-416 4.75-125 6.15- 91	17-130 5.60-95 5.76-73	16-405 6.22-111 4.85- 15	15-109 6.05-87 6.04-84
22-130 6.13- 70 5.63-116	23-109 5.64-101 6.65- 12	24-405 7.24-115 5.95-170	25-416 6.30-101 5.75- 15	26-593 5.36- 99 5.21-105	27-407 6.20-127 6.74-194	28-406 5.17-104 6.62-117
35-593 5.80-93 6.13-73	34-130 5.78- 98 5.38-102	33-416 5.85- 8 5.73-33	32-405 5.67-132 4.59- 83	31-406 5.86-144 5.75- 98	30-109 3.85-105 5.89- 88	29-407 6.18-169 5.54 -90

12x7=84 ft.

≠ First no.=plot no.;second no.=varietal designation, and last two pairs of numbers equal percentage of resin and dry weight of shrub from the two plants.

Least Squares Estimates of Partial Regression Coefficients and their Variances.

One unit per plot

If a single observation is made on each plot of a randomized complete blocks design, then the linear model

$$X_{ij} = \mu + \tau + \rho_j + e_{ij}$$

may be assumed to represent the yield of any plot if the effects are additive and the parameters are expressed as μ, τ_i, and ρ_j, where μ represents the population mean value, τ_i = effect common to i th treatment, ρ_j = effect common to j th replicate, and e_{ij} is the effect common to the ith treatment in the jth block. The above linear equation may be more recognizable if written in the form

$$X_{ij} = \mu + \tau_i X'_{1i} + \rho_j X'_{2j} + e_{ij} ,$$

where X_1 and X_2 have the values of 0 or 1. X'_{1j} takes on the value 1 in all cells of the 2-way classification where τ_i is present and zero elsewhere; likewise, X'_{2j} has the value 1 in replicate j and zero elsewhere.

The Least Squares estimates of the parameters, μ, τ_1, τ_2, ..., τ_v, ρ_1, ρ_2, ..., ρ_n, are obtained as before, i.e. by differentiation of the residual sum of squares with respect to the estimates. The residual sum of squares is

$$\sum_{i=1}^{v} \sum_{j=1}^{n} (X_{ij} - \hat{\mu} - t_i - r_j)^2 = R \qquad ,$$

$$\frac{\partial R}{\partial \hat{\mu}} = -2\Sigma\Sigma(X_{ij} - \hat{\mu} - t_i - r_j) = 0 ,$$

$$\frac{\partial R}{\partial t_i} = -2\sum_j (X_{ij} - \hat{\mu} - t_i - r_j) = 0 ,$$

$$\frac{\partial R}{\partial r_j} = -2\sum_i (X_{ij} - \hat{\mu} - t_i - r_j) = 0 ,$$

and $\hat{\mu}$, t_i, and r_j are the solutions of the equations which make the residual sum of squares a minimum. This set of differential equations leads to the following set of normal equations:

Equation for $\hat{\mu}$:

$$\Sigma\Sigma X_{ij} = X_{..} = n\sum_i t_i + v\sum_j r_j + nv\hat{\mu} \quad .$$

Equations for treatment effects $t_i, \ldots , t_v$

$$\Sigma X_{1j} = X_{1.} = nt_1 + \sum_j r_j + n\hat{\mu} \quad ,$$

$$\sum_j X_{2j} = X_{2.} = nt_2 + \sum_j r_j + n\hat{\mu} \quad ,$$

$$\vdots$$

$$\sum_j X_{vj} = X_{v.} = nt_v + \sum_j r_j + n\hat{\mu} \quad .$$

Equations for replicate effects, $r_1, r_2, \ldots , r_n$:

$$X_{.1} = \sum_i t_i + vr_1 + v\hat{\mu} \quad ,$$

$$X_{.2} = \Sigma t_i + vr_2 + v\hat{\mu} \quad ,$$

$$\vdots$$

$$X_{.n} = \Sigma t_i + vr_n + v\hat{\mu} \quad .$$

In order to obtain unique solutions for the $v + n + 1$ partial regression coefficients, the following restrictions are imposed

$$\Sigma t_i = 0$$

$$\Sigma r_j = 0 \quad .$$

Now, the least squares estimate of the experimental mean is

$$\hat{\mu} = \frac{1}{nv}\left(-n\Sigma t_i - v\Sigma r_j + X_{..}\right) = \frac{X_{..}}{nv} = \bar{x}$$

of t_i,

$$t_i = \frac{X_{i.}}{n} - \bar{x} = \bar{x}_{i.} - \bar{x}$$

and of r_j,

$$r_j = \frac{X_{.j}}{v} - \bar{x} = \bar{x}_{.j} - \bar{x} \quad .$$

The variance of $\hat{\mu}$ is

$$E[\hat{\mu}-\mu]^2 = E[\hat{\mu}^2]-\mu^2 = E\left[\frac{X_{..}}{rv}\right]^2 - \mu^2$$

$$= E\left[\frac{nv\mu + n\Sigma\tau_i + v\Sigma\rho_j + \Sigma\Sigma e_{ij}}{nv}\right] - \mu^2$$

$$= \mu^2 + \frac{\sigma_e^2}{nv} - \mu^2 = \frac{\sigma_e^2}{nv}$$, since it is assumed $\Sigma\tau_i = \Sigma\rho_j = 0$.

The variance of any t_i is,

$$E(t_i - \tau_i)^2 = E\left[\frac{X_{i.}}{n} - \hat{\mu}\right]^2 - \sigma_\tau^2$$

$$= E\left[\frac{n\mu + n\tau_i + \Sigma\rho_j + \sum_j e_{ij}}{n} - \frac{nv\mu + n\Sigma\tau_i + v\Sigma\rho_j}{nv} + \frac{\Sigma\Sigma e_{ij}}{nv}\right]^2 - \sigma_\tau^2$$

$$= E\left[\tau_i + \frac{\sum_j e_{ij}}{n} - \frac{\Sigma\Sigma e_{ij}}{nv}\right]^2 - \sigma_\tau^2$$

$$= \sigma_\tau^2 + \frac{\sigma_e^2}{n} - \frac{\sigma_e^2}{nv} - \sigma_\tau^2 = \frac{(v-1)}{nv}\sigma_e^2$$, since $E[\tau_i^2] = \sigma_\tau^2$.

In a like manner the variance of any r_j is $\frac{\sigma_e^2(n-1)}{nv}$,
the covariance of any $t_i t_{i'}$ $(i \neq i')$ or of any $r_j r_{j'}$ $(j \neq j')$ is $-\sigma_e^2/nv$, and the covariance of $t_i r_j$, $t_i\hat{\mu}$, or of $r_j\hat{\mu}$ is equal to zero.

In some cases the variances or covariances of the Least Squares estimates are not desired but rather the expectation or average value of a sum of squares after fitting certain constants. In this case, it is assumed that the t_i and r_j are a random sample from populations with mean zero and $E[t_i^2] = \sigma_\tau^2$ and $E[r_j^2] = \sigma_\rho^2$, respectively (see Crump, 1946). In obtaining the expectation or average value of various mean squares, the restriction that $\Sigma\tau_i = \Sigma\rho_j = 0$ is not imposed.

The reduction in the total sum of squares due to fitting the estimated constants $\hat{\mu}$, t_i and r_j, where $X_{ij}=\mu+\tau_i+\rho_j+e_{ij}$, is

$$E\left[\Sigma\Sigma X_{ij}^2 - \hat{\mu}X_{..} - \frac{\Sigma t_i X_{i.}}{n} - \frac{\Sigma r_j X_{.j}}{v}\right]$$

$$= E\left[\Sigma\Sigma X_{ij}^2 - \frac{\Sigma X_{i.}^2}{n} - \frac{\Sigma X_{.j}^2}{v} + \frac{X_{..}^2}{nv}\right]$$

$$= E\left[\Sigma\Sigma(\mu+\tau_i+\rho_j+e_{ij})^2 - \Sigma_i\frac{(n\mu+n\tau_i+\rho_1+\ldots+\rho_n+e_{i1}+\ldots+e_{in})^2}{n}\right.$$

$$-\Sigma_j\frac{(v\mu+\tau_1+\ldots+\tau_v+v\rho_j+e_{1j}+\ldots+e_{vj})^2}{v}$$

$$\left.+\frac{(nv\mu+n(\tau_1+\ldots+\tau_v)+v(\rho_1+\ldots+\rho_n)+e_{11}+\ldots+e_{rv})^2}{rv}\right]$$

$$= nv\mu^2 + nv\sigma_\tau^2 + nv\sigma_\rho^2 + nv\sigma_e^2 - nv\hat{\mu}^2 - nv\sigma_\tau^2 - v\sigma_\rho^2 - v\sigma_e^2$$

$$-nv\mu^2 - n\sigma_\tau^2 - nv\sigma_\rho^2 - n\sigma_e^2 + nv\hat{\mu}^2 + n\sigma_\tau^2 + v\sigma_\rho^2 + \sigma_e^2$$

$$= (nv - v - n + 1)\sigma_e^2 = (n-1)(v-1)\sigma_e^2 .$$

The reduction in the sum of squares due to fitting the t_i is the reduction due to fitting the $\hat{\mu}$ and r_j minus the reduction due to fitting the $\hat{\mu}$, t_i and r_j,

$$E\left[\left(\Sigma\Sigma X_{ij}^2 - \hat{\mu}X_{..} - \frac{\Sigma r_j X_{.j}}{v}\right) - \left(\Sigma\Sigma X_{ij}^2 - \hat{\mu}X_{..} - \frac{\Sigma r_j X_{.j}}{v} - \frac{\Sigma t_i X_{i.}}{n}\right)\right]$$

$$= E\left[\frac{\Sigma t_i X_{i.}}{n}\right] = E\left[\frac{\Sigma X_{i.}^2}{n} - \frac{X_{..}^2}{nv}\right]$$

$$= nv\mu^2 + nv\sigma_\tau^2 + v\sigma_\rho^2 + v\sigma_e^2 - nv\mu^2 - n\sigma_\tau^2 - v\sigma_\rho^2 - \sigma_e^2$$

$$= (v-1)(\sigma_e^2 + n\sigma_\tau^2) .$$

The above expectation is for the variety sum of squares. Likewise, the expectation of the replicate sum of squares is

$$E\left[\frac{\Sigma X_{.j}^2}{v} - \frac{X_{..}^2}{nv}\right] = (n-1)(\sigma_e^2 + v\sigma_\rho^2)$$

and of the total sum of squares is

$$E\left[\Sigma\Sigma X_{ij}^2 - \frac{X_{..}^2}{rv}\right] = n(v-1)\sigma_\tau^2 + v(n-1)\sigma_\rho^2 + (nv-1)\sigma_e^2 .$$

<u>k units per plot.</u>

If k observations are made on each cell of a two-way classification, the linear model

$$X_{ijh} = \mu + \tau_i + \rho_j + \tau\rho_{ij} + e_{ijh}$$

may be assumed to represent the yield of any observation, where μ represents the population mean value, τ_i = effect common to the ith treatment, ρ_j = effect common to the jth replicate, $\tau\rho_{ij}$ = an effect common to the ith treatment in the jth replicate, e_{ijh} = effect peculiar to ijh th observation, $i = 1,2,\ldots,v$, $j=1,2,\ldots,n$, and $h = 1,2,\ldots,k$.

The Least Squares solutions of the parameters μ, τ_i, ρ_j, and $\tau\rho_{ij}$ are obtained as before, thus

$$\frac{\partial R}{\partial \hat{\mu}} = -2\sum_{ijh}\sum\sum (X_{ijh} - \hat{\mu} - t_i - r_j - rt_{ij}) = 0$$

$$\frac{\partial R}{\partial t_i} = -2\sum_{jh}\sum (X_{ijh} - \hat{\mu} - t_i - r_j - rt_{ij}) = 0$$

$$\frac{\partial R}{\partial r_j} = -2\sum_{ih}\sum (X_{ijh} - \hat{\mu} - t_i - r_j - rt_{ij}) = 0$$

$$\frac{\partial R}{\partial rt_{ij}} = -2\sum_{h} (X_{ijh} - \hat{\mu} - t_i - r_j - rt_{ij}) = 0$$

where

$$R = \sum_{ijh}\sum\sum (X_{ijh} - \hat{\mu} - t_i - r_j - rt_{ij})^2 ,$$

and $\hat{\mu}$, t_i, r_j, and rt_{ij}, are the estimates making the residual sum of squares a minimum. The above set of differential equations leads to the following set of normal equations:

Equation for $\hat{\mu}$:

$$X_{...} = nk\Sigma t_i + vk\Sigma r_j + k\Sigma\Sigma rt_{ij} + nvk\hat{\mu}$$

Equations for t_i:

$$X_{1..} = nkt_1 + k\Sigma(r_j + rt_{1j}) + nk\hat{\mu}$$

$$X_{2..} = nkt_2 + k\Sigma(r_j + rt_{2j}) + nk\hat{\mu}$$

$$\vdots$$

$$X_{v..} = nkt_v + k\Sigma(r_j + rt_{vj}) + nk\hat{\mu}$$

Equations for r_i:

$$X_{.1.} = k\Sigma(t_i + rt_{i1}) + vkr_1 + vk\hat{\mu}$$

$$X_{.2.} = k\Sigma(t_i + rt_{i2}) + vkr_2 + vk\hat{\mu}$$

$$\vdots$$

$$X_{.n.} = k\Sigma(t_i + rt_{in}) + vkr_n + vk\hat{\mu}$$

Equations for rt_{ij}:

$$X_{11.} = k(t_1+r_1+rt_{11} + \hat{\mu})$$

$$X_{12.} = k(t_1+r_2+ rt_{12} + \hat{\mu})$$

$$\vdots$$

$$X_{vn} = k(t_v+ r_n + rt_{vn} + \hat{\mu})$$

In order to obtain unique solutions for the $n+ v + nv + 1$ partial regression coefficients, the following restrictions are imposed

$$\Sigma t_i = 0$$

$$\Sigma r_j = 0$$

$$\Sigma\Sigma rt_{ij} = 0$$

With these restrictions, the Least Squares estimates are

$$\hat{\mu} = \frac{X_{...}}{nvk} = \bar{\bar{x}}$$

$$t_i = \frac{X_{i..}}{nk} - \hat{\mu} = \bar{x}_{i.} - \bar{\bar{x}}$$

$$r_j = \frac{X_{.j.}}{vk} - \hat{\mu} = \bar{x}_{.j} - \bar{\bar{x}}$$

$$rt_{ij} = \frac{X_{ij.}}{k} - t_i - r_j - \hat{\mu} = \bar{x}_{ij} - \bar{x}_{i.} - \bar{x}_{.j} + \bar{\bar{x}} .$$

The variances of the above Least Squares estimates may be obtained as before and are left as an exercise for the student to do.

The reduction in the total sum of squares due to fitting the constants $\hat{\mu}$, t_i, r_j, and rt_{ij} has the expectation:

$$E\left[\Sigma\Sigma\Sigma X_{ijh}^2 - \hat{\mu}X_{\cdots} - \Sigma t_i X_{i\cdot\cdot} - \Sigma r_j X_{\cdot j\cdot} - \Sigma\Sigma rt_{ij} X_{ij\cdot}\right]$$

$$= E\left[\sum_{ijh}\Sigma\Sigma X_{ijh}^2 - \sum_{ij}\Sigma\Sigma\frac{X_{ij\cdot}^2}{k}\right]$$

$$= E\left[\sum_{ij}\Sigma\Sigma\left(\sum_h X_{ijh}^2 - \frac{X_{ij\cdot}^2}{k}\right)\right]$$

$$= E\sum_{ij}\Sigma\Sigma\left[\sum_h (\mu + \tau_i + \rho_j + \tau\rho_{ij} + e_{ijh})^2 - \frac{(k\mu + k\tau_i + k\rho_j + k\rho\tau_{ij} + e_{ij1} + \cdots + e_{ijk})^2}{k}\right]$$

$$= nvk(\mu + \sigma_\tau^2 + \sigma_\rho^2 + \sigma_{\tau\rho}^2 + \sigma_e^2) - nv(k\hat{\mu}^2 + k\sigma_\tau^2 + k\sigma_\rho^2 + k\sigma_{\tau\rho}^2 + \sigma_e^2)$$

$$= nv(k-1)\sigma_e^2.$$

In a like manner the expectation of reductions in the sum of squares due to the t_i, r_j, and rt_{ij}, respectively, are

$$E\left[\frac{\Sigma X_{i\cdot\cdot}^2}{nk} - \frac{X_{\cdots}^2}{nvk}\right] = (v-1)(\sigma_e^2 + k\sigma_{\rho\tau}^2 + nk\sigma_\tau^2),$$

$$E\left[\frac{\Sigma X_{\cdot j\cdot}^2}{vk} - \frac{X_{\cdots}^2}{nvk}\right] = (n-1)(\sigma_e^2 + k\sigma_{\rho\tau}^2 + vk\sigma_\rho^2),$$

and

$$E\left[\frac{\Sigma\Sigma X_{ij\cdot}^2}{k} - \frac{\Sigma X_{i\cdot\cdot}^2}{nk} - \frac{\Sigma X_{\cdot j\cdot}^2}{vk} + \frac{X_{\cdots}^2}{nvk}\right] = (v-1)(n-1)(\sigma_e^2 + k\sigma_{\rho\tau}^2) .$$

For the case of disproportionate numbers in the cells of the two-way classification the reader is referred to the Ph.D. Theses by Federer, June, 1948, and Henderson, December, 1948, Iowa State College.

LATIN SQUARE DESIGNS

by

W.T.Federer

V-1. Introduction

In randomized complete block designs the restriction is imposed that all treatments or varieties must appear together in the compact blocks or units an equal or proportional number of times rather than being alloted at random over the whole experimental area. The latter situation refers to the design of the completely randomized design. For the latin square design two restrictions are imposed, namely, that for an experimental area divided into rows and columns, each treatment must appear once in a row and once in a column. Thus for latin squares, the treatments are grouped into replicates in two ways, once in rows and once in columns. Through the elimination of row and column effects from the within treatment variation, the residual or error variance may be considerably reduced. The effect of the removal of the row and column variance on the residual variance will be illustrated later after construction and field design of latin squares has been discussed.

Latin square designs have a wide variety of applications in experimental work. They are used in industrial, laboratory, field, and greenhouse experimentation from comparing a group of varieties or fertilizer treatments to testing biological assays and from comparing worker differences in the laboratory to comparing weaving processes. Tippett (1934, Manchester Statistical Society) illustrates an exceptional use of the latin square (in reality a graeco-latin square) in tracing the origin of a defective mechanical part in a cotton mill. Fisher (1942, Design of Experiments) discusses this design. Cochran and Cox (1944) cite a wide variety of uses of the latin square design in experimentation, (Yates and Watson, 1934, Empire Journal Exp. Agric.; Cochran and Watson, 1936, Empire Jour. Exp. Agric.; Main and Tippett, 1941, Shirley inst. Memoirs; Chen, Bliss, and Robbins, 1942, Jour. Pharm. and Exp. Ther., et. al.). Taylor(1949, Ph. D. Thesis, Cornell Univ.) has effectively used the latin square design in determining the various factors affecting differences in bioelectric potential between two portions of the stem in plants; an example from this soils pyhsics problem is discussed later, Examples V-1 and V-3.

One has but to consult researchers to determine the popularity of the latin square design. Despite its popularity the latin square design in practical only for 5 to 12 treatments unless more than one square are used in which case it is suitable for fewer treatments. For the 2 x 2, 3 x 3, and 4 x 4 latin squares there are zero, 2, and 6 degrees of freedom associated with the residual sum of squares and with such few degrees of freedom in the error term it is imperative that more than a single latin square should be used. Likewise, since the latin square design requires as many replicates as there are treatments, the design is seldom used for more than 10 to 12 treatments. With regard to the above comments on the use of latin square design and with regard to the high precision (standard error less than 2 percent of the mean) frequently obtained, Fisher (1942, Design of Expts.) sums this up with the statement "If experimentation were only concerned with the comparison of four to eight treatments or varieties, it (the latin square design) would therefore be not merely the principal but almost the universal design employed".

V-2. Construction and Arrangements

For the discussion on the construction of latin squares it is advantageous to define or explain some of the terminology used in connection with these designs (see Fisher and Yates, 1948).

(i) standard square - A square is said to be standard if the first row and first column are ordered. There are as many standard squares for a k x k latin square as there are types which cannot be converted into one another by a reshuffling of rows and columns.

(ii) conjugate square - Two standard square are conjugate if the rows of one are the columns of the other.

(iii) self-conjugate square - A square is self-conjugate if its arrangement in rows and columns is the same.

(iv) adjugate set - By permuting with each other the three categories, rows, columns, and letters, six sets (not necessarily all different) are formed. The resulting sets are said to be adjugate.

(v) self-adjugate set - A set is self-adjugate if a permutation of the three categories, columns, rows, and letters results in the same set.

For the 2 x 2 latin square there is only the one standard square,

A	B
B	A

.

The two conjugate squares for the above standard square result in the same arrangement as given above. This means that the 2 x 2 latin square is also self-conjugate since the letters in a row are the same as those in the corresponding column. By interchanging rows with columns, columns with treatments, and treatments with rows three latin square arrangements are obtained. The conjugate of each of the above three sets may be obtained resulting in the six adjugate sets. These sets give the single square for the 2 x 2 latin square and hence, the 2 x 2 latin square is self-adjugate.

Likewise, for the 3 x 3 latin square there is only one standard square,

A	B	C
B	C	A
C	A	B

.

The square is self conjugate since the arrangement of the letters in rows and columns is the same. There are, however, 12 possible arrangements for the 3 x 3 latin square:

A	B	C
B	C	A
C	A	B

A	C	B
B	A	C
C	B	A

B	C	A
C	A	B
A	B	C

B	A	C
C	B	A
A	C	B

C	B	A
A	C	B
B	A	C

C	A	B
A	B	C
B	C	A

A	B	C
C	A	B
B	C	A

A	C	B
C	B	A
B	A	C

B	C	A
A	B	C
C	A	B

B	A	C
A	C	B
C	B	A

C	B	A
B	A	C
A	C	B

C	A	B
B	C	A
A	B	C

.

There are 3! (3-1)! = 12 arrangements for the 3 x 3 latin square, of which 11 are nonstandard squares.

Four standard square are possible for the 4 x 4 latin square,

A	B	C	D
B	A	D	C
C	D	B	A
D	C	A	B

A	B	C	D
B	C	D	A
C	D	A	B
D	A	B	C

A	B	C	D
B	D	A	C
C	A	D	B
D	C	B	A

A	B	C	D
B	A	D	C
C	D	A	B
D	C	B	A

All 4 standard 4 x 4 latin squares are self-conjugate. For each standard square there are 4! (4-1)! = 144 possible arrangements, resulting in a total of 576 possible arrangements of the 4 standard squares. Of the 576 arrangements 572 are non-standard squares and the remaining are the 4 standard squares.

For the 5 x 5 latin square there are 25 standard squares, their conjugates, and six self-conjugate squares resulting in 56 standard squares. Also, there are 56(5!)(4!) = 56(2880) = 161,280 possible arrangements.

The number of possible arrangements increases rapidly as the size of the latin square increases. It is obvious then why the possible arrangements for all k x k latin squares have not been tabulated. Fisher and Yates (1948) have given the standard squares for the 4 x 4 and 5 x 5 latin squares and the five conjugates pairs of transformation sets and the 12 sets containing conjugates for the 6 x 6 latin squares. Norton (1939, Annals of Eugenics) has tabulated the 562 sets from which it is possible to generate the 16,927,968 standard squares for the 7x7 latin square. To date all the standard squares for higher ordered latin squares have not been tabulated.

V-3. Randomization

In designing a latin square experiment it is desired to choose, at random, one of the possible arrangements. The procedure is quite simple for the 2x2 latin square since of the two arrangements,

A	B
B	A

B	A
A	B

one is chosen by the toss of a coin or from a table of random numbers. The letters A and B represent two treatments under consideration. Likewise, for a 3x3 latin square with the treatments A, B, and C one of the 12 arrangements listed above is chosen at random.

All possible arrangements of the 2x2 and 3x3 latin squares may be found in several references. The 576 arrangements of the 4x4 latin square have been tabulated by K. Pearson (Tables for Statisticians and Biometricians, Part II). The remainder have not been enumerated to date. Therefore, another method for selecting a random arrangement must be provided. In accordance with the rules for obtaining latin square arrangements as set forward by Fisher and Yates (1948) and Cochran and Cox

(1944) the following procedure may be utilized for obtaining latin square designs for experiments:

(i) <u>2x2 latin square</u> - Randomize the arrangement of the columns or alternatively select one of the two arrangements at random.

(ii) <u>3x3 latin square</u> - Randomize the arrangement of the 3 columns and of the last two rows, or alternatively, select one of the 12 arrangements at random.

(iii) <u>4x4 latin square</u> - Select one of the four standard squares at random and then randomize the arrangement of the columns and the last three rows. The procedure of selecting one of the 576 arrangements at random may be used instead since it results in the same arrangements.

(iv) <u>5x5 latin square</u> - Select one of the 56 standard squares at random and then randomize the arrangement of the five columns and the last four rows,resulting in one of the 161,280 arrangements.

(v) <u>6x6 latin square</u> - Select one of the 9408 standard squares at random and randomize the arrangement of the columns and the last five rows or alternatively select at random one of the sets enumerated by Fisher and Yates (1948) in proportion to the number of standard squares possible in the set and then randomize the allotment of the letters to the treatments, the arrangement of the columns, and the arrangement of the rows.

(vi) <u>7x7 latin square</u> - Select one of the 16,927,968 standard squares at random and then arrange all columns and the last six rows at random or alternatively follow the second plan for the 6x6 latin square which results in the same thing.

(vii) <u>8x8 and higher latin squares</u> -Select one of the tabulated squares or construct one and then arrange the columns and the rows at random and assign the letters to the treatments at random.

The procedure given in (vii) may be used to construct the 5x5 and larger latin square arrangements and often is in practice. However, it must be remembered that this method does not result in all possible arrangements since certain configurations are excluded. Cochran and Cox (1944) state that uhless latin squares are used very frequently the number of arrangements are sufficiently large for experimental plans. Thus, little harm from this procedure is apt to result unless the latin squares are used extensively.

Yates (1933, Emp. Jour. of Exp. Agri.) has discussed the theoretical basis for randomizations of latin squares. The student is referred to the above reference for a further discussion of this topic.

V-4. Experimental Lay-out

The general conception is that the latin square design should occupy a square or nearly square experimental area. In practice this is generally true for field experiments, i.e., the rows are laid out perpendicular to the columns, for example:

Row Number	Column Number 1	2	3
1	A	C	B
2	C	B	A
3	B	A	C

The purpose of the latin square design in field and laboratory experiments is to control variation in two directions such as down the field and across the field or across the greenhouse bench and along the bench. However, it is not necessary to design the experiment as described above. In some instances it may be desirable to keep the treatments in a row in a compact block in such a way that the blocks are the rows and the order within the blocks represents the columns. Such an experimental design might be illustrated by the following:

Row 1 or Block 1			Row 2 or Block 2			Row 3 or Block 3			
A	C	B	C	B	A	B	A	C	or

Row 1	A
	C
	B
Row 2	C
	B
	A
Row 3	B
	A
	C

The above design might be used on a single row or set of rows in a grape vineyard, where treatment A represents no spray or check, treatment B represents spray 1, and treatment C represents spray 2. The sprayer could be equiped with two tanks and the various treatments applied as indicated in the design.

In some instances more replication is desired. The procedure here is to randomly select the number of arrangements of the latin square desired. Suppose that nine replicates for the three treatments A, B, and C are desired. The field design could be of the following form for three rows (or three sets of rows in a grape vineyard:

A	C	A
C	A	B
B	B	C
C	B	B
B	C	C
A	A	A
B	A	C
A	B	A
C	C	B

or for the three locations, farms, or positions:

Square I

A	C	B
C	B	A
B	A	C

Square II

C	A	B
B	C	A
A	B	C

Square III

A	B	C
B	C	A
C	A	B

Modifications of latin square designs reult in other configurations depending upon the nature of the experiment and the experimental material. The first of the two designs listed immediately above might be useful in bakery or cookery experiments, which are conducted over a period of days. Since it might be possible to bake only three cakes per day and since the worker may tire as the day progresses, it would be desirable to have each kind of cake baked in all three orders of baking. The 3x3 latin square design would satisfy these requirements. The whole experiment could be repeated on a second set of three days.

V-5. Statistical Analysis for a Single Latin Square with a Single Determination per Plot

The breakdown of the total degrees of freedom in the analysis of a kxk latin square design is:

Source of Variation	Degrees of Freedom
Rows	$k - 1$
Columns	$k - 1$
Treatments	$k - 1$
Error or residual	$(k-1)(k-2)$
Total	$k^2 - 1$

The row sum of squares is obtained by squaring the row totals, $X_{i..}$, and dividing by k and then subtracting the correction term equal to the grand total, $X_{...}$, squared and divided by k^2, i.e.

$$\frac{X^2_{1..} + X^2_{2..} + \ldots + X^2_{k..}}{k^2} - \frac{X^2_{...}}{k^2}$$

$$= \bar{X}_{1..}X_{1..} + \bar{X}_{2..}X_{2..} + \ldots + \bar{X}_{k..}X_{k..} - \bar{X}\,X_{...} ,$$

where $X_{i..}$ = row means and $\bar{X}$ = experiment mean. In a similar manner the treatment and column sums of squares are obtained as

$$\frac{X^2_{..1} + \ldots + X^2_{..k}}{k} - \frac{X^2_{...}}{k^2}$$

$$= \bar{X}_{..1}X_{..1} + \ldots + \bar{X}_{..k}X_{..k} - \bar{X}\,X_{...}$$

and

$$\frac{X^2_{.1.} + \ldots + X^2_{.k.}}{k} - \frac{X^2_{...}}{k^2}$$

$$= \bar{X}_{.1.}X_{.1.} + \ldots + \bar{X}_{.k.}X_{.k.} - \bar{X}\,X_{...}$$

respectively, where $X_{..h}$ and $\bar{X}_{..h}$ represent the treatment totals and means, respectively, and where $X_{.j.}$ and $\bar{X}_{.j.}$ represent the column totals and means, respectively. The total sum of squares with k^2-1 degrees of freedom is obtained by squaring the k^2 determinations, X_{ijh}, and subtracting the correction term,

$$\sum_{i,j,h} X^2_{ijh} - \frac{X^2_{...}}{k^2} .$$

The error or residual sum of squares is obtained by subtraction of the row, column, and treatment sums of squares from the total,

$$\Sigma X^2_{ijh} - \frac{\Sigma X^2_{i..}}{k} - \frac{\Sigma X^2_{.j.}}{k} - \frac{\Sigma X^2_{..h}}{k} + \frac{2X^2_{...}}{k^2} .$$

The estimated standard error of a difference between two means may be obtained from the formula

$$\sqrt{\frac{2(\text{Error mean square})}{k}}$$

In the event that one of the treatments yields much more variable results than do the other k-1 treatments in the latin square design, Cochran and Cox (1944) have given a method for determining the error for the more variable treatment and the error for testing the differences among the remaining k-1 treatments which have approximately the same amount of variation. In addition they (Cochran and Cox, 1944) have described a procedure for calculating a missing value and for making comparisons among the means. Yates (1933, Emp. Jour. Exp. Agric.) has given an approximate method for treating several missing plots in the latin square. Yates (1936, Jour. Agric. Sc.) has discussed the analysis when either a row, column, or treatment is missing entirely while Yates and Hale (1939, Suppl. Jour. Roy. Stat. Soc.) have given a method for analyzing the results from a latin square in which two or more rows, columns, or treatments are missing.

Example V-1. A 4x4 latin square design (Taylor, Ph.D. Thesis, 1949, Cornell Univ.) was set up to compare the effects of four light intensities (D = dark or zero, L = 500, M = 900, and H = 1200 foot-candles of light) on the difference in bioelectric potential (in millivolts) between a point on the stem of the bean plant and the point at which the nutrient solution made contact with the stem. Since the difference in bioelectric potential required a period of 1 to $1\frac{1}{2}$ hours to become stablized after a change in light intensities, it was necessary to wait two hours after changing light intensities in order to obtain a reliable measure of the difference in potential in the two points measured on the stem of the bean plant. This meant that, at most, three complete readings per day could be obtained but it was thought advisable to keep the plant under ordinary greenhouse conditions prior to starting the readings. Therefore, the first treatment was applied at 10:00 A.M. and the reading was recorded at noon. The second treatment was started immediately and the corresponding reading taken at 2:00 P.M. Likewise, the third and fourth treatment readings were recorded at 4:00 and 6:00 P.M. respectively. Now, it was thought that time of day might have an effect on differences in bioelectric potential and, therefore, it would be necessary to have the treatments (light intensities) applied once at each of the four times. A period of four days was required to run the experiment. The time of day was considered to be the row effect and the day of the week the column effect.

In reality, two readings were recorded for each of three plants but the mean reading is the figure recorded in Table V-1. The individual readings are recorded in Table V-3 and the complete analysis is discussed under Example V-3. A different set of plants was used each day.

The total sum of squares is obtained by squaring the 16 mean readings in Table V-1 and subtracting the correction term,

$$53.3^2 + 51.3^2 + \ldots + 43.5^2 - \frac{739.8^2}{16} = 1190.4975.$$

Table V-1. Mean Differences in Bioelectric Potential (millivolts) between stem and nutrient solution for three bean plants with two determinations on each plant under four light intensties (D = zero, L = 500, M = 900, and H = 1200 foot-candles of light) arranged in a 4 x 4 latin square.

Time of Day	Day of Week: Wednesday	Thursday	Friday	Saturday	Total
Noon	53.3	51.3	39.0	41.7	185.3
2:00 P.M.	61.5	59.8	49.8	44.8	215.9
4.00 P.M.	45.2	41.5	51.3	40.0	178.0
6:00 P.M.	44.3	23.8	49.0	43.5	160.6
Total	204.3	176.4	189.1	170.0	739.8

Treatment	Totals	Means
D	195.7	48.92
L	185.4	46.35
M	185.5	46.38
H	173.2	43.30
Total	739.8	46.24

Analysis of Variance

Source of Variation	d.f.	Sum of Squares	Mean Square
Time of day (rows)	3	399.8125	133.2708
Day of week (columns)	3	172.0625	57.3542
Light Intensity (treatments)	3	63.5325	21.1775
Error or residual	6	555.0900	92.5150
Total	15	1190.4975	---

The row (time of day), column(day of week), and treatment (light intensities) sums of squares were obtained as follows:

$$\frac{185.3^2 + \ldots + 160.6^2}{4} - \frac{739.8^2}{16} = 399.8125,$$

$$\frac{204.3^2 + \ldots + 170.0^2}{4} - \frac{739.8^2}{16} = 172.0625,$$

and

$$\frac{195.7^2 + \ldots + 173.2^2}{4} - \frac{739.8^2}{16} = 63.5325.$$

The error or residual sum of squares is obtained by subtraction,

$$1190.4975 - 399.8125 - 172.0625 - 63.5325 = 555.0900.$$

The experimenter may have been somewhat startled at the results obtained from the analysis of variance in Table V-1. The treatment mean square is smaller, but not significantly so, than any of the others. Neither of the mean squares for time of day or day of the week exhibit any unusual variability.

The next step in examining the experimental results could be to obtain the linear regressions for the various sets of means. Even this doesn't appear to be very fruitful. The next step might be to compute the coefficient of variation,

$$v = \frac{\sqrt{92.5150}}{46.24} = 20.8 \text{ percent},$$

which appears to be rather high for experimental work. Therefore, the differences between the treatment, row, or column means may still be real but the experimental material or methods were too variable to detect these differences. The next step might be to study the experimental material and procedure. This was done and it was found that the variation among readings on an individual plant was very small but that the variation among plants was quite large. Also, it was found that plants with high potential readings tended to remain high and vice versa. With this information, the plants were divided into homogeneous groups with regard to magnitude of bioelectric potential readings and then the differences in the groups was confounded with day to day differences by applying the treatments to a different group each day.

The standard error of a treatment, row, or column mean is

$$s_{\bar{x}} = \sqrt{\frac{92.5150}{4}} = 4.809,$$

the standard error of a mean difference is

$$s_d = \sqrt{\frac{2(92.5150)}{4}} = 6.801 = s_{\bar{x}}\sqrt{2}$$

the least or minimum significant difference between two paired means is

$$s_d\, t_{.05(6\text{ d.f.})} = 6.801(2.447) = 16.64,$$

and the least significant difference between the largest and smallest of the means is

$$3.65\, s_d = 3.65(6.801) = 24.82,$$

where 3.65 is the value listed for $n = 4$, $P = .05$, Table 5.5 Snedecor, 1946. The above statistics may have little meaning for this example but are presented to illustrate the computations. Usually a non-significant F value would be the end of the computations unless a coefficient of variation was wanted. If the mean square for treatments had been significantly greater than the error mean square and if the treatments were unrelated, Tukey's (1949) method of testing the differences among the ranked means would be appropriate.

However, with these specific treatments and rows and perhaps columns, individual comparisons (linear, quadratic, and cubic effects) would be appropriate.

The efficiency of this latin square compared to what it would have been had a completely randomized design been used is

$$\frac{(k-1)(\text{col. m.s.} + \text{row m.s.}) + [(k-1) + (k-1)(k-2)][\text{residual m.s.}]}{\text{residual m.s. } [2(k-1) + (k-1) + (k-1)(k-2)]}$$

$$= \frac{3(57.3542 + 133.2708) + (3+6)(92.5150)}{(92.5150)(15)}$$

$$= \frac{93.6340}{92.5150} = 101.2 \text{ percent.}$$

Thus, the reduction in block size from 16 to 4 plots and the elimination of variation in two directions resulted in an increase in precision of 1.2 percent. This slight increase is not all real since it is necessary, for absolute accuracy, to take into account the difference in degrees of freedom associated with the two mean squares. Cochran and Cox (1944) discuss these adjustments, which are

$$\frac{s_2^2(n_1+1)(n_2+3)}{s_1^2(n_1+3)(n_2+1)} = \frac{93.6340(6+1)(12+3)}{92.5150(6+3)(12+1)} = 90.8 \text{ percent,}$$

where s_1^2 and s_2^2 are the two error variances and n_1 and n_2 are the corresponding degrees of freedom associated with each error variance. This correction should have been made on the efficiencies computed in Chapter IV and should be used whenever the degrees of freedom associated with the error mean squares are less than 20.

The efficiency of this latin square relative to what it would have been had the rows been used as replicates is

$$\frac{(k-1)(\text{col.m.s.}) + [(k-1) + (k-2)(k-1)](\text{residual m.s.})}{\text{residual m.s. } [(k-1) + (k-1) + (k-2)(k-1)]}$$

$$= \frac{3(57.3542) + (3 + 6)(92.5150)}{92.5150\ (\cancel{15})\ 12} = \frac{66.9798}{92.5150} = 72.4 \text{ percent,}$$

which when adjusted for the difference in degrees of freedom is

$$\frac{66.9798(6+1)(9+3)}{92.5150(6+3)(9+1)} = 67.6 \text{ percent.}$$

The efficiency of this latin square relative to what it would have been had the columns or day of the week been used as replicates is

$$\frac{(k-1)(\text{row m.s.}) + [(k-1) + (k-2)(k-1)](\text{residual m.s.})}{\text{residual m.s.}[(k-1) + (k-1) + (k-2)(k-1)]}$$

$$= \frac{3(133.2708) + (3+6)(92.5150)}{92.5150(15)} = \frac{82.1632}{92.5150} = 88.8 \text{ percent.}$$

The efficiency adjusted for difference in the degrees of freedom is $\dfrac{82.1632(6+1)(9+3)}{92.5150(6+3)(9+1)} = 82.9$ percent.

The efficiency of this latin square is somewhat lower than is usually obtained in experimentation.

V-6. Statistical Analysis for a Group of Latin Squares with a Single determination per Plot

In some cases it may be desirable to have more than a single latin square at a single location or to have a single latin square at several locations. For the 2x2, 3x3, and sometimes 4x4 latin squares, it is often desirable to have two or more squares at a location in order to have sufficient degrees of freedom in the error sum of squares. The procedure of designing an experiment in more than one latin square has already been discussed. The breakdown of the total degrees of freedom in the analysis of variance follows:

Source of Variation	Degrees of Freedom
Squares (or locations)	s-1
Rows within squares	s(k-1)
Columns within squares	s(k-1)
Treatments	k-1
Treatments X squares	(s-1)(k-1)
Residual within squares	s(k-1)(k-2)
Total	sk^2-1

The "treatment X squares" sum of squares may be pooled with "residual within squares" sum of squares if there is no treatment-square interaction. If the squares are planted at different locations then, for some hypotheses, t may be appropriate to use the treatment X square mean square to test the treatment differences. Fisher (1942, Design of Expts., Sec. 65) discusses the analysis for a group of latin squares and the approporiate error mean square for testing the differences among treatment means for various hypotheses. The analysis for two 4x4 latin squares has been discussed by Wishart (Field trials: their layout and statistical analysis. Imp. Bur. Pl. Br. and Gen. Cambridge).

Example V-2. As an illustration two 3x3 latin squares(Table V-2) were superimposed on corn uniformity trial data (Zuber, 1940) for which the yields were recorded as pounds of ear corn per 4x5 hill plot. The distance between hills within the row and between rows was 3.5 feet, resulting in a plot size of 14' x 17.5' and the square size of 42' x 52.5'. The total experimental area was 84' x 52.5'.

Table V-2. Two 3x3 latin squares, row, column, and treatment totals and the analyses of variance. Yield in pounds of ear corn per 4x5 hill plot.

	Square I			Total	Square II			Total
	A 33.9	C 32.1	B 33.1	99.1	C 29.2	A 31.1	B 29.6	89.9
	C 28.7	B 31.2	A 34.4	94.3	B 32.3	C 30.8	A 31.1	94.2
	B 30.4	A 30.5	C 29.7	90.6	A 28.1	B 31.3	C 30.4	89.8
Total	93.0	93.8	97.2	284.0	89.6	93.2	91.1	273.9

Treatment	Square I	Square II	Total
A	98.8	90.3	189.1
B	94.7	93.2	187.9
C	90.5	90.4	180.9
Total	284.0	273.9	557.9

Source of Variation	Square I			Square II			Square I and II			
	d.f.	s.s.	m.s.	d.f.	s.s	m.s.	d.f.	s.s.	m.s.	
Squares	—	—	—	—	—	—	1	5.6672	5.6672	
Rows	2	12.1089	6.0544	2	4.2067	2.1034	4	16.3156	4.0789	
Columns	2	3.3155	1.6578	2	2.1800	1.0900	4	5.4955	1.3739	
Treatments	2	11.4822	5.7411	2	1.8067	.9034	2	6.5377	3.2688	
Treatments X squares	—	—	—	—	—	—	2	6.7512	3.3756	2.5022
Error	2	3.5356	1.7678	2	4.7266	2.3633	4	8.2622	2.0656	
Total	8	30.4422		8	12.9200		17	49.0294		

The analysis of variance was obtained for each square separately and then the results were combined. The separate analyses present no additional work, unless the individual mean squares are obtained, and may indicate the source of large variations. As a general rule, it would be wise to study the individual analyses in connection with the combined analysis. All three analyses of variance are given in Table V-2 along with the individual and total yields.

The total sum of squares for Square I is

$$33.9^2 + \ldots + 29.7^2 - \frac{284.0^2}{9} = 8,992.22 - 8961.7778$$

$= 30.4422$, for Square II it is

$$29.2^2 + \ldots + 30.4^2 - \frac{273.9^2}{9} = 8,348.61 - 8,335.6900$$

$= 12.9200$, and for both squares it is

$$8,992.22 + 8,348.61 - \frac{(284.0 + 273.9)^2}{18}$$

$= 17,340.83 - 17,291.8006 = 49.0294$.

The sums of squares for rows, columns, and treatments in Square I are, respectively,

$$\frac{99.1^2 + 94.3^2 + 90.6^2}{3} - \frac{284.0^2}{9} = 12.1089,$$

$$\frac{93.0^2 + 93.8^2 + 97.2^2}{3} - \frac{284.0^2}{9} = 3.3155,$$

and

$$\frac{98.8^2 + 94.7^2 + 90.5^2}{3} - \frac{284.0^2}{9} = 11.4822$$

and in Square II are, respectively,

$$\frac{89.9^2 + 94.2^2 + 89.8^2}{3} - \frac{273.9^2}{9} = 4.2067,$$

$$\frac{89.6^2 + 93.2^2 + 91.1^2}{3} - \frac{273.9^2}{9} = 2.1800,$$

and

$$\frac{90.3^2 + 93.2^2 + 90.4^2}{3} - \frac{273.9^2}{9} = 1.8067.$$

The treatment sum of squares for both squares is

$$\frac{189.1^2 + 187.9^2 + 180.9^2}{6} - \frac{557.9^2}{18} = 6.5377.$$

The error sums of squares for Square I and II are obtained by subtraction. Error or residual sum of squares within squares is obtained by addition of the error sum of squares for each of the squares, $3.5356 + 4.7266 = 8.2622$. The treatment X square sum of squares is obtained either from the treatments and squares 2x3 table of treatment totals or by subtracting the treatment sum of squares from the treatment within square sum of squares, $11.4822 + 1.8067 - 6.5377 = 6.7512$. Lastly, the sum of squares between squares is obtained from the individual square correction terms,

$$\frac{284.0^2 + 273.9^2}{9} - \frac{557.9^2}{18} = 5.6672.$$

As a partial check the sums of squares in the combined analysis should add to the total sum of squares.

The residual within square and treatment X square sums of squares were pooled since in this experiment they are estimates of the same variance. None of the mean squares are significantly larger than the error mean square. In practice the computations would usually stop here but for illustrative purposes a number of statistics will be computed.

The coefficient of variation is

$$v = \frac{18\sqrt{2.5022}}{557.9} = 5.1 \text{ percent.}$$

The standard error of a difference of two treatment totals is

$$s_d = \sqrt{2x6x2.5022} = 5.480,$$

and the least significant difference between two treatment totals is

$$s_d\, t_{.05(6\text{ d.f.})} = (5.480)(2.447) = 13.41.$$

The efficiency of this latin square design relative to what it would have been had a completely randomized design been used is

$$\frac{1(5.6672) + 4(4.0789) + 4(1.3739) + 2(3.3756) + (2+4)(2.0656)}{2.0656\ (1+4+4+2+2+4)}$$

$= \frac{2.7425}{2.0656} = 133$ percent, where 3.3756 is the treatment X square mean square and 2.0656 is the error within squares mean square.

The efficiency of the latin square relative to what it would have been had a completely randomized design been used in each of the two squares is

$$\frac{4(4.0789) + 4(1.3739) + (2+4)(2.0656)}{2.0656(14)}$$

$$= \frac{2.4432}{2.0656} = 118 \text{ percent.}$$

The efficiency of the latin square design relative to what it would have been had the rows been used as the replicates within each of the squares is

$$\frac{4(1.3739) + (2+4)(2.0656)}{2.0656(10)} = \frac{1.7889}{2.0656} = 87 \text{ percent.}$$

The efficiency of the design relative to what it would have been had the columns been used as replicates within each of the squares is

$$\frac{4(4.0789) + (2+4)(2.0656)}{2.0656(10)} = \frac{2.8709}{2.0656} = 139 \text{ percent}$$

In order to obtain the relative efficiencies adjusted for the difference in degrees of freedom for the various comparisons made above, each of the percentages should be multiplied by the factor $\frac{(n_1+1)(n_2+3)}{(n_1+3)(n_2+1)}$, where n_1 and n_2 are the degrees of freedom associated with the two error variances. The four percentages,133, 118, 87, and 139 adjusted for the differences in number of degrees of freedom associated with the two variances are:

$$133\,\frac{(4+1)(15+3)}{(4+3)(15+1)} = 107 \text{ percent},$$

$$118\,\frac{(4+1)(14+3)}{(4+3)(14+1)} = 96 \text{ percent},$$

$$87\,\frac{(4+1)(12+3)}{(4+3)(12+1)} = 72 \text{ percent},$$

and

$$139\,\frac{(4+1)(12+3)}{(4+3)(12+1)} = 115 \text{ percent}.$$

Example V-3. Several variations of latin squares are possible and some of these will be discussed in a later chapter. The example of the present section is a latin square with more than a single unit per plot. The original data with three plants per day exposed to each of four light intensities and two readings on each plant under each of the light intensities are recorded in Table V-3. A different set of three plants was used on each of the four days. The order of applying light intensity treatments was at random with the restriction that each of the treatments must occur in each of the orders over a four day period. The arrangement of the treatments (light intensities are given in Table V-3 along with the various totals used in obtaining the analysis of variance in Table V-4.

Table V-3. Differences in bioelectric potential (millivolts) between stem and nutrient solution for bean plants under four light intensity treatments (D = zero, L = 500, M = 900, and H = 1200 foot-candles of light) arranged in a 4x4 latin square.

		Wednesday Plant Number			Thursday Plant Number			Friday Plant Number			Saturday Plant Number			Row Total
		1	2	3	1	2	3	1	2	3	1	2	3	
		Treatment H			Treatment L			Treatment M			Treatment D			
Noon	1st det.	64	65	35	56	34	64	38	37	44	32	46	47	
	2nd det.	60	64	32	56	34	64	38	34	43	32	46	47	
	Total	124	129	67	112	68	128	76	71	87	64	92	94	
Cell total				320			308			234			250	1112
		Treatment M			Treatment D			Treatment L			Treatment H			
2:00 PM	1st det.	59	63	62	67	56	57	59	54	38	36	51	49	
	2nd. det.	58	65	62	65	58	56	59	52	37	34	50	49	
	Total	117	128	124	132	114	113	118	106	75	70	101	98	
Cell total				369			359			299			269	1296
		Treatment D			Treatment M			Treatment H			Treatment L			
4:00 PM	1st det.	15	62	56	53	42	31	62	49	45	34	49	37	
	2nd det.	20	60	58	52	41	30	60	48	44	35	49	36	
	Total	35	122	114	105	83	61	122	97	89	69	98	73	
Cell total				271			249			308			240	1068
		Treatment L			Treatment H			Treatment D			Treatment M			
6:00 PM	1st det.	26	54	54	24	23	22	52	44	52	48	54	30	
	2nd det.	27	52	53	28	24	22	52	42	52	46	53	30	
	Total	53	106	107	52	47	44	104	86	104	94	107	60	
Cell total				266			143			294			261	964
Totals		329	485	412	401	312	346	420	360	355	297	398	325	4440
Column Total				1226			1059			1135			1020	4440

In making the analysis, it must be remembered that the three plants used each day were subjected to the four treatments. Another arrangement could have been to use three different plants in each of 16 cells of the 4x4 latin square, resulting in a total of 48 plants rather than the 12 used. This procedure was impractical due to the amount of time required for setting up the apparatus to obtain readings on differences in bioelectric potentials between the stem of a bean plant and the nutrient solution in which its roots were submerged. In this case the breakdown of the total degrees of freedom would be:

Source of Variation	Degrees of Freedom
Rows (time of day)	3
Columns (day of week)	3
Treatments (light intensities)	3
Error (experimental)	6
Among plants within columns	2x16 = 32
Between readings on same plants	1x48 = 48
Total	95

But such was not the case and the further partitioning of the variation among plants within days with 8 degrees of freedom out of the 32 listed above is possible (see Table V-4). If it would have been possible to distinguish between the first and second determinations or readings of the difference in bioelectric potential, then a single degree of freedom from the 48 could have been segregated. Also, it would have been possible to obtain the various interactions of first and second readings with other factors. Since the two readings are considered to be measures of the same thing, no further partitioning of the 48 degrees of freedom was considered necessary.

Table V-4. Analysis of variance of the data in Table V-3.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Ave. value of Mean Square
Time of day (rows)	3	2403.3333	801.1111	
Day of week (cols)	3	1032.5833	344.1944	
Light intensities (treatments)	3	375.5833	125.1944	
Experimental Error	6	3337.1668	556.1945	$\sigma_\delta^2+2\sigma_\pi^2+6\sigma_e^2$
Among plants within cells	32	8437.3333	263.6667	$\sigma_\delta^2+2\sigma_\pi^2$
Among plants in columns	8	3034.1667	379.2708	
Remainder	24	5403.1666	225.1319	
Between readings on the same plant	48	68.0000	1.4167	σ_δ^2
Total	95	15654.0000	—	

The sums of squares for the analysis of variance in Table V-4 are obtained in much the same manner as for previous examples. The total sum is obtained by squaring the 96 determinations and subtracting the correction term, $\sum_{i,j,k,l,m} X_{ijklm}^2 - \frac{X_{.....}^2}{96}$

$$= 64^2 + 65^2 + \ldots + 53^2 + 30^2 - \frac{4440^2}{96} = 15{,}654.0000.$$

The row, column, and treatment sums of squares are, respectively,

$$\frac{1112^2 + 1296^2 + 1068^2 + 964^2}{3x2x4 = 24} - \frac{4440^2}{96} = 2403.3333,$$

$$\frac{1226^2 + 1059^2 + 1135^2 + 1020^2}{24} - \frac{4440^2}{96} = 1032.5833,$$

and $$\frac{1174^2 + 1113^2 + 1113^2 + 1040^2}{24} - \frac{4440^2}{96} = 375.5833,$$

The experimental error sum of squares is obtained by subtracting the row, column, and treatment sum of squares from the sum of squares of the $k^2 = 16$ cell total squared, i.e.

$$\frac{320^2 + 308^2 + \ldots + 294^2 + 261^2}{6} - \frac{4440^2}{96} - (2403.3333 + 1032.5833$$

$$+ 375.5833) = 212{,}498.6667 - 205{,}350.0000 - (3811.4999)$$

$$= 7148.6667 - 3811.4999 = 3337.1668.$$

The sum of squares associated with the variation among plant totals in each of the 16 cells is

$$\frac{124^2 + 129^2 + 67^2}{2} - \frac{320^2}{6} + \cdots + \frac{94^2 + 107^2 + 60^2}{2} - \frac{261^2}{6}$$

$$= 220{,}936.0000 - 212{,}498.6667 = 8437.3333.$$

The sum of squares attributable to the variation among plants within days is

$$\frac{329^2 + 485^2 + 412^2}{8} - \frac{1226^2}{24} + \ldots + \frac{297^2 + 398^2 + 325^2}{8} - \frac{1020^2}{24}$$

$$= 209{,}416.7500 - 206{,}382.5833 = 3034.1667.$$

The subtraction of the above sum of squares from that for variation among plant totals in each of the 16 cells results in a remainder sum of squares that is a composite of several effects (that is, within column sums of squares for plant X row, plant X treatment, and plant X row X treatment), thus,

$$8437.3333 - 3034.1667 = 5403.1666.$$

The sum of squares of the dfferences among readings on the same plant is obtained as follows:

$$64^2 + 60^2 - \frac{124^2}{2} + 65^2 + 64^2 - \frac{129^2}{2} + \ldots + 30^2 + 30^2 - \frac{60^2}{2}$$

$$= 221{,}004.0000 - 220{,}936.0000 = 68.0000 = \frac{(64-60)^2}{2} + \ldots + \frac{(30-30)^2}{2}.$$

In an experiment designed in this manner it is possible to test several hypotheses. The experimental error is used to test the variation among treatment means. In this particular case the treatment mean square is less than the experimental error mean square which indicates more uniformity among the treatment means than might be expected in a population with error variances of the magnitude found in this experiment. The suggestions for improving the experiment are given in the discussion of example V-1.

The F test of the experimental error residual mean square and the mean square associated with the differences among plants with the cells of the 4x4 latin square is

$$F = \frac{556.1945}{263.6667} = 2.11,$$ which is slightly lower than the tabulated F value, 2.40, at the 5 percent level of probability for 6 and 32 degrees of freedom.

One could test the hypotheses of no differences among the plant means within days by the F test,

$$F = \frac{379.2708}{225.1319} = 1.68.$$

The corresponding F value at the 5 percent point for 8 and 24 degrees of freedom is equal to 2.36. The variation among plant means for each day could be tested in a like manner to determine if any group of three plants may be considered as unusually variable.

The variance attributable to the differences between readings on the same plant, equal to 1.4167, is extremely small in comparison with the remaining mean squares. The obvious conclusion is that one reading per plant would be sufficient for all practical purposes and that more homogeneous groups of plants are required. If this is impossible then more plants per cell and more replicates of the treatments are required to obtain standard errors of a mean that are relatively small.

The standard error of a treatment mean is

$$\sqrt{\frac{\hat{\sigma}_{\delta}^2 + 2\hat{\sigma}_{\pi}^2 + 6\hat{\sigma}_{e}^2}{2 \times 3 \times 4}} = \sqrt{\frac{556.1945}{24}} = 4.814.$$

If k equals the number of replicates, p equals the number of plants, and d equals the number of determinations or readings, the variance of a treatment mean from a design such as this is

$$\frac{\hat{\sigma}_{\delta}^2}{dpk} + \frac{\hat{\sigma}_{\pi}^2}{pk} + \frac{\hat{\sigma}_{e}^2}{k} .$$

Using the estimates of $\hat{\sigma}_{\delta}^2$, $\hat{\sigma}_{\pi}^2$, and $\hat{\sigma}_{e}^2$ obtained from the data in Table V-4, the above formula becomes,

$$\frac{1.4167}{dpk} + \frac{263.6667 - 1.4167}{2(pk)} + \frac{556.1945 - 263.6667}{6k}$$

$$= \frac{1.4167}{dpk} + \frac{131.1250}{pk} + \frac{48.7546}{k} .$$

For k = 8, p = 1, and d = 1 the above expression is equal to 22.6620, which is smaller than the variance of the treatment mean actually obtained, 23.1748 and only one-third as many readings would be recorded. This method of reallocating various items does not take into account the additional cost of having more replicates. Yates and Zacopanay (1935, Jour. Agric. Sci.) have discussed this problem in detail and the student is advised to read this classic paper for further information on the subject of sampling.

Problem V-1. Compute the standard error of a treatment total and the corresponding least or minimum significant differences for the data of Table V-1.

Problem V-2. For the data in Table V-1, how many duplications of the 4x4 latin square would be required to obtain a standard error of a treatment mean equal to less than 5 percent of the mean?

Problem V-3. Compute the efficiency of the randomized complete blocks design relative to the completely randomized design adjusted for the difference in number of degrees of freedom for examples IV-1 and IV-2.

Problem V-4. Find the efficiency of the latin square design in Table 11.11 of Snedecor (1946) relative to a completely randomized design and to the two randomized complete blocks obtained when thecolumns are used as replicates and when the rows are used as replicates. Discuss briefly the effect of reducing the block size from 25 plots to 5. Obtain the efficiencies adjusted for the difference in degrees of freedom in the two error variances.

Problem V-5. In example IV-2 discuss briefly the effect on the variance of a treatment mean of using:

10 replicates, 1 plant per plot
5 replicates, 4 plants per plot
10 replicates, 2 plants per plot
20 replicates, 1 plant per plot.

Without bothering to adjust the variances of a treatment mean for the difference in degrees of freedom, compute the efficiencies of the above designs relative to the design used in Example IV-2.

Problem V-6. Compute the coefficients of variation for examples V-1, V-2, V-3.

Problem V-7. Compute the relative efficiencies for example V-3. What is the effect of reducing the block size from 16 to 4 plots and removing the variation due to time of day and day of week?

Problem V-8. Discuss briefly the relationships between the analyses of variance in Tables V-1 and V-4.

V-8. Least Squares Estimates and Average Value of Mean Squares for Latin Square Designs

In the following discussion, it is assumed that treatments, columns, rows, and squares represent random samples from their respective populations.

One Unit Per Plot

If a single observation is made on each plot of a latin square design, then the yield of the ijhth observation may be expressed as

$$X_{ijh} = \mu + \rho_i + \lambda_j + \tau_h + e_{ijh}$$

where i, j, h = 1, 2, ..., k, μ represents the population mean, ρ_i an effect common to the ith row, λ_j an effect common to the jth column, τ_h an effect common to the hth treatment, and e_{ijh} an effect common to the ijhth observation, if it is assumed that yield of any observation is expressible as the sum of several independent linear effects.

The Least Squares estimates of the 3k + 1 parameters, μ, $\rho_1, \ldots, \rho_k, \lambda_1, \ldots, \lambda_k, \tau_1, \ldots, \tau_k$, are obtained by partial differentiation of the residual sum of squares with respect to the 3k + 1 estimates of these parameters, setting the resulting equations equal to zero, and solving for the set of estimates. The residual sum of squares after fitting the 3k+1 constants is

$$\sum_{i,j,h} (X_{ijh} - \hat{\mu} - r_i - c_j - t_h)^2$$

and the normal equations after differentiation are

$$X_{...} = k\Sigma r_i + k\Sigma c_j + k\Sigma t_h + k^2\hat{\mu}$$

$$X_{1..} = kr_1 + \Sigma c_j + \Sigma t_h + k\hat{\mu}$$

$$\vdots$$

$$X_{k..} = kr_k + \Sigma c_j + \Sigma t_h + k\hat{\mu}$$

$$X_{.1.} = \Sigma r_i + kc_k + \Sigma t_h + k\hat{\mu}$$

$$\vdots$$

$$X_{.k.} = \Sigma r_i + kc_k + \Sigma t_h + k\hat{\mu}$$

$$X_{..1} = \Sigma r_i + \Sigma c_j + kt_1 + k\hat{\mu}$$

$$\vdots$$

$$X_{..k} = \Sigma r_i + \Sigma c_j + kt_k + k\hat{\mu}$$

The r_i, c_j, t_h, and $\hat{\mu}$ are the estimates which make the residual sum of squares a minimum. Now in order to obtain a unique solution the following restrictions are necessary:

$$\sum_i r_i = \sum_j c_j = \sum_h t_h = 0.$$

With the above conditions, then,

$$\hat{\mu} = \bar{x} = X_{...}/k^2$$

$$r_i = X_{i..}/k - \hat{\mu} = \bar{x}_{i..} - \bar{x},$$

$$c_j = X_{.j.}/k - \hat{\mu} = \bar{x}_{.j.} - \bar{x},$$

and $$t_h = X_{..h}/k - \hat{\mu} = \bar{x}_{..h} - \bar{x}.$$

The variances of the Least Squares estimates may be obtained as before.

The reduction in sum of squares due to fitting $\hat{\mu}$ has the same expectation as the expected value of the correction term for the latin square,

$$E[\hat{\mu}X_{...}] = E\left[\frac{X^2_{...}}{k^2}\right] = E\left[\frac{\sum_{ijh}(\mu+\rho_i+\lambda_j+\tau_h+e_{ijh})]^2}{k^2}\right.$$

$$= \frac{E[k^2\mu+k(\rho_1+\ldots.+\rho_k)+k(\lambda_1+\ldots.+\lambda_k)+k(\tau_1+\ldots.+\tau_k)+\sum_{ijh} e_{ijh}]^2}{k^2}$$

$$= k^2\mu^2 + k\sigma^2_\rho + k\sigma^2_\lambda + k\sigma^2_\tau + \sigma^2_e.$$

The total sum of squares has the expectation

$$E[\sum_{ijh} X^2_{ijh}] = \sum_{ijh} E[\mu+\rho_i+\lambda_j+\tau_h+e_{ijh}]^2$$

$$= k^2(\mu^2+\sigma^2_\rho+\sigma^2_\lambda+\sigma^2_\tau+\sigma^2_e).$$

The reduction in the sum of squares due to fitting the r_i, c_j, t_h, and $\hat{\mu}$ is

$$E[\Sigma X^2_{ijh} - \hat{\mu}X_{...} - \Sigma r_i X_{i..} - \Sigma c_j X_{.j.} - \Sigma t_h X_{..h}]$$

$$= E\left[\Sigma X^2_{ijh} - \frac{\Sigma X^2_{i..}}{k} - \frac{\Sigma X^2_{.j.}}{k} - \frac{\Sigma X^2_{..h.}}{k} + \frac{2X^2_{...}}{k^2}\right]$$

$$= k^2(\mu^2+\sigma^2_\rho+\sigma^2_\lambda+\sigma^2_\tau+\sigma^2_e) - (k^2\mu^2+k^2\sigma^2_\rho+ k\sigma^2_\lambda+ k\sigma^2_\tau+ k\sigma^2_e) - (k^2\mu^2+ k\sigma^2_\rho+k^2\sigma^2_\lambda+k\sigma^2_\tau+k\sigma^2_e) - (k^2\mu^2+k\sigma^2_\rho+k\sigma^2_\lambda+k^2\sigma^2_\tau+k\sigma^2_e) + 2(k^2\mu^2+ k\sigma^2_e+k\sigma^2_\lambda+k\sigma^2_\tau+\sigma^2_e) = (k-1)(k-2)\sigma^2_e,$$

which is the residual error variance times the degrees of freedom.

The reduction in sum of squares due to fitting the r_i only is the reduction due to fitting $\hat{\mu}$, r_i, c_j, and t_h minus the reduction due to fitting $\hat{\mu}, c_j$, and t_h which for the orthogonal case has the expectation

$$E[\Sigma r_i X_{i..}] = E\left[\frac{\Sigma X^2_{i..}}{k} - \frac{X^2_{...}}{k^2}\right]$$

$$= k^2\mu^2+k^2\sigma^2_\rho+k\sigma^2_\lambda+k\sigma^2_\tau+k\sigma^2_e - (k^2\mu^2+k\sigma^2_\rho+k\sigma^2_\lambda+k\sigma^2_\tau+\sigma^2_e) = (k-1)(\sigma^2_e+k\sigma^2_\rho),$$

with k-1 degrees of freedom.

In a like manner, the reduction in sum of square due to fitting the c_j or the t_h have the respective expectations,

$$(k-1)(\sigma^2_e + k\sigma^2_\lambda) \quad \text{or} \quad (k-1)(\sigma^2_e + k\sigma^2_\tau).$$

The total sum of squares after fitting $\hat{\mu}$ has the expectation,

$$E\left[\sum_{ijh} X^2_{ijh} - \frac{X^2_{...}}{k^2}\right]$$

$$= (k^2-1)\sigma_e^2+(k-1)k\sigma_\tau^2+(k-1)k\sigma_\rho^2+(k-1)k\sigma_\lambda^2,$$

which is the sum of the expectations for the treatment, row, column, and residual sum of squares.

<u>s squares of kxk latin squares</u>

The linear model for s groups or squares of kxk latin squares is $X_{ijhg} = \mu+\delta_i+\tau_j+\delta\tau_{ij}+\rho_{ih}+\lambda_{ig}+e_{ijhg}$, where μ represents the population parameter for the mean, δ_i = an effect common to i^{th} square, τ_j = effect common to j^{th} treatment, $\delta\tau_{ij}$ = an effect common to the j^{th} treatment in the i^{th} square, ρ_{ih} = effect common to h^{th} row in the i^{th} square, λ_{ig} = effect common to the g^{th} column in the i^{th} square, e_{ijhg} = effect common to $ijhg^{th}$ observation, i = 1, 2, ..., s, j = 1, 2, ..., k, h = 1, 2, ..., k, and g = 1, 2, ..., k.

The Least Squares estimates are obtained from the following sets of normal equations:

$$X_{....} = k^2\Sigma d_i+sk\Sigma t_j+k\Sigma\Sigma dt_{ij}+k\Sigma\Sigma r_{ih}+k\Sigma\Sigma c_{ig}+sk^2\hat{\mu}$$

$$X_{1...} = k^2 d_1+k\Sigma t_j+k\Sigma dt_{1j}+k\Sigma r_{1h}+k\Sigma c_{1g}+k^2\hat{\mu}$$

$$\vdots$$

$$X_{s...} = k^2 d_s+k\Sigma t_j+k\Sigma dt_{sj}+k\Sigma r_{sh}+k\Sigma c_{sg}+k^2\hat{\mu}$$

$$X_{.1..} = k\Sigma d_i+skt_1+k\Sigma dt_{i1}+\Sigma\Sigma r_{ih}+\Sigma\Sigma c_{ig}+sk\hat{\mu}$$

$$\vdots$$

$$X_{.k..} = k\Sigma d_i+skt_k+k\Sigma dt_{ik}+\Sigma\Sigma r_{ih}+\Sigma\Sigma c_{ig}+sk\hat{\mu}$$

$$X_{11..} = kd_1+kt_1+kdt_{11}+\Sigma r_{1h}+\Sigma c_{1g}+k\hat{\mu}$$

$$\vdots$$

$$X_{sk..} = kd_s+kt_k+kdt_{sk}+\Sigma r_{sh}+\Sigma c_{sg}+k\hat{\mu}$$

$$X_{1.1.} = kd_1+\Sigma t_j+\Sigma dt_{ij}+kr_{11}+\Sigma c_{1g}+k\hat{\mu}$$

$$\vdots$$

$$X_{s.k.} = kd_s+\Sigma t_j+\Sigma dt_{sj}+kr_{sk}+\Sigma c_{sg}+k\hat{\mu}$$

$$X_{1..1} = kd_1+\Sigma t_j+\Sigma dt_{1j}+\Sigma r_{1h}+kc_{11}+k\hat{\mu}$$

$$\vdots$$

$$X_{s..k} = kd_s+\Sigma t_j+\Sigma dt_{sj}+\Sigma r_{sh}+kc_{sk}+k\hat{\mu}$$

after imposing the restrictions

$$\Sigma d_i = \Sigma t_j = \sum_h r_{ih} = \sum_g c_{ig} = \sum_i td_{ij} = \sum_j dt_{ij} = 0 \text{ ,}$$

the estimates are

$$\hat{\mu} = \bar{x} = X_{....} / sk^2 \text{ ,}$$

$$d_i = (X_{i...} / k^2) - \bar{x} = \bar{x}_{i...} - \bar{x} \text{ ,}$$

$$t_j = (X_{.j..} / sk) - \bar{x} = \bar{x}_{.j..} - \bar{x} \text{ ,}$$

$$r_{ih} = (X_{i.h.}/ k) - \bar{x}_{i...} \text{ ,}$$

$$c_{ig} = (X_{i..g}/ k) - \bar{x}_{i...} \text{ ,}$$

$$dt_{ij} = (X_{ij..}/k) - (X_{i...}/k^2) - (X_{....}/sk) + \bar{x} \text{ .}$$

The total sum of squares corrected for the mean or the sum of squares after fitting $\hat{\mu}$ has the expectation

$$E\left[\Sigma X^2_{ijhg} - \frac{X^2_{....}}{sk^2}\right] = \sum_{ijhg} E[\mu+\delta_i+\tau_j+\delta\tau_{ij}+\rho_{ih}+\lambda_{ig}+e_{ijhg}]^2$$

$$- \frac{1}{sk^2} E[sk^2\mu+k^2(\delta_1+\ldots+\delta_s)+sk(\tau_1+\ldots+\tau_k)+k(\delta\tau_{11}+\ldots+\delta\tau_{sk})$$

$$+k(\rho_{11}+\ldots+\rho_{sk})+k(\lambda_{11}+\ldots+\lambda_{sk})+\Sigma\Sigma\Sigma\Sigma e_{ijhg}]^2$$

$$= sk^2(\mu^2+\sigma^2_\delta+\sigma^2_\tau+\sigma^2_{\delta\tau}+\sigma^2_\rho+\sigma^2_\lambda+\sigma^2_e) - (sk^2\mu^2+k^2\sigma^2_\delta+sk\sigma^2_\tau+k\sigma^2_{\delta\tau}+k\sigma^2_\rho+k\sigma^2_\lambda+\sigma^2_e)$$

$$= (sk^2-1)\sigma^2_e+(sk-1)k\sigma^2_\rho+(sk-1)k\sigma^2_\lambda+(sk-1)k\sigma^2_{\delta\tau}+sk(k-1)\sigma^2_\tau+k^2(s-1)\sigma^2_\delta.$$

The sum of squares for treatments has the expectation

$$E[\Sigma t_j X_{\cdot j \cdot \cdot}] = E\left[\frac{\Sigma X^2_{\cdot j \cdot \cdot}}{sk} - \frac{X^2_{\cdot \cdot \cdot \cdot}}{sk^2}\right]$$

$$= \frac{1}{sk} \sum_j E[sk\mu + k\Sigma\delta_i + sk\tau_j + k\sum_i \delta\tau_{ij} + \sum_{ih}\Sigma\rho_{ih} + \sum_{ig}\Sigma\lambda_{ig} + \sum_{ihg}\Sigma\Sigma e_{ijgh}]^2 - \frac{E(X^2_{\cdot \cdot \cdot \cdot})}{sk^2}$$

$$= sk^2\mu^2 + k^2\sigma^2_\delta + sk^2\sigma^2_\tau + k^2\sigma^2_{\delta\tau} + k\sigma^2_\rho + k\sigma^2_\lambda + k\sigma^2_e - sk^2\mu^2 - k^2\sigma^2_\delta - sk\sigma^2_\tau - k\sigma^2_{\delta\tau} - k\sigma^2_\rho$$

$$-k\sigma^2_\lambda - \sigma^2_e = (k-1)(\sigma^2_e + k\sigma^2_{\delta\tau} + sk\sigma^2_\tau),$$ with $(k-1)$ degrees of freedom.

The squares sum of squares has the expectation

$$E[\Sigma d_i X_{i \cdot \cdot \cdot}] = E\left[\sum_i \frac{X^2_{i \cdot \cdot \cdot}}{k^2} - \frac{X^2_{\cdot \cdot \cdot \cdot}}{sk^2}\right]$$

$$= \frac{1}{k^2} \Sigma E[k^2\mu + k^2\delta_i + k\Sigma\tau_j + k\Sigma\delta\tau_{ij} + k\Sigma\rho_{ih} + k\Sigma\lambda_{ig} + \Sigma\Sigma\Sigma e_{ijhg}]^2 - \frac{E[X^2_{\cdot \cdot \cdot \cdot}]}{sk^2}$$

$$= sk^2\mu^2 + sk^2\sigma^2_\delta + sk\sigma^2_\tau + sk\sigma^2_{\delta\tau} + sk\sigma^2_\rho + sk\sigma^2_\lambda + s\sigma^2_e - sk^2\mu^2 - k^2\sigma^2_\delta - sk\sigma^2_\tau$$

$$-k\sigma^2_{\delta\tau} - k\sigma^2_\rho - k\sigma^2_\lambda - \sigma^2_e$$

$$= (s-1)(\sigma^2_e + k\sigma^2_\lambda + k\sigma^2_\rho + k\sigma_{\delta\tau}^2 + k^2\sigma^2_\delta),$$ with $(s-1)$ degrees of freedom.

The treatment X square sum of squares has the expectation

$$E\left[\sum_{ij}\Sigma \frac{X^2_{ij \cdot \cdot}}{k} - \sum_i \frac{X^2_{i \cdot \cdot \cdot}}{k^2} - \sum_j \frac{\Sigma X^2_{\cdot j \cdot \cdot}}{sk} + \frac{X^2_{\cdot \cdot \cdot \cdot}}{sk^2}\right]$$

$$= (s-1)(k-1)(\sigma^2_e + k\sigma^2_{\delta\tau}).$$

The row within squares, column within squares, and residual within squares sums of squares have the respective expectations

$$s(k-1)(\sigma^2_e + k\sigma^2_\rho), \quad s(k-1)(\sigma^2_e + k\sigma^2_\lambda), \text{ and } s(k-1)(k-2)\sigma^2_e.$$

kxk latin square with p items per cell and d determinations on each item

If it is assumed that the treatments, rows, columns, items, and determinations are random samples from their respective populations and that the yield of the ijhgfth observation may be expressed as the sum of the several independent effects, that is,

$$X_{ijhgf} = \mu+\rho_i+\lambda_j+\tau_h+e_{ijh}+\pi_{ijhg}+\delta_{ijhgf},$$

where μ = population mean, ρ_i = effect common to ith row, λ_j= effect common to jth column, τ_h = effect common to the hth treatment, e_{ijh} = effect common to ijhthcell, π_{ijhg} = effect common to the gth item in the ijhth cell, δ_{ijhgf} = effect common to the ijhgfth determination, i,j,h,=1,2,...,k, g=1,2,...,p, and f = 1, 2, ..., d. For this example a new sample of items are used in each cell of the kxk latin square and determination f for one item has nothing in common with the fth determination on another item.

The Least Squares estimates may be obtained as before and are left as an exercise for the student to do. Also, the following expectations may be verified by the student:

Source of Variation	Degrees of Freedom	Average Value of Mean Square
Rows	k-1	$\sigma_\delta^2 + d\sigma_\pi^2 + dp\sigma_e^2 + kdp\sigma_\rho^2$
Columns	k-1	$\sigma_\delta^2 + d\sigma_\pi^2 + dp\sigma_e^2 + kdp\sigma_\lambda^2$
Treatments	k-1	$\sigma_\delta^2 + d\sigma_\pi^2 + dp\sigma_e^2 + kdp\sigma_\tau^2$
Residual	(k-1)(k-2)	$\sigma_\delta^2 + d\sigma_\pi^2 + dp\sigma_e^2$
Items within cells	$k^2(p-1)$	$\sigma_\delta^2 + d\sigma_\pi^2$
Determinations on same item	$pk^2(d-1)$	σ_δ^2
Total	dpk^2-1	

In the event that a new sample of items is used for each column (see Example V-3), the linear model is

$$X_{ijhgf} = \mu + \rho_i + \lambda_j + \tau_h + e_{ijh} + \pi_{jg} + \alpha_{ijhg} + \delta_{ijhgf}$$

where the effects and subscripts are the same as before except for π_{jg} and α_{ijhg}. π_{jg} is the effect common to the g^{th} item in the j^{th} column, α_{ijhg} is an effect common to the $ijhg^{th}$ observation, and $g = 1, 2, \ldots, p$ in each column.

For this case, the expectations for the various mean squares are:

Source of Variation	Degrees of Freedom	Average Value of Mean Square
Rows	k-1	$\sigma_\delta^2+d\sigma_\alpha^2+dp\sigma_e^2+dpk\sigma_\rho^2$
Columns	k-1	$\sigma_\delta^2+d\sigma_\alpha^2+dk\sigma_\pi^2+dp\sigma_e^2+dpk\sigma_\lambda^2$
Treatments	k-1	$\sigma_\delta^2+d\sigma_\alpha^2+dp\sigma_e^2+dpk\sigma_\tau^2$
Residual	(k-1)(k-2)	$\sigma_\delta^2+d\sigma_\alpha^2+dp\sigma_e^2$
Items within cols	k(p-1)	$\sigma_\delta^2+d\sigma_\alpha^2+dk\sigma_\pi^2$
Remainder	k(p-1)(k-1)	$\sigma_\delta^2+d\sigma_\alpha^2$
Determinations on same item	$pk^2(d-1)$	σ_δ^2
Total	dpk^2-1	———

The correction term has the expectation

$$E\left[\frac{X^2_{\cdot\cdot\cdot\cdot\cdot}}{dpk^2}\right] = \frac{1}{dpk^2}\,E[dpk^2\mu+dpk(\Sigma\rho_i+\Sigma\lambda_j+\Sigma\tau_h) + dp\Sigma\Sigma\Sigma e_{ijh}$$

$$+dk\Sigma\Sigma\pi_{jg} + d\Sigma\Sigma\Sigma\Sigma\alpha_{ijhg} + \Sigma\Sigma\Sigma\Sigma\Sigma\delta_{ijhgf}]^2$$

$$= dpk^2\mu^2+dpk(\sigma_\rho^2+\sigma_\lambda^2+\sigma_\tau^2)+dp\sigma_e^2+dk\sigma_\pi^2+d\sigma_\alpha^2+\sigma_\delta^2 = CT.$$

The items within columns sum of squares with k(p-1) degrees of freedom has the expectation

$$E\left[\frac{\Sigma\Sigma X^2_{\cdot j\cdot g\cdot}}{dk} - \frac{\Sigma_j X^2_{\cdot\cdot j\cdot\cdot\cdot}}{dpk}\right]$$

$$= \frac{1}{dk}\sum_{jg}\Sigma E[dk\mu+d\Sigma\rho_i+dk\lambda_j+d\Sigma\tau_h+d\sum_{ih}\Sigma e_{ijh}+dk\pi_{jg}+d\sum_{ih}\Sigma\alpha_{ijhg}+\sum_{ihf}\Sigma\Sigma\delta_{ijhgf}]^2$$

$$- \frac{1}{dpk} \sum_j E \left[dpk(\mu+\lambda_j)+dp(\Sigma\rho_i+\Sigma\tau_h+\sum_{ih}\Sigma e_{ijh})+dk\sum_g \pi_{jg}+d\sum_{ihg}\Sigma\Sigma\alpha_{ijhg} + \sum_{ihgf}\Sigma\Sigma\Sigma\delta_{ijhgf}\right]^2$$

$$= dpk^2\mu^2+dpk\sigma^2_\rho+dpk^2\sigma^2_\lambda+dpk\sigma^2_\tau+dpk\sigma^2_e+dpk^2\sigma^2_\pi+dpk\sigma^2_\alpha+kp\sigma^2_\delta - [dpk^2\mu^2 + dpk^2\sigma^2_\lambda+dpk(\sigma^2_\rho+\sigma^2_\tau+\sigma^2_e)+dk^2\sigma^2_\pi+dk\sigma^2_\alpha+k\sigma^2_\delta]$$

$$= k(p-1)[\sigma^2_e+d\sigma^2_\alpha+dk\sigma^2_\pi].$$

The column sum of squares has the expectation

$$E\left[\frac{1}{dpk}\cdot\Sigma X^2_{.j...}\right] - CT = (k-1)(\sigma^2_\delta+d\sigma^2_\alpha+dp\sigma^2_e+dk\sigma^2_\pi+dpk\sigma^2_\lambda),$$

with (k-1) degrees of freedom.

The expectation of the row sum of squares is

$$E\left[\frac{1}{dpk}\Sigma X^2_{i....}\right] - CT = \frac{1}{dpk}\sum_i E\left[dpk(\mu+\rho_i)+dp(\Sigma\lambda_j+\Sigma\tau_h+\sum_{jh}\Sigma e_{ijh}) + d\sum_{jg}\Sigma\pi_{jg}+d\sum_{jhg}\Sigma\Sigma\alpha_{ijhg}+\Sigma\Sigma\Sigma\Sigma\delta_{ijhgf}\right]^2 - CT$$

$$= dpk^2(\mu^2+\sigma^2_\rho)+dpk(\sigma^2_\lambda+\sigma^2_\tau+\sigma^2_e)+dk\sigma^2_\pi+dk\sigma^2_\alpha+k\sigma^2_\delta - CT$$

$$= (k-1)(\sigma^2_\delta+d\sigma^2_\alpha+dp\sigma^2_e+dpk\sigma^2_\rho),$$

with (k-1) degrees of freedom.

The remaining expectations are obtained similarly and are left as an exercise for the student.

Chapter VI

THE PLANNING OF EXPERIMENTS AS RELATED TO THE CHOICE OF TREATMENTS AND THE FACTORIAL EXPERIMENT

by

W.T.Federer

VI-1. The Conduct of Experiments and the Choice of Treatments

The foregoing chapters have dealt mostly with the construction, lay-out, and statistical analysis of some of the simpler and more widely used experimental or observational designs. The merits and faults of each of the designs were discussed. Before proceeding to more complicated experimental designs it is necessary to bring in some new statistical concepts; in particular, those related to "factorial" arrangements and experiments. The new ideas are developed via the procedure for selecting the treatments to be included in the experiment.

In many experiments success or failure may depend more upon the selection of treatments for comparisons to be made than upon the design. Of course, the selection of both the design and of the treatmehts is important and neither should be slighted in planning the experiment. For example, the design of the experiment might be a latin square and the treatments new methods or new varieties. The design may be quite appropriate, but the selection of the treatments may include only new methods or varieties affording no comparisons (assuming these comparisons are desired) with the standard method or check variety. On the other hand, the proper choice of treatments may have been made but a poor choice of the design, such as a systematic, might invalidate the results.

Other illustrations could be cited but the only one that merits considerable discussion is the group of treatments involving two or more levels or kinds of two or more substances or factors, such as:

(i) light, oxygen, and temperature
(ii) temperature and length of storage
(iii) spacings and rates of planting
(iv) levels of two or more fertilizers
(v) levels of ingredients and methods of mixing
(vi) levels of ingredients and baking temperatures
(vii) levels of proteins and carbohydrates in feeding trials
(viii) levels of insecticides or fungicides and varieties or species
(ix) methods of teaching and schools
(x) length and type of mixings

A group of treatments which contain two or more levels of two or more factors or subdtances is known as a factorial arrangement. As indicated by the various types of factorials tested above, the factorial arrangement of treatments covers a wide variety of experiments.

For the successful conclusion of an experiment several items, of which the choice of treatments and of the design represent only a phase in the planning of an experiment, are of considerable importance. The experiment may be planned satisfactorily but many other things could go wrong in the conduct of an experiment. The experimental site may be located in the path of floods or made variable in other ways; the measurements could be recorded unreliably, thereby vitiating the results; a poor choice of size and shape of plot and replicate and number of replicates may have bearing on the successful conclusion of an experiment, etc.

Most of the requirements for good scientific experimentation (see Dewey, Churchman, et. al.) are given below:

i) Formulation of hypotheses

In the formulation of hypotheses the experimenter should have a clear and well-defined concept of why the experiment is being conducted. No experiment should be started from some "half-baked idea" and allowed to grow "like Topsy". The lack of formulation of pertinent questions and objects is a waste of both the experimenter's time and the funds appropriated for the conduct of his research projects.

ii) A careful and logical analysis of the problem generated by the hypothesis

The experimenter should make a complete review of the literature related to the questions that are asked. He may also want to consult with other experimenters in this field. It is very improbable that anyone could be working on a project so new that no literature, even in a related field, is available.

iii) Use of the deductive method to design how to effect the solution of the problem.

This involves a detailed outline of the experiment with available funds, equipment, personnel, methods, and so forth. The factors should be expressed in quantitative terms such as dollars and cents and hours whenever possible. In light of this detailed outline it is possible to determine the most efficient design for available resources.

iv) Control of personal equation

If the personal element is not controlled the results of an experiment may be completely misleading. To illustrate this, suppose that an experimenter takes two observations on each of his treatments and always discards the lower one. The results will have little meaning. Likewise, the personal element may enter in various other ways. For example, in disease readings, plant vigor, quality grade, and other subjective scorings, the experimenter may rate the treatment of his preference a higher grade than it actually should have. In any subjective measurement the personal element must be controlled in order to obtain unbiased estimates of the actual values.

v) Rigorous and exact experimental procedure with collection of data pertinent to the subject

In a recent survey set up by the Extension Service the objects were clearly defined and well formulated. However, after the experiment was completed it was found that no data had been taken relative to two of the objectives of the study. Thus, even though the experimenter had practised good experimentation up to this point, he failed because the necessary measurements relative to the objectives were not taken. Likewise, in a field or greenhouse experiment haphazard collection of the data would make the experiment meaningless. The old adage: "The chain is no stronger than its weakest link" applies here as well as in the other steps considered essential to sound experimental procedure.

vi) Sound and logical reasoning as to how the results bear on the trial hypothesis and in the formulation ofgeneralizations

In analyzing the results of an experiment, the experimenter should consider all possible evidence. An analysis of variance table is not an analysis but rather a reduction of the data. Conclusions do not end with the calculation of F values!

vii) A complete and careful report of the data and methods of analysis so that others may check methods and hypotheses

This does not mean that one should present in a report everything concerning the experiment. However, one should give enough of the summary data so that others may test various hypotheses for themselves. By giving an account of the experimental procedure and statistical methods used the reader may decide for himself whether or not he considers the procedure sound. In connection with the statistical tools used, the author should not elaborate on the methods but should refer the reader to the source of that particular statistical tool.

VI-2. The Factorial Experiment

At this point some definitions should be made and some facts noted. Firstly, the factorial experiment should not be referred to as a design. Any of the experimental designs discussed so far or others may be used for the factorial experiment. The choice of treatments, not of the design, determines whether or not the experiment is a factorial. Secondly, the factors are designated by small letters, a, b, c, etc., while the effects or yields of the levels of a factor are denoted by capital letters, A, B, C, etc. Thirdly, if there are p levels, p a prime number, of a factor, say a, then there are p effects, A_0, A_1, ..., A_{p-1}, in a factorial experiment. Fourthly, all the units are used to evaluate the effects for a particular factor. Fifthly, the lowest or zero level of a factor need not be zero or none but it is the lowest level of the factor considered pertinent to the experiment, e.g.,

50 pounds equals zero or the lowest level and 150 pounds equals one or the highest level in the comparison of two levels of a factor. Lastly, the interaction of factors needs to be defined both by words and with symbols. In other words, the interaction of two factors is the failure of the levels of one factor, say a, to retain its relative order of performance throughout all levels of the second factor, say b. For two levels, zero and one, of the two factors a and b, the failure of the yields from the zero and one levels of factor a, say a_o and a_1, on the one level of b, say b_1, to be of the same relative magnitude as on the zero level of b, say b_o, is a measure of the interaction of the factors a and b. Symbolically, the interaction of the two factors a and b at zero and one levels is $AB = (a_1b_1 - a_ob_1) - (a_1b_o - a_ob_o)$.
The symbolical representation of interactions will be further exemplified with specific examples.

The criticisms of factorial experiments have been discussed in detail by Yates (1937) in his classic paper on factorial experiments entitled "The Design and Analysis of Factorial Experiments". Therefore, the so-called "faults" of factorial experiments are not discussed herein. Instead, the advantages as given by Yates (1937) are stressed.

In an experiment the two levels of the factors a and b are to be compared with the check or zero level of both factors, say a_ob_o . In evaluating the response or effect of the upper level over the lower level of b, say a_ob_1 vs. a_ob_o, only the plots involving b_o and b_1 are used. In an experiment containing the three treatments a_ob_o, a_1b_o, and a_ob_1 two-thirds of the plots are used to evaluate the effect of the upper over the lower level of the factor a or b. Now, for three levels of a and b the treatments would be a_ob_o, a_1b_o, a_2b_o, a_ob_1, a_ob_2 and only three-fifths of the plots are used in evaluating the effect of the three levels of factor a or b. As the number of levels are increased a smaller proportion of the total plots will be used to evaluate the effects. The two-level case utilizes the largest proportion of the total plots for evaluating responses of the various levels of factors.

Alternatively, the treatments a_ob_o and a_1b_o could be included in one experiment and the treatments a_ob_o and a_ob_1 could be placed in a second experiment. This type of design would include a larger experimental area than the above and would give less information on the comparison of the treatments a_1b_o and a_ob_1.

Is it possible to use all plots in an experiment to evaluate treatment responses or effects? The answer is yes if a factorial arrangement of the treatments is used. Now, a factorial arrangement includes all combinations of the levels of the different factors. This means that four treatments, a_ob_o, a_1b_o, a_ob_1, a_1b_1, are included instead of three, a_ob_o, a_1b_o, a_ob_1, to study the response of the two levels of the factors a and b. The response of the upper level of a over the lower level of a is given as, $A = a_1b_o - a_ob_o + a_1b_1 - a_ob_1$. The two levels of a are compared on the two levels of b and the effect A is the average effect over the two levels of the b factor. Thus, in addition to using all the plots, the effect A is evaluated over a wider range of conditions. The correct choice of treatments then has allowed the experimenter to make the most efficient use of the results. The effect B is evaluated in a like manner, thus

$$B = a_0b_1 - a_0b_0 + a_1b_1 - a_1b_0 .$$

The choice of the treatments as described immediately above results in information on the interaction of the factors a and b, $AB = (a_1b_1 - a_1b_0) - (a_0b_1 - a_0b_0)$.

The advantages of a factorial experiment may be summarized as

i) All plots are utilized in evaluating treatment responses resulting in the most efficient use of resources.

ii) The effects A, B, etc., are evaluated over a wider range of conditions with the minimum outlay of resources.

iii) An interaction of the factors is obtainable.

VI-3. The Factorial Experiment - 2^n Series

Although some experimenters may prefer to think of the effects and interactions in terms of the I, J, W, X, Y, Z, etc. effects outlined by Yates (Tech. Comm. No. 35, 1937), it has been found that the modulo notation (see Kempthorne, Biometrika, 1948, and Kempthorne and Federer, Biometrics, 1948) is extremely useful for the more complex factorial experiments and for confounding certain unimportant effects with block differences (see following chapters). The method has been found to be useful in the design and analysis of incomplete block designs (see Kempthorne and Federer, Biometrics, 1948-9).

The modulo notation is suitable for p = a prime number

(2,3,5,7,11,13,17,etc.) or power of a prime number. In this notation, the number system is from zero to p-1, whereas the number system used in computational work is from zero to nine. For modulo p, the following relationship among numbers exist.

$$
\begin{aligned}
0 &= p = 2p = \dots = qp \\
1 &= p+1 = 2p+1 = \dots = qp+1 \\
2 &= p+2 = 2p+2 = \dots = qp+2 \\
&\vdots \\
p-1 &= 2p-1 = 3p-1 = \dots = qp-1
\end{aligned}
$$

The subscripts of the factors a, b, c, etc. are i, j, h, etc., where $i = 0, 1, \dots, p-1$, $j = 0, 1, \dots, p-1$, $h = 0, 1, \dots, p-1$, etc. For a $p^n = 2^2$ factorial the levels of the main effects and interactions are

$$
\begin{aligned}
(A)_{i=0} &= a_0b_0 + a_0b_1 = 00 + 01 \\
(A)_{i=1} &= a_1b_0 + a_1b_1 = 10 + 11 \\
(B)_{j=0} &= a_0b_0 + a_1b_0 = 00 + 10 \\
(B)_{j=1} &= a_0b_1 + a_1b_1 = 01 + 11 \\
(AB)_{i+j=0} &= a_0b_0 + a_1b_1 = 00 + 11 \\
(AB)_{i+j=1} &= a_0b_1 + a_1b_0 = 01 + 10
\end{aligned}
$$

and the effects and interactions are

$$
\begin{aligned}
A &= (A)_1 - (A)_0 = (10+11) - (00+01) \\
B &= (B)_1 - (B)_0 = (01+11) - (00+10) \\
AB &= (AB)_0 - (AB)_1 = (00+11) - (01+10)
\end{aligned}
$$

It may appear strange to the student to compare the levels of of the two-factor interaction effect AB in the opposite direction. Upon close examination it will be found that this is not the case since the difference in the two levels of one factor compared at the two levels of the second factor is the interaction

$$
\begin{aligned}
AB &= a_1\text{present } [b_1-b_0] - a_0\text{present } [b_1-b_0] \\
&= a_1b_1 - a_1b_0 - a_0b_1 + a_0b_0 \\
&= 11 + 00 - 10 - 01
\end{aligned}
$$

Also, the interaction of AB is equal to the interaction BA,

$$\begin{aligned} BA &= b_1\text{present } [a_1 - a_o] - b_o\text{present } [a_1 - a_o] \\ &= b_1a_1 - b_1a_o - b_oa_1 + b_oa_o \\ &= a_1b_1 - a_ob_1 - a_1b_o + a_ob_o \\ &= 11 + 00 - 01 - 10 = AB = (AB)_o - (AB)_1 \,. \end{aligned}$$

The above comparisons correspond to the table of plus and minus signs used prevalently in statistical references (Yates, 1937; Snedecor, 1946; Fisher, 1942; etc.),

Effect	Combination of Treatments a_ob_o	a_1b_o	a_ob_1	a_1b_1
A	-	+	-	+
B	-	-	+	+
AB	+	-	-	+
Total	+	+	+	+

Thus $A = -a_ob_o + a_1b_o - a_ob_1 + a_1b_1 = 10 - 00 + 11 - 01$, etc.

The sums of squares for the comparison of the main effects and interaction from n replicates are

$$\frac{[(A)_1-(A)_o]^2}{4n} = \frac{(X_{.11} + X_{.10} - X_{.00} - X_{.01})^2}{n[1^2+1^2+(-1)^2+(-1)^2]} ,$$

$$\frac{[(B)_1-(B)_o]^2}{4n} = \frac{(X_{.11} + X_{.01} - X_{.00} - X_{.10})^2}{n(1+1+1+1)} ,$$

and $$\frac{[(AB)_o-(AB)_1]^2}{4n} = \frac{(X_{.11} + X_{.00} - X_{.10} - X_{.01})^2}{n(1+1+1+1+1)} ,$$

where $X_{.oo}$, $X_{.o1}$, $X_{.1o}$, and $X_{.11}$ are the totals for treatments 00, 01, 10, and 11 respectively.

The replicate and error sum of squares are obtained in the usual manner.

Example VI-1. The yields of sugar beets in tons per acre were obtained for seven treatments in a randomized complete blocks design with six replicates (see Table 12.13, Snedecor, 1946). Four of the seven treatments formed a 2^2 factorial arrangement of two levels of p = superphosphate and k = muriate of potash. The yields for the four treatments $p_o k_o$ = 00, $p_1 k_o$ = 10, $p_o k_1$ = o1, and $p_1 k_1$ = 11 in the six blocks are presented in Table VI-1.

Table VI-1. Yields of sugar beets (tons per acre) obtained with four fertilizer treatments in a randomized complete blocks design.

Treatment Combination		Block Number 1	2	3	4	5	6	
None	= 00	2.45	2.25	4.38	4.35	3.42	3.27	20.12
Superphosphate	= 10	6.71	5.44	4.92	5.23	6.74	4.74	33.78
Muriate of potash	= 01	3.22	4.14	2.32	4.42	3.23	4.00	21.38
Both	= 11	6.34	5.44	5.22	8.00	6.96	6.96	38.92
Totals		18.72	17.27	16.84	22.00	20.40	18.97	114.20

The coefficients of the treatment totals for calculating the main effects and interaction are given below:

Effects	Treatment and treatment totals 00 20.12	10 33.78	01 21.38	11 38.92	Sum
Total	+	+	+	+	114.20
P	-	+	-	+	31.20
K	-	-	+	+	6.40
PK	+	-	-	+	3.88

For example, the interaction PK is obtained as

$$20.12 - 33.78 - 21.38 + 38.92 = 3.88.$$

The treatment sum of squares is

$$\frac{(20.12)^2 + (33.78)^2 + (21.38)^2 + (38.92)^2}{6} - \frac{(114.20)^2}{24}$$

= 42.8939, with three degrees of freedom. Since the above comparisons form an orthogonal set, the individual sums of squares for the effects corrected for the mean each with one degree of freedom (the comparison of two quantities) add to the total for treatments thus,

$$\frac{(31.20)^2}{6(4)} + \frac{(6.40)^2}{6(4)} + \frac{(3.88)^2}{6(4)} = 40.56000 + 1.70667$$

$$+ 1.70667 + 0.62727 = 42.89394.$$

The sums of squares are summarized in the following analysis of variance table.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Replicates	5	4.6727	0.9345	
Treatments	3	42.8939	14.2980	18.22
P	1	40.56000		51.69
K	1	1.70667		2.17
PK	1	0.62727		0.80
Residual	15	11.7711	0.7847	
Total	23	59.3377		

The largest and only significant effect is due to the difference in the two levels of superphosphate indicating that the significance among the four treatment totals is due to the single degree of freedom associated with the comparison of the two levels of superphosphate.

Individual errors may be computed for each comparison, i.e., the interaction of the three effects P. K, and PK with replicates yields three error terms each with five degrees of freedom (see Snedecor, 1946, Section 15.7). The pooled error was used in this experiment since the individual error variances are considered to be estimates of the same residual error variance σ_e^2.

The coefficient of variation is

$$\frac{24\sqrt{0.7847}}{114.20} = 18.6 \text{ percent.}$$

The standard error of an effect total is

$\sqrt{(12)(2)(.7847)} = 4.34$, since there are twelve items involved in each of the sums $(A)_1$ and $(A)_0$, etc.

The least or minimum significant difference, which is applicable to each of the effects, is

$$2.131\sqrt{(12)(2)(.7847)} = 9.25 .$$

This statistic is quite appropriate and useful for comparing all the effects since the null hypotheses tested are: A = 0, B = 0, and AB = 0. The above use of an lsd has probably been the reason for the misuse of this statistic in comparing unrelated treatments, such as the highest versus the lowest, etc.

One further fact should be noted in connection with the later use of factorial arrangements in confounding. Any treatment total (or mean) may be obtained from the combination of the main effects and interactions. Thus, the total for treatment 00 is $\frac{114.20 - 31.20 - 6.40 + 3.88}{4} = 20.12$,

for 10 $\frac{114.20 + 31.20 - 6.40 - 3.88}{4} = 33.78$

for 01 $\frac{114.20 - 31.20 + 6.40 - 3.88}{4} = 21.38$,

and for 11 $\frac{114.20 + 31.20 + 6.40 + 3.88}{4} = 38.92$

$$= \frac{\text{Total} + A + B + AB}{4}$$

$$= \frac{(A)_1 + (B)_1 + (AB)_0}{2} - \frac{2(\text{Total})}{4} .$$

In general, $ij = \frac{(A)_i + (B)_j + (AB)_{i+j}}{2} - \frac{2(\text{Total})}{4}$

The next factorial arrangement in the 2^n series is the 2^3 which involves three factors a, b, and c each at two levels in all combinations. The eight treatment combinations are:

000, 100, 010, 110, 001, 101, 011, 111

and the levels of the main effects and interactions are obtained from the following combination of treatments:

$$
\begin{aligned}
(A)_{i=o} &= 000 + 010 + 001 + 011 \\
(A)_{i=1} &= 100 + 110 + 101 + 111 \\
(B)_{j=o} &= 000 + 100 + 001 + 101 \\
(B)_{j=1} &= 010 + 110 + 011 + 111 \\
(AB)_{i+j=o} &= 000 + 110 + 001 + 111 \\
(AB)_{i+j=1} &= 100 + 010 + 101 + 011 \\
(C)_{h=o} &= 000 + 100 + 010 + 110 \\
(C)_{h=1} &= 001 + 101 + 011 + 111 \\
(AC)_{i+h=o} &= 000 + 010 + 101 + 111 \\
(AC)_{i+h=1} &= 100 + 110 + 001 + 011 \\
(BC)_{j+h=o} &= 000 + 100 + 011 + 111 \\
(BC)_{j+h=1} &= 010 + 110 + 001 + 101 \\
(ABC)_{i+j+h=o} &= 000 + 110 + 101 + 011 \\
(ABC)_{i+j+h=1} &= 100 + 010 + 001 + 111
\end{aligned}
$$

The main effects and interactions are the contrasts of two sums, thus

$$
\begin{aligned}
A &= (A)_1 - (A)_o \\
B &= (B)_1 - (B)_o \\
AB &= (AB)_o - (AB)_1 \\
&\vdots \\
ABC &= (ABC)_1 - (ABC)_o
\end{aligned}
$$

In a like manner the effects may be obtained from the following table:

Effect	Treatment combinations and totals							
	000 $X_{.000}$	100 $X_{.100}$	010 $X_{.010}$	110 $X_{.110}$	001 $X_{.001}$	101 $X_{.101}$	011 $X_{.011}$	111 $X_{.111}$
Total	+	+	+	+	+	+	+	+
A	-	+	-	+	-	+	-	+
B	-	-	+	+	-	-	+	+
AB	+	-	-	+	+	-	-	+
C	-	-	-	-	+	+	+	+
AC	+	-	+	-	-	+	-	+
BC	+	+	-	-	-	-	+	+
ABC	-	+	+	-	+	-	-	+

,

where the totals of each column are added or subtracted as indicated. For example,

$$AC = X_{.000}-X_{.100}+X_{.010}-X_{.110}-X_{.001}+X_{.101}-X_{.011}+X_{.111}=(AC)_0-(AC)_1.$$

The sum of squares for AC is $\frac{[(AC)_0 - (AC)_1]^2}{n(1+1+1+1+1+1+1+1)}$, where

n equals the number of replicates involved in obtaining the totals $X_{.ijh}$. The remaining sums of squares for the effects are obtained similarly.

A third computational method for obtaining the main effects and interactions has been presented by Yates (1937) and Snedecor (1946). The steps are presented in the following table:

Treatment	Yield	Col. 1	Col. 2	Col. 3	Effect
000	$X_{.000}$	$X_{.000}+X_{.100}=s_1$	s_1+s_2	$s_1+s_2+s_3+s_4$	Total
100	$X_{.100}$	$X_{.010}+X_{.110}=s_2$	s_3+s_4	$s_5+s_1+s_7+s_8$	A
010	$X_{.010}$	$X_{.001}+X_{.101}=s_3$	s_5+s_6	$s_2-s_1+s_4-s_3$	B
110	$X_{.110}$	$X_{.011}+X_{.111}=s_4$	s_7+s_8	$s_6-s_5+s_8-s_7$	AB
001	$X_{.001}$	$X_{.100}-X_{.000}=s_5$	s_2-s_1	$s_3+s_4-s_1-s_2$	C
101	$X_{.101}$	$X_{.110}-X_{.010}=s_6$	s_4-s_3	$s_7+s_8-s_5-s_6$	AC
011	$X_{.011}$	$X_{.101}-X_{.001}=s_7$	s_6-s_5	$s_4-s_3-s_2+s_1$	BC
111	$X_{.111}$	$X_{.111}-X_{.011}=s_8$	s_8-s_7	$s_8-s_7-s_6+s_5$	ABC

The sum of the items in a row of Column 3 yields the effects listed in the last column. The same combinations of treatments for the effects are used as in the two preceding computational methods.

Example VI-2. An experiment on the fertilizing of potatoes was conducted at Wimblington, England in 1934 (Yates, 1937, No. 35, p 9). Three fertilizers, nitrogen, potash, and manure or dung, each at two levels were included in all combinations. The eight treatments are:

Sulphate of Ammonia (n) Sulphate of Potash (k) Dung (d)

$$\left\{\begin{matrix}\text{none}\\ 0.45\text{ cwt. nitrogen}\\ \text{per acre}\end{matrix}\right\} \text{ X } \left\{\begin{matrix}\text{none}\\ 1.12\text{ cwt. }K_2O/\text{acre}\end{matrix}\right\} \text{ X } \left\{\begin{matrix}\text{none}\\ 8\text{ tons/acre}\end{matrix}\right\}$$

or

$$\begin{aligned}
000 &= n_0k_0d_e = (1)\\
100 &= n_1k_0d_0 = n\\
010 &= n_0k_1d_0 = k\\
110 &= n_1k_1d_0 = nk\\
001 &= n_0k_0d_1 = d\\
101 &= n_ik_0d_1 = nd\\
011 &= n_0k_1d_1 = kd\\
111 &= n_1k_1d_1 = nkd\ .
\end{aligned}$$

A randomized complete blocks design was used with four replicates. The individual plot yields, field plan, and replicate and treatment totals are given in Table VI-2.

Table VI-2. Field plan, plot yields in pounds of potatoes per 1/60 acre plot, replicate, and treatment totals (variety number in parentheses).

Replicate I				Replicate II			
(110)	(011)	(001)	(101)	(011)	(001)	(010)	(110)
291	398	312	373	407	324	272	306
(000)	(010)	(100)	(111)	(100)	(111)	(101)	(000)
101	265	106	450	89	449	338	106
(001)	(000)	(101)	(011)	(101)	(110)	(100)	(001)
323	87	324	423	361	272	103	324
(110)	(010)	(100)	(111)	(010)	(000)	(111)	(011)
334	279	128	471	302	131	437	445
Replicate III				Replicate IV			

Replicate Totals

I	2296
II	2291
III	2369
IV	2375
Total	9331

Treatment Totals

000	425	001	1283
100	426	101	1396
010	1118	011	1673
110	1203	111	1807
	Total		9331

The effects and the sum of squares of the effects are computed as explained earlier. These are:

Effect	Treatment number and total yield (000) 425	(100) 426	(010) 1118	(110) 1203	(001) 1283	(101) 1396	(011) 1673	(111) 1807	Sum of +'s	-'s	Total for Effect
Total	+	+	+	+	+	+	+	+	9331	0	9331
N	−	+	−	+	−	+	−	+	4832	4499	333
K	−	−	+	+	−	−	+	+	5801	3530	2271
NK	+	−	−	+	+	−	−	+	4718	4613	105
D	−	−	−	−	+	+	+	+	6159	3172	2987
ND	+	−	+	−	−	+	−	+	4746	4585	161
KD	+	+	−	−	−	−	+	+	4331	5000	−669
NKD	−	+	+	−	+	−	−	+	4634	4697	−63

The sum of the pluses for the N effect results in the total for $(N)_1 = 4832$. Likewise, the sum of the minuses yields

$(N)_0 = 4499$. $N = (N)_1 - (N)_0 = 333$. The sum of squares for the N effect is $(333)^2/\ 4(1+1+1+1+1+1+1+1) = 3465.3$. The remaining effects and sums of squares are computed similarly.

Just as with the 2^2 factorial, the treatment totals may be computed from the effects. Thus, the total for treatment 000 is $(9331-333-2271+105-2937+161-669+63)/8 = 425$. Also, the total for treatment 000 may be computed from the levels of the effects,

$$\frac{(N)_0+(K)_0+(NK)_0+(D)_0+(ND)_0+(KD)_0+(NKD)_0}{4} - \frac{6(\text{Total})}{8}$$

$$= \frac{4499+3530+4718+3172+4746+4331+4697}{4} - \frac{6(9331)}{8} = \frac{3400}{8} = 425.$$

The analysis of variance for the data of Table VI-2 is

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Replicates	3	774.09	258.03	--
Treatments	7	458717.97	65531.14	188.83
N	1	3465.28		9.99
K	1	161170.03		464.43
NK	1	344.53		--
D	1	278817.79		803.44
ND	1	810.03		2.33
KD	1	13986.28		40.30
NKD	1	124.03		--
Error	21	7287.66	347.03	
Total	31	466779.72	--	

The least significant difference for the effect totals is

$$2.080\ \sqrt{(4)(4)(2)(347.03)} = 219.19.$$

In converting the treatment total yields to long tons per acre the conversion factor 60/(2240)(4) was used and in converting the total effects to long tons per acre Yates (1937) used the conversion factor 60/(2240)(16). Both the total effects and the lsd are multiplied by the last conversion factor to obtain the results in tons per acre.

The 2^4 factorial arrangement of treatments involves four factors, a, b, c, and d, each at two levels. The 16 treatment combinations are designated as:

0000	0010	0001	0011
1000	1010	1001	1011
0100	0110	0101	0111
1100	1110	1101	1111

The 15 treatment degrees of freedom may be partitioned into 15 independent comparisons with single degrees of freedom, thus for n replicates

Source of Variation	Degrees of Freedom	Sum of Squares
A	1	$[(A)_1-(A)_0]^2/16n$
B	1	$[(B)_1-(B)_0]^2/16n$
AB	1	$[(AB)_0-(AB)_1]^2/16n$
C	1	$[(C)_1-(C)_0]^2/16n$
AC	1	$[(AC)_0-(AC)_1]^2/16n$
BC	1	$[(BC)_0-(BC)_1]^2/16n$
ABC	1	$[(ABC)_1-(ABC)_0]^2/16n$
D	1	$[(D)_1-(D)_0]^2/16n$
AD	1	$[(AD)_0-(AD)_1]^2/16n$
BD	1	$[(BD)_0-(BD)_1]^2/16n$
ABD	1	$[(ABD)_1-(ABD)_0]^2/16n$
CD	1	$[(CD)_0-(CD)_1]^2/16n$
ACD	1	$[(ACD)_1-(ACD)_0]^2/16n$
BCD	1	$[(BCD)_1-(BCD)_0]^2/16n$
ABCD	1	$[(ABCD)_0-(ABCD)_1]^2/16n$

The three and four factor or second and third order interactions may have little meaning in some factorial experiments. If this is true then these interactions may be pooled with the experimental error. This fact may be quite useful in setting up an experiment. For example, it was desired to observe the effects of the fertilizer treatments - two levels of the four factors, nitrogen(n), potash(k), phosphorous(p), and lime(l), in all combinations - on the composition of the vegetation growing in peat and bog lands in the Adirondacks. The 16 fertilizer treatments were to be applied in 1949 and the effect on kind and amount of vegetation observed over a period of years. Since it is difficult to transport the fertilizer to the experimental area, it was decided to use one replicate of the 16 treatments. Several checks would be included and the variation among the checks and the interactions for three and four factors would be used as error. The breakdown of the degrees of freedom in the analysis of variance for the 16 treatments and four additional checks placed at random over the experimental area is:

Source of Variation	Degrees of Freedom	Source of Variation	Degrees of Freedom	
N	1	L	1	
K	1	NL	1	
NK	1	KL	1	
P	1	PL	1	
NP	1	Among 5 checks	4	9
NK	1	3 and 4 factor interactions	5	
		Total	19	

For such a design, information is available for all main effects and two factor or first order interactions and nine degrees of freedom are available for error sum of squares.

As a further precaution check plots were placed between each plot to control (by covariance) some of the variation over the experimental area. Even with the covariance analysis eight degrees of freedom are available for the error sum of squares.

Fisher (1942, Section 41) suggests this same type of design for a 2^6 factorial experiment with the 3, 4, 5, and 6 factor interactions to be used as error.

The concept of a generalized interaction has been discussed in detail by Yates (1937) and by Fisher (1942, Section 45.1). As illustrated the interaction of A and B is AB. Likewise, the interaction of A and BC is ABC and of A and AB is B. The latter example AxAB = A^2B = B reduced modulo two illustrates the fact that 0 and 1 are the only elements of this number system and that $A^2 = A^0 = 1$ since any number to the zero power is unity. Some further examples will illustrate the method and principles involved:

ABC x BCD = AD
ABC x DEF = ABCDEF
ADE x AFG = DEFG
BDF x BCFG = CDG
BDF x ABC = ACDF
AB x BC = AC
C x BCDF = BDF
ABCD x ABC = D

The concept of generalized interactions is useful for clarifying the construction of various incomplete block designs.

VI-4. The Factorial Experiment - 3^n series

The 3^n series is constructed in much the same way as the 2^n. There are n factors each at three levels in a 3^n factorial arrangement. The number system here is $0 = 3 = 6 = \ldots$, $1 = 4 = 7 = \ldots$, $2 = 5 = 8 = \ldots$ For the case of the 3^2 factorial, factors a and b are at the 0, 1, and 2 levels and in all possible combinations resulting in the nine treatments 00, 01, 02, 10, 11, 12, 20, 21, 22. The 0, 1, and 2 levels of effects A and B are:

$$(A)_0 = 00 + 01 + 02$$
$$(A)_1 = 10 + 11 + 12$$
$$(A)_2 = 20 + 21 + 22$$
$$(B)_0 = 00 + 10 + 20$$
$$(B)_1 = 01 + 11 + 21$$
$$(B)_2 = 02 + 12 + 22$$

The comparison among the three levels of the factors A and B from n replicates $\dfrac{(A)_0^2+(A)_1^2+(A)_2^2}{3n} - \dfrac{[(A)_0+(A)_1+(A)_2]^2}{9n}$

and $\dfrac{(B)_0^2+(B)_1^2+(B)_2^2}{3n} - \dfrac{[(B)_0+(B)_1+(B)_2]^2}{9n}$

yields sums of squares with two degrees of freedom each. Among the nine treatments there are eight degrees of freedom and thus there are four degrees of freedom for the interaction of the factors a and b. It is possible to partition these four degrees of freedom into two separate portions, AB = J effect in Yates notation and AB^2 = I effect in Yates notation. This partitioning may have little or no meaning biologically but is very important in constructing incomplete block experiments. The interaction of A x B = AB and A x B^2 = AB^2.

The three levels of AB and AB^2 are (see Yates, 1937, p 40)

$$(AB)_{i+j=0} = 00 + 12 + 21 = [J_1]$$
$$(AB)_{i+j=1} = 01 + 10 + 22 = [J_2]$$
$$(AB)_{i+j=2} = 02 + 11 + 20 = [J_3]$$
$$(AB^2)_{i+2j=0} = 00 + 11 + 22 = [I_1]$$
$$(AB^2)_{i+2j=1} = 02 + 10 + 21 = [I_2]$$
$$(AB^2)_{i+2j=2} = 01 + 12 + 20 = [I_3]$$

since the notation is reduced modulo three and 0 = 3, 1 = 4, and 2 = 5.

The first letter in the interaction has the coefficient of unity and the second letter has either unity or 2 = 3-1. As the student may observe the notation is merely an extension of that used for the 2^n series where the partitioning of the sums of squares was in blocks of 2-1 = single degrees of freedom. For the 3^n series the partitioning of the sums of squares is in groups of 3-1 = 2 degrees of freedom. This system may be extended for p = 5, 7, 11, 13, etc. (see Kempthorne, Biometrika, 1948 and Kempthorne and Federer, Biometrics, 1948).

The concept of a generalized interaction carries over to the 3^n series or to the p^n series in general. Several examples of interactions, reduced modulo three, and the first factor to the first power are presented below:

$$AB \times AB^2 = A^2B^3 = A^4B^6 = AB^0 = A$$
$$AB \times A^2B^3 = A^3B^4 = B$$
$$A \times AB = A^2B = A^4B^2 = AB^2$$
$$A \times A^2B^2 = A^3B^2 = B^2 = B$$
$$A \times B = AB$$
$$A \times B^2 = AB^2$$

$$AB \times CD = ABCD$$
$$AB \times C^2D^2 = ABC^2D^2$$
$$AB^2 \times CD^2 = AB^2CD^2$$
$$AB^2 \times C^2D^4 = AB^2C^2D^4 = AB^2C^2D$$
$$ABC^2 \times CD^2 = ABC^3D^2 = ABD^2$$
$$ABC^2 \times C^2D^4 = ABC^4D^4 = ABCD$$
$$ABCD \times B^2C^2D^2 = AB^3C^3D^3 = A$$
$$ABCD \times B^4C^4D^4 = AB^2C^2D^2$$

As in the 2^n series the total for treatment ij may be obtained from the formula,

$$\frac{(A)_i + (B)_j + (AB)_{i+j} + (AB)^2_{i+2j}}{3} - \frac{3(\text{Total})}{9},$$

involving levels of the main effects and interactions.

Example VI-3. Two replicates of a randomized complete blocks design were used by R.H.Walker (unpublished notes by Miss. G. M. Cox) to compare the effect of three levels of nitrogen and phosphorous in all combinations on the yield of grass from soils of Philadelphia Flat, Monti National Forest. The data are presented in Table VI-3.

Table VI-3. Yields of grass (grams) for nine fertilizer treatments from two replicates and totals

Phosphorous		Nitrogen n_0	n_1	n_2	Total
p_0	Rep.I	18.7	20.8	22.3	
	Rep.II	17.5	20.5	22.9	
	Sum	36.2	41.3	45.2	122.7
p_1	Rep. I	19.2	18.8	24.9	
	Rep. II	21.3	23.5	24.2	
	Sum	40.5	42.3	49.1	131.9
p_2	Rep. I	20.8	22.0	25.6	
	Rep. II	20.5	24.0	27.1	
		41.3	46.0	52.7	140.0
Total		118.0	129.6	147.0	394.6

Rep.	Totals
I	193.1
II	201.5
Sum	394.6

$$\boxed{\begin{aligned} (NP)_0 &= 131.3 \\ (NP)_1 &= 134.5 \\ (NP)_2 &= 128.8 \\ (NP^2)_0 &= 131.2 \\ (NP^2)_1 &= 131.7 \\ (NP^2)_2 &= 131.7 \end{aligned}}$$

The sum of squares among the three levels of nitrogen with two degrees of freedom is

$$\frac{(N)_0^2+(N)_1^2+(N)_2^2}{n(3)} - \frac{[(N)_0+(N)_1+(N)_2]^2}{9n}$$

$$= \frac{118.0^2 + 129.6^2 + 147.0^2}{6} - \frac{394.6^2}{18}$$

$$= 8721.5267 - 8650.5089 = 71.0178.$$

The interaction sum of squares is computed from the 3x3 table of treatment totals, thus

$$\frac{36.2^2 + \ldots + 52.7^2}{2} - \frac{118.0^2 + 129.6^2 + 147.0^2}{6}$$

$$- \frac{122.7^2 + 131.9^2 + 140.0^2}{6} + \frac{394.6^2}{18} = 2.7489,$$

or directly from the levels of interactions

$$\frac{(NP)_0^2+(NP)_1^2+(NP)_2^2}{2(3)} - \frac{394.6^2}{18} + \frac{(NP^2)_0^2+(NP^2)_1^2+(NP^2)_2^2}{2(3)} - \frac{394.6^2}{18}$$

$$= \frac{131.3^2+134.5^2+128.8^2}{6} - \frac{394.6^2}{18} + \frac{131.2^2+131.7^2+131.7^2}{6} - \frac{394.6^2}{18}$$

$$= 2.7211 + .0278 = 2.7489.$$

The analysis of variance for the data in Table VI-3 follows:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Replicates	1	3.9200	3.9200	2.29
Treatments	8	98.7411	12.3426	7.21
N	2	71.0178	35.5089	20.75
P	2	24.9744	12.4872	7.30
NP	4	2.7489	0.6872	0.40
Error	8	13.6900	1.7112	
Total	17	116.3511		

The eight treatment degrees of freedom may be partitioned into linear and quadratic effects if desired (see Snedecor, 1946, Chapter 15). The table of coefficients for the linear and quadratic effects of nitrogen and phosphorous, N_L, N_Q, P_L, and P_Q is

Effect	Treatment 00 36.2	01 40.5	02 41.3	10 41.3	11 42.3	12 46.0	20 45.2	21 49.1	22 52.7	Sum	Divisors
N_L	−	−	−	0	0	0	+	+	+	29.0	6(2)
N_Q	+	+	+	−2	−2	−2	+	+	+	5.8	18(2)
P_L	−	0	+	−	0	+	−	0	+	17.3	6(2)
P_Q	+	−2	+	+	−2	+	+	−2	+	−1.1	18(2)
N_LP_L	+	0	−	0	0	0	−	0	+	2.4	4(2)
N_LP_Q	−	+2	−	0	0	0	+	−2	+	3.2	12(2)
N_QP_L	−	0	+	+2	0	−2	−	0	+	3.2	12(2)
N_QP_Q	+	−2	+	−2	+4	−2	+	−2	+	−9.2	35(2)

For this breakdown of the treatment degrees of freedom, the following sums of squares were obtained:

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Treatments	8	98.7411	12.3426
N_L	1	70.0833	
N_Q	1	.9344	
P_L	1	24.9408	
P_Q	1	0.0336	
N_LP_L	1	0.7200	
N_LP_Q	1	0.4300	
N_QP_L	1	0.4300	
N_QP_Q	1	1.1800	
Error	8	13.6900	1.7112

The standard error of difference between two treatment totals is $\sqrt{2(2)(1.7112)} = 2.6162$.

The standard error of a difference between two levels of an effect is $\sqrt{2(3)(2)(1.7112)} = 4.5315$.

It may be desired to test three factors a, b, and c in all combinations of three levels per factor. The resulting 27 treatments may be included in a randomized complete blocks or other design. The treatment combinations would be

000	001	002
100	101	102
200	201	202
010	011	012
110	111	112
210	211	212
020	021	022
120	121	122
220	221	222

The levels of the effects in terms of the treatment combinations are:

$(A)_0$ = 000 + 001 + 002 + 010 + 011 + 012 + 020 + 021 + 022
$(A)_1$ = 100 + 101 + 102 + 110 + 111 + 112 + 120 + 121 + 122
$(A)_2$ = 200 + 201 + 202 + 210 + 211 + 212 + 220 + 221 + 222
$(B)_0$ = 000 + 001 + 002 + 100 + 101 + 102 + 200 + 201 + 202
$(B)_1$ = 010 + 011 + 012 + 110 + 111 + 112 + 210 + 211 + 212
$(B)_2$ = 020 + 021 + 022 + 120 + 121 + 122 + 220 + 221 + 222
$(AB)_0$ = 000 + 001 + 002 + 120 + 121 + 122 + 210 + 211 + 212
$(AB)_1$ = 010 + 011 + 012 + 100 + 101 + 102 + 220 + 221 + 222
$(AB)_2$ = 020 + 021 + 022 + 110 + 111 + 112 + 200 + 201 + 202
$(AB^2)_0$ = 000 + 001 + 002 + 110 + 111 + 112 + 220 + 221 + 222
$(AB^2)_1$ = 020 + 021 + 022 + 100 + 101 + 102 + 210 + 211 + 212
$(AB^2)_2$ = 010 + 011 + 012 + 120 + 121 + 122 + 200 + 201 + 202
$(C)_0$ = 000 + 010 + 020 + 100 + 110 + 120 + 200 + 210 + 220
$(C)_1$ = 001 + 011 + 021 + 101 + 111 + 121 + 201 + 211 + 221
$(C)_2$ = 002 + 012 + 022 + 102 + 112 + 122 + 202 + 212 + 222
$(AC)_0$ = 000 + 010 + 020 + 102 + 112 + 122 + 201 + 211 + 221
$(AC)_1$ = 001 + 011 + 021 + 100 + 110 + 120 + 202 + 212 + 222
$(AC)_2$ = 002 + 012 + 022 + 101 + 111 + 121 + 200 + 210 + 220

$(AC^2)_0$ = 000 + 010 + 020 + 101 + 111 + 121 + 202 + 212 + 222
$(AC^2)_1$ = 002 + 012 + 022 + 100 + 110 + 120 + 201 + 211 + 221
$(AC^2)_2$ = 001 + 011 + 021 + 102 + 112 + 122 + 200 + 210 + 220
$(BC)_0$ = 000 + 012 + 021 + 100 + 112 + 121 + 200 + 212 + 221
$(BC)_1$ = 001 + 010 + 022 + 101 + 110 + 122 + 201 + 210 + 222
$(BC)_2$ = 002 + 011 + 020 + 102 + 111 + 120 + 202 + 211 + 220
$(BC^2)_0$ = 000 + 011 + 022 + 100 + 111 + 122 + 200 + 211 + 222
$(BC^2)_1$ = 002 + 010 + 021 + 102 + 110 + 121 + 202 + 210 + 221
$(BC^2)_2$ = 001 + 012 + 020 + 101 + 112 + 120 + 201 + 212 + 220
$(ABC)_0$ = 000 + 012 + 021 + 102 + 111 + 120 + 201 + 210 + 222
$(ABC)_1$ = 001 + 010 + 022 + 100 + 112 + 121 + 202 + 211 + 220
$(ABC)_2$ = 002 + 011 + 020 + 101 + 110 + 122 + 200 + 212 + 221
$(ABC^2)_0$ = 000 + 011 + 022 + 101 + 112 + 120 + 202 + 210 + 221
$(ABC^2)_1$ = 002 + 010 + 021 + 100 + 111 + 122 + 201 + 212 + 220
$(ABC^2)_2$ = 001 + 012 + 020 + 102 + 110 + 121 + 200 + 211 + 222
$(AB^2C)_0$ = 000 + 011 + 022 + 102 + 110 + 121 + 201 + 212 + 220
$(AB^2C)_1$ = 001 + 012 + 020 + 100 + 111 + 122 + 202 + 210 + 221
$(AB^2C)_2$ = 002 + 010 + 021 + 100 + 112 + 120 + 200 + 211 + 222
$(AB^2C^2)_0$ = 000 + 012 + 021 + 101 + 110 + 122 + 202 + 211 + 220
$(AB^2C^2)_1$ = 002 + 011 + 020 + 100 + 112 + 121 + 201 + 210 + 222
$(AB^2C^2)_2$ = 001 + 010 + 022 + 102 + 111 + 120 + 200 + 212 + 221

The comparisons among three levels of each of the $p^2 + p + 1 = 13$ effects yields 13 sums of squares each with two degrees of freedom. The following breakdown of the 26 treatment degrees of freedom is possible.

Source of Variation	Degrees of Freedom	
Treatments	26	
Main effects		
A	2	
B	2	
C	2	
2-factor interactions		
AxB: AB	2	4
AxB: AB^2	2	
AxC: AC	2	4
AxC: AC^2	2	
BxC: BC	2	4
BxC: BC^2	2	

3-factor interaction

$$\text{AxBxC}\begin{cases} ABC = JJ & 2 \\ ABC^2 = II & 2 \\ AB^2C = JI & 2 \\ AB^2C^2 = IJ & 2 \end{cases} \Big\} \; 8$$

In a factorial experiment with no confounding of effects the two factor and three factor interactions would not be partitioned into the separate parts. The partitioning is useful in the construction and analysis of incomplete block experiments. The sums of squares are obtained as before. For example, the sum of squares for the AB^2C effect from n replicates is

$$\frac{(AB^2C)_0^2 + (AB^2C)_1^2 + (AB^2C)_2^2}{9\ n} - \frac{(\text{Total})^2}{27n},$$

since 9n yields made up each total for the level of an effect and 27 n yields make up the grand total.

VI-5. <u>The Factorial Experiment - 4^n, 5^n, pxq, pxqxr series.</u>

The 4^2 factorial may be considered either as a 2^4 or as a 4x4. The former consideration would be useful in the construction and analysis of an incomplete block design. The latter consideration would be necessary if two factors, each at four levels, were tested in all combinations. For example, four methods of mixing cakes and four types of baking powder might be tried in all combinations. The breakdown of the 15 treatment degrees of freedom from k replicates follows:

Source of Variation	Degrees of Freedom	
Replicates	(k-1)	
Treatments	15	
Methods of mixing		3
Types of baking powder		3
Methods x types		9
Error	15(k-1)	
Total	16k-1	

The interaction sum of squares is obtained from the 4x4 table of treatment totals.

In another type of 4^n factorial experiment (see Snedecor, 1946, Chap.15) the experimenter may be interested in paritioning the main effect degrees of freedom into linear, quadratic, and cubic effects. In a like manner, it is possible to obtain the linear x linear, linear x quadratic, quadratic x linear, linear x cubic, etc. comparisons by partitioning the interaction sum of squares into individual degrees of freedom.

Since five is a prime number, the 5^n series may be handled in much the same manner as the 2^n and 3^n series. The breakdown of the degrees of freedom for a 5^2 factorial experiment with k replicates of a randomized complete blocks design is:

Source of Variation	Degrees of Freedom	
Replicates	k-1	
Treatments	24	
A		4
B		4
AB		4
AB^2		4
AB^3		4
AB^4		4
Error	24(k-1)	
Total	25k-1	

For each level of an effect 5k yields are available. Therefore, the sum of squares for the A effect is

$$\frac{(A)_0^2 + (A)_1^2 + (A)_2^2 + (A)_3^2 + (A)_4^2}{5k} - \frac{(\text{Total})^2}{25k}$$

In some instances it may be desirable to have p levels of one factor, say a, and q of another, say b. In this case the breakdown of the degrees of freedom for an experiment in which the pq treatments were compared in k replicates of a randomized complete blocks design is

Source of Variation	Degrees of Freedom	
Replicates	k-1	
Treatment	pq-1	
A		p-1
B		q-1
AxB		(p-1)(q-1)
Error	(k-1)(pq-1)	
Replicates x A		(k-1)(p-1)
Replicates x B		(k-1)(q-1)
Replicates x A x B		(k-1)(p-1)(q-1)
Total	kpq-1	

The error sum of squares may be partitioned for all factorials just as in the above experiment.

As a further illustration of factorial experiments, suppose that three factors, a, b, and c are at p, q, and r levels respectively and are to be tested in all combinations. The design is k replicates of a randomized complete blocks. The breakdown of the total degrees of freedom in the analysis of variance is

Source of Variation	Degrees of Freedom	
Replicates = R	k-1	
Treatments = T	pqr-1	
A		p-1
B		q-1
AxB		(p-1)(q-1)
C		r-1
AxC		(p-1)(r-1)
BxC		(q-1)(r-1)
AxBxC		(p-1)(q-1)(r-1)
R x T	(k-1)(pqr-1)	
Total	kpqr-1	

From the foregoing general examples the analysis of other factorial experiments is straightforward. The design could be a completely randomized, a randomized complete blocks, or a latin square. The partitioning of the treatment degrees of freedom from a factorial experiment follows the methods set forward in this chapter.

One concept that is evident in every factorial experiment should be reemphasized before proceeding further. The factorial arrangement allows for many more replicates than other arrangements. This concept is one of "hidden" replication. For example, 2^3 treatments are tested in four randomized complete blocks. There are four replicates on the individual treatments <u>but</u> there are 4 x 4 = 16 replicates for testing the two levels of any effect. For 3 x 3 treatments in a 9 x 9 latin square, there are 9 x 3 = 27 replicates available for comparing the three levels of an effect. The idea of <u>hidden</u> replication in a factorial experiment should not be overlooked even if the experimenter is only mildly or not at all interested in an interaction.

For a more comprehensive discussion of factorial experiments, the student is referred to Yates' classic paper, The Design and Analysis of Factorial Experiments, Technical Communication No. 35, Imperial Bureau of Soil Science, 1937.

Problem VI-1. Complete the following tables from the data given.

Yields of Wheat in a 2^3 Factorial Experiment laid down in a Latin Square. Nitrogen (n), Phosphorous (p), and Potassium (k) were tried at Two Levels, None and Some. (1) indicates no fertilizer.

p 18.8	n 12.2	np 18.3	k 15.8	nk 11.4	(1) 11.5	npk 19.4	pk 18.9
n 12.9	nk 7.3	pk 17.4	npk 17.2	p 19.7	k 12.0	np 19.0	(1) 15.6
nk 10.7	np 17.5	n 10.4	p 18.0	(1) 9.8	npk 16.6	pk 17.5	k 14.3
pk 18.3	k 12.6	npk 14.2	(1) 12.2	n 11.4	np 14.5	p 16.9	nk 16.1
np 17.9	(1) 12.8	nk 13.3	n 11.3	pk 16.5	p 15.6	k 10.9	npk 16.7
k 14.9	pk 18.2	(1) 12.8	np 17.1	npk 15.8	n 9.5	nk 8.9	p 20.6
npk 19.0	p 18.9	k 11.2	pk 17.1	np 17.9	nk 8.6	(1) 10.2	n 14.5
(1) 17.5	npk 20.4	p 20.8	nk 16.4	k 16.8	pk 18.5	n 13.6	np 23.0

Treatment Sums

(1)	102.4	p	149.3	np	145.2	pk	?
n	95.8	k	108.5	nk	92.7	npk	?

Preliminary Analysis of Variance

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Rows	7	?	?
Columns	7	?	?
Treatments	7	513.79	73.4
Error	42	?	?
Total	?	?	

Source	d.f.	s.s.	m.s.	F	Effects
Treatments	7	513.789			
N	1	13.690		6.25	-29.6
P	1	488.410		223.02	176.8
K	1	1.501			-9.8
NP	1	?			15.2
NK	1	?	2.338	1.07	?
PK	1	?			?
NPK	1	1.626			?
Error	42		2.190		

F_{05} (42, 1 d.f.) = 4.07

F_{01} (42, 1 d.f.) = 7.27

Effects of Nitrogen in Wheat Experiment

Treatment	Sum of Yields	N	NP	NPK
(1)	102.4	-6.6	?	?
n	95.8			
p	149.3	?		
np	145.2			
k	108.5	?	?	
nk	92.7			
pk	142.4	-3.1		
npk	139.3			
Total		-29.6	15.2	?

Treatments		K	NK
(1)	102.4	6.1	-9.2
k	108.5		
n	95.8	-3.1	
nk	92.7		
p	149.3	?	?
pk	142.4		
np	145.2	?	
npk	139.3		
Totals		-9.8	?

Treatments		P	KP
(1)	102.4	?	
p	149.3		?
k	108.5	?	
pk	142.4		
n	95.8	49.4	
np	145.2		- 2.8
nk	92.7	46.6	
npk	139.3		
		176.8	?

Compute the lsd for a treatment mean, for an effect mean, and for an effect total.

Problem VI-2. The following data were copied from class notes distributed by Miss G.M.Cox at Iowa State College. For purposes of illustration it is assumed that the $32 = 2^5$ treatments were allotted to the 32 plots in the experimental area at random.

2^5 Experiment on Spartan Barley H.R.Meldrum
Mt. Pleasant Field

Series III - Treatment and Results - 1938

Date of Seeding: April 12

Treatments: April 12
- 20 percent superphosphate - 120 lbs. per acre
- Muriate of potash - 24 lbs. per acre
- Ammonium sulphate - 29.5 lbs. per acre
- Complete fertilizer (n+p+k) equivalent to 150 lbs. per acre of 4-16-8

Harvesting:
- Date - July 7
- Method - 3(5 x 5') samples - 1/580.8 acre
- Weight per bushel - 48 lbs.

check = no treatment   n = ammonium sulphate
k = muriate of potash   l = limestone
p = superphosphate   m = manure

Results:

Plot	Treatment	Yield in bu. per acre	Plot	Treatment	Yield in bu. per acre
1	ck	24.6	17	m	37.9
2	k	30.3	18	mk	32.6
3	p	27.6	19	mp	34.4
4	n	25.8	20	mn	38.9
5	l	24.9	21	ml	31.3
6	pk	33.6	22	mpk	34.9
7	nk	34.4	23	mnk	35.9
8	lk	31.0	24	mlk	33.6
9	np	26.5	25	mnp	28.3
10	lp	31.8	26	mlp	37.0
11	ln	21.2	27	mln	32.2
12	npk	31.3	28	mnpk	36.3
13	lpk	36.3	29	mlpk	33.6
14	lnk	24.9	30	mlnk	35.2
15	lnp	28.3	31	mlnp	36.7
16	lnpk	21.9	32	mlnpk	37.4

Analysis of Variance

Source	d.f.	s.s.	m.s.
Treatments (main effect and interactions)	15	559.1338	37.28
Remainder	16	178.9362	11.18
Total	31	738.0700	

Comment of experimenter: Red clover seeded with barley and a fair stand was obtained. Barley stand was rather un-uniform, making sampling difficult. All phosphate plots showed advanced maturity at harvest time. Clover looked good in October with some incresed growth noted on manure and phosphate plots. Woods bad on entire series.

Complete the following analysis of Variance

Source of Variation	d.f.		s.s.	m.s.
K	1			40.05
P	1			14.04
N	1			12.75
L	1			8.00
M	1			
KP	1			
KN	1	15	559.1338	
KL	1			
KM	1			
PN	1			
PL	1			
PM	1			
NL	1			
NM	1			
LM	1			
Remainder	16		178.9362	11.18
Total	31		738.0700	---

Also, partition the remaining 16 degrees of freedom into single degrees of freedom. Do they form a homogeneous set of variances? Do you agree with the comments of the experimenter that increased growth was noted on manure and phosphate plots?

Problem VI-3. Six replicates of a randomized complete blocks design were used to compare nine treatments. Eight of the treatments received a lime application in addition to one of the eight combinations of three factors, n, p, and k each at two levels. Thus, the nine treatments represent a 2^3 factorial arrangement of the factors n, p, and k plus a check. Do you agree with the following breakdown of the total degrees of freedom? Why or why not?

Source of Variation	Degrees of Freedom	
Replicates	5	
Treatments	8	
Ch vs. others		1
LN		1
LP		1
LK		1
LNP		1
LNK		1
LPK		1
LNPK		1
Error	40	
Total	53	

What coefficients with what signs are required for the above comparisons? Answer by completing the following table:

Effect	ch.	l	ln	lp	lnp	lk	lnk	lpk	lnpk	Divisor
Ch.vs.others										
LN										
LP										
LNP										
LK										
LNK										
LPK										
LNPK										

If E = error mean square, what is the standard error of difference for the comparison of lime versus no lime? What is the lsd for an effect total? for an effect mean?

CONFOUNDING IN FACTORIAL EXPERIMENTS

by

W. T. Federer

VII-1. Confounding - Use and Types

The number of treatment combinations increases rapidly as the number of factors and (or) levels of the factor is increased. For over 10-12 treatment combinations the latin square design becomes impractical and as the number of treatment combinations increases it becomes exceedingly difficult to select replicates for randomized complete block designs which are relatively homogeneous within. Because the variation within replicates tends to increase as the replicate size increases and thus increase the experimental error variance, it is desirable to keep block sizes small. In order to retain relatively small block sizes for large numbers of treatments, only a portion of the treatment combinations are included in a block. The resulting blocks are called incomplete blocks. By a device known as confounding (see Yates, 1937) the necessity of including all treatments in each block (or row and column in a latin square) is side-stepped.

The whole block or replicate is divided into the desired number of incomplete blocks. By removing the variation among incomplete blocks (freed of treatment effects) within replicates, the experimental error mean square is often much smaller than what it would have been for a randomized complete blocks design resulting in more precise comparisons among some treatments. The segregation of the blocks within replicate sum of squares results in a decrease in thedegrees of freedom associated either with the error or treatment sums of squares. This means that, in some cases, information among some treatment comparisons may be mixed up with or confounded with the interblock information. If that particular treatment comparison is of little or no value, then this feature may be included in the incomplete block design. Indiscriminant confounding may result in complete loss of information on the comparisons or contrasts of greatest importance. This means that the experimenter should confound with incomplete block differences only those contrasts or comparisons of little or no importance.

If an effect, A, is of little or no interest, it may be confounded with the incomplete block differences in all replicates. This system of confounding is known as complete confounding. For example, it may be desirable to have incomplete blocks of four plots in a 2^3 factorial experiment with three replicates and the effect ABC may be of little or no importance. The treatments in an incomplete block will be one of these two groups:

$(ABC)_1$	$(ABC)_0$
001	000
010	011
100	101
111	110

Thus the comparison between the incomplete blocks is also the comparison between $(ABC)_1$ and $(ABC)_0$.

If an effect is confounded with incomplete block differences in replicate I, a second effect in replicate II, and a third effect or one of the first two in replicate III, these effects are partially confounded with incomplete block differences, i.e. they are confounded with incomplete block differences in some replicates and unconfounded in others. Some information is available on all comparisons but some comparisons are more accurately determined since they are made in all replicates instead of only a portion of the replicates.

The term balanced confounding (Cochran and Cox, 1944) has been reserved for the incomplete blocks design for which all effects of a certain order, say all two factor interactions, are confounded with incomplete block differences an equal number of times. In the present manuscript this type of design will be defined to be a partially balanced one. If all two factor and three factor interactions are confounded with incomplete block differences, the design is still a partially balanced one. Likewise, if all effects are confounded with incomplete block differences an equal number of times the design is said to be balanced. The addition of the term partially balanced (equals Cochran's and Cox's, 1944, term of balanced) was deemed to be necessary for the discussion of a group of incomplete blocks designs which are known as lattices (see Chapter XI).

In order to make efficient use of confounding, it is necessary to comprehend the advantages and disadvantages as discussed by Cochran and Cox (1944, p.42). The advantages arise from the reduction in the experimental error -

resulting from the use of a block which is more homogeneous or which can be subjected to more uniform technique than the complete replicate. "Without some idea of the amount of this reduction, a realistic decision cannot be made on the question of confounding. If applicable uniformity data have been collected, the experimenter may compare the variability within the incomplete blocks with the variability within replicates. In addition from the results of an experiment in which confounding has been employed, it is usually possible to estimate what the experimental error would have been if confounding had not been used."* In estimating average gains in precision from confounding, Yates (1935, Suppl. Jour. Royal Stat. Soc.) has shown that a relatively large number of experiments is required and that little reliance can be placed on the gain in precision obtained from a single experiment.

The disadvantages in confounding are:

(i) The reduction in number of replicates for the confounded comparisons
(ii) The increase in number and complexity of calculations

In regard to the first disadvantage, no comparison should be completely confounded unless there is considerable evidence to show that the comparison has little or no value. The experimenter should ascertain the relative importance of each of the comparisons and partially confound those of less importance.

The increase in computations from the use of confounded designs may be small or large depending upon the

*Cochran and Cox, 1944

type of confounding used. Cochran and Cox (1944) state that "as usual, it is a good practice to study the method of analysis before the experiment is commenced". This bit of advice cannot be stressed too strongly. The experimenter may save himself considerable time and anxiety if he writes out the analysis prior to running the experiment.

VII-2. Complete Confounding

In some instances the higher ordered interactions or even the main effects (see Chapter VIII on split plot designs) may have little or no meaning and it is decided to confound these comparisons with block differences in all replicates. An example of confounding the ABC interaction of a 2^3 factorial experiment in all three replicates of a randomized complete blocks design was given in the previous section. The field plan for this design may be the following:

Replicate I		Replicate II		Replicate III	
000	111	010	101	001	011
110	010	001	011	111	000
101	001	111	101	100	101
011	100	100	000	010	110
I_a	I_b	II_b	II_a	III_b	III_a

All treatment combinations in incomplete blocks I_b, II_b, and III_b are those which make up $(ABC)_1$ and those in blocks I_a, II_a, and III_a make up $(ABC)_0$. The groups are alloted to the incomplete blocks at random and the treatment combinations within each incomplete block are alloted to the plots at random. The comparison between incomplete block totals in a replicate also represents the contrast between $(ABC)_1$ and $(ABC)_0$. The partitioning of the degrees of freedom in the analysis of variance follows:

Source of variation	Degrees of Freedom		Mean Square
Replicates	2		
ABC	1		$\} E_b$
Replicates x ABC	2		E_b'
Treatments	6		
A		1	
B		1	
AB		1	
C		1	
AC		1	
BC		1	
Residual	12		E_e
Total	23		

The contrast of the levels $(ABC)_1$ and $(ABC)_0$ is rather ill determined since only three replicates are available for the contrast and since the replicates x ABC mean square, which is the error term for testing the above comparison, is determined with only two degrees of freedom. Thus, most of the information on the ABC effect has been sacrificed at the expense of having blocks of four instead of blocks of eight plots. The increase in precision due to confounding (ignoring the difference in the degrees of freedom for r replicates of the incomplete and complete blocks designs) is obtained from the formula

$$\frac{E_e'}{E_e} = \frac{(r-1+1)E_b' + [6+6(r-1)]E_e}{[r-1+1+6+6(r-1)]E_e} = \frac{(E_b'+6E_e)}{7E_e} ,$$

where E_b' and E_e are the two error mean squares in the previous example and where the interaction is not assumed negligible. In the event that the interaction is assumed negligible the efficiency of the incomplete blocks relative to the randomized complete blocks design is

$$\frac{E_e'}{E_e} = \frac{rE_b + [6+6(r-1)E_e}{[r+6+6(r-1)]E_e} = \frac{E_b+6E_e}{7E_e}$$

, where E_b is the incomplete blocks within replicate mean square. The experimenter should usually use the former measure of efficiency since no assumptions are made relative to the magnitude of the confounded effect.

An example illustrating the computational procedure for an incomplete blocks design with complete confounding of the ABC interaction has been presented by Yates (1937, section 4b) and will not be discussed herein. The main effects and two factor interaction effects are computed in the same manner as for an experiment in which there is no confounding. The three factor interaction effect is omitted if the assumption is made that it is zero. The treatment totals, however, require adjustments since the completely confounded effect is now zero and the adjusted mean for treatment ijh is equal to

$$\frac{(A)_i+(B)_j+(AB)_{i+j}+(C)_h+(AC)_{i+h}+(BC)_{j+h}-5/2(\text{total})}{4}$$

$$= \frac{A+B+AB+C+AC+BC+\text{total}}{8} .$$

For the example, on page 20 of Yates (1937) article on factorial experiments, the adjusted yeilds for treatment 111 is

$$\frac{4838+5301+4718+6159+4746+4331+(9331)/2 - 3(9331)}{4}$$

$$= \frac{333+2271+105+2987+161-669+9331}{8} = 1814.875$$ which equals

$1814.875(60) \big/ (2240)4 = 12.1532$ long tons per acre.

The other adjusted yields are obtained similarly.

For other examples of complete confounding of some effects, the reader is referred to "The Design and Analysis of Factorial Experiments" by Yates (1937) and to later chapters of this manuscript. The chapter on split plot designs and the chapter on graeco-latin squares and quasi-latin squares contain illustrations of complete confounding of some effects.

In the event that the groups of treatments making up the comparions $(ABC)_1$ and $(ABC)_0$ were not allotted to the incomplete blocks ar random, i.e., the field lay-out was of the following form by design:

Replicate I		Replicate II		Replicate III	
000	111	101	010	001	011
110	010	011	001	111	000
101	001	101	111	100	101
011	100	000	100	010	110
I_a	I_b	II_a	II_b	III_a	III_b

the breakdown of the total degrees of freedom would be:

Source of variation	Degrees of freedom	
Blocks	5	
Treatments	6	
A		1
B		1
AB		1
C		1
AC		1
BC		1
Residual	12	
Total	23	

It is not possible to partition the five degrees of freedom for the blocks sum of squares since the design was systematic.

VII-3. Partial Confounding

It may be desirable to obtain at least partial information on all effects in a factorial experiment for which the number of treatment combinations is large and at the same time to keep the block size small. A scheme of confounding may be followed in which some effects are confounded in one replicate, other effects in the second replicate, and still others in the remaining replicates. The scheme of partial confounding allows for at least partial information on all effects.

In a 2^3 factorial experiment with four replicates information may be desired on all seven effects. It may be considered that blocks of eight plots cover too much heterogeneity and that blocks of four plots would be much more efficient. The main effects A, B, and C are of most interest with the secondary interest on the AB, AC, BC, and ABC interactions. Complete confounding of any of the interactions may be undesirable. The interaction ABC could be confounded with block differences in replicate I, AC in replicate II, BC in replicate III, and AB in replicate IV. Some such field plan as the following might be obtained:

Interaction Confounded	ABC		AC		BC		AB	
Block	I_a	I_b	II_b	II_a	III_a	III_b	IV_a	IV_b
	001	101	000	011	000	101	001	011
	100	011	111	110	100	010	110	100
	111	110	010	001	111	110	000	010
	010	000	101	100	011	001	111	101
	Rep. I		Rep. II		Rep. III		Rep. IV	

where the effect confounded is allotted to the replicate at random and the level of the effect to the incomplete block within a replicate at random. The treatments within an incomplete block being assigned to the plots at random. For such a design the effects AB, AC, BC, and ABC are estimated from the three replicates in which they are unconfounded. Thus, the interactions AB, AC, BC, and ABC are evaluated from 24 plots instead of the entire 32 plots as are the main effects A, B, and C. The amount of information, ignoring interblock comparisons, is then $3/4$ on the interactions.

The analysis for a 2^3 design with confounding in the four replicates as given above is illustrated by Yates, 1937, p. 21-23. The breakdown of the total degrees of freedom is

Source of variation	d.f.
Blocks (ignoring treatments)	7
Main effects (ignoring blocks)	3
Interactions (from unconfounded reps)	4
Error	17
Total	31

In the event that the information on interblock comparisons (see Chapter XI on lattice designs) is utilized, the breakdown of total degrees of freedom would be of the form:

Source of variation	d.f.	Expectation of mean sq.
Replicates	3	
Blocks (elim. treatment effects)	4	$\sigma_e^2 + \frac{3}{4} 4\sigma_b^2$
Treatments (ignoring block effects)	7	
Error	17	σ_e^2
Total	31	

In the former case the treatment totals are obtained from the formula

$$\frac{\text{Total} + A + B + C + AB + AC + BC + ABC}{8}$$

$$= \frac{(A)_i + (B)_j + (C)_h + (AB)_{i+j} + (AC)_{i+h} + (BC)_{j+h} + (ABC)_{i+j+h} - 3\,\text{Total}}{4}$$

where the interactions are evaluated from the three replicates in which they are unconfounded. In the latter case, the interactions are evaluated with variance $1/w' = \sigma_e^2 + 4\sigma_b^2$ in the replicates in which they are confounded with blocks and with variance $1/w = \sigma_e^2$ in the replicates in which they are unconfounded with block differences. The weighted level of interaction ABC would be

$$\frac{w'(ABC)_{i+j+h} \text{ in rep. I} + w(ABC)_{i+j+h} \text{ in reps. II, III, IV}}{w' + 3w} .$$

The other weighted levels of interactions are obtained similarly. The weighted effects are then inserted in the formula

$$\frac{(A)_i+(B)_j+(C)_h}{4} + \frac{[(AB)_{i+j}+(AC)_{i+h}+(BC)_{j+h}+(ABC)_{i+j+h}]_{wt'd}}{4} - \frac{3}{4}\text{Total}$$

to obtain the adjusted treatment totals for r replicates.

Neglecting interblock information the standard error of the totals for the main effects (Yates, 1937) is

$$\hat{\sigma}_e \sqrt{(2)(4)(r)}$$

and of the interactions confounded in one replicate out of four in z=1, 2, ... sets of four replicates

$$\hat{\sigma}_e \sqrt{2(4)(z)(4-1)} .$$

For a further discussion of this example and of the subject of confounding the reader is referred to Yates' "Design and Analysis of Factorial Experiments" (1937).

In the above example three replicates might have been selected instead of four, all two factor interactions could have been confounded with the incomplete block differences. The selection of replicates II, III, and IV would result in such an arrangement. This design, like the design with four replicates, would have been partially balanced since all two factor interactions are confounded an equal number of times. Likewise, five replicates may be selected so that ABC is confounded in replicates I and V and AB, AC, and BC are confounded with block differences in replicates IV, II, and III respectively. Such a design would be partially balanced. If, on the other hand, full information is desired on the interaction AB and the main effect in an experiment with four replicates, ABC could be confounded with block differences in replicates I and IV, AC in replicate II, and BC in replicate III. This is an unbalanced design since all interactions of the same order are not confounded equally. The breakdown of the total degrees of freedom, neglecting interblock information, is:

Source of variation	d.f.	m.s.
Replicates	3	
Blocks within reps (ignoring treat).	4	E_b
Main effects (ignoring blocks)	3	
Interaction (from unconfounded reps)	4	
Error	17	E_e
Total	31	

and making use of interblock information is:

Source of variation	d.f.		m.s.	Expectation of mean sq.
Replicates	3			
Blocks eliminating treatments	4		E_b	$\sigma_e^2 + 3/4(4\sigma_b^2)$
component (a)		1		$\sigma_e^2+4\sigma_b^2$
component (b)		1		$\sigma_e^2 + 2\sigma_b^2$
component (c)		2		$\sigma_e^2 + 3\sigma_b^2$
Treatments (ignoring blocks)	7			
Error	17		E_e	σ_e^2
Total	31			

where component (a) is the sum of squares for the interaction of levels of effect ABC with replicates I and IV; component (b) is the sum of squares for the comparison of the levels of effect ABC in replicates I and IV with the unconfounded levels in replicates II and III; component (c) is the sum of squares for the comparison of the levels of effects AC and BC in the replicate in which the effect is confounded with block differences with the corresponding levels of the effect in the replicates in which they are unconfounded with incomplete block differences.

The precision (see Cochran and Cox, 1944, p.60) of the above incomplete blocks design, assuming the confounded interactions are negligible, is:

$$\frac{4E_b + (7+17)E_e}{(4+7+17)E_e} = \frac{(E_b+6E_e)}{7E_e}$$

and not assuming the confounded effects negligible is:

$$\frac{4[(4E_b-E_e)/3] + (7+17)E_e}{(4+7+17)E_e} = \frac{4E_b + 17E_e}{21E_e}$$

The above formula does not consider the information on interblock comparisons in which case the precision is:

$$(4E_b+17E_e)/21 \Big/ \frac{4E_e}{7}\left\{\frac{1}{2 + 2w'/w} + \frac{2}{3 + w'/w} + \frac{4}{4}\right\},$$

where $w = 1/E_e$ and $w' = \dfrac{3}{4E_b-E_e}$.

Example VII-1. A 3x2x2 factorial arrangement of applications of nitrogen and clippings in the fall and spring was conducted by M.L.Peterson (Ph.D. thesis, Iowa State College, 1945) to study the effect on yields of hay from alfalfa. The treatments were:

a_o = no nitrogen
a_1 = 60 pounds of nitrogen applied on March 15
a_2 = 30 lbs. of nitrogen on Sept. 15 and 30 lbs. on Mar.15
b_o = no spring deferment of clipping
b_1 = spring deferment of clipping
c_o = no fall deferment of clipping
c_1 = fall deferment of clipping

in all combinations. The twelve combinations were planted in three replicates with incomplete blocks of six plots. The interactions ABC and BC were partially confounded with incomplete block differences. The specified group of treatment combinations were assigned to the incomplete blocks within a replicate at random and the treatment combinations were assigned to the plots within the incomplete block in a random manner. The systematic arrangement of the treatment combinations and the yields of alfalfa in grams per plot are given in Table VII-1. The treatment combinations are arranged in a partially balanced incomplete blocks design in such a manner that the BC and ABC interactions are partially confounded with block differences in each replicate. Interaction BC is confounded as little as possible in this design.

The statistical analysis for the 3x2x2 factorial experiment on alfalfa yields is first made ignoring the confounding (see page 39, Yates, 1937) i.e., assuming the twelve treatments

Table VII-1. Systematic arrangement of yields (gms) from a 3x2x2 factorial experiment in three replicates with incomplete blocks of six plots. The interactions BC and ABC are partially confounded with block differences.

	Replicate I Block number				Replicate II Block number				Replicate III Block number			
	I_a		I_b		II_a		II_b		III_a		III_b	
	T*	Y**	T	Y	T	Y	T	Y	T	Y	T	Y
	001	294	000	249	000	232	001	267	000	308	001	386
	010	226	011	340	011	254	010	235	011	288	010	310
	100	403	101	523	101	523	100	410	100	617	101	750
	111	520	110	404	110	404	111	450	111	730	110	562
	200	370	201	457	200	411	201	485	201	565	200	630
	211	487	210	481	211	436	210	399	210	539	211	830
Σ B.		2300		2454		2260		2246		3047		3468
Σ Rep.				4754				4506				6515
Grand Total												15775

* Treatment combinations are designated by the subscript ijh of the treatment combination $a_i b_j c_h$, where i=0,1,2; j=0,1; and h=0,1.
** Yield

were completely randomized within blocks of 12 plots. Most of the calculations are required for the analysis of the incomplete blocks design and also, the reader can observe the differences in the analyses for the randomized complete blocks and the incomplete blocks design. The totals (Table VII-2) for the 12 treatment combinations are obtained in the usual manner. Summing the treatment totals over the factor a, the totals in column 4 are obtained. The four figures in column 5 are obtained as follows:

$$789 + 771 = 1560$$
$$947 + 882 = 1829$$
$$771 - 789 = -18$$
$$882 - 947 = -65$$

The figures in columns 6, 7, and 8 are obtained in the same manner from columns 2, 3, and 4 respectively. Columns 9, 10, 11, and 12 are obtained by the same procedure applied to columns 5, 6, 7, and 8, respectively. For example, the

Table VII-2. Treatment combination totals and calculations for estimating the main effects and interaction effects from the 3x2x2 factorial experiment and alternative tables for computing the interaction sums of squares. (Treatment combination $a_i b_j c_h$=ijh. The dot in the subscript means - summed up over the subscript replaced by the dot.)

	Yield (gms/plot)				Sums and differences				Effects			
	a_o	a_1	a_2	Sum	a_o	a_1	a_2	Sum	a_o	a_1	a_2	Sum
$b_o c_o$	789	1430	1411	3630	1560	2800	2830	7190	3389	6296	6090	15775=total
$b_1 c_o$	771	1370	1419	3560	1829	3496	3260	8585	-83	-156	254	15= B
$b_o c_1$	947	1796	1507	4250	-18	-60	8	-70	269	696	430	1395= C
$b_1 c_1$	882	1700	1753	4335	-65	-96	246	85	-47	-36	238	155=BC
Totals	3389 $=(A)_o$	6296 $=(A)_1$	6090 $=(A)_2$	15775 =total								

	oj.	oj.	oj.	Total
io.	1736	3226	2918	$7880=(B)_o$
i1.	1653	3070	3172	$7895=(B)_1$
Total	3389 $=(A)_o$	6296 $=(A)_1$	6090 $=(A)_2$	15775=Total

	o.h	1.h	2.h	Total
i.o	1560	2800	2830	$7190=(C)_o$
i.1	1829	3496	3260	$8585=(C)_1$
Total	3389 $=(A)_o$	6296 $=(A)_1$	6090 $=(A)_2$	15775=Total

	.jo	.j1	Total
.oh	3630	4250	$7880=(B)_o$
.1h	3560	4335	$7895=(B)_1$
Total	7190 $=(C)_o$	8585 $=(C)_1$	15775=Total

	ojh	1jh	2jh	Total
.oo	789	1430	1411	3630
i1o	771	1370	1419	3560
io1	947	1796	1507	4250
i11	882	1700	1753	4335
Total	3389 $=(A)_o$	6296 $=(A)_1$	6090 $=(A)_2$	15775=Total

figure 15 in column 12, which is the B effect = $(B)_1 - (B)_0$, is obtained from column 8 as: 85 + (-70) = 15.
or as: -83 - 156 + 254 =15.

Several checks for the computations in the top portion of Table VII-2 are available. The sum of each of the columns 1, 2, 3, and 4 should equal the first figure of columns 9, 10, 11, and 12. Likewise, the sum of the numbers over the factor a serves as a check to the figures obtained by the sum and differences.

The four tables at the bottom of Table VII-2 may be used to compute the AB, AC, BC, and ABC interactions or they may be computed directly from columns 9 to 12 of the top part of Table VII-2. The sums of squares of the main effects and interaction effects are:

$$A: \frac{(A)_0^2+(A)_1^2+(A)_2^2}{12} - \frac{G^2}{36} = \frac{3389^2+6296^2+6090^2}{12} - \frac{15775^2}{36} = 438,569.[illegible]$$

$$B: \frac{(B)_0^2+(B)_1^2}{18} - \frac{G^2}{36} = \frac{[(B)_1-(B)_0]^2}{36} = \frac{15^2}{36} = \qquad 6.25$$

$$C: \frac{(C)_0^2+(C)_1^2}{18} - \frac{G^2}{36} = \frac{[(C)_1-(C)_0]^2}{36} = \frac{1395^2}{36} = \qquad 54,056.25$$

$$AB: \frac{(Ba_0)^2+(Ba_1)^2+(Ba_2)^2}{12} - \frac{B^2}{36} = \frac{(-83)^2+(-156)^2+(254)^2}{12} - \frac{15^2}{36}$$

$$= \qquad 7,972.17$$

$$AC: \frac{(Ca_0)^2+(Ca_1)^2+(Ca_2)^2}{12} - \frac{C^2}{36} = \frac{269^2+696^2+430^2}{12} - \frac{1395^2}{36}$$

$$= \qquad 7,750.17$$

$$BC(\text{uncorrected}): \frac{(BC)_0^2+(BC)_1^2}{18} - \frac{G^2}{36} = \frac{[(BC)_0-(BC)_1]^2}{36} = \frac{155^2}{36}$$

$$= \qquad 667.36$$

ABC(uncorrected): $\dfrac{(BCa_0)^2+(BCa_1)^2+(BCa_2)^2}{12} - \dfrac{BC^2}{36}$

$$= \frac{(-47)^2+(-36)^2+238^2}{12} - \frac{155^2}{36} = 4,345.06$$

Sum of the above sums of squares = 513,366.32. As a check the treatment conbination sum of squares is

$$\frac{789^2+1430^2+\ldots+1700^2+1753^2}{3} - \frac{15775^2}{36} = 513,366.31 .$$

The total, replicate, and error sums of squares are obtained in the usual manner. The analysis of variance, neglecting the effects due to confounding, is presented in Table VII-3a.

The sums of squares for BC and ABC contain some effects due to block differences. The next step is to eliminate the block effects from the estimates of $(BC)_0$, $(BC)_1$, (BCa_0), (BCa_1), and (BCa_2) and from the corresponding sums of squares. These sums of squares added to those for effects A, B, C, AB, and AC yields the treatment sum of squares adjusted for block effects (Table VII-3b). Yates (1937) adjusts the BC effect by adding a correction to the unadjusted effect thus:

$$\begin{aligned} 3Q &= 3[BC(\text{unadjusted})]+I_b-I_a+II_b-II_a+III_b-III_a \\ &= [BC(\text{unadj})]+I_b-I_a+[BC(\text{unadj})]+II_b-II_a+[BC(\text{unadj})] \\ &\qquad +III_b-III_a \\ &= 155+2454-2300+155+2246-2260+155+3468-3047 \\ &= 3(155)+154-14+421 = 1026. \end{aligned}$$

The adjusted sum of squares for BC is $1026^2/288 = 3655.12$.

Before proceeding further an explanation of the divisors, etc., is in order. In the first place, the quantity Q is unaffected by block differences which is apparent from the following, where the treatment yield used is designated by

Table VII-3a. Analysis of variance for the 3x2x2 factorial experiment of Table VII-1, neglecting the effects due to confounding.

Source of Variation	d.f.		s.s.		m.s.
Replicates	2		199,964.06		
Treatments(ignoring blocks)	11		513,366.32		
A		2		438,569.06	
B		1		6.25	
C		1		54,056.25	
AB		2		7,972.17	
AC		2		7,750.17	
BC (uncorrected)		1		667.36	
ABC (uncorrected)		2		4,345.06	
Residual	22		89,123.26		4051.06
Total	35		802,453.64		

Table VII-3b. Analysis of variance for incomplete blocks design

Source of Variation	d.f.		s.s.		m.s.	
Replicates	2		199,964.06		99,982.03	
Blocks within reps (ign.tr.)	3		16,762.75		5,587.58	
Treatments (elim.blocks)	11		513,350.72			
A		2		438,569.06		219,284.53
B		1		6.25		6.25
C		1		54,056.25		54,056.25
AB		2		7,972.17		3,986.08
AC		2		7,750.17		3,875.08
BC' (corrected)		1		3,655.12		3,655.12
ABC' (corrected)		2		1,341.70		670.85
Error	19		72,376.11		3,809.27	
Total	35		802,453.64			

the treatment number and the replicate number by I, II, and III:

$$
\begin{aligned}
3Q &= 3BC(\text{unadj}) + I_b - I_a + II_b - II_a + III_b - III_a \\
&= 3(000+001+100+111+200+211-001-010-101-110-201-210)(I+II+III) \\
&\quad +(000+011+100+111+200+211-001-010-101-110-201-210)(I) \\
&\quad +(001+010+100+111+201+210-000-011-101-110-200-211)(II) \\
&\quad +(001+010+101+110+200+211-000-011-100-111-201-210)(III) \\
&= [000(3I + 3II + 3III + I - II - III) \\
&\quad + 011(3I + 3II + 3III + I - II - III) \\
&\quad + 100(3I + 3II + 3III - I + II - III) \\
&\quad + 111(3I + 3II + 3III - I + II - III) \\
&\quad + 200(3I + 3II + 3III - I - II + III) \\
&\quad + 211(3I + 3II + 3III - I - II + III)] \\
&\quad - [001(3I + 3II + 3III + I - II - III) \\
&\qquad + 010(3I + 3II + 3III + I - II - III) \\
&\qquad + 101(3I + 3II + 3III - I + II - III) \\
&\qquad + 110(3I + 3II + 3III - I + II - III) \\
&\qquad + 201(3I + 3II + 3III - I - II + III) \\
&\qquad + 210(3I + 3II + 3III - I - II + III)] \\
&= [000(4I + 2II + 2III) + 011(4I + 2II + 2III) \\
&\quad + 100(2I + 4II + 2III) + 111(2I + 4II + 2III) \\
&\quad + 200(2I + 2II + 4III) + 211(2I + 2II + 4III)] \\
&\quad - [001(4I + 2II + 2III) + 010(4I + 2II + 2III) \\
&\qquad + 101(2I + 4II + 2III) + 110(2I + 4II + 2III) \\
&\qquad + 201(2I + 2II + 4III) + 210(2I + 2II + 4III)] \\
&= (BC)_0\text{adjusted} - (BC)_1\text{adjusted} = BC \text{ adjusted.}
\end{aligned}
$$

From the above it is apparent that the quantity 3Q is the difference between two quantities each of which is composed of 48 plot yields. Therefore, the quantity $3Q/48 = Q/16$ is the BC effect adjusted for the effects of incomplete blocks on a single plot basis. The divisor for the sum of squares of the quantity Q is $\frac{2(6)}{3^2}(4^2+2^2+2^2) = 32$ and for

3Q is $2(6)(4^2+2^2+2^2) = 288$. The sum of squares for the BC effect adjusted for block differences is

$$\frac{1}{32} Q^2 = \frac{1}{288} (3Q)^2 = \frac{(1026)^2}{288} = 3655.12$$

which may be compared with the BC effect and sum of squares from the unconfounded experiment. The BC effect on a single plot basis is $BC/18 = [(BC)_0-(BC)_1]/18$ and the sum of squares is $[(BC)_0-(BC)_1]^2/36$.

The error variance for the BC effect for the unconfounded experiment is $\frac{2\hat{\sigma}_e^2}{18} = \frac{1}{9}\hat{\sigma}_e^2$ and is $\frac{2}{16}\hat{\sigma}_e^2 = \frac{1}{8}\hat{\sigma}_e^2$ in an experiment with the confounding as described above. The loss in information due to confounding <u>if there is no reduction in the error variance</u> is $1 - \frac{1}{9}/\frac{1}{8} = 1 - \frac{8}{9} = \frac{1}{9}$, since the relative information from the two designs is $\frac{1}{9}\hat{\sigma}_e^2 \Big/ \frac{1}{8}\hat{\sigma}_e^2 = \frac{8}{9}$. If there is a sizeable reduction in the error mean square, then the BC interaction may be estimated more accurately in the incomplete blocks design than in the randomized complete blocks experiment even though it is partially confounded with incomplete block differences.

The estimate of the ABC interaction is obtained similarly. This interaction is expressible as the variation of the BC interaction over the three levels of the factor a. The notation (BCa_0) means that the BC interaction is estimated at the zero level of factor a. In a like manner (BCa_1) and (BCa_2) represent the BC interaction at the second and third levels of the factor. The comparison among the three quantities corrected for the mean yields the ABC sum of squares with two degrees of freedom. Since these quantities contain block differences in addition to the effect, it is necessary to adjust them. The adjusted quantities are:

$$3R_0 = 3(BCa_0)\text{ unadj.} + I_a - I_b + II_b - II_a + III_b - III_a$$
$$= 3(-47) - 154 - 14 + 421 = 112$$

$$3R_1 = 3(BCa_1)\text{unadj.} + I_b - I_a + II_a - II_b + III_b - III_a$$
$$= 3(-36) + 154 + 14 + 421 = 481$$

$$3R_2 = 3(BCa_2)\text{unadj.} + I_b - I_a + II_b - II_a + III_a - III_b$$
$$= 3(328) + 154 - 14 - 421 = 433.$$

As a partial check the sum $3R_0 + 3R_1 + 3R_2 = 3Q$

$$= 112 + 481 + 433 = 1026.$$

The adjusted BC effects at the three levels of a on a single plot basis are: $\frac{1}{10}(3R_0 - Q + 3R_1 - Q + 3R_2 - Q)$

$$= \frac{1}{10}\left(112 - \frac{1026}{3} + 481 - 342 + 433 - 342\right)$$

$$= -23.0 + 13.9 + 9.1 = 0$$

The coefficient 10 in the denominator may be verified by the process given above for adjusting the BC effect. In the unconfounded experiment the BCa_o effect on a single plot basis would be $\frac{1}{6}(BCa_0)\text{unadj} - \frac{BC}{18} = \frac{1}{6}\left(-47-\frac{155}{3}\right)$, the BCa_1 effect would be $\frac{1}{6}(BCa_1)\text{unadj} - \frac{BC}{18} = \frac{1}{6}\left(-36 - \frac{155}{3}\right)$, and the BCa_2 effect would be $\frac{1}{6}(BCa_2)\text{unadj} - \frac{BC}{18} = \frac{1}{6}\left(238 - \frac{155}{3}\right)$, with the error variance $\frac{2}{6}\hat{\sigma}_e^2 = \frac{1}{3}\hat{\sigma}_e^2$. In this incomplete blocks experiment the error variance of the adjusted effects is $V(R_i\text{dev.}) = \frac{3\text{x}2}{10}\hat{\sigma}_e^2 = \frac{3}{5}\hat{\sigma}_e^2$. Hence, the ratio of the relative information on the effects BCa_0, BCa_1, and BCa_2 deviations in the two designs is $1/3 \neq 3/5 = 5/9$, if there is no reduction in the error variance. The loss in information is $1 - 5/9 = 4/9$. Yates (1937) points out that the loss in information on each degree of freedom confounded adds up to the single degree of freedom confounded with block differences, $1\left(\frac{1}{9}\right) + 2\left(\frac{4}{9}\right) = 1$. This feature is a property of balanced arrangements.

The sum of squares for the ABC effect adjusted for block differences is $\frac{1}{60}[(3R_0-Q)^2 + (3R_1-Q)^2 + (3R-Q)^2]$

$$= \frac{1}{60}[(-230)^2 + 139^2 + 91^2] = 1341.70.$$

The treatment sum of squares adjusted for block differences is the sum of the effect sums of squares unaffected by block differences plus the sums of squares for the partially confounded effects adjusted for block differences thus:

$$(438{,}569.06 + 6.25 + 54{,}056.25 + 7972.17 + 7750.17)$$
$$+ (3655.12 + 1341.70) = 513{,}350.72.$$

The blocks sum of squares within replicates is

$$\frac{(I_b - I_a)^2}{2k} + \frac{(II_b - II_a)^2}{2k} + \frac{(III_b - III_a)^2}{2k}$$

$$= \frac{154^2}{12} + \frac{(-14)^2}{12} + \frac{421^2}{12} = 16{,}762.75.$$

The sums of squares are summarized in the analysis of variance in Table VII-3b.

The blocks within replicates sum of squares, adjusted for treatment effects, is obtained from the following relation of sums of squares:

blocks unadjusted + treatments adjusted = blocks adjusted + treatments unadjusted. Therefore, the sum of squares for blocks within replicates adjusted for treatments = blocks unadjusted + treatments adjusted - treatments unadjusted = 16,762.75 + 513,350.72 - 513,366.32 = 16,747.15 with the resulting mean square of 16,747.15/3 = 5,582.38.

The efficiency of this incomplete block design relative to what it would have been had a complete block of 12 plots been used is: $\frac{3E_b + 11\hat{\sigma}_e^2}{(3+11)\hat{\sigma}_e^2} = \frac{3(5582.38) + 11(3809.27)}{14(3809.27)} = 110$ percent, or an increase in efficiency of 10 percent. In this case it is not assumed that the confounded effects are negligible.

In the event that it is desired to partition the 11 treatment degrees of freedom into 11 independent contrasts, it is possible to do so. The two degrees of freedom for the A effect can be partitioned into linear and quadratic effects and the interactions of these contrasts with the other factors yields the 11 comparisons listed below:

Effect	Treatment combinations												Divisors
	000	100	200	010	110	210	001	101	201	011	111	211	
A_L	-	0	+	-	0	+	-	0	+	-	0	+	3(8)
A_Q	+	-2	+	+	-2	+	+	-2	+	+	-2	+	3(24)
B	-	-	-	-	-	-	+	+	+	+	+	+	3(12)
C	-	-	-	+	+	+	-	-	-	+	+	+	3(12)
BC	+	+	+	-	-	-	-	-	-	+	+	+	3(12)
A_LB	-	0	+	+	0	-	+	0	-	-	0	+	3(8)
A_LC	+	0	-	-	0	+	+	0	-	-	0	+	3(8)
A_LBC	-	0	+	+	0	-	+	0	-	-	0	+	3(8)
A_QB	-	+2	-	-	+2	-	+	-2	+	+	-2	+	3(24)
A_QC	-	+2	-	+	-2	+	-	+2	-	+	-2	+	3(24)
A_QBC	+	-2	+	-	+2	-	-	+2	-	+	-2	+	3(24)
Total	+	+	+	+	+	+	+	+	+	+	+	+	3(8)

A fuller discussion of confounding in factorial experiments may be found in Yates (1937) "Design and Analysis of Factorial Experiments".

Problem VII-1. What design would be most appropriate given the following information?

Only nine roasts can be cut from each animal. The experimenter wishes to study the effect upon tenderness of freezing and storage temperatures and length of storage of roasts. He uses the following in all combinations:

Storage temperatures	0, 10, and 15 degrees
Freezing temperatures	0, -10, and -20 degrees
Length of storage	30, 90, and 180 days

Write out the breakdown of the total degrees of freedom and set up the design. What would the design and analysis be if only six roasts per animal were available and only two freezing temperatures, 0 and -20, were of interest?

Problem VII-2. The following is a field plan for a 2^3 factorial experiment. What effects are confounded in each of the replicates?

I		II		III	
ac	c	ab	c	(1)	bc
(1)	abc	a	(1)	ab	a
bc	b	abc	bc	c	b
ab	a	ac	b	abc	ac

Write out the subdivision of the total degrees of freedom in the analysis of variance.

Problem VII-3. For a 2^3 factorial experiment with four replicates, give the appropriate subdivision of the total degrees of freedom and the field design for

(i) a randomized incomplete blocks design in which the AB interaction is completely confounded in all replicates

(ii) a randomized incomplete blocks design in which the
ABC effect is confounded in replicate 1
BC effect is confounded in replicate 2

AC effect is confounded in replicate 3
AB effect is confounded in replicate 4.

Problem VII-4. Compute the sums of squares for the linear and quadratic components for the factor a in example VII-1 and partition the sums of squares for the AB, AC, and ABC effects into single degrees of freedom. Is there any change in the interpretation of the effects? Compute the standard error for each of the 11 comparisons.

FACTORIAL EXPERIMENTS WITH MAIN EFFECTS CONFOUNDED SPLIT PLOT AND SPLIT BLOCK DESIGNS

by

W.T.Federer

VIII-1. The Split Plot Design

The very nature of the levels of one factor, say a, may be such as to exclude the use of small plots or units or the experimenter may know that these levels usually yield differently. In such circumstances the levels of factor a, a_0, a_1, ..., a_{n-1}, may be laid out in a randomized complete blocks, latin square or other design. Since the whole plots are large by necessity or design, it may be desirable to compare levels of another factor, say b, on each plot, the several levels b_0, b_1, ..., b_{q-1}, being allotted to the split or sub plots of each whole plot at random. Such an arrangement would compare to a factorial arrangement of the factors a and b with n and q levels, respectively, in which the main effect A with (n-1) degrees of freedom was completely confounded with incomplete block or whole plot differences. The analogy between the incomplete block designs in the preceding chapter and the split plot design, which is an incomplete block design, should now be apparent.

If the whole plots are laid out in a randomized complete blocks design with 3 = r replicates, 4 = n levels of the factor a, and 3 = q levels of the factor b, the field design and the breakdown of the treatment degrees of freedom would be as presented in Table VIII-1.

Since the levels of factor a are compared in three rep-

Table VIII-1. Field lay-out for a split plot design

Replicate I

a_3	a_1	a_2	a_0
b_2	b_2	b_1	b_1
b_0	b_0	b_2	b_2
b_1	b_1	b_0	b_0

Replicate II

a_1	a_0	a_2	a_3
b_1	b_0	b_0	b_1
b_2	b_2	b_1	b_2
b_0	b_1	b_2	b_0

Replicate III

a_1	a_3	a_0	a_2
b_2	b_2	b_0	b_1
b_1	b_0	b_2	b_0
b_0	b_1	b_1	b_2

Analysis of variance

Source of Variation	d.f.
Whole plots	
Replicates	2
A	3
Error (a)	6
Split plots	
B	2
A x B	6
Error (b)	16
Total	35

licates of a randomized complete blocks design, error (a) or the replicates x A interaction mean square is the appropriate error for comparing the variation among levels. Likewise, error (b), which is a composite of the interaction sums of squares for replicates x B and replicates x A x B, is the appropriate error mean square for testing the A x B interaction and for testing the B effect in some instances (see section 65, Fisher, Design of Experiments). The replicates x B and replicates x A x B effects are confounded with each other and are not separable as such. Even though the calculation of these two interactions is possible, they are estimates of the same quantity and hence should be pooled together in the error (b) sum of squares, which may appropriately be called a sub plot x replicates equals B x replicates sum of squares within whole plots or levels of factor a.

The above discussion brings up the question - how does this incomplete block design, the split plot, compare with a randomized complete blocks design with regard to the contrasts on the main effects and the interactions? In making this comparison several points need to be considered. The B and AB effects are usually estimated more accurately than the whole plot treatments or the A effects, since the variation within incomplete blocks is usually smaller than between the incomplete blocks. Also, the number of degrees of freedom available for whole plot comparisons is smaller than for sub or split plot comparisons. Since the average standard error of a difference is the same for both the incomplete blocks and randomized complete blocks design, there is no gain in accuracy by using the incomplete block design. The increased accuracy on the B and AB effects is obtained by sacrificing accuracy on the A effects. Also, both sets of comparisons have fewer degrees of freedom available for the error variances which makes the randomized complete blocks design somewhat superior for all comparisons.

The efficiency of the split plot design relative to the randomized complete blocks design on the B and AB comparison is

$$\frac{[(n-1)+(n-1)(r-1)]E_a+[(q-1)+(q-1)(n-1)+n(r-1)(q-1)]E_b}{(nqr-r)E_b} = \frac{E_e'}{E_b},$$

where n = number of whole plot treatments, q = number of split plot treatments, r = number of replicates, E_a = error (a) mean square, and E_b = error (b) mean square. On the other hand, the efficiency on the A effects or the whole plot comparisons would be decreased, the formula in this case being E_e'/E_a.

In some instances, depending upon number of replicates and the experimental conditions, the whole plots may be arranged in a latin square design. By so doing, the comparisons on the A effects or whole plot contrasts may be more precise than if the nq combinations of the factors a and b had been laid out in a randomized complete blocks design since the latin square design is often more efficient than the randomized complete blocks experiment. The breakdown of the total degrees of freedom in the analysis of variance would be:

Source of variation	d.f.	m.s.
Rows	n-1	E_r
Columns	n-1	E_c
A = whole plot treatments	n-1	
Error (a)	(n-1)(n-2)	E_e
B = sub plot treatments	q-1	
AB	(n-1)(q-1)	
Error (b)	n(r-1)(q-1)	E_b
Total	nrq-1	

If the rows (or columns) have been used as replicates in the randomized complete blocks design for whole plot treatments, the efficiency of the latin square arrangement would be

$$\frac{E_c + (k-1)E_e}{kE_e} = \frac{E_a}{E_e}.$$

The efficiency of this design for the whole plot treatments to what it would be had the nq combinations been laid out in randomized complete blocks design is E_e' / E_e, where E_e' is defined as

$$\frac{(n-1)E_a + n(q-1)E_b}{(nq-1)} = E_e'$$

Other variations of the split plot design would be to arrange the split plot treatments in each of the orders within the replicates if there were as many split plot treatments as whole plot treatments. If it is suspected that order of split plot treatments has an effect then this design might prove useful. On the other hand, if the magnitude of the order effect is small, then the ordering within replicates results in a decrease in the number of degrees of freedom associated with error (b). This would tend to make the comparisons less precise. For example, the illustrative design in Table VIII-1 could have been designed as in Table VIII-2 where the split plot treatments occupy all orders in the three replicates. Four of the 12 arrangements of a 3 x 3 latin square were selected at random and laid out in each of the four treatments a_o, a_1, a_2 , and a_3. The order within whole plot treatments must be used so as not to confound a part of the interaction AB. The breakdown of the total degrees of freedom in the analysis of variance is also presentin Table VIII-2.

Example VIII-1. During the spring of 1944 a seed germination test on 49 varieties of guayule was conducted in the greenhouse at Capitola, California. Four seed treatments were applied to lots of seed from the varieties. The treatments

Table VIII-2. Field lay-out for a split plot design with the whole plots in randomized blocks arrangement and the split plot in a latin square arrangement within whole plot treatments.

Replicate I

a_3	a_1	a_2	a_0
b_2	b_2	b_0	b_2
b_1	b_0	b_2	b_0
b_0	b_1	b_1	b_1

Replicate II

a_1	a_0	a_2	a_3
b_0	b_1	b_2	b_1
b_1	b_2	b_1	b_0
b_2	b_0	b_0	b_2

Replicate III

a_1	a_3	a_0	a_2
b_1	b_0	b_0	b_1
b_2	b_2	b_1	b_0
b_0	b_1	b_2	b_2

Analysis of variance

Source of variation	d.f.
Replicates	2
A = whole plot treatments	3
Error (a)	6
B = sub plot treatments	2
AB	6
Orders within treatments	8
Residual = error (b)	8
Total	35

are as follows:

(i) 1943 collected seed was threshed but not treated with sodium hypochlorite = b_0

(ii) 1943 collected seed was neither threshed nor treated with sodium hypochlorite = b_1

(iii) 1942 collected seed was not threshed but treated with sodium hypochlorite = b_2

(iv) 1943 collected seed was not threshed but treated with sodium hypochlorite = b_3

Since the seed treatments and the interaction of varieties and seed treatments were considered to be more important than variety mean germinations, a split plot design with varieties as whole plots and with six replicates was used. The whole plot was a greenhouse flat subdivided into four split plots. One hundred seeds were planted in each split plot. The data recorded were number of plants emerged.

For an illustrative example eight varieties, a_0, a_1..., a_7, were selected from the first three replicates of this planting. The number of plants emerging from 100 seeds and the variety and seed treatment designation are recorded in Table VIII-3 for the eight varieties in the three replicates. The necessary totals for the analysis of variance are given in Table VIII-3 also.

The correction term is

$$\frac{X_{...}^2}{\text{total no. of plots}} = \frac{2429^2}{8(3)(4)} = 61{,}458.76 = CT.$$

The total sum of squares is

$$12^2 + 10^2 + \ldots + 11^2 + 15^2 - CT = 98{,}195 - CT = 36{,}736.24.$$

The replicate sum of squares is

$$\frac{825^2 + 781^2 + 823^2}{8(4) = 32} - CT = 38.58 \text{ (2 d.f.)}.$$

The variety sum of squares is

$$\frac{296^2 + 322^2 + \ldots + 333^2}{4(3)=12} - CT = 763.16 \quad \text{(7 d.f.)}.$$

The variety by replicate or error (a) sum of squares is

$$\frac{97^2 + 89^2 + \ldots + 101^2 + 110^2}{4} - 38.58 - 763.16 - CT$$

$$= 63{,}637.75 - 38.58 - 763.16 - 61{,}458.76 = 2{,}178.99 \quad \text{(14 d.f.)}.$$

The seed treatment sum of squares is

$$\frac{1340^2 + 334^2 + 481^2 + 274^2}{3(8) = 24} - CT = 30{,}774.28 \quad \text{(3 d.f.)}.$$

The treatment by variety sum of squares is

$$\frac{199^2 + 190^2 + \ldots + 30^2 + 36^2}{3} - CT - 763.16 - 30{,}774.28$$
$$= 2{,}620.13 \text{ (21 d.f.)}.$$

The error (b) or treatment by replicate plus treatment by replicate by variety sum of squares with 2(3+21) = 48 degrees of freedom is

$$36{,}736.24-38.58-763.16-2{,}178.99-30{,}774.28-2{,}620.13=1{,}162.84.$$

Since some contrasts among the seed treatments were of interest, the three degrees of freedom for seed treatments were partitioned into individual degrees of freedom. The b_0 seed treatment was compared with the mean of the remaining three. This contrast accounted for the major proportion of the sum of squares for treatments, thus

$$\frac{[3(1340)-334-481-274]^2}{24(9+1+1+1)} = 29{,}829.03 .$$

The sum of squares for the comparison of the unthreshed untreated = b_1 mean with the mean of b_2 and b_3 is

$\dfrac{[2(334)-481-274]^2}{24(4+1+1)} = 52.56$, and for the comparison of the two unthreshed seed collections in different years treated with sodium hypochlorite is $\dfrac{(481-274)^2}{24(1+1)} = 892.69$.

Another comparison of interest would be to compare the treated and untreated samples for 1943 collected seed, thus:

$$\frac{(334-274)^2}{24(1+1)} = 75.00.$$

The interaction mean square for varieties by seed treatments is much more variable, $F = 124.77/24.22 = 5.15 > F_{01}$,

Table VIII-3. Number of plants germinating from 100 seeds for each of four seed treatments on each of eight guayule varieties in a split-plot design of three replicates and totals for the analysis of variance.

Seed treatment designation	
threshed, untreated	$= b_0$
unthreshed, untreated	$= b_1$
unthreshed, treated 1942	$= b_2$
unthreshed, treated 1943	$= b_3$

Variety designation	
Strain no.	Code
42438	a_0
42441	a_1
42444	a_2
42447	a_3
42457	a_4
42468	a_5
42471	a_6
42478	a_7

Replicate I

	a_0b_1 12	a_2b_3 10	a_3b_0 52	a_5b_2 28	a_4b_2 9	a_1b_1 26	a_7b_3 9	a_6b_1 12	
	a_0b_2 13	a_2b_0 51	a_3b_3 13	a_5b_3 14	a_4b_3 12	a_1b_2 27	a_7b_1 14	a_6b_2 26	
	a_0b_0 66	a_2b_1 8	a_3b_2 19	a_5b_1 8	a_4b_0 45	a_1b_0 77	a_7b_2 30	a_6b_3 15	
	a_0b_3 6	a_2b_2 20	a_3b_1 4	a_5b_0 59	a_4b_1 20	a_1b_3 15	a_7b_0 49	a_6b_0 56	
Total	97	89	88	109	86	145	102	109	825

Replicate II

	a_3b_2 16	a_6b_0 38	a_7b_3 15	a_4b_1 13	a_0b_3 12	a_1b_2 5	a_5b_1 8	a_2b_1 16	
	a_3b_1 15	a_6b_2 16	a_7b_0 41	a_4b_3 12	a_0b_0 63	a_1b_0 47	a_5b_2 32	a_2b_2 30	
	a_3b_3 9	a_6b_1 16	a_7b_2 28	a_4b_0 51	a_0b_2 13	a_1b_1 11	a_5b_3 21	a_2b_0 81	
	a_3b_0 40	a_6b_3 8	a_7b_1 20	a_4b_2 10	a_0b_1 10	a_1b_3 4	a_5b_0 66	a_2b_3 14	
Total	80	78	104	86	98	67	127	141	781

Table VIII-3 continued

Replicate III

	a_6b_3 7	a_7b_2 36	a_5b_0 49	a_4b_2 12	a_3b_2 7	a_2b_2 29	a_0b_1 13	a_1b_1 18	
	a_6b_2 24	a_7b_1 25	a_5b_2 29	a_4b_0 52	a_3b_0 59	a_2b_1 14	a_0b_3 7	a_1b_0 66	
	a_6b_1 16	a_7b_0 54	a_5b_3 16	a_4b_1 16	a_3b_1 11	a_2b_3 10	a_0b_0 70	a_1b_2 11	
	a_6b_0 45	a_7b_3 12	a_5b_1 8	a_4b_3 11	a_3b_3 7	a_2b_0 63	a_0b_2 11	a_1b_3 15	
Total	92	127	102	91	84	116	101	110	823

Variety and treatment totals

Treatments	Varieties - a_0	a_1	a_2	a_3	a_4	a_5	a_6	a_7	Total
b_0	199	190	195	151	148	174	139	144	1340
b_1	35	55	38	30	49	24	44	59	334
b_2	37	43	79	42	31	89	66	94	481
b_3	25	34	34	29	35	51	30	36	274
Total	296	322	346	252	263	338	279	333	2429

* *

than expected on the hypothesis of no interaction. The variety x treatment sum of squares may be partitioned into three sets of seven degrees of freedom, each corresponding to the interaction of the three contrasts with varieties. All are significant at the one percent level of probability even though the contrast of threshed with unthreshed accounts for a larger proportion of the interaction sum of squares. The calculation of these sums of squares is left as an exercise for the reader (see Chapter 15, Snedecor, p.46).

The correct error term for testing variety differences is error (a) <u>if it is assumed that these particular seed</u>

Table VIII-4. Analysis of Variance of data in Table VIII-3.

Source of Variation	d.f.	Sum of Squares	Mean Square
Whole plot analysis			
Replicates	2	38.58	19.29
Varieties = A	7	763.16	109.02
Error (a)	14	1,377.25	98.38
Split or sub-plot analysis			
Treatments = B	3	30,774.28	10,258.09
b_0 vs. $b_1+b_2+b_3$	1	29,829.03	
b_1 vs. b_2+b_3	1	52.56	
b_2 vs. b_3	1	892.69	
AB	21	2,620.13	124.77
A x b_0 vs. $b_1+b_2+b_3$	7	1,456.72	208.10
A x b_1 vs. b_2+b_3	7	632.94	90.42
A x b_2 vs. b_3	7	530.47	75.78
Error (b)	48	1,162.84	24.22
Total	95	36,736.24	

* *

<u>treatments are not a random sample of seed treatments but are the only ones of interest</u>. The F test of the hypothesis of no difference among varieties in the germination of 1200 seeds with the four seed treatments b_0, b_1, b_2, and b_3 is

$$F = \frac{109.02}{98.38} = 1.11$$ which is near the mean value of F

($E(F) = 14/12 = 7/6$). The replicate mean square is small but not significantly so, $F = \frac{98.38}{19.29} = 5.10$, which is less than the tabulated F (=19.42) value at the 5 percent level of probability.

The variety by seed treatment interaction is highly significant and, therefore, indicates that the varieties reacted differently to the four seed treatments. The correct error variance for testing the hypothesis of zero interaction is the error (b) mean square.

The choice of the experimental error variance for comparing the variation among mean germination for the four seed treatments depends upon the hypothesis being tested (see Fisher, 1942, sec. 65, and Chapter IV, p.13 to 14). If the eight varieties constitute a random sample from a population of guayule varieties and a single seed treatment is being recommended for all guayule varieties, then the AB interaction mean square is the appropriate error for making an F test, thus $F = \frac{10,258.09}{124.77} = 82.216 > F_{01} = 4.87$. The treatment mean yields being much more variable than expected under the null hypothesis, the contrast b_0 with the other three accounts for most of the variation but the contrast of b_2 with b_3 is significantly more variable than ordinarily expected. If, on the other hand, these eight guayule varieties represented the only varieties of interest and the recommended seed treatment is for the eight varieties, then the error (b) mean square equal to 24.22 is the correct error for comparing the seed treatment means. In either case the interpretation is the same. A third situation may arise in which it is assumed that the eight varieties constitute a random sample of guayule varieties but a different seed treatment will be recommended for each variety since there is known to be a variety by seed treatment interaction. In this case the correct experimental error is the error (b) mean square.

Cochran and Cox (1944) have given standard errors of a mean difference for the several comparisons that may be made among the 4 x 8 = 32 seed treatment and variety combinations. The standard error of a difference between two variety means on a split plot basis is, where E_a = error (a) and E_b = error (b) mean squares.

$$s_d = \sqrt{\frac{2E_a}{rq}} = \sqrt{\frac{2(98.38)}{3(4)}} = 4.049.$$

The standard error of a difference between two seed treatment means on a single plot basis, for the first hypothesis cited above, is

$$s_d = \sqrt{\frac{2(\text{interaction m.s.})}{n\ r}} = \sqrt{\frac{2(124.77)}{8(3)}} = 3.224$$

and for the second and third cases given above, is

$$s_d = \sqrt{\frac{2E_b}{nr}} = \sqrt{\frac{2(24.22)}{8(3)}} = 1.421.$$

The standard error of a difference between two b or seed treatment means at one level of the factor a or varieties is

$$s_d = \sqrt{\frac{2E_b}{r}} = \sqrt{\frac{2(24.22)}{3}} = 4.009.$$

The standard error of a difference between two a or variety means at the same level of the factor b or for the same seed treatment is

$$s_d = \sqrt{\frac{2[(q-1)E_b+E_a]}{rq}} = \sqrt{\frac{2[3(24.22)+98.38]}{3(4)}} = 5.348.$$

Cochran and Cox (1944) warn that all the above standard errors are appropriate <u>only if the a or whole plot treatments</u> have been randomized within the complete blocks. If a systematic arrangement of the varieties had been used, then only the second and third standard errors of a mean difference would be applicable. The fourth standard error of a mean difference would be appropriate if the individual errors comprising E_b were all estimates of the same error variance σ_e^2. The first and fifth standard errors would not be applicable for a systematic arrangement of the varieties. Cochran and Cox(1944) state that because of these complications that "systematic arrangements of the a (whole plot) treatments should be avoided wherever possible."

The efficiency of this split plot design relative to what it would have been had all 32 combinations been allotted at random to the 32 plots in each replicate on the B and AB comparisons is

$$\frac{(7+14)(98.38) + (3+21+48)(24.22)}{93(24.22)} = \frac{40.97}{24.22} = 169 \text{ percent}$$

and for the A comparisons is $\dfrac{40.97}{98.38} = 42$ percent. On the former comparisons a gain in precision of 69 percent was obtained while for the latter comparisons, the variety comparisons, this design was less than half as efficient as the randomized complete blocks.

Example VIII-2. Iowa is divided into four regions - North, North Central, South Central, and South - with three districts per region for purposes of corn yield trials. Since Iowa is approximately square in shape and the districts are roughly equal in area and shape, the state is divided into four strips, approximately equal in area, from north to south. District I is designated as the group of counties in northwestern Iowa, District II the northernmost group of counties in central Iowa, and District III the group of counties in northeastern

Iowa. The other districts, IV to XII, are set up in a like manner. Yield tests on corn are conducted annually in each district. All entries in the trials within a region are the same but may differ from region to region. The entries from year to year seldom remain the same although a few are tested over more than a one year period.

In Districts I and II six doublecross corn hybrids were tested in 1942 and also in 1943. The yield data for 1942 are given in Table VIII-5 and for 1943 under Problem VIII-2. The trials were grown on a farm (supposedly selected at random) within each district. Each farm is designated as a centre, place, or location and for year 1942 there is a sample of two locations. The design was a randomized complete blocks with four replicates in each district each year. The plot size was 2 x 10 hills of corn with three plants per hill. The data recorded were yield of ear corn per 2 x 10 hill plot. The replicates are numbered I, II, III and IV in District I and V, VI, VII, and VIII in District II since replicate I in District I has nothing in common with the first replicate in District II.

The analysis of variance for each of the districts and for the combined analysis is presented in Table VIII-6. The separate analyses should always be made prior to pooling the results. Hidden features of the data may be brought to light in the individual analyses that are obscured in the pooled analysis. Outside of computing the mean squares, there are no additional computations necessary for the individual analyses that are not required for the pooled analysis.

The sum of the individual correction terms minus the

Table VIII-5. Yield data (pounds of ear corn per 2 x 10 hill plot) for six doublecross corn hybrids planted in Districts I and II of Iowa in 1942. Systematic arrangement of the yields in the four reps.

Doublecross designation	District I Replicate Number I	II	III	IV	District I Total	District II Replicate Number V	VI	VII	VIII	District II Total	Doublecross totals
1-1	34.6	33.4	36.5	33.0	137.5	33.1	24.6	33.8	34.6	126.1	263.6
2-2	34.5	39.1	35.4	35.6	144.6	46.4	36.9	36.3	45.3	164.9	309.5
4-3	30.1	30.8	35.0	33.3	129.2	32.3	38.7	37.5	37.6	146.1	275.3
15-43	31.3	29.3	29.7	33.2	123.5	37.5	39.2	39.1	34.1	149.9	273.4
8-38	32.8	35.7	36.0	34.0	138.5	31.2	40.8	46.1	44.1	162.2	300.7
7-39	30.7	35.5	35.3	30.6	132.1	35.8	38.2	38.8	39.6	152.4	284.5
Total	194.0	203.8	207.9	199.7	805.4	216.3	218.4	231.6	235.3	901.6	1707.0

Table VIII-6. Analyses of variance of the data in Table VIII-5

Source of Variation	District I d.f.	s.s.	m.s.	District II d.f.	s.s	m.s.
Replicates	3	17.61	5.87	3	44.71	14.90
Hybrids	5	70.36	14.07	5	240.65	48.13
Error	15	56.27	3.75	15	287.33	19.16
Total	23	144.24	-	23	572.69	-
Correction for mean	1	27,027.88		1	33,870.11	-
Uncorrected sum of sqs.	24	27,172.12	-	24	34,442.80	-

Combined Analysis

Source of variation	d.f.	s.s.	m.s.
Locations	1	192.80	192.80
Reps within locations	6	62.32	10.39
Hybrids	5	191.51	38.30
Hybrids x locations	5	119.50	23.90
Hyb x reps within locations	30	343.60	11.45
Total	47	909.73	-
Correction for mean	1	60,705.19	-
Uncorrected sum of squares	48	61,614.92	-

overall correction for the mean yields the sum of squares attributable to years,

$$27{,}027.88 + 33{,}870.11 - 60{,}705.19 = 192.80,$$

with a single degree of freedom. The total sum of squares corrected for the mean is

$$27{,}172.12 + 34{,}442.80 - 60{,}705.19 = 909.73.$$

The variety x replicate within location or place sum of squares is the sum of sums of squares for the individual analyses, $56.27 + 287.33 = 343.60$, with $15 + 15 = 30$ degrees of freedom.

The replicates within location sum of squares is the sum of the replicate sum of squares for the separate analyses,

$17.61 + 44.71 = 62.32$, with $3 + 3 = 6$ degrees of freedom.

The variety within locations sum of squares, $70.36 + 240.65 = 311.01$, contains the variety or hybrid and the hybrid x location sums of squares. The former is

$$\frac{263.6^2 + 309.5^2 + \ldots + 284.5^2}{8} - 60{,}705.19 = 191.51$$

and the latter is $311.01 - 191.51 = 119.50$, where each sum of squares has five degrees of freedom. The sum of the above sum of squares should add to the total sum of squares within rounding errors.

Tests of significance may be made in the manner discussed under example VIII-1. If it is assumed that the fields on which the six hybrids were planted represent a random sample of fields or locations and it is desired to recommend one hybrid for the area comprised by Districts I and II, then the interaction mean square, 23.900, is an unbiased estimate of the experimental error for comparing the differences among hybrid means. The F test, $F = \frac{38.302}{23.900} = 1.60$, indicates little evidence for rejecting the null hypothesis. However, further inquiry into the data indicates that the relatively large, though not significant at the five percent level of probability, interaction mean square is mainly due to the relative difference in yields for hybrids 1-1 and 15-43 at the two locations. Hybrid 2-2 was highest at both locations and hybrid 8-38 was second highest at both locations.

The error variances at the two places were tested by Bartlett's χ^2 test for homogeneity of variances:

$$\chi^2 = 2.3026[30\log 11.453 - 15 \log 3.75 - 15 \log 19.16]$$
$$= 9.03 \text{ with one degree of freedom,}$$

indicating heterogeneity of the error variances. Despite this, the pooled error may be considered the best estimate of the local experimental error since the coefficients of variation,

$$\frac{24\sqrt{3.75}}{805.4} = 6 \text{ percent for District I}$$

and

$$\frac{24\sqrt{19.16}}{901.6} = 12 \text{ percent for District II,}$$

are expected to be about 8-12 percent for corn yield trials in Iowa and District I appears to have a smaller error mean square than would ordinarily be expected. Unless some explanation is available as to the reason for the difference in the two error variances the experimenter may justifiably regard the pooled error mean square, 11.453, as the appropriate one to use in making tests of significance.

In making recommeddations for the area for which a random sample of two fields are available, the standard error of a difference between two hybrid totals is

$$s_d = \sqrt{2(4)(2)(23.900)} = 19.555$$

and for making recommendations for each place separately is

$$s_d = \sqrt{2(4)(2)(11.453)} = 13.537.$$

VIII-2. The Split Block or Two-Way Whole Plots Design

In some cases treatments a_i and treatments b_j comparisons may be of relatively little interest compared to the interaction AB and it is difficult to conduct an experiment in the manner described for the split plot design as illustrated in example VIII-1. The sub-plot treatments may be laid out in strips across all whole plot treatments. A design of this type has been called a split block (Leonard and Clark, 1939), two-way whole plots (Cox, lecture notes, 1942), or a design with the sub-units in strips (Cochran and Cox, 1944). Leonard and Clark (1939, p.214-6) have given a numerical example on corn uniformity trial data illustrating the analysis for this design.

Two field plans and the breakdown of the degrees of freedom in the analysis of variance for each design are given in Table VIII-7(a) and Table VIII-7(b). The first table illustrates the design and analysis for an experiment in which the main effects are in randomized complete blocks designs with each of the levels of one factor running across all levels of the second factor. The comparisons among levels of both the a and b factors are made on three replicates and three replicates only. The standard error of a difference between two means on a plot basis among the a_i levels is

$$\sqrt{\frac{2\ E_a}{rq}} = \sqrt{\frac{2E_a}{9}}$$

and among the b_j levels is

$$\sqrt{\frac{2\ E_b}{rn}} = \sqrt{\frac{2\ E_b}{12}}$$

where r = number of replicates, q = number of levels of factor b and n = number of levels of factor a. In the second design the order of the b treatments within a replicate are taken into account. This type of design may be used to advantage where there is a gradient from one replicate to another and when the number of replicates equals the number of treatments. Since there are only two degrees of freedom associated with the error sum of squares in a 3 x 3 latin square it may be inadvisable to use this design for three replicates. The standard errors of a mean difference are obtained by the same formula given above for the design in Table VIII-7(a).

The F test of the A, B, and AB effects employs E_a, E_b, and E_c respectively, as the experimental error mean squares. It is expected that the error (c) mean square, E_c, will generally be smaller than E_a or E_b since it represents intrablock or intra whole plot variation while the other two represent inter block or inter whole plot variation. Thus, the AB interaction is estimated more precisely than are the main effects A or B. The increased accuracy on the AB interaction is obtained by sacrificing accuracy on the whole plot comparisons. If the nq = 12 treatments of Table VIII(a) had been completely randomized within each of the replicates, the A and B effects

Table VIII-7. Field designs and breakdown of degrees of freedom for a split block experiment with three levels of factor b, four levels of factor a, and three replicates

(a) Factors a and b in randomized complete blocks design

		a_3	a_1	a_0	a_2
	b_2				
Rep I	b_0				
	b_1				

		a_1	a_0	a_3	a_2
	b_2				
RepII	b_1				
	b_0				

		a_2	a_0	a_3	a_1
	b_1				
RepIII	b_2				
	b_0				

Source of variation	d.f.	m.s.
Replicates	2	
A	3	
Reps x A = Error(a)	6	E_a
B	2	
Reps x B = Error(b)	4	E_b
AB	6	
Error(c)	12	E_c
Total	35	

(b) Factor a in randomized complete blocks and factor b in latin square arrangement.

		a_3	a_1	a_0	a_2
	b_2				
Rep I	b_0				
	b_1				

		a_1	a_0	a_3	a_2
	b_1				
Rep II	b_2				
	b_0				

		a_2	a_0	a_3	a_1
	b_0				
RepIII	b_1				
	b_2				

Source of variation	d.f.	m.s.
Replicates	2	
A	3	
Reps x A = Error(a)	6	E_a
B	2	
Order with replicates	2	
Error(b)	2	E_b
AB	6	
Error(c)	12	E_c
Total	35	

would usually be more accurately estimated and the AB interaction less accurately than with the present design.

Despite the importance of some main effect comparisons, it may be impractical to design an experiment in any way other than in a split block design. In a pasture experiment at Albia, Iowa (Hughes, Iowa State College), the effect of eight methods of cultivation and four cutting treatments (time and height of cutting) was studied to observe the effect of the treatments on forage yields. Regular farm machinery was used in the experiment thereby necessitating the use of large plots. Four replicates of the 32 treatment combinations were used. The entire experiment would be spread over a large area if 32 plots were included in each randomized complete block. Therefore, it was decided to have smaller plots by using a split block design, the first replicate of which follows:

		Methods of cultivation							
		2	8	6	5	1	4	3	7
Treatments	b								
	c								
	a								
	d								

The use of smaller plots allow the choice of a more homogeneous area for the complete block and would require less forage to be weighed and handled. Another advantage of this type of design is that the minimum amount of turning of farm machinery is required.

The disadvantage of the above design is the same as for all split block designs, i.e., the main effects A and B are estimated with the least precision and the AB interaction with the most. It must be remembered that there are only four replicates on cutting treatments and on methods of cultivation.

VIII-3. The Split Split Plot Design

Several levels of three factors in all combinations (an n x p x q factorial arrangement of the factors) may be of interest to an experimenter. The factor a is of little interest b somewhat more, and c and the interactions with the factor c are quite important. The incomplete block containing the comparison of the levels of factor c and the levels of the interactions with c should be as homogeneous as possible since these comparisons are to be measured with the most precision.

The field design and analysis are presented in Table VIII-8. The levels of the factor a are randomized within each complete block or replicate. Within each level of factor a the levels of b are allotted to the split plots at random and then within each level of b the levels of the factor c are allotted to the split split plots at random.

Under the hypothesis that these are the only levels of the factors a, b, and c of interest, the experimental error for the A effects mean square is E_a, for the B and AB effects is E_b, and for the C, AC, BC, and ABC effects is E_c. In general it is expected that $E_c < E_b < E_a$ and that the precision with which the split split plot comparisons are measured is greater than for the other comparisons. The B and AB comparisons bear the same relationship to the A comparisons as they

Table VIII-8. Field design and breakdown of the degrees of freedom in the analysis of variance for a split split plot design of nqp = 4x3x2 = 24 combinations of the factors a, b, and c in three replicates.

Replicate I

a_3	a_1	a_0	a_2
c_0 b_0 c_1	c_0 b_0 c_1	c_1 b_1 c_0	c_0 b_1 c_1
c_1 b_1 c_0	c_0 b_2 c_1	c_1 b_2 c_0	c_1 b_2 c_0
c_1 b_2 c_0	c_1 b_1 c_0	c_0 b_0 c_1	c_0 b_0 c_1

Replicate II

a_1	a_0	a_3	a_2
c_1 b_0 c_0	c_0 b_1 c_1	c_1 b_0 c_0	c_1 b_2 c_0
c_1 b_1 c_0	c_0 b_0 c_1	c_1 b_2 c_0	c_0 b_1 c_1
c_1 b_2 c_0	c_1 b_2 c_0	c_1 b_1 c_0	c_1 b_0 c_0

Replicate III

a_2	a_0	a_3	a_1
c_1 b_1 c_0	c_0 b_1 c_1	c_1 b_0 c_0	c_0 b_2 c_1
c_1 b_0 c_0	c_0 b_2 c_1	c_1 b_1 c_0	c_0 b_1 c_1
c_0 b_2 c_1	c_0 b_0 c_1	c_0 b_2 c_1	c_1 b_0 c_0

Source of variation	d.f.	m.s.
Replicates	2	
A	3	
Reps x A = error(a)	6	E_a
B	2	
AB	6	
Error(b)	16	E_b
C	1	
AC	3	
BC	2	
ABC	6	
Error(c)	24	E_c
Total	71	

do in an ordinary split plot design.

The standard errors of a difference between two means o a split split plot basis (Cochran and Cox, 1944) are: for the comparison of two levels of factor a

$$\sqrt{\frac{2\ E_a}{qpr}}\ ,$$

for the comparison of two levels of factor b

$$\sqrt{\frac{2\ E_b}{npr}}\ ,$$

for the comparison of two levels of factor b at the same level of factor a

$$\sqrt{\frac{2\ E_b}{pr}}\ ,$$

for the comparison of two levels of factor a at the same level of factor b

$$\sqrt{\frac{2[(q-1)E_b + E_a]}{rqp}}$$

for the comparison of two levels of factor c

$$\sqrt{\frac{2\ E_c}{nqr}}$$

for the comparison of two levels of c at the same level of a

$$\sqrt{\frac{2\ E_c}{rq}}$$

for the comparison of two levels of a at the same level of c

$$\sqrt{\frac{2[q(p-1)E_c + (q-1)E_b + E_a]}{rq^2p}}$$

for the comparison of two levels of c at the same level of b

$$\sqrt{\frac{2\ E_c}{rn}}$$

and for the comparison of two levels of b at the same level of c

$$\sqrt{\frac{2[(p-1)E_c + E_b]}{rnp}}\,.$$

The split split plot design may be necessary because of the nature of the experimental material rather than because of the desire of the experimenter for more precision on some of the comparisons by sacrificing accuracy on others. If this is true, the experimenter may change the plot shape and incomplete block shapes in order to increase the accuracy on the other factors. For example, suppose that it is impossible to lay out the levels of a in any other than large plots. The experimenter can choose the shape of whole plots (usually long and narrow) and the shape of the replicate (usually square in the absence of any knowledge of soil variation) so as to have the best comparisons possible on the factor a. Or the factor b may be of considerable importance and in this case the whole plot should be as nearly square as possible with the split plots rectangular. This gives the best comparisons on the B and AB effects. In the third instance, if the c factor and interactions are of most importance then the split plot should be square or nearly so with the split split plots being rectangular in shape. From the above considerations, then, the experimenter may change the precision on certain comparisons by changing plot shape even though the experimental material requires a split split plot design.

Goulden (1939, p 151-159) illustrates the analysis for a design of this type with four replicates, two wheat varieties, (a_0 and a_1), ten split plot treatments composed of wet and dry applications of five seed dusts for root rot (b_0, b_1, B_2, ..., b_{10}) and the split plot divided in half one portion of which was inoculated with the organism for root rot of wheat and the other half was not inoculated (c_0 and c_1). The whole experiment was a nqp = 2 x 10 x 2 [in reality a 2(2x5)(2) factorial] factorial experiment.

Vittum (1948, see Query 11) has used the split block design to advantage in using variety yield trials to study the effect of fertilizers or insecticides on yields.

VIII-4. Some Variations of Split Plot Designs

Some slight modifications of the split plot design are illustrated in Queries 10 and 13. The number of replicates at the various locations were different for the examples in both queries but this feature does not complicate the analysis unduly. As long as the number of replicates for the treatments or varieties remains the same at each location, the feature of proportionality is not affected.

Queries 12 and 15 present a more complicated variation of the split plot and split block designs, respectively. In these cases the latin square arrangements are used for both the whole plot treatments and split plot treatments. These are but two of the designs involving latin square arrangements with the split plot design as used by M.T.Vittum of the Geneva Experiment Station. The analysis for the more complicated designs suggested by Vittum has not been solved as yet.

When the split plot treatments represent a factorial arrangement of treatments, the split plot treatments may be put into incomplete blocks within the whole plot. Confounding some split plot comparisons with incomplete blocks may increase the accuracy on the A or whole plot comparisons. Cochran and Cox (1944, p. 78-80) illustrate the method of confounding, design, and breakdown of the degrees of freedom for the case where the split plot treatments form a 2^3 factorial arrangement.

In some instances variation in split plot designs are due to errors in laying out the experiment. Examples of this have been found in the course of consulting. The design illustrated in Table VIII-9 was presented to the statistician as a split plot design. Upon further inquiry it was found that the "split plot treatments" or methods of application were laid out in strips across all whole plots or fungicides and that the arrangement of methods of application was systematic in the three replicates. This design should have been called a split block design with one set of whole plots arranged systematically in all replicates. Due to the error in laying out the experiment, one of the comparisons of most interest, methods of applications = B effect, was lost entirely as there is no suitable error for testing the B effect mean square. The A effect or fungicides = whole plot treatments are tested with the same precision as obtained in any split plot or split block design. The AB interaction is tested with the same precision as it is in the ordinary split block design. Thus, the net result of this design is that no information is available on the B effect, the A effect is

Table VIII-9. Example of a split block design with one of the whole plot treatments arranged systematically in the three replicates (Fungicides = a_0, a_1, a_2, ..., a_8; methods of application = b_0, b_1, b_2).

Rep I

	a_2	a_5	a_8	a_4	a_7	a_1	a_0	a_6	a_3
b_0									
b_1									
b_2									

Rep II

	a_1	a_7	a_4	a_8	a_0	a_3	a_2	a_5	a_6
b_0									
b_1									
b_2									

Rep III

	a_4	a_0	a_2	a_5	a_8	a_3	a_1	a_7	a_6
b_0									
b_1									
b_2									

Source of variation		d.f.	m.s.	
Replicates		2	-	
A = fungicides		8	-	F test
Reps x A = error(a)		16	E_a	
B = methods	confounded	6	-	
Reps x B				
AB		16	-	F test
AB x reps = error(b)		32	E_b	
Total		80		

compared in three replicates in a randomized complete blocks design with an estimated experimental error equal to E_a, and the AB effect is measured the most accurately and is tested with the error (b) mean square = E_b. The statistical consultant should be cautioned to always determine exactly what the design is rather than what it is supposed to be. Three designs of the above type were found within a single year and all were presented to the consultant as bona fide split plot designs.

The split plot design was used effectively by Gowe (Cornell, 1948-49) in a poultry breeding experiment to study the length of fertility of sperm in the oviduct of two strains of chickens, Cornell and Kimber. The breakdown of the total degrees of freedom was quite different from the ordinary split plot due to the nature of the experimental material. Two pens representing replicates were available. The whole plot treatments were eight cocks, four of the Kimber strain and four of the Cornell strain. The eight cocks were used in both pens and each cock was mated to four dams two of which were of the Cornell strain and the other two of the Kimber strain. Four different dams were used for each cock resulting in a total of 32 dams in each pen.

The breakdown of the total degrees of freedom for the design is given in Table VIII-10.

The split plot treatments were not the same for all whole plot treatments and the 16 degrees of freedom in the split plot analysis represents the failure of the two samples of two hens of the same strain in the pens mated with the same

Table VIII-10. Breakdown of degrees of freedom for Gowe poultry breeding experiment.

Source of variation	d.f.		m.s.
Whole Plot Analysis			
Pens	1		-
Cocks	7		-
Between strains		1	-
Within Cornell strain		3	-
Within Kimber strain		3	-
Pens x cocks = error(a)	7		E_a
Split Plot Analysis			
Between strains for dams	1		-
Strains dams x strains cocks	1		-
Error(b)	14		E_b
Within Split Plots Analysis			
Between two Cornell dams on same male in same pen	16		-
Between two Kimber dams on same male in same pen	16		-
Total	63		

* *

cock to react the same with regard to length of fertility of the sperm in the oviduct resulting in eight degrees of freedom for the samples of Cornell dams and eight for the Kimber.

After removing the effect due to strains of the dams and the interaction of strains dams by strains cocks, there are 14 remaining degrees of freedom for error(b) sum of squares which was regarded as the experimental error sum of squares. The within split plot sums of squares are obtained in the usual manner. The variability among dams and cocks in the Cornell strain was significantly largerthan in the Kimber strain.

Another variation of the split plot design may be introduced when the comparison among some split or split split plots represent dummy comparisons since the treatments are identical on both plots. Homeyer (lecture notes, 1946, Iowa State College) discussed an example involving two methods of application (c_0 and c_1), three fertilizers (b_0, b_1, and b_2) and four rates of planting (a_0, a_1, a_2, and a_3) planted in three replicates. The field design was a conventional split split plot design with the rates of planting as the whole plots, the fertilizers (equal to none, 100 lbs., and 200 lbs.) as the split plots, and methods of application as the split split plot. Clearly, it is impossible to make two methods of application of no fertilizer! The comparisons among these two split split plots treated alike represent intra-split plot variation and should be included in the error(c) sum of squares. The breakdown of the total degrees of freedom for a design of this type is as given in Table VIII-11.

In obtaining the BC interaction sum of squares only the levels of fertilizer b_1 and b_2 are used resulting in an interaction sum of squares with one degree of freedom. If three levels of b were used two degrees of freedom would be available for interaction but one of the two degrees of freedom represents a dummy comparison and is included in the error(c) sum of squares. Likewise, there are three degrees of freedom from six ABC interaction degrees of freedom which represent dummy comparisons. These three are included in error(c). The resulting error(c) sum of squares has 28 degrees of freedom instead of the 24 in the ordinary split split plot design. The BC and ABC interactions are evaluated on 2/3 of the plots rather

Table VIII-11. Breakdown of degrees of freedom for Homeyer application-fertilizer-planting experiment

Source of variation		d.f.	m.s.
Whole plot analysis			
Replicates		2	-
Rates of planting = A		3	-
Rates x reps = error(a)		6	E_a
Split plot analysis			
Levels of fertilizers = B		2	-
AB		6	-
Error(b)		16	E_b
Split split plot analysis			
Methods of application = C		1	-
AC		3	-
BC		1	-
ABC		3	-
Error(c)	1+3+24 or 12+16 =	28	E_c
Total		71	-

* *

than all the plots as they are in the ordinary split split plot design.

Problem VIII-1. Using Paulson's (see Chapter IV, p.7) formula obtain the approximate probability of obtaining an F value as large or larger than

$$F = \frac{10{,}258.09 \text{ (with 3 d.f.)}}{124.77 \text{ (with 21 d.f.)}} = 82.216$$

$$F = \frac{10{,}258.09 \text{ (with 3 d.f.)}}{24.22 \text{ (with 48 d.f.)}} = 423.538$$

$$F = \frac{29{,}829.03 \text{ (with 1 d.f.)}}{124.77 \text{ (with 21 d.f.}} = 239.072$$

$$F = \frac{52.56 \text{ (with 1 d.f.}}{24.22 \text{ (with 48 d.f.)}} = 2.170$$

Check the closeness of the approximate probability values with those obtained from the t tables, remembering F (1,nd.f.) $=t^2$(with n.d.f.).

Problem VIII-2. 1943 yield data were obtained on the same six corn hybrids as used in example VIII-2. The yields in pounds of ear corn from a 2 x 10 hill plot for the six hybrids from four replicates of a randomized complete blocks design are given below in a systematic arrangement of replicates and varieties for Districts I and II. Compute the analysis of variance for the above data. Compute the analysis of variance for years 1942 and 1943 for each district and obtain the the combined analysis over both districts and both years.

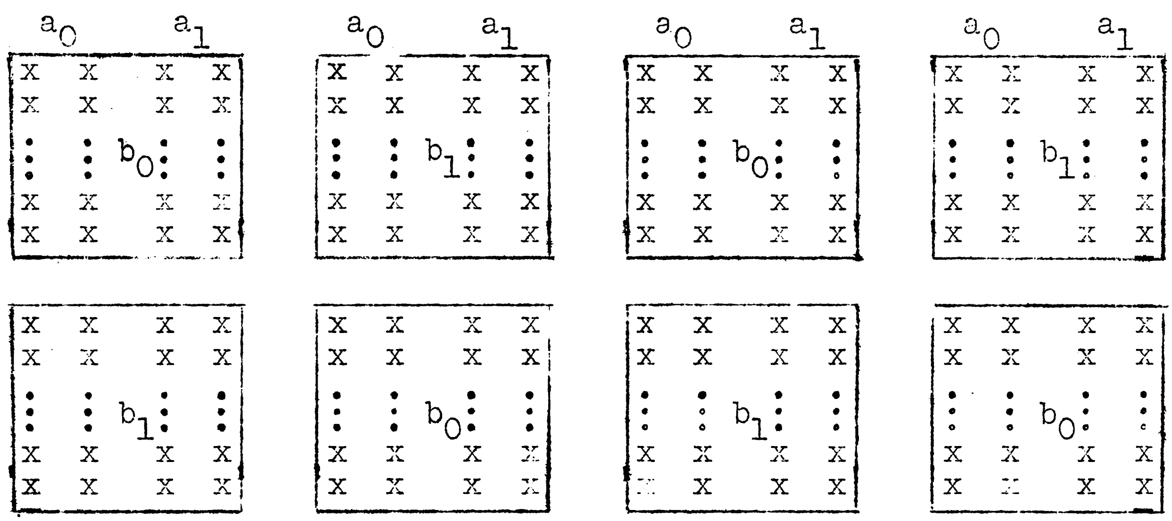
Table for Problem VIII-2. Systematic arrangement of replicates and varieties of corn hybrid yield data for Districts I and II for 1943

Doublecross designation	District I					District II					Doublecross totals
	Replciate Number					Replicate Number					
	I	II	III	IV	Total	I	II	III	IV	Total	
1-1	37.5	36.6	34.9	33.2	142.2	24.2	27.7	26.5	25.4	103.8	246.0
2-2	37.2	37.0	34.5	33.8	142.5	28.7	31.1	27.4	29.5	116.7	259.2
4-3	28.7	32.2	31.0	28.5	120.4	25.8	24.4	19.9	20.5	90.6	211.0
15-43	34.7	32.7	31.0	30.7	129.1	25.5	32.1	28.8	21.8	108.2	237.3
8-38	40.3	37.0	37.0	36.6	150.9	28.3	26.2	27.3	24.9	106.7	257.6
7-39	34.1	33.2	32.8	31.6	131.7	28.1	29.6	26.2	24.5	108.4	240.1
Total	212.5	208.7	201.2	194.4	816.8	160.6	171.1	156.1	146.6	634.4	1451.1

Problem VIII-3. Apply Tukey's test for ranked means to each of the four separate analyses at each district in each year, the data of example VIII-1, and the combined data over both years and both districts.

Problem VIII-4. Apply Tukey's test to the data in District I and District II, separately, for example VIII-2.

Problem VIII-5. The following design of an experiment conducted in 1948 at Cornell University was used in an orchard:



Rep I   Rep II   Rep III   Rep IV

The a_0 and a_1 treatments were systematically arranged in all four replicates. Likewise, the b_0 and b_1 treatments were arranged in the manner shown by design and not by chance. The a_0 treatment represents no dusting treatment and a_1 represents a dusting treatment. The b_0 and b_1 treatments (fertilizers) arranged in the manner shown because the experimenter wished them to be "representative". What effects are confounded and what effects are unconfounded? Is it correct to use the

F test to compare the dusting mean square, the fertilizer mean square, or the interaction mean square? Why or why not? Also, it was argued that no other design could have been used since the fertilizer and dusting applications were made in a farmer's orchard and one must use the farmer and regular farm machinery for these operations. Do you agree or disagree on the reasons for designing the experiment in this manner? Why or why not?

Problem VIII-6. In a grazing experiment with 24 dairy cows, two replicates in a pasture were available and it was desired to study the effect of three grazing treatments on equal areas of land - continuous grazing, 12 days of no grazing, and then 12 days of grazing, two days of grazing and then two days of no grazing - with four cows per grazing treatment. In addition, two of the four cows on each grazing treatment would be fed concentrates and the other two would not. Set up a design for such an experiment conducted over a three year period and "key" out the degrees of freedom in the analysis of variance. What are the correct error mean squares for testing the variation among the various means under the hypothesis stated by you?

CROSS-OVER DESIGNS

by

W. T. Federer

A design combining the features of latin squares and randomized complete blocks has been used for comparing two to four treatments in some dairy husbandry and biological studies. This design could also be used to advantage in psychological research. A group of latin squares may be less efficient than the <u>cross-over</u>, <u>change-over</u>, <u>switchback</u>, or <u>reversal</u> design. Like a latin square the cross-over design has two restrictions imposed on the randomization of the "treatments" to the "plots" or individual units. The treatments are all included in each replicate or group. The individual plots are rated from "superior" to "inferior" in each replicate or group. The second restriction is that each treatment must be applied to each kind or category, superior to inferior, in the replicates an equal number of times.

In the simplest case consider two treatments, A = supplemental feeding and B = no supplemental feeding, to 2p = 12 dairy cows. The 12 cows are first grouped into 6 = p pairs = replicates of two cows each so that the members of a pair are as nearly alike as possible. The members of each pair are then rated either as superior or inferior. The treatments A and B are then allotted to the members of a pair at random with the <u>restriction</u> that <u>half</u> of the "<u>superior</u>" cows will receive treatment A and the other half, treatment B. The same is true for the inferior cows. The experimental design for the six replicates or the six pairs of like cows would be of the following nature:

Rows	Columns (Pairs or replicates) I	II	III	IV	V	VI
Superior=1	B	B	A	A	B	A
Inferior=2	A	A	B	B	A	B

If the experiment had been conducted as three 2 x 2 latin squares the design would be of the following nature:

Rows	Square I		Square II		Square III	
	Columns					
Cow 1	A	B	A	B	B	A
Cow 2	B	A	B	A	A	B

where no rating of the two cows in a pair is made. The difference between the cross-over design and sets of latin squares is illustrated by the following breakdown of degrees of freedom in the respective analyses of variance.

Cross-over design

Source of variation	d.f.
Columns or pairs	5
Rows or superior vs. inferior	1
Treatments	1
Residual = error	4
Total	11

Three 2x2 latin squares

Source of variation	d.f.
Squares	2
Columns in squares	3
Rows in squares	3
Treatments	1
Tr. x squares=error	2
Total	11

The main difference lies in the fact that there are more degrees of freedom associated with the error sum of squares in the cross-over design and there is a less complete elimination of row effects. This design is quite suitable when row differences are approximately equal in all replicates in which case most of the row effects are removed by the single degree of freedom for rows and the error mean square is no larger than for the latin square. Also, there are more degrees of freedom available for error.

The cross-over design may be used for any number of treatments with the condition that the number of replicates must be a multiple of the number of treatments. Cochran and Cox (1944) state that it would probably be inadvisable to use the cross-over design in preference to a latin square if there are more than four treatments.

If in the example above the 12 dairy cows could be classified into two groups of six cows each with the cows in each group possessing no distinguishable traits with regard to being better or poorer, then a randomized complete blocks design of the following nature may be preferable:

	Treatment application	
Cow no.	Group I	Group II
1	A	B
2	A	A
3	B	A
4	B	B
5	A	A
6	B	B

The resulting breakdown of the total degrees of freedom is:

Source of variation	Degrees of freedom	
Groups	1	
Treatments	1	
Treatments x groups	1	9
Among treatments within groups	8	
Total	11	

For the case of no treatment x group interaction, the sum of squares may be pooled with the within group sum of squares resulting in an error mean square with nine degrees of freedom. If the variation among cows within a group is likely to be small, then the above design results in an error mean of approximately the same magnitude as the latin square error mean square but it would have nine degrees of freedom as compared to four for the cross-over design and two for the three 2x2 latin squares.

As a second example of a cross-over design suppose that the lactation period for dairy cows is divisible into four periods with "rest periods" in between to remove "carry-over" effect (Cochran, Autrey, and Cannon, 1946 give a method for adjusting for carry-over effects). Then for two treatments, A and B, applied to two pairs of two similar cows, where the members of a pair are classified as "better" and "poorer", the experimental design would be of the form:

	Pair I		Pair II	
Period	Better	Poorer	Better	Poorer
1	A	B	A	B
2	B	A	B	A
3	B	A	A	B
4	A	B	B	A

The breakdown of the total degrees of freedom in the analysis of variance is

Source of variation	Degrees of freedom
Periods	3
Pairs	1
Treatments	1
Treatment x period within pairs	6
Treatment x pair	1
Period x pair	3
Total	15

The last three sum of squares may be pooled, if they are estimates of the same variance, to obtain an error mean square with ten degrees of freedom.

H. L. Lucas, University of North Carolina, presented a paper entitled "Designs in Animal Science Research" at the Auburn Conference on Statistics Applied to Research in the Social Sciences, Plant Sciences, and Animal Sciences held on September 7-8, 1948. The following excerpts of this paper were taken from the Proceedings of this conference, pages 82-85:

" '<u>Designs for Change-Over Trials</u>'

As mentioned previously, the possibility of carry-over effects must be considered in setting up change-over trials. There are some situations, however, in which the investigator is quite certain from previous experience, or is rather certain from the nature of the treatments, that carry-over effects do not exist or are negligible. For this reason, the various designs will first be discussed assuming that no carry-over effects exist. Modifications introduced in order that carry-over effects may be efficiently estimated will then be taken up. It might be noted that covariance is not ordinarily used for the purpose of error reduction in change-over trials.

Randomized blocks designs:

As was mentioned previously, time trends in behaviour may sometimes not be expected. In this event, designs of the randomized blocks type may well be used in change-over trials. Here, each animal, or pen of animals, is a separate block and all treatments are administered to each animal (or pen) in a random order. The sequence for each animal is chosen independently of all others. The designs would seem to have special use in metabolism trials, especially with mature animals, and in studies where the response is measured by the blood level of some factor which ordinarily does not exhibit time trends.

Incomplete block designs:

Usually the number of treatments which a single animal can receive during the course of an experiment is limited by several factors. If the number of treatments to be studied exceeds the number which can be administered to a single animal, then where no time trends are expected, incomplete blocks designs are indicated.

The simple switchover or reversal design:

This is the simplest of those change-over designs which yield control on time trends. It involves two treatments only, and two sequences of treatment, as follows:

	Sequence	
	I	II
Period I	1	2
Period II	2	1

where the arabic numerals represent the treatments. The group of animals available is simply allotted at random, half to each sequence. This design might be considered as a 2 x 2 Latin Square. The only distinction between this and the Latin Square is that in the latter the animals would first be paired according to expected time trend and then one member of each pair would be allotted at random. The 2 x 2 Latin Square will be superior to the simple switch-over only if an effective reduction in error is accomplished by the pairing. I might note that in dairy cattle experiments, at least, the pairing may not be especially effective, but I do not wish to make a general statement on this point.

<u>The double-reversal design</u>:

This design, like the simple switch-over design, basically involves only two treatments and two sequences of treatments. Three periods are, however, used, as follows:

	Sequence I	Sequence II
Period I	1	2
Period II	2	1
Period III	1	2

I might note that the best analysis for this design is a little out of the ordinary, since it is made on the quadratic term of the time trend. That is, one first computes for each animal a quantity, Q, where

$$Q = Y_I - 2Y_{II} + Y_{III}$$

and Y_I = performance in period I

Y_{II} = performance in period II

Y_{III} = performance in period III

These quantities are then subjected to analysis. The reversal process may be continued through 4, 5, or even more periods. I do not see any particular advantage in this, however. Brandt (Iowa Res. Bul. 234, 1938) has described the analysis of the double-reversal and extended designs.

It might be noted that the double-reversal design yields very small experimental errors in the case of dairy cattle. A partial explanation for this is that the error is purely the variance of the quadratic term of the time trend, whereas, in the other change-over designs, the error involves the variance of the linear term. In dairy cattle, the variance of the linear term of the lactation curve is greater than the variance of the quadratic. The difference in these two variances does not appear, however, to explain all of the greater precision of the double-reversal. I am at a loss as to what explains the rest of it.

One would like to take advantage of the high precision of this design. It's use is rather limited, however, because basically it compares only two treatments. To overcome this difficulty to some extent, Seath (J. Dairy Sci. 27:159, 1944) devised a double reversal design for a 2 x 2 factorial set of treatments. The design is as follows:

	Sequence			
	I	II	III	IV
Period I	a1	b2	a2	b1
Period II	b2	a1	b1	a2
Period III	a1	b2	a2	b1

where a and b represent the two levels of one factor, and 1 and 2 the two levels of the other factor. The main effects of the two factors are tested against the pure quadratic term variance. The interaction, however, is tested with a larger error.

Latin Square designs:

These designs are sometimes referred to as round-robins by animal science men. The 2 x 2 design has already been mentioned. I think the larger Latin Square designs are more useful but there is a limitation to the size which may be used. This may vary for different studies. One does not like to make a period length too short for fear of missing treatment effects, yet, an animal may be used only so long on a given experiment. In work with milking dairy cattle the most useful Latin Squares appear to be the 3 x 3, 4 x 4, and 5 x 5. Occasionally, however, larger ones may have a place. For digestion studies I think the larger ones might be used very often.

To discuss certain points about these designs let us consider the 3 x 3 case, which is as follows:

	Animal I	Animal II	Animal III
Period I	1	2	3
Period II	2	3	1
Period III	3	1	2

The first thing to note is that there are several 3 x 3 squares, and in setting up an experiment, one of the several should be selected at random. (This is not true when carry-over effects are suspected. In this case only certain ones are usable.) In the case of the larger squares the number to choose from may be very great. A second point of importance is that the design will, except perhaps in the case of the larger squares, need to be replicated. This will be required to obtain sufficient degrees of freedom for error, and desirably low errors for treatment effects. Note that for a single 3 x 3 square there are only two degrees of freedom for error, and the error of a treatment mean is only one third the experimental error. When replicating, an independently selected square should be used for each replication.

There is another point which may be quite important. Animals may inherently vary quite widely with respect to the slope of their time trends in behaviour. This variation in slope is an important factor in experimental error. If the animals can be segregated into groups, such that each group is fairly uniform with regard to slope, then a large portion of the variation in slope can be removed from error as "square by period" interaction. In the case of milking cows, it is well known that the rate of decline in production rate is fairly highly correlated with initial production rate. Segregation into production level groups will, therefore, usually yield substantial reductions in error. In the case of the 3 x 3 square, the three highest producers would form the first square, the three next highest producers, the next square, and so on.

*   *   *   *   *

<u>On the matter of carry-over effects</u>:

If carry-over effects exist, an estimate of them can be made in all of the previous designs except the simple switch-over and the 2 x 2 Latin squares. Because of non-symmetry in the confounding of carry-over effects with direct effects and animals, the designs not only may provide poor estimates of the carry-over effects, but also the computations involved may be rather tedious. In the case of the Latin square designs this difficulty is easily overcome. Cochran et al (J. Dairy Sci. 24:937, 1941) have discussed this problem and have given designs and methods of analysis for the 3 x 3 and 4 x 4 squares. The fundamental requirement of symmetry is satisfied if one uses a set of orthogonal squares, rather than selecting a number of squares at random. In the case of the 3 x 3 design, the set consists of two squares; for the 4 x 4, three squares (it also happens that the requirement of symmetry may be satisfied by a single 4 x 4 square); and for the 5 x 5, four squares. The symmetry obtained may be illustrated by the 3 x 3 set.

	Square I			Square II		
Animal	I	II	III	IV	V	VI
Period I	1	2	3	1	2	3
Period II	2	3	1	3	1	2
Period III	3	1	2	2	3	1

Note that each diet is followed by every other diet a constant number of times. Thus, the degree of confounding of direct and residual effects is uniform. This makes for an easy analysis. There are, however, certain undesirable features about the design which might bear comment.

We note that carry-over effects do not occur in Period I. Therefore, the amount of replication is less for the carry-over effects than for the direct effects, the ratio of replication here being 2 to 3. Further, the carry-over effects are partially confounded with cows. This renders the effective replication on carry-over effects less than the actual. As a result of these two factors, the residual effects are estimated with considerably less accuracy than are the direct effects.

Another undesirable feature stems from the fact that the estimates of the direct effect of a given treatment and the carry-over effect of the same treatment are positively correlated. In many instances an investigator may be interested in the direct effect plus the carry-over effect. This quantity estimates the treatment effect which would have been observed during the second period if a given treatment were administered two consecutive periods instead of only one. It may actually be of more practical importance than either the direct or the carry-over effects per se. Since the variance of a sum is the sum of the variances and twice the covariances of the quantities summed, the variance of direct plus carry-over is disconcertingly high in these designs.

It happens that the covariance between direct and residual effects can be eliminated and the variances of the two effects made more nearly equal by the introduction of a single feature to the design. This is the addition of an extra period to the experiment as follows:

	Square I			Square II		
Animal	I	II	III	IV	V	VI
Period I	1	2	3	1	2	3
II	2	3	1	3	1	2
III	3	1	2	2	3	1
IV*	3	1	2	2	3	1

*Note that this may be considered as simply doubling the length of period III. Actually, which period is doubled should be determined at random and separately for each square. The doubled period is, however, considered as two periods in the analysis.

We note now that each treatment is followed by all treatments including itself. Thus the covariance between direct and carry-over effects is zero. Although the replication is still less for carry-over than for direct effects, the ratio has been increased to 3 to 4. Further the direct effects instead of the carry-over effects are now confounded with cows. This makes the effective replication ratio closer to one; for this design it is 3 to 3.72."

Cochran and Cox (1944, page 82) discuss the design and breakdown of the total degrees of freedom for the split plot design for which the sub-units or split plot treatments are in a cross-over design. Their (Cochran and Cox, 1944) discussion on page 82, section 4.8, of their mimeographed manuscript follows:

"4.8 Sub-unit treatments in a cross-over design.

This variant of the previous design (section 2.5) may be used when the number of replicates is a multiple of the number of sub-unit treatments. Suppose that there are six replicates of the design in table[VIII-2]On the six units which receive $\underline{a_0}$, the treatments $\underline{b_0}$, $\underline{b_1}$, $\underline{b_2}$, are arranged in a cross-over design (section 2.5), and similarly for $\underline{a_0}$, $\underline{a_1}$, ... with a new randomization for each whole-unit treatment.

Each whole-unit treatment contributes two degrees of freedom for rows as against four for the Latin squares. If the differences among rows are substantially constant in all six replicates, the cross-over design is preferable, since more degrees of freedom are available for error (b). When there are only two sub-unit treatments the cross-over design provides twice as many degrees of freedom as the Latin squares. The Latin squares may give a smaller error (b) if the row differences change from one replicate to the next. Thus the choice between the two designs depends on (i) the amount by which the differences among rows vary from replication to replication and (ii) the relative numbers of degrees of freedom for error(b).

The sub-division of the sub-unit degrees of freedom for the cross-over design is as follows:

Table 4.14 Partition of sub-unit degrees of freedom for a cross-over design

	d.f.
Rows	$\alpha(\beta-1)$
Sub-unit treatments	$(\beta-1)$
Sub-unit x whole-unit treatments	$(\alpha-1)(\beta-1)$
Error (b)	$\alpha(\beta-1)(r-2)$
Total	$r\alpha(\beta-1)$

Notation: α = number of whole unit treatments
β = number of sub-unit treatments
r = number of replicates."

For a more complete discussion of this design, the reader is referred to:

Brandt, A.E. Tests of significance in reversal or switch-back trials. Iowa Agric. Exp. Sta. Res. Bul. No. 234, 1938.

Fieller, E.C. The biological standardization of insulin Suppl. Jour. Roy. Stat. Soc., 7:1-64, 1940.

Cochran, W.G., Autrey, K.M., and Cannon, C.Y. A double change-over design for dairy cattle feeding experiments. Jour. Dairy Sc. 24:937-951. 1941.

Seath, D.M. Jour. Dairy Sc. 27:159, 1944.

Lucas, H.L. Designs in animal science research. Proc. Auburn Conf. on Stat. Applied to Res. in Soc. Sc., Pl. Sc., and Animal Sc., p 77-86, 1948.

* *

Problem IX-1. Complete the computations suggested in examples 15.22 and 15.23, Snedecor, Statistical Methods, 1946, and discuss the results of the two experiments involved.

Problem IX-2. Complete the design for the 24 cycles suggested by Cochran, Autrey, and Cannon, Jour. Dairy Sc. 24:949, 1941.

Problem IX-3. Run the analysis for the experiment cited in the above reference, ignoring the fact that one of the cows in group 3 became sick. Compare your results with those of Cochran, Autrey, and Cannon.

GRAECO, QUASI, PLAID, HALF PLAID, and OTHER VARIATIONS OF THE LATIN SQUARES

by

W. T. Federer

X-1. Latin Squares With Split Plots

Yates (1937, p.81) illustrates the use of a 7x7 latin square to compare 14 varieties. In each cell of the latin square a pair of varieties are included. Thus, the resulting design is a 7x7 latin square with split plots of two varieties. The comparison of varieties appearing together as pairs and of varieties which are not paired in split plots are not, in general, of equal accuracy. Precision on the former comparisons is gained by sacrificing accuracy on the latter group. Yates (1937, p.82) has suggested that the graeco latin square (in the following section) may be used to avoid the complication described above.

In the event that the experimenter has several families or groups = n (from 5 - 10, preferably) with several individuals = q per family and the comparisons among lines in different families is not as important as the comparison among lines within a family, then the latin square design with split plots may be used to advantage. Suppose that 5 = n families with 10 = q lines per family are to be compared in a 5x5 = kxk latin square with split plots of 10 plots. The family is the whole plot. The breakdown of the total degrees of freedom in the analysis of variance is presented in Table X-1.

Table X-1. Breakdown of the total degrees of freedom for five families in a latin square design with split plots of ten lines.

Source of variation	Degrees of	Mean square
Whole plot analysis		
Rows	4	
Columns	4	
Families	4	
Error(a)	12	E_a
Split plot analysis		
Among lines within family 1	9	
Among lines within family 2	9	
Among lines within family 3	9	
Among lines within family 4	9	
Among lines within family 5	9	
Error(b)	180	E_b
Total	249	

* * * * * * * * * * * * * * *

From the discussion in the previous chapter, the standard error of a difference for two line means of the same family is:

$$\sqrt{\frac{2\ E_b}{5}} = \sqrt{\frac{2\ E_b}{k}},$$

where k refers to the size of the kxk latin square used. The standard error of a difference between two line means not occurring together in a split plot is

$$\sqrt{\frac{2[(10-1)E_b + E_a]}{5(10)}} = \sqrt{\frac{2[(q-1)E_b + E_a]}{kq}},$$

where k and q are as defined previously.

Cochran (1940, p.19-20) has discussed the use of this design to some extent and suggests that if the latter comparison is of equal importance then one should choose a more efficient design (for example the lattice, Chapter XI) for the experiment.

X-2. Graeco Latin Squares

In an ordinary latin square every treatment occurs in every row and in every column. The treatments are usually designated by Latin letters, A, B, C, etc. A second set of treatments, say Greek letters = α, β, γ, etc, may be superimposed on the Latin letter set of treatments in the latin square in such a manner that the Greek letters appear once in every row, and once in every column, and once with every Latin letter. For example the sole 3x3 graeco latin square possible is (Fisher, Design of Expts., Sec. 35):

Aα	Bβ	Cγ
Bγ	Cα	Aβ
Cβ	Aγ	Bα

The partitioning of the eight degrees of freedom for the nine cells follows:

Rows	2
Columns	2
Latin letters	2
Greek letters	2

In order to observe the relationship of the above to confounding in factorial experiments let A = 0, B = 1, C = 2, α = 0, β = 1, γ = 2, then the graeco latin square becomes

00	11	22
12	20	01
21	02	10

The partitioning of the eight degrees of freedom then is:

	d.f.
Rows = comparison among $(AB^2)_0$, $(AB^2)_1$, and $(AB^2)_2 = AB^2$	2
Columns = " " $(AB)_0$, $(AB)_1$, and $(AB)_2 = AB$	2
Latin letters = comp. among $(A)_0$, $(A)_1$, and $(A)_2 = A$	2
Greek letters = " " $(B)_0$, $(B)_1$, and $(B)_2 = B$	2

Thus the AB^2 effect is completely confounded with row differences and AB with column differences in the above graeco latin square. The A effect is the Latin letters and the B effect the Greek letters. There is nothing left unspecified in the 3x3 graeco latin square resulting in zero degrees of freedom and zero sum of squares for residual or error variance since the four comparisons are independent. The above illustrates the orthogonality of the two latin squares involved in the 3x3 graeco latin square.

Though there is only one 3x3 graeco latin square there are 72 arrangements possible. There are 12 arrangements possible for a 3x3 latin square (see Chapter V) and the three Greek letters may be permuted among themselves in six ways, resulting in 72 arrangements.

In using the 3x3 graeco latin square one of the 72 arrangements should be selected at random. An alternative method is to select one of the 12 arrangements of the 3x3 latin squares and then assign the Greek letters to the treatments at random.

As stated in Chapter V there are four standard 4x4 latin squares and a total of 576 arrangements.

Of the four standard squares only one

A	B	C	D
B	A	D	C
C	D	A	B
D	C	B	A

yields a graeco latin square (Fisher, Design of Expts., Sec35) and this in two ways:

$A\alpha$	$B\beta$	$C\gamma$	$D\delta$
$B\delta$	$A\gamma$	$D\beta$	$C\alpha$
$C\beta$	$D\alpha$	$A\delta$	$B\gamma$
$D\gamma$	$C\delta$	$B\alpha$	$A\beta$

$A\alpha$	$B\beta$	$C\gamma$	$D\delta$
$B\gamma$	$A\delta$	$D\alpha$	$C\beta$
$C\delta$	$D\gamma$	$A\beta$	$B\alpha$
$D\beta$	$C\alpha$	$B\delta$	$A\gamma$

There are 144 = 4! 3! arrangements for the above 4x4 standard latin square, 24 ways of permuting the Greek letters among themselves, and two orthogonal graeco latin squares resulting in a total of 6912 = 144 x 2 x 24 arrangements.

Fisher (1942) forms the following 4x4 hyper graeco latin square (see Yates, 1937, p. 84) by letting one of the sets of Greek letters = the numbers or suffixes 1, 2, 3, and 4:

$A1\alpha$	$B2\beta$	$C3\gamma$	$D4\delta$
$B4\gamma$	$A3\delta$	$D2\alpha$	$C1\beta$
$C2\delta$	$D1\gamma$	$A4\beta$	$B3\alpha$
$D3\beta$	$C4\alpha$	$B1\delta$	$A2\gamma$

Again it is possible to set up the analogy between the hyper graeco latin square and confounding in factorial experiments. The 16 cells may be considered as the treatment combinations obtained from four factors each at two levels or a 2^4 factorial arrangement in which the following scheme of association with the 4x4 hyper graeco latin square is possible.

Effects in 2^4 factorial	Item of hyper graeco latin square	Degrees of freedom
A, BC, ABC	Rows	3
B, AD, ABD	Columns	3
C, BD, BCD	Latin letters	3
D, AC, ACD	Numbers	3
AB, CD, ABCD	Greek letters	3

Among the four row totals, etc. there are three degrees of freedom to correspond to the three degrees of freedom associated with A, BC, and ABC, etc.

In laying out a 4x4 graeco latin square one of the 144 arrangements of the latin square should be chosen. Then select one of the two types of graeco latin squares at random and assign the Greek letters to the treatments at random. This results in the same thing as selecting one of the 6912 arrangements at random. Likewise for the 4x4 hyper graeco latin square, there are 2x144x24x24 arrangements of which one should be selected at random. Alternatively, one of the graeco latin squares may be selected randomly and then the treatments assigned to the numbers at random.

Fisher (1942, sec. 35) states that of the 56 standard squares for the 5x5 latin square only six standard squares yield graeco latin squares and each of the six yield three different squares which do not differ merely in the randomization of the Greek letters. He (Fisher 1942) states that there are $3x6x24x120^2$ 5x5 graeco latin squares, $6x6x24x120^2$ 5x5 hyper graeco latin squares with Latin letters, Greek letters and numbers, and $6x6x24x120^4$ different hyper graeco latin squares for Latin letters, Greek letters, first number suffix, and second number suffix.

The 5x5 hyper graeco latin square design of Greek letters and one suffix may be likened to the Knut Vik square (Fisher, 1942, p.76) where each treatment (Latin letter) appears once in a row, once in a column, once in the diagonal in one direction and once in a diagonal in the opposite direction or to a 5^2

factorial with the following association among the effects and the Latin or Greek letters and the numbers:

Effect of 5^2 factorial	Item in hyper graeco latin square	Degrees of freedom
A	Latin letters	4
B	Greek letters = first diagonal	4
AB	Rows	4
AB^2	Columns	4
AB^3	Numbers = second diagonal	4
AB^4	Residual	4

Any other relationship among the effects and the categories of the hyper graeco latin square desired may be substituted for the one above. The relationship usually will depend upon the nature of the experimental material. The association presented above is merely to illustrate the relationship between the factorial experiment and the hyper graeco latin square.

In the event that a second set of numbers or suffixes (Fisher, 1942) is used, it would correspond to the AB^4 effect. Thus all items in the 5x5 latin square would be specified and there would be zero degrees of freedom and sum of squares for the residual or error.

Fisher and Yates (1934) have established the fact that no 6x6 graeco latin square exists by enumerating all the actual types of squares which occur. Fisher and Yates (1948) list the six orthogonal 7x7 latin squares, any pair of which yields a graeco latin square, the seven orthogonal 8x8 latin squares, and the eight 9x9 orthogonal latin squares. Cochran and Cox (1944) state that graeco latin squares have been constructed for all numbers of treatments from 3 to 13 with the exception of six and ten and that they will include the 11x11, 12x12, 13x13 graeco latin squares in their forthcoming book. It is suspected from the nature of the modulo notation that graeco latin squares may not be possible for the 14x14 and 15x15 since these numbers represent mixed primes, i.e. 2x7=14 and 3x5=15 just the same as do the numbers 2x3=6 and 2x5=10. However, since two is the only even prime number, combinations of two and other prime numbers such as 3, 5, 7, etc. may yield properties which are not common to numbers such as 3x5, 3x7, 5x7, etc. since these are all odd prime numbers. This phase of prime number theory needs investigation.

Dunlop (J. Agric. Sci., 1933) has suggested a 5x5 graeco latin square for use in pig feeding experiments. Suppose that five pigs each from five litters are available. Five feeding pens each with five feeding crates are to be used with one pig per feeding crate. Furthermore, five feeding treatments are to be compared. Using a 5x5 graeco latin square design, designate the litters as the rows, the feeding pens as the columns, the five feeding treatments as A, B, C, D, and E = Latin letters, and the feeding position in the pen as the Greek letters α, β, γ, δ, and ε. The extra control on feeding positions was thought necessary because the first and fifth crates differed in their construction and because the pigs in the end crates have less "company" while feeding. In addition the heaviest pig from each of the five litters was assigned to the first feeding pen, the second heaviest to the second etc. The columns then were used to control pen differences and weight differences simultaneously.

The partitioning of the total degrees of freedom follows:

Source of variation	Degrees of freedom	
Rows = litters	4	(k-1)
Columns = pens and weights	4	(k-1)
Greek letters = feeding positions	4	(k-1)
Latin letters = feeding treatments	4	(k-1)
Residual = error for comparing treat.	8	(k-1)(k-3)
Total	24	k^2-1

Tippett (1934, Manchester Stat. Soc.) has illustrated the use of a 5x5 hyper graeco latin square in determining which part of the winding weft in a cotton mill was defective. Fisher (1942, Design of Expt.,35.1) discusses this example in connection with his discussion on graeco latin squares.

As a further illustration of the use of graeco latin squares, Yates (1937, p. 82) has discussed the use of the 7x7 graeco latin square to compare the yields of 14 varieties. Each variety in a pair appears with one member of each of the other six pairs in the other rows and columns, corresponding to the relationship between Greek and Latin letters.

In all graeco latin squares randomizations may be obtained by randomizing all rows, all columns, all Latin letters, and all Greek letters. For larger graeco latin squares some of the arrangements will be excluded by this method but the number of arrangements possible will probably be large enough unless graeco latin squares are used extensively and it is desired to summarize the results from all graeco latin square designs.

Graeco latin square designs may be particularly effective in controlling or locating sources of variation in the fields of Soil Physics, Physiology, Psychology, Physics, Chemistry, and others.

X-3. Quasi Latin Squares

For some cases it is possible to use the latin square design in factorial experiments for which certain effects are to be confounded and to use latin squares which are smaller, than the total number of treatments. Yates (1937) has called this design a quasi latin square.

Suppose that it is desired to compare 2^3 treatments and to use the latin square design. Without any confounding, this would require an 8x8 latin square. Yates (1937) has given a design for 2^3 treatments in two 4x4 latin squares by arranging the four treatment combinations, 000, 110, 101, and 011 in one 4x4 latin square and the treatments 100, 101, 001, and 111 in the second 4x4 latin square. The three factor interaction ABC is completely confounded with the differences between squares. Yates (1937) notes that this design "has the defect that any differences in response to one of the factors [say a] in the two squares will give rise to an apparent interaction between the remaining factors"[b and c]. He (Yates, 1937) suggests that this defect may be overcome by interlacing the two squares. The paired columns of two squares are assigned to the column positions at random. For example, the two 4x4 latin squares might be arranged as follows:

Square I

000	011	110	101
101	110	011	000
011	000	101	110
110	101	000	011

Square II

001	010	111	100
111	100	001	010
100	001	010	111
010	111	100	001

and after interlacing the respective pairs of columns (or rows) some such field arrangement as the following might be obtained:

Pair I		Pair II		Pair III		Pair IV	
001	000	010	011	111	110	101	100
111	101	100	110	001	011	000	010
100	011	001	000	010	101	110	111
010	110	111	101	100	000	011	001

The analysis of variance (Yates, 1937) is the same as for the case in which the two squares have not been interlaced, thus:

Source of Variation	Degrees of freedom	
Squares = ABC	1	
Rows in squares	6	
Columns in squares	6	
Treatments in squares	6	
A		1
B		1
AB		1
C		1
AC		1
BC		1
Error in squares	12	
Total	31	

With the above arrangement it is possible to obtain a test of significance for the ABC effect. The comparison among the two members of a pair is the contrast of $(ABC)_0$ with $(ABC)_1$; there are four replicates for this comparison. The breakdown of the square and column degrees of freedom may more appropriately be presented in the following manner:

Source of variation	Degrees of freedom
Replicates or pairs	3
ABC	1
Error for ABC = reps. x ABC	3

The remainder of the analysis is as given before. Some information is available on all effects but the error for the ABC interaction is estimated with only three degrees of freedom and, hence, is not very reliable.

A part of the efficiency of the latin square may be lost by the interlacing (Yates, 1937) but this is probably negligible as compared to the defect of false interaction mentioned above.

If it is desirable to completely confound the ABC interaction with incomplete block differences as in the above example then some other scheme of confounding may be followed. Yates (1937) and Cochran and Cox (1944) suggest a design in which 1/2 of the relative information is retained on the interactions, AB, AC, BC, and ABC, and full information on the main effects, A, B, and C. The field layout and analysis is illustrated in the following example.

Example X-1. A 2^3 soil treatment test with split plot of two varieties of soybeans was conducted by Martin Weiss (Iowa State College and U.S.D.A., B.P.I., 1939) at Muscatine, Iowa. This example was used by Miss G. M. Cox in her lectures at Iowa State College and is discussed again in the mimeographed text "Experimental Designs" by Cochran and Cox(1944).

The 2^3 soil treatments were

000 = check or nothing added
100 = 3000 pounds of limestone
010 = 400 pounds of 0-20-0 phosphorous fertilizer
001 = 150 pounds of 0-0-50 potash fertilizer
110 = 3000 lbs. of limestone plus 400 lbs. of 0-20-0
101 = 3000 lbs. of limestone plus 150 lbs. of 0-0-50
011 = 400 lbs. of 0-20-0 plus 150 lbs. of 0-0-50
111 = 3000 lbs. of limestone plus 400 lbs. of 0-20-0 plus 150 lbs. of 0-0-50.

The eight treatments were tested in two 4x4 latin squares with the PK effect confounded with the first two rows in square I and with the last two columns of square II, the LP effect with the last two columns of square I and the first two rows of square II, LPK with the first two rows of square I and the first two columns of square II, and LK with the last two rows of square I and the last two columns of square II. The yields are in bushels per acre per variety and since the yields per soil treatment plot or whole plot represents the yields of two soybean varieties the data are double the bushel yield per acre. The data presented in Table X-2 represent whole plot totals. The analysis of variance is obtained in the manner described in Chapter VII. The unadjusted totals for the seven effects, the adjustments for confounding and the adjusted effect totals are presented in Table X-3.

To remove the confounded portion of the LP interaction, it is necessary to subtract the row and column effect from the unadjusted LP total thus,

$$35.7 - [239.0-240.1+255.8-236.4] = 35.7 - 18.3 = 17.4.$$

Table X-2. Eight soil treatments on soybeans compared in two 4x4 latin squares at Muscatine, Iowa, 1939 by Martin Weiss. (from G. M. Cox's lecture notes at Iowa State College). Interaction confounded is shown in table, treatment combination number is in parenthesis, yield is twice the acre yield in bushels, and analysis of variance.

Square I

	PK		LP			Totals
	(011) 58.6	(110) 69.3	(000) 61.5	(101) 62.7	LPK	252.1
	(111) 68.5	(010) 63.1	(001) 62.4	(100) 64.6		258.6
	(000) 63.5	(101) 67.3	(111) 66.0	(010) 56.5	LK	253.3
	(100) 58.1	(001) 58.1	(110) 65.9	(011) 52.6		234.7
Total	248.7	257.8	255.8	236.4		998.7

Square II

	LPK		LK			Total
	(100) 66.2	(101) 60.5	(010) 60.0	(011) 53.4	LP	240.1
	(001) 57.9	(000) 62.3	(111) 60.2	(110) 58.6		239.0
	(111) 64.1	(011) 61.3	(000) 63.2	(100) 59.2	PK	247.8
	(010) 58.4	(110) 61.8	(101) 67.0	(001) 61.9		249.1
Total	246.6	245.9	250.4	233.1		976.0

Analysis of Variance

Source of variation	d.f.	Sum of squares	Mean square
Squares	1	16.10	8.05
Rows	6	100.90	16.82
Columns	6	112.72	18.78
Main effects, L, P, and K	3	146.43	---
LP	1	18.92	18.92
LK	1	0.06	0.06
PK	1	0.33	0.33
LPK	1	8.27	8.27
Error	11	101.86	9.26
Total	31	505.59	--

Table X-3. Effect totals and adjustments for confounding.

Effect	Unadj. effect total	Adjustment for confounding	Adjusted effect total
L	65.3	none	65.3
P	-18.1	none	-18.1
K	-9.7	none	-9.7
LP	35.7	-18.3	17.4
LK	34.9	-35.9	-1.0
PK	-8.1	10.4	2.3
LPK	-4.3	-7.2	-11.5

In the manner described in Chapter VII it may be verified that the divisor for the adjusted interaction totals is 16. The sum of squares for the adjusted LP interaction is

$$\frac{17.4^2}{16} = 18.92$$

and the sum of squares for the main effects, which is unadjusted the divisor is 32 thus,

$$\frac{65.3^2 + (-18.1)^2 + (-9.7)^2}{32} = 146.43 .$$

Suppose that a two way LP table of means is desired. The adjustments for the means are obtained from Table X-3. The adjustment for the LP effect is

$$\frac{17.4}{16} - \frac{35.7}{32} = -0.03 ,$$

which is added to the means for 000 and 110 and subtracted from the means for treatments 100 and 010. The table of adjusted means for LK, PK, and LPK are obtained similarly.

Yates (1937) gives the plans for a number of quasi latin squares in his paper entitled "The Design and Analysis of Factorial Experiments": Cochran and Cox (1944) give a number of the same plans and some others. The description of the quasi latin squares available are given in Table X-4. Since the additional two plans given by Cochran and Cox (1944) are not generally available they are given in Example X-1 and Table X-5. From the discussions in Chapter VI and VII the reader should be able to set up schemes of confounding which are appropriate for the particular experiment in which he is interested.

For a more complete description of quasi latin squares, the reader is referred to the forthcoming book on Experimental Designs by Cochran and Cox and to Yates (1937).

X-4. Hlaf Plaid Latin Squares

Half plaid latin squares are in themselves nothing but quasi latin squares in which the main effects rather than the interactions have been confounded with rows or columns. Yates (1937) called them half plaid latin squares because of their relationship to the plaid latin squares discussed in the next section. In plaid latin squares one set of main effects is confounded with rows and another with columns and the result after randomization gives"a typical Scotch plaid" (Yates,1937) effect. Also, the half plaid latin square may be regarded as a combination of the <u>split plot</u> design and the <u>quasi latin square</u> while the plaid latin square is a combination of the <u>split block</u> design and the <u>quasi latin square</u>.

Table X-4. Quasi latin square plans given by Yates (1937) and Cochran and Cox (1944)

Quasi latin square and effects confounded	Table No. in Yates	Plan no. in Cochran and Cox
2^3 in a 4x4 latin squares with ABC confounded with columns and BC with rows	26	---
2^3 in two 4x4 latin squares with ABC completely confounded with squares	27	5.1b
2^3 in two 4x4 latin squares with AB, AC, BC, and ABC partially confounded with rows and columns	--	5.1a (see Ex.X-1.)
2^4 in an 8x8 latin square with ABCD confounded with columns and the three factor interactions partially confounded with rows	--	5.2
2^5 in an 8x8 latin square with interactions ACE, BCD, ABDE, ACD, BDE, ABCE, ABC, ADE, BCDE, ABD, BCE, and ACDE partially confounded with row and column differences	32	5.3
2^6 in an 8x8 latin square with various interactions confounded with row and column differences	33	5.4
3^3 in a 9x9 latin square with one of the interactions ABC, ABC^2, AB^2C, or AB^2C^2 completely confounded with columns and another with rows	50	5.5
3^4 in a 9x9 latin square with various interactions confounded with row and column differences	51	5.6
$4x2^2$ in an 8x8 latin square	--	5.7
3^2x2 in a 6x6 latin square	70	---
$6x2^3$ in a 6x6 latin square	79	---

Table X-5. Plan 5.2 from Cochran and Cox (1944) for 2^4 factorial treatments in an 8x8 latin square

ABCD

0010	1111	0100	1001	1000	0101	1110	0011	ABC
1101	0000	0111	0110	1011	1010	0001	1100	
0001	0110	0000	1111	0100	0011	1101	1010	ABD
0111	1001	1011	0101	1110	1100	0010	0000	
1000	0101	0010	1100	0001	1111	1011	0110	ACD
1110	1010	1101	0011	0111	0000	0100	1001	
0100	1100	0001	1010	0010	1001	0111	1111	BCD
1011	0011	1110	0000	1101	0110	1000	0101	

* * * * * * * * *

The notation for a half plaid latin square differs slightly from that used previously. Thus, in half plaid latin square notation $2x(2^2)$ means that the factor a is confounded with row (or column) differences and the factors b and c are the four treatment combinations in the 4x4 latin square. The systematic design for the A effect confounded with columns and the ABC effect with rows is:

$(A)_0$		$(A)_1$		
010	001	100	111	$(ABC)_1$
001	010	111	100	
000	011	110	101	$(ABC)_0$
011	000	101	110	

In setting out a $2x2^2$ half plaid latin square Cochran and Cox (1944) state that at least two squares = four replicates of eight treatments should be used and that the rows and columns should be permuted at random. Thus a field design would be of the nature:

Square I				Square II			
011	101	110	000	000	011	101	110
001	111	100	010	001	010	100	111
000	110	101	011	010	001	111	100
010	100	111	001	011	000	110	101

The statistical analysis would be:

Source of variation	Degrees of freedom two squares		Degrees of freedom s squares	
Squares	1		(s-1)	
Rows within squares	6		3s	
A		1		1
Error for A		5		(3s-1)
Columns within squares	6		3s	
ABC		1		1
Error for ABC		5		(3s-1)
B, C, BC, AB, AC	5		5	
Error for B, C, BC, AB, and AC	13		(9s-5)	
Total	31		16s-1	

Yates (1937) points out that it is "not permissible" to arrange the A effects in complete replicates but that "the rows and columns must be completely randomized among themselves, as in quasi-latin squares with confounded interactions".

Yates (1937) gives the plan for a half plaid latin square design for $4x2^3$ treatments (Table 80) in an 8x8 latin squares for $3x3^3$ treatments (Table 83) in a 9x9 latin square. In addition to these two half plaid latin squares Cochran and Cox (1944) give a number of other half plaid squares in plans 5.8 to 5.12. These plans have been reproduced in Table X-6, since they are not otherwise immediately available.

The reader is referred to Query 14 for an illustrative example of the breakdown of the total degrees of freedom in a $4x2^3$ half plaid latin square.

Table X-6. Plans and partitioning of total degrees of freedom for half plaid latin squares as presented by Cochran and Cox (1944) in plans 5.8 to 5.12, inclusive.

Plan 5.8 — $2x2^2$ design in a 4x4 half-plaid square

ABC

(-)	b	c	(1)	bc	
(=)	c	b	bc	(1)	
a	(1)	bc	b	c	A
a	bc	(1)	c	b	

k squares (2k replicates)

Squares		(k-1)
Rows		3k
A	1	
Error	(3k-1)	
Columns		3k
B, C, BC, AB, AC		5
Error		(9k-5)
Total		(16k-1)

Plan 5.9 — (3x2)x2 design in a 6x6 half plaid square

c	ab					
0	01	00	11	10	21	20
0	10	11	20	21	00	01
0	20	20	00	01	10	11
1	00	01	10	11	20	21
1	11	10	21	20	01	00
1	21	20	01	00	11	10
	Ia	Ib	IIa	IIb	IIIa	IIIb

Rows		5
C	1	
Error	4	
Columns		5
A, B, AB, AC		7
BC		1'
ABC		2'
Error		15
Total		35

Plan 5.10 — 3x(3x2) design in a 6x6 half-plaid square

Sq.I-AB,ABC partially conf.

a	bc						
0	20	10	00	21	11	01	
0	11	01	21	10	00	20	
1	00	20	10	01	21	11	A
1	21	11	01	20	10	00	
2	01	21	11	00	20	10	
2	10	00	20	11	01	21	
	Ia	Ib	Ic	IIa	IIb	IIc	

Sq.II-AB,ABC partially conf.

a	bc						
0	10	20	00	11	21	01	
0	21	01	11	20	00	10	
1	00	10	20	01	11	21	A
1	11	21	01	10	20	00	
2	20	00	10	21	01	11	
2	01	11	21	00	10	20	
	IIIa	IIIb	IIIc	IVa	IVb	IVc	

Square I alone (2reps)

Rows		5
A	2	
Error	3	
Columns		5
B, C, BCm AC		7
AB		4'
ABC		4'
Error		10
Total		35

Both squares (4 reps)

Squares		1
Rows		10
A	2	
Error	8	
Columns		10
B, C, BC, AC		7
AB		4'
ABC		4'
Error		35
Total		71

Plan 5.11 2x2^3 design in an 8x8 half-plaid square

ABCD confounded

	-	(1)	bc	bc	cd	b	c	d	bcd
	-	bc	(1)	cd	bd	d	bcd	b	c
	-	bd	cd	(1)	bc	bcd	d	c	b
A	-	cd	bd	bc	(1)	c	b	bcd	d
	a	b	c	d	bcd	(1)	bd	cd	bc
	a	c	b	bcd	d	bc	cd	bd	(1)
	a	d	bcd	b	c	cd	(1)	bc	bd
	a	bcd	d	c	b	bd	bc	(1)	cd

Rows		7
{A	1	
}Error	6	
Columns		7
B, C, -D		3
AB, AC, AD, BC, BD, CD		6
ABC, ABD, ACD, BCD		4
Error		36
Total		63

Plan 5.12 2x2^4 design in an 8x8 half-plaid square

ABD, BCE, ACDE confounded

	-	(1)	bd	bc	cd	ce	bcde	be	de
	-	bcd	c	d	bce	bde	e	cde	b
A	-	ce	bcde	be	de	(1)	bd	bc	cd
BCDE ABCDE	-	bde	e	cde	b	bcd	c	d	bce
	a	bc	cd	ce	bcde	be	de	(1)	bd
	a	d	b	bcd	c	cde	bce	bde	e
	a	be	de	(1)	bd	bc	cd	ce	bcde
	a	cde	bce	bde	e	d	b	bcd	c

Rows		7
{A	1	
{Error	6	
Columns		7
Main effects		4
Two-factor interactions		10
Three-factor "		8
Four-factor "		3
Error		24
Total		63

X-5. Plaid Latin Squares

As explained in the previous section in the plaid latin squares one set of main effects is confounded with row differences and a second set with columns differences. The notation is an extension of that for half plaid latin squares. For example in a $2x2x2^2$ the 4x4 plaid latin square is

	a_0		a_1	
b_0	1	2	3	4
	4	3	1	2
b_1	3	4	2	1
	2	1	4	3

where the numbers 1, 2, 3, and 4 refer to the 2^2 treatment combinations of the factors c and d. Using the factorial notation of Chapter VI the above 4x4 latin square is

	$(A)_0$		$(A)_1$	
$(B)_0$	0000	0001	1010	1011
	0011	0010	1000	1001
$(B)_1$	0110	0111	1101	1100
	0101	0100	1111	1110

and the effects confounded with the various columns are (indicated by the symbol x) given below:

Effect	Row number 1	2	3	4	Column number 1	2	3	4	d.f. confounded
A					x	x	x	x	1
B	x	x	x	x					1
AC	x	x	x	x					1
BD							x	x	1/2
ABC	x	x	x	x					1
ABD							x	x	1/2
BCD					x	x			1/2
ABCD					x	x			1/2
Total d.f.confounded -									6

Table X-7. 8x8 plaid latin squares for $2x2x2^4$ designs as given by Cochran and Cox (1944) as plans 5.13 and 5.14.

Plan 5.13 $2x2x(2^3)$ design in an 8x8 plaid square

B, ACDE, ABCDE confounded

		-	-	-	-	b	b	b	b
	-	(1)	e	cd	cde	ce	c	de	d
	-	ce	c	de	d	cd	e	(1)	cde
A	-	cd	cde	(1)	e	de	d	ce	c
BCD	-	de	d	ce	c	(1)	cde	cd	e
ABCD	a	e	(1)	cde	cd	c	ce	d	de
	a	c	de	d	ce	e	(1)	cde	cd
	a	cde	cd	e	(1)	d	de	c	ce
	a	d	ce	c	de	cde	cd	e	(1)

Plan 5.14 $2x2x(2^4)$ design in an 8x8 plaid square

B, ACDE, CEF, ABCD, BCEF, ADEF, ABDEF

		-	-	-	-	b	b	b	b
A	-	e	cde	df	cf	cd	(1)	cef	def
BEF	-	f	cdf	de	ce	cdef	ef	c	d
CDF	-	cd	(1)	cef	def	e	cde	df	cf
ABEF	-	cdef	ef	c	d	f	cdf	de	ce
ACDF	a	df	cf	e	cde	cef	def	cd	(1)
BCDE	a	de	ce	f	cdf	c	d	cdef	ef
ABCDE	a	cef	def	cd	(1)	df	cf	e	cde
	a	c	d	cdef	ef	de	ce	f	cdf

Breakdown of degrees of freedom for the above plans

Plan 5.13

Rows		7
A	1	
Error	6	
Columns		7
B	1	
Error	6	
Main effects		3
Two-factor interactions		10
Three-factor "		9
Four-factor "		3
Error		24
Total		63

Plan 5.14

Rows		7
A	1	
Error	6	
Columns		7
B	1	
Error	6	
Main effects		4
Two-factor interactions		15
Error (from high-order interactions)		30
Total		63

Thus effects B, AC, and ABC are completely confounded with row differences and A with column differences. Effects BD, ABD, BCD, and ABCD are partially confounded with column differences with one half relative information on each. The degrees of freedom confounded add up to the six degrees of freedom for rows and columns.

Yates(1937) gives a 9x9 plaid latin square for a $3x3x3^2$ factorial experiment (Table 83) and Cochran and Cox (1944) give an 8x8 plaid latin square for a $2x2x2^3$ factorial and for a $2x2x2^4$ factorial. These two 8x8 plaid latin squares have been reproduced in Table X-7.

As with all quasi factorial experiments the rows and columns should be completely randomized.

The reader is referred to Yates'(1937) "Design and Analysis of Factorial Experiments" for a further discussion of these designs.

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Problem X-1. In section 36 of Fishers' "Design of Experiments", it is suggested that the reader describe the fourth fascist and the Welsh lawyer. Specify all traits about the 16 passengers. Is your specification unique or is there more than one way of specifying the 16 passengers?

Problem X-2. In example X-1 show all calculations for the sums of squares, effects, and adjusted means.

INCOMPLETE BLOCK DESIGNS - THE LATTICES

by
W.T.Federer

XI-1 Introduction

The designs discussed thus far may not be suitable for comparing a large number of varieties or treatments. This is especially true if equal accuracy is desired on all comparisons. In response to this need for more efficient designs, Yates (19, 20, 21, 22, 23, 24, 25) set forward a whole group of incomplete block designs known as the lattices (12, 13, 14). These incomplete block designs make use of within block (intrablock) and between or among block (interblock) variances for the comparison of the various treatments. Also, different varieties are compared in incomplete blocks in the different replicates in contrast to the arrangement in a split plot design where a higher degree of accuracy on split plot comparisons are obtained by sacrificing accuracy on whole plot comparisons.

In constructing lattice designs the treatments or varieties are designated in the manner described for factorial arrangements. For example, $3^2 = k^2$ treatments are numbered 00, 01, 02, 10, 11, 12, 20, 21, and 22 and even though the nine varieties or treatments are not a factorial arrangement of the factors a and b, they may be likened to a factorial arrangement for purposes of design and analysis. The resulting main effects and interactions are called <u>pseudo main effects</u> and <u>pseudo interactions</u> to distinguish them from the factorial experiment. In the above example, then, the following pseudo effects are availalbe - A, B, AB, and AB^2 - with the three levels for each effect. In constructing the lattice designs, various effects are confounded with incomplete block (or row and column) differences in the different replicates with the confounding spread as equally as possible among the pseudo effects. The analogy between confounding in factorial experiments and lattice designs is apparent from the examples that follow.

The two chief disadvantages of lattice designs are the additional computations requried and the fact that lattice designs are not available for all numbers. However, the addition or subtraction of a few varieties is usually all that is required to obtain a lattice of the desired size. Also, computational procedures may be greatly simplified by the use of punched card machines (12) and other computational devices.

The chief advantage of lattice designs is the fact that a large number of treatments or varieties may be compared and the block size may be kept small. Another advantage of lattice designs lies in the fact that they may be analyzed as <u>randomized complete</u> blocks designs (22) even though the varieties were planted in an incomplete block design. The latter feature adds considerably to the utility of lattice designs and it means that in no case can the lattice designs be <u>less</u> efficient than the randomized complete blocks design [except for a small loss in information due to estimating the weights (3)].

Also, it should be pointed out that if there are large differences in the variety yields it may be inadvisable to use lattice designs since the variances may be related to the means and thereby invalidate the use of a pooled error. In such cases, the widely divergent groups should be included in separate experiments or in a split plot design with the groups as whole plots. Weiss and Cox (17a) state that "the partial confounding of variety differences with block effects makes it unwise to employ this type of design (lattices) when comparing varieties which have an extremely large range in yields".

XI-2. Classification of Lattice Designs

In general, lattice designs may be divided into two broad groups, Group I comprising the lattice designs (Table XI-1) which do not form complete replicates in the field and Group II, the lattices forming complete replicates. The former group is particularly useful when the number of treatments is less than 20 and if the experiment is conducted in a laboratory, greenhouse, or factory. For such cases the requirement that the treatment comparisons be in complete replicates may not be as essential as it is for field experiments. In field experiments it is not unusual to have missing plots and this would complicate the analysis considerably. Also, some of the more variable treatments may need to be excluded from the analysis and this is quite simple when the lattice designs are in complete replicates, i.e. a randomized complete blocks analysis is used on the varieties of interest, the remainder being excluded from the analysis.

Yates (25) discusses the analysis and has worked out some numerical examples for the balanced lattice designs in Table XI-1. The analysis has been developed for partially balanced lattice designs for the same numbers of treatments and incomplete block size as for the balanced group in Table XI-1 but with different numbers of replicates. These results (George Brown, Iowa State College, 1946) have not been published to date. It is possible that they may be included in the works of the Indian Statisticians, Bose, Mau, and Rao,in Sankya. (The last issues of Sankya were not available to check this point). The reader is referred to Yates' paper (25) for illustrative examples of the analysis for the balanced lattice designs which do not form complete replicates.

The second group of lattice designs, those which form complete replicates, comprise a large number of different types. Although not explicitly stated, a classification of the lattice designs of Group II was implied when Kempthorne and Federer (13,14) set forward the general theory for all prime-power lattice designs. The classification was extended to include all lattice designs of Group II and is presented in Table XI-2.

The term dimensional was used to refer to the power of the number k in the k^n lattice design. This term was adopted by Yates (22) in his discussion of the three-dimensional lattice known as the cubic lattice (12, 16) in which all the combinations of levels of three factors were likened to the intersections of the coordinates of a cube which is in three dimensions. Also, all combinations of two factors may be likened to the intersections of the lines on ordinary graph paper which is in two dimensions. Also, a n-dimensional lattice may be thought of as all intersections of the lines in n dimensional space.

The simplest lattice designs, those of Group I and the

Table XI-1. Balanced lattice designs not forming complete replicates (copied from Table 6.5 of Cochran and Cox, 1944, mimeographed)

Number of treatments	No. of units per incomplete block	Available no of replicates
4	3	3, 6, 9
5	2	4,8
5	3	6
5	4	4,8
6	2	5,10
6	3	5,10
6	4	10
6	5	5,10
7	2	6
*7	3	3,6,9
7	4	4,8
7	6	6
8	7	7
9	2	8
9	4	8
9	5	10
9	6	8
9	8	8
10	2	9
10	3	9
10	4	6
10	5	9
10	6	9
10	9	9
11	2	10
11	5	5,10
11	6	6
11	10	10
13	3	6
*13	4	4,8
13	9	9
15	3	7
17	7	7
15	8	8
16	6	6
16	10	10
19	3	9
19	9	9
19	10	10
21	3	10
*21	5	5,10
25	4	8
28	4	9
28	7	9
*31	6	6
37	9	9
41	5	10
*57	8	8
*73	9	9
*91	10	10
*133	12	12

*May also be designed as a Youden square.

Table XI-2. Classification of lattice designs in complete replicates. Number of entries and replicates required for the various designs.

Name of design	No. of entries	No. of replicates n = any integer	References on design & analysis
Two-dimensional with k^2 entries			
<u>One-restrictional</u>			
Simple or double lattice	k = any integer	$2n^{\oplus}$	2,3,8,12,13,14,16,26
Triple lattice	k = any integer	$3n^{\oplus}$	2,3,8,12,13,14,16,26
Quadruple lattice	k = 4,5,7,8,9,11,12,13,16,...	$4n^{\oplus}$	2,9,13,14
⋮			
Balanced lattice	k = 2,3,4,5,7,8,9,11,13,16,...	$(k+1)n^{\oplus}$	2,12,13,14,18,25,26
<u>Two-restictional</u>			
Semi-balanced lattice square	k = 3,5,7,9,11,13	$(k+1)n/2$	2,4,13,14,24,26
Balanced lattice square	k = 2,3,4,5,7,8,9,11,13	$(k+1)n^{\oplus}$	2,4,13,14,24,26
Unbalanced lattice square	k = 6,10,12	$3n^{\oplus}$	1,7,13,14
	k = any integer	number dependent on $k^{\oplus}$	13,14
Two-dimensional with pq entries			
<u>One-restrictional</u>			
Rectangular simple lattice	p and q = any integers, preferable to have p=q-1	2n	10,11,26
Rectangular triple lattices		3n	11a,26
Three-dimensional with k^3 entries			
<u>One-restrictional - k^2 blocks of k</u>			
Cubic lattice	k = any integer	$3n^{\oplus}$	13,14,22,26
Quartic lattice	k = a prime number	$4n^{\oplus}$	5, 13, 14
Quintic lattice	k = a prime number	$5n^{\oplus}$	5, 13, 14
⋮			
Balanced	k = a prime number	$(k^2+k+1)n^{\oplus}$	
<u>Two-restrictional k blocks of k of k</u>			
Unpublished to date although theory is given in references 13 and 14			
<u>Three-restrictional</u>			
kxk lattice square with split plot of k	k = any integer	$3n^{\oplus}$	13,14,15
k kxk lattice squares - unpublished although theory is given in references 13 and 14			

Table XI-2 continued

Four-dimensional with k^4 entries			
One-restrictional			
k^3 blocks of k entries	worked for k = prime number but probably suitable for k = any integer	4n⊕	6,13,14
k^3 blocks of k entries	k = prime number	any number	6,13,14
Two-restrictional			
k^2 blocks of k of k k blocks of k^2 of k k block of k of k^2	see references 13 and 14 for theory no worked examples		
Three-restrictional			
k^2 kxk lattice square kxk lattice squares with split plots of k^2 k block of k of k of k entries	see references 13 and 14 for theory no worked examples		
Four-restrictional			
kxk lattice squares with split plot of kxk lattice squares ⋮	see references 13 and 14 for theory no worked examples		
n-dimensional with p^n entries			
One-restrictional			
p^n entries in blocks of p^s –	a 2^5 in blocks of 2^2 example is being worked; otherwise see references 13 and 14 for general theory. $s = 1, 2, \ldots < n$. Minimum number of replicates equals n/s or nearest integer above if $n/s \neq$ integer		
n-dimensional with pqr...s entries –	nothing published to date although reference 26 mentions this design		

⊕ Some of the designs are partially balanced and some are balanced. Following the theory of references 13 and 14 the analysis for any number of replicates may be obtained without much extra trouble.

two-dimensional one-restrictional lattices of Group II are subject to a single restriction, i.e. that certain groups of varieties appear together in an incomplete block. Lattice square designs are subject to two restrictions, similar to latin squares, in that comparisons of specified groups of varieties are made within columns and within rows. The three and four restrictional designs are subject to three and four restrictions in the grouping of the varieties.

Within each of the n-dimensional s-restrictional designs there are balanced, partially balanced, and unbalanced lattice designs. Balanced lattice designs have the feature that every variety or treatment is compared with every other variety an equal number of times in the incomplete blocks (or rows and columns). In order for balance to be attained and for every variety to be compared with every other variety once and only once in the incomplete blocks, it is necessary to have k+1 replicates for k^2 varieties, k^2+k+1 replicates for k^3 varieties, k^3+k^2+k+1 replicates for k^4 varieties, etc. and k must be a prime number or power of a prime number.

In partially balanced lattice designs the varieties are compared in incomplete blocks either once or zero times. For unbalanced lattices comparisons in the incomplete block are unequal in number and are different than for the partially balanced lattices.

The term rectangular lattice has been used by Harshbarger (10, 11, 11a) to indicate that there are pq factorial combinations rather than k^2. Also, he (10, 11, 11a) proposed this term to contrast with the notation used by Goulden (9) who designates a lattice design for k^2 varieties as a <u>square lattice.</u> The present notation (Table XI-2) is not inconsistent with that already used even though it is in a slightly different form.

The two-dimensional one-restrictional partially balanced lattice design in two replicates has been known as the <u>simple lattice</u> or the <u>lattice</u>. In order to be consistent with the notation for the triple lattice (3) and the quadruple lattice (9) this design should be called a <u>double</u> lattice.

For the three-dimensional designs the notation cubic, quartic, etc. was used to be consistent with the prevalent use of the name cubic lattice.for the lattice design with k^3 varieties in sets of three replicates.

Youden (27) introduced another set of incomplete block designs known as <u>Youden squares</u>. The designs combine the features of latin squares and balanced incomplete block designs and may be classified as <u>incomplete latin squares</u>. Every variety appears once with every other variety in the incomplete block and the incomplete blocks are the columns in a latin square except that some of the rows are omitted. For example, the Youden square for seven varieties or treatments is

7	1	2	3	4	5	6
1	2	3	4	5	6	7
3	4	5	6	7	1	2

If four more rows were added a 7x7 latin square could be formed. Every variety <u>occurs with every other variety in one of the columns</u> and with all varieties in each row. The reader is referred to another source (27) for a discussion of this design. Reference to this design was necessary since some of the balanced incomplete block designs of Table XI-1 may also be Youden squares.

XI-3. Two-dimensional One-restrictional Lattices for k^2 Varieties

Probably the most understandable method of presenting the procedure for designing and analyzing lattice designs is with numerical examples. This is the method followed and a number of examples are presented.

Example XI-1. Double lattice with two replicates.

Suppose that is desired to set up a double lattice design for $k^2 = 3^2$ varieties or treatments. The nine varieties are numbered 00, 01, 02, 10, 11, 12, 20, 21, and 22. The levels of pseudo effects are obtained by summing the yields for certain varieties. The relationship between the varieties and levels of pseudo effects are:

Level of pseudo effect	Varieties
$(A)_0$	00 + 01 + 02
$(A)_1$	10 + 11 + 12
$(A)_2$	20 + 21 + 22
$(B)_0$	00 + 10 + 20
$(B)_1$	01 + 11 + 21
$(B)_2$	02 + 12 + 22
$(AB)_0$	00 + 12 + 21
$(AB)_1$	01 + 10 + 22
$(AB)_2$	02 + 11 + 20
$(AB^2)_0$	00 + 11 + 22
$(AB^2)_1$	02 + 10 + 21
$(AB^2)_2$	01 + 12 + 20

Since the pseudo main effects are no more important than the pseudo interactions, it matters little which are confounded with incomplete block differences. Therefore, for simplicity, let the A pseudo effect be confounded with incomplete block differences in one replicate and let B be confounded in the second replicate. The resulting X and Y arrangements (this notation is followed because of its prevalent use in statistical literature) are:

Arrangement X

00	01	02	$(A)_0$
10	11	12	$(A)_1$
20	21	22	$(A)_2$

Arrangement Y

00	10	20	$(B)_0$
01	11	21	$(B)_1$
02	12	22	$(B)_2$

The AB and AB^2 effects are unconfounded with incomplete block differences in both arrangements and the A and B effects are completely confounded in the X and Y arrangements. Some information is available on all treatment comparisons even though the comparisons among treatments within the incomplete block are more accurate than the comparisons of treatments not appearing together in an incomplete block.

The method of randomization is as follows:

(i) assign the numbers $ij = 00, 01, \ldots, 22$ to the varieties at random.

(ii) assign the levels of the effects or groups of varieties to the incomplete blocks at random

(iii) assign the varieties within the incomplete block to the plots at random.

After randomization a plan such as that presented in Table XI-3 might be obtained. The yields are synthetic and were chosen to facilitate the analysis. However, they might represent yields in pounds from a 2 x 10 hill plot for inbred lines of corn or some other such experiment. The arrangements were allotted to the replicates at random, the treatments or varieties making up the levels of the A pseudo effect in the X arrangement = $(X)_0$, $(X)_1$, and $(X)_2$ were assigned to the incomplete blocks at random and the varieties within an incomplete block group to the plots at random. The same procedure was followed in obtaining the field randomization for the Y arrangement. The incomplete block totals in replicate I are designated as $(Y)_0$, $(Y)_1$, and $(Y)_2$ corresponding to the $(B)_0$, $(B)_1$, and $(B)_2$ pseudo effects respectively in replicate I. Thus, the sum of the yields of varieties 02, 12, and 22 in Replicate I is the $(B)_2$ level of pseudo effect B for replicate I,

$$3 + 2 + 6 = 11 = (Y)_2.$$

In Table XI-4 the variety totals from replicates I and II are presented in such a way that the levels of the A effect may be obtained by summing over the rows and the levels of the B effect by summing over the columns. For example,

$$\begin{aligned}(A)_0 &= (8 + 6) + (3 + 2) + (3 + 4)\\ &= 14 + 5 + 7 = 26\end{aligned}$$

and

$$\begin{aligned}(B)_1 &= (3 + 2) + (7 + 3) + (3 + 2)\\ &= 5 + 10 + 5 = 20.\end{aligned}$$

The next step is to copy the incomplete block totals, i.e., $(X)_i$ and $(Y)_j$, from Table XI-3 in the appropriate places. $(X)_0$ is

Table XI-3. Yields per plot for a double lattice experiment with $k^2 = 3^2$ synthetic treatments in two replicates. Entries or varieties in parentheses.

Replicate I
(Y arrangement)

(00)	(20)	(10)	(02)	(12)	(22)	(21)	(11)	(01)
8	5	3	3	2	6	3	7	3

Block Σ $(Y)_0=16$ $(Y)_2=11$ $(Y)_1=13$

Replicate Σ 40

Replicate II
(X arrangement)

(21)	(20)	(22)	(10)	(11)	(12)	(01)	(02)	(00)
2	2	7	3	3	3	2	4	6

Block Σ $(X)_2=11$ $(X)_1=9$ $(X)_0=12$

Replicate Σ 32

Table XI-4. Total yields and other totals required for the analysis of the double lattice experiment presented in Table XI-3.

	variety numbers and totals			$(A)_i$	$(X)_i$	$(A)_i-2(X)_i$	c'_x
	(00) 14	(01) 5	(02) 7	26	12	2	.24
	(10) 6	(11) 10	(12) 5	21	9	3	.37
	(20) 7	(21) 5	(22) 13	25	11	3	.37
Totals $(B)_j$	27	20	25	72	32	8	
$(Y)_j$	16	13	11	40			
$(B)_j-2(Y)_j$	-5	-6	3	-8		0	
c'_y	-.61	-.73	.37				

Table XI-5. Analysis of variance for the data of Table XI-3

Randomized complete blocks analysis

Source of variation	d.f.	s.s.	m.s.
Replicates	1	3.56	3.56
Varieties or treatments	8	49.00	6.125
Residual	8	13.44	$1.680 = E'_e$
Total	17	66.00	

Double lattice analysis

Source of variation	d.f.*	d.f.	s.s.	m.s.
Replicates	1	1	3.56	3.56
Varieties (ignor.blocks)	k^2-1	8	49.00	6.125
Blocks (elim. var)	$2(k-1)$	4	8.22	$2.055=E_b$
Among $(A)_i-2(X)_i$	$(k-1)$	2	1.111	
Among $(B)_j-2(Y)_j$	$(k-1)$	2	7.111	
Intrablock = residual	$(k-1)^2$	4	5.22	$1.305=E_e$
Total	$2k^2-1$	17	66.00	

* General case

placed next to $(A)_0$, $(X)_1$ to $(A)_1$, etc. This means that the group of varieties which were together in the incomplete block are placed next to sum of the totals of the same varieties. The next step is to compute the quantities $(A)_i-2(X)_i$ and $(B)_j-2(Y)_j$ which correspond directly to the rkc_x and rkc_y values in various references (3, 12, 16). The second $(A)_i-2(X)_i$ value in Table XI-4 is $26 - 2(12) = 2$. The computations necessary to obtain the last column and row of Table XI-4 will be explained later.

The sums of squares for the randomized complete blocks analysis (top part of Table XI-5) should always be computed first since all the sums of squares are required for the analysis of a double lattice experiment. The latter analysis consists of partitioning the randomized complete blocks residual of error sum of squares into two portions

(i) that due to the variation among incomplete blocks after the varietal effect has been removed = blocks eliminating varietal effect

(ii) intrablock or that due to residual variation after removing varietal, complete blocks or replicates, and incomplete block effects.

The blocks (eliminating varietal effect) sum of squares is computed as the variation among the quantities

$(A)_i-2(X)_i$ and $(B)_j-2(Y)_j$ thus:

$$\frac{\sum_{i=0}^{2}[(A)_i-2(X)_i]^2}{k(l+1)} + \frac{\{\Sigma[(A)_i-2(X)_i]\}^2}{2k^2} + \frac{\sum_{j=0}^{2}[(B)_j-2(Y)_j]^2}{k(l+1)} - \frac{\{\Sigma[(B)_j-2(Y)_j]\}^2}{2k^2}$$

$$= \frac{2^2 + 3^2 + 3^2}{2(3)} - \frac{8^2}{18} + \frac{(-5)^2 + (-6)^2 + 3^2}{2(3)} - \frac{(-8)^2}{18}$$

$$= 1.111 + 7.111 = 8.222.$$

Upon further examination it will be discovered that the quantities $(A)_i-2(X)_i$ and $(B)_j-2(Y)_j$ represent the comparison of the totals of the varieties which appear together in an incomplete block with the totals of the <u>same</u> varieties in the replicate in which these varieties do not appear together in an incomplete block. Alternatively, these quantities represent the comparison of the levels of the pseudo effects in the replicates in which the effect is <u>unconfounded</u> with incomplete block differences with the levels in the replicate in which the effect is <u>confounded</u> with incomplete block differences. The latter way of viewing these sums of squares explains the divisor for the sums of squares. Each quantity compared is composed of k yields and the sums of squares of coefficients is $1^2 + (-1)^2 = 2$.

The intrablock error sum of squares is obtained by subtracting the blocks (eliminating variety) sum of squares from the randomized complete blocks error sum of squares, $13.44 - 8.22 = 5.22$ with $(2-1)(k^2-1) - 2(k-1) = (k-1)^2 = 4$ degrees of freedom.

The sums of squares and mean squares are presented in Table XI-5.

The average standard error variance of a mean difference between any two treatments for any lattice has been given in general terms by Kempthorne and Federer (13). Briefly, the average standard error variance of a mean difference is derived in this manner. The levels of the effects in the unconfounded replicates are estimated with variance σ_i^2/k^2 and in the confounded replicates with variance $(\sigma_i^2 + k\sigma_b^2)/k^2$ where E_e is an estimate of σ_i^2 and $2E_b-E_e$ is an estimate of $\sigma_i^2+k\sigma_b^2$. Therefore, the amount of information on intrablock comparisons is $w = 1/E_e = 1/1.305 = 0.7663$. and on interblock comparisons is $w' = 1/(2E_b-E_e) = 1/[2(2.055)-1.305] = 0.3565$.

In general the average standard error variance of a mean difference is

$$\frac{2}{k+1}\left\{\frac{2}{w+w'} + \frac{k-1}{2w}\right\} = \frac{2E_e}{k+1}\left\{\frac{2}{1+w'/w} + \frac{k-1}{2}\right\}$$

$$= E_e \left\{1 + \frac{2k}{k+1}\mu\right\} = 1.305 \left\{1 + \frac{2(3)}{3+1}(0.122)\right\} = 1.544,$$

where $\mu = \dfrac{w-w'}{k(w+w')} = 0.122.$

For the case of two replicates, the above is also the average effective error variance.

The efficiency of this double lattice relative to the randomized complete blocks design is the ratio of the two effective error variances in percent,

$$\frac{1.680}{1.544} = 109 \text{ percent or a}$$

gain in efficiency of nine percent. If the gain in efficiency is small (less than 10 to 15 percent), the resulting standard error and the unadjusted means should be used. If the gain in efficiency is larger than 15 to 20 percent the double lattice analysis should be used and the treatment or variety means should be adjusted. In the present example, the unadjusted means will differ little from the adjusted means due to the small gain in efficiency but the means will be adjusted for illustrative purposes.

The first step in obtaining adjusted means is to multiply the $(A)_i-2(X)_i$ and $(B)_j-2(Y)_j$ values by $\mu = \dfrac{w-w'}{k(w+w')} = 0.122.$ The resulting values are entered in the last column and last row, respectively, of Table XI-4. For example, the second c'_x value is $\mu[(A)_1-2(X)_1] = 0.122(2) = 0.24$

and the last c'_y value is $\mu[(B)_2-2(Y)_2] = 0.122(3) = 0.37.$

The second step in adjusting the means is to add corrections c'_x and c'_y to the corresponding total and divide by two, the number of replicates. The adjusted mean for variety 01 is $1/2[5 + 0.24 - 0.73] = 2.26$ and for variety 22 is

$1/2[13 + 0.37 + 0.37] = 6.87.$

The remainder of the adjusted means (Table XI-6) are obtained in a similar manner.

Table XI-6. Adjusted totals and means for the experiment in Table XI-3.

Variety number	Unadjusted totals	Sum of adjustments	Adjusted totals	Adjusted means
00	14	-.37	13.63	6.82
01	5	-.49	4.51	2.26
02	7	.61	7.61	3.80
10	6	-.24	5.76	2.88
11	10	-.36	9.64	4.82
12	5	.74	5.74	2.87
20	7	-.24	6.76	3.38
21	5	-.36	4.64	2.32
22	13	.74	13.74	6.87
Total	72	.03	72.03	36.02

In order to understand the various adjustments and sums of squares it is helpful to obtain these quantities in another manner. For the second method of analysis it is necessary to obtain the levels of the pseudo effects for each replicate (Table XI-7). These effects are obtained by adding the necessary variety yields for each. The $(AB)_1$ in replicate I is

$(AB)_1 = (10) + (01) + (22) = 3 + 3 + 6 = 12.$

The blocks (eliminating variety effect) sum of squares is the sum of squares of the differences of the levels of the effects confounded in one replicate and the corresponding level of the effect in the replicate in which it is unconfounded,

Table XI-7. Weighted and unweighted effects on a total means per k plot basis.

Level of effect	Rep. I*	Rep. II*.	Unweighted for both reps.	Weighted for both reps.
$(A)_0$	14	$\underline{12}$	13.0	13.3650
$(A)_1$	12	$\underline{9}$	10.5	11.0475
$(A)_2$	14	$\underline{11}$	12.5	13.0475
$(B)_0$	$\underline{16}$	11	13.5	12.5875
$(B)_1$	$\underline{13}$	7	10.0	8.9051
$(B)_2$	$\underline{11}$	14	12.5	13.0475
$(AB)_0$	13	11	12.0	12.0000
$(AB)_1$	12	12	12.0	12.0000
$(AB)_2$	15	9	12.0	12.0000
$(AB^2)_0$	21	16	18.5	18.5000
$(AB^2)_1$	9	9	9.0	9.0000
$(AB^2)_2$	10	7	8.5	8.5000
Total	160 $=(3+1)40$	128 $=(3+1)32$	144.0 $=\frac{(3+1)72}{2}$	144.0001

*Underline indicates that level of the effect is confounded with incomplete block differences in the specified replicate.

$$\frac{(14-12)^2 + (12-9)^2 + (14-11)^2}{3(1+1)} - \frac{(40-32)^2}{9(1+1)}$$

$$+ \frac{(11-16)^2 + (7-13)^2 + (14-11)^2}{3(1+1)} - \frac{(32-40)^2}{9(1+1)} = 8.22.$$

The intrablock error sum of squares may be obtained as the interaction of levels of the effects with the replicates in which the effects are unconfounded. The only effects unconfounded in more than one replicate are AB and AB^2. The interaction of levels of these two effects with replicates yields 2+2=4 degrees of freedom and a sum of squares of

$$\frac{11^2+13^2+12^2+12^2+9^2+15^2}{3 = k} - \frac{32^2+40^2}{9 = k^2} - \frac{24^2+24^2+24^2}{2k = 6} + \frac{72^2}{2k^2=18}$$

$$\frac{16^2+21^2+9^2+7^2+10^2}{3 = k} - \frac{32^2+40^2}{9 = k^2} - \frac{37^2+18^2+17^2}{2k = 6} + \frac{72^2}{2k^2=18} = 5.22.$$

The intrablock error sum of squares need not be obtained by subtraction but may be computed as above. The analysis of variance is the same as that presented in Table XI-5. The weights w and w' and the standard errors are computed similarly.

Before computing the adjusted means it is necessary to obtain the weighted levels of the pseudo effects. This weighting is necessary because the partially confounded effects are estimated with different variances in the two replicates. In the replicates where the effect is confounded with incomplete block differences, the levels of the effect are estimated with a variance $\sigma_i^2 + k\sigma_b^2 = 1/w'$. The unconfounded effects are estimated with variance $\sigma_i^2 = 1/w$. Weighting the level of the effects inversely to the variance with which it is estimated, the weighted levels of the effects are obtained. The weighted level of the $(A)_0$ effect is

$$\frac{w'(A)_{OX}+w(A)_{OY}}{w'+w} = \frac{0.3565(12)+0.7663(14)}{0.3565+0.7663} = 13.3650.$$

The remaining levels of effects are computed similarly and are given in the last column of Table XI-7.

The adjusted means are obtained by using the weighted effects in the last column of Table XI-7 thus

$$ij = \frac{[(A)_i+(B)_j]_{wt'd}}{k} + \frac{(AB)_{i+j}+(AB^2)_{i+2j}}{k} - \frac{(\text{Total})}{2k}$$

which for variety 00 is

$$\frac{13.3650 + 12.5875 + 12.0000 + 18.5000}{3} - \frac{72}{6} = 6.82$$

and for variety 02 is

$$\frac{13.3650 + 13.0475 + 12.0000 + 9.000}{3} - \frac{72}{6} = 3.80$$

The remaining adjusted means are computed similarly and should agree with those in the last column of Table XI-6 within rounding errors.

Example XI-2. Double Lattice with four replicates.

In the event that two or more sets of the double lattice experiment are planted, only one additional sum of squares is required for the analysis. The numerical example in Table XI-8 is for four replicates or 2 = q sets of a double lattice. The first set is X_1 and Y_1 and the second set, X_2 and Y_2. The method of analysis is general for 4, 6, 8, ..., 2q replicates. The double lattice experiment with 2, 4, 6, etc. replicates is partially balanced and if one of the arrangements is omitted the design becomes unbalanced resulting in slight complications in the analysis.

In designing a double lattice experiment with four replicates, different randomizations are required (see Table XI-8 for the field randomization) for the two sets. The X and Y arrangements of any set are kept together with the arrangements being allotted to the replicate at random.

The yield data for $k^2 = 3^2$ varieties in two sets = four replicates of a double lattice design are presented in Table XI-8. The incomplete block totals, $(X_1)_i = A_i$ effect in replicate II, $(Y_1)_j = (B)_j$ effect in replicate I, $(X_2)_i = (A)_i$ effect in replicate III, and $(Y_2)_j = (B)_j$ effect in replicate IV, and the replicate totals are given (Table XI-8).

Table XI-9 is similar to Table XI-4 except that the totals are from four replicates instead of two. The $(A)_i$ and $(B)_j$

Table XI-8. Yields from four replicates of a double lattice experiment. (Entry or variety numbers in parantheses.)

Replicate I
(Y_1 arrangement)

(00)	(20)	(10)	(02)	(12)	(22)	(21)	(11)	(01)
8	5	3	3	2	6	3	7	3

Block Σ $(Y_1)_0=16$ $(Y_1)_2=11$ $(Y_1)_1=13$

Replicate total - 40

Replicate II
(X_1 arrangement)

(21)	(20)	(22)	(10)	(11)	(12)	(01)	(02)	(00)
2	2	7	3	3	3	2	4	6

Block Σ $(X_1)_2=11$ $(X_1)_1=9$ $(X_1)_0=12$

Replicate total - 32

Replicate III
(Y_2 arrangement)

(22)	(02)	(12)	(01)	(21)	(11)	(10)	(00)	(20)
5	4	4	2	2	5	3	7	4

Block Σ $(Y_2)_2=13$ $(Y_2)_1=9$ $(Y_2)_0=14$

Replicate total - 36

Replicate IV
(X_2 arrangement)

(00)	(01)	(02)	(21)	(22)	(20)	(12)	(10)	(11)
8	3	4	2	7	3	3	3	5

Block Σ $(X_2)_0=15$ $(X_2)_2=12$ $(X_2)_1=11$

Replicate total - 38

Field arrangement

Rep. I	Rep. II
Rep. IV	Rep. III

Table XI-9 Table of total yields and other totals required for the analysis of the double lattice experiment in Table XI-8.

	Variety numbers and totals			$(A)_i$	$(X_1)_i+(X_2)_i = (X)_i$	$(A)_i-2(X)_i$	c'_x
	(00) 29	(01) 10	(02) 15	54	15+12=27	0	.000
	(10) 12	(11) 20	(12) 12	44	11+ 9=20	4	.400
	(20) 14	(21) 9	(22) 25	48	12+11=23	2	.200
$(B)_j$	55	39	52	146	70	6	
$(Y_1)_j+(Y_2)_j=(Y)_j$	14+16=30	9+13=22	13+11=24	76			
$(B)_j-2(Y)_j$	-5	-5	4	-6		0	
c'_y	-.500	-.500	.400				

effects are obtained by summing the row and column yields respectively. The second from the last column is the sum of the incomplete block totals from replicates II and III or the replicates in which the A effect was confounded with incomplete block differences. The second from last row was obtained from the incomplete block totals of replicates I and IV. The next to the last column and row are obtained as in Table XI-4, while the last column and row are computed after completeing the analysis of variance table (Table XI-10).

The component (b) sum of squares is obtained as the comparison of the level of the effect in the confounded replicates and in the unconfounded replicates and is the same comparison made with two replicates of the double lattice except that the divisors are altered for the number of replicates thus

$$\sum_{i=0}^{k-1} \frac{[(A)_i-2(X)_i]^2}{rk} - \frac{[\Sigma[(A)_i-2(X)_i]]^2}{rk^2} + \frac{\Sigma[(B)_j-2(Y)_j]^2}{rk} - \frac{[\Sigma(B)_j-2(Y)_j]^2}{rk^2}$$

$$= \frac{0^2+4^2+2^2}{12} - \frac{6^2}{36} + \frac{(-5)^2+(-5)^2+4^2}{12} - \frac{(-6)^2}{36} = 5.17.$$

Table XI-10 Analyses of variance for the data of Table XI-8

Randomized Complete Blocks Analysis

Source of variation	d.f.	s.s.	m.s.
Replicates	3	3.89	1.297
Varieties	8	96.89	12.111
Residual	24	19.11	0.796
Total	35	119.89	

Double Lattice Analysis

Source of variation	d.f.⊕	d.f.	s.s.	m.s.
Replicates	$r-1$*	3	3.89	1.297
Varieties (ignor. blocks)	k^2-1	8	96.89	12.111
Blocks (elim. varieties)	$r(k-1)$	8	8.62	$1.078=E_b$
Component (a)	$(k-1)(r-2)$	4	3.45	
Component (b)	$2(k-1)$	4	5.17	
Intrablock	rk^2-k^2-rk+1	16	10.49	$0.656=E_e$
Total	rk^2-1	35		

⊕ General case
* The number of replicates is a multiple of two.

The component (a) sum of squares is the interaction of the levels of the effects with the replicates in which the effects are confounded. Thus, the interaction sums of squares for levels of effect A with replicates II and III and of levels of effect B with replicates I and IV are, respectively,

$$\frac{15^2+12^2+11^2+9^2+12^2+11^2}{k=3} - \frac{32^2+38^2}{k^2=9} - \frac{27^2+20^2+23^2}{2k=6} + \frac{70^2}{2k^2=18}$$

$$+ \frac{14^2+16^2+9^2+13^2+11^2}{k=3} - \frac{40^2+36^2}{k^2=9} - \frac{30^2+22^2+24^2}{2k=6} + \frac{76^2}{2k^2=18}$$

$= 0.34 + 3.11 = 3.45$, with $2(r/2-1)(k-1)=4$ degrees of freedom.

The intrablock error is obtained by subtracting the total of the components (a) and (b) sums of squares = blocks eliminating variety sum of squares from the randomized complete blocks error sum of squares,

$$19.11 - 3.45 - 5.17 = 10.49.$$

The remaining sums of squares and mean square are given in Table XI-10.

The weights for adjusting the variety totals are obtained as

$$w' = \frac{r-1}{rE_b-E_e} = \frac{3}{4(1.078)-0.656} = 0.821$$

$$w = \frac{1}{0.656} = 1.524,$$

and

$$\mu = \frac{w-w'}{k(w+w')} = \frac{q(E_b-E_e)}{k(qE_b+(q-1)E_e)} = \frac{0.844}{0.8436} = 0.1000.$$

The average effective error variance is

$$E_e\left[1+\frac{2(k)}{k+1}\mu\right] = 0.656\left[1+\frac{2(3)}{(4)}(.1000)\right] = 0.7544.$$

The efficiency of the double lattice to the randomized complete blocks design is $0.796/0.7544 = 106$ percent.

The average standard error of a mean difference between two adjusted means is

$$\sqrt{\frac{2E_e}{r}\left[1+\frac{2(k)}{k+1}\mu\right]} = \sqrt{\frac{.7544}{2}} = .614.$$

The standard error of a mean difference for two varieties appearing in the same incomplete block is

$$\sqrt{\frac{2E_e(1+\mu)}{r}} = \sqrt{\frac{0.656(1+.1000)}{2}} = .600.$$

The standard error of a mean difference for two varieties not compared in an incomplete block is

$$\sqrt{\frac{2E_e(1+2\mu)}{r}} = \sqrt{\frac{0.656(1+.2000)}{2}} = .628.$$

Table XI-11. Adjusted totals and means for the double lattice experiment in Table XI-8.

Variety Number	Unadjusted totals	Sum of adjustments	Adjusted totals	Adjusted means
00	29	-.500	28.500	7.125
01	10	-.500	9.500	2.375
02	15	.400	15.400	3.850
10	12	-.100	11.900	2.975
11	20	-.100	19.900	4.975
12	12	.800	12.800	3.200
20	14	-.300	13.700	3.425
21	9	-.300	8.700	2.175
22	25	.600	25.600	6.400
Total	146	0.000	146.000	36.500

The adjustments for the totals are obtained by multiplying the $A_i-2(X)_i$ and the $(B)_j-2(Y)_j$ values by μ to obtain the c'_x and c'_y values. These values are then added to the corresponding totals in the same manner as for the previous example to obtain the adjusted totals and means (Table XI-11).

The intrablock error sum of squares may be computed directly as the interaction of levels of unconfounded effects with the replicates in which the effects are unconfounded. Effects AB and AB^2 are unconfounded in all four replicates. Hence the interaction sum of squares for levels of effects AB and AB^2 with replicates will yield $2(4-1)(k-1) = 12$ degrees of freedom. The effect A is unconfounded in replicates I and IV and B in replicates II and III. The interaction sum of squares from the levels of effects A and B with replicate yields $2(k-1)(2-1) = 4$ degrees of freedom. The total of the interaction sums of squares yields 16 degrees of freedom for the intrablock error sum of squares.

Example XI-3. Triple Lattice The triple lattice design is constructed similarly to the double lattice. The X and Y arrangements are constructed in the same manner but another arrangement, Z, is added. The triple lattice experiment may be used for 3, 6, 9, ..., 3q replicates. The AB (or AB^2, AB^3, or any other) effect is confounded with incomplete block differences in the Z arrangement. For the $k^2=3^2$ triple lattice, the effects A, B, and AB^2 (or AB) are each confounded with incomplete block differences in one arrangement and unconfounded in the other two while AB (or AB^2) is unconfounded in all three arrangements.

The randomization procedure for the triple lattice follows that for the double lattice, i.e.

(i) assign the variety numbers to the varieties at random

(ii) assign the arrangements to the replicates and the levels of the effects to the incomplete blocks

(iii) assign the varieties within the incomplete blocks to the plots at random

For more than one set of the triple lattice proceed in the same manner as described for the double lattice.

The field arrangement and yields (synthetic) for a $3^2 = k^2$ triple lattice with q = 1 set of three replicates is given in Table XI-12. The incomplete block totals in replicates I, II, and III represent the $(Y)_j=(B)_j$ effect in replicate I, $(Z)_h=(AB^2)_h$ effect is replicate II, and $(X)_i=(A)_i$ effect in replicate III. The replicate totals of $3^2 = k^2$ plot yields are given also (Table XI-12).

The next step in the analysis is to obtain the variety totals and arrange them in the X, Y, and Z groupings. Summing the rows in the first part of Table XI-13 results in the $(A)_i$ levels of effect A. The column totals are the levels of the B effect. The last part of Table XI-13 is required to obtain the totals for the levels of the AB^2 effect, i.e. the row totals yields the $3 = k$ levels of effect AB^2.

The $(A)_i-3(X)_i$, $(B)_j-3(Y)_j$, and $(AB^2)_h-3(Z)_h$ quantities represent the comparisons of the levels of the confounded effect from one replicate with the unconfounded effect from the other two replicates. The sums of squares of these quantities = component (b) sum of squares

$$\frac{\Sigma[(A)_i-3(X)_i]^2}{qk(1+1+4)} - \frac{\left\{\Sigma[(A)_i-3(X)_i]\right\}^2}{6qk^2} + \frac{\Sigma[(B)_j-3(Y)_j]^2}{6qk} - \frac{\left\{\Sigma[(B)_j-3(Y)_j]\right\}^2}{6qk^2}$$

$$+ \frac{\Sigma[(AB^2)_h-3(Z)_h]^2}{6qk} - \frac{\left\{\Sigma[(AB^2)_h-3(Z)_h]\right\}^2}{6qk^2} = \frac{5^2+5^2+4^2}{3(6)} - \frac{14^2}{54}$$

$$+ \frac{(-7)^2+(-9)^2+6^2}{18} - \frac{(-10)^2}{54} + \frac{(-3)^2+(-1)^2+0^2}{18} - \frac{(-4)^2}{54} = 7.66.$$

The component (a) sum of squares is the total of the interaction sum of squares for levels of the effect and the replicates in which the effect is confounded. For a single set, $q = 1$, the component (a) sum of squares does not exist. For two or more sets, the component (a) sum of squares may be computed similarly to that for the double lattice in example XI-2.

The sum of the component (a) and (b) sum of squares yields the blocks (eliminating varietal effect) sum of squares,

$0.00 + 7.66 = 7.66$, with $3q(k-1) = r(k-1) = 3(3-1) = 6$ degrees of freedom.

The intrablock error sum of squares is obtained by subtracting the blocks (eliminating variety) sum of squares from the randomized complete blocks error sum of squares,

$$14.82 - 7.66 = 7.16$$

with $(r-1)(k^2-1) - r(k-1) = rk^2-k^2-rk+1 = 10$ degrees of freedom.

The sums of squares and mean squares are given in Table XI-14.

The weights, w and w', for the triple lattice design are obtained from the intrablock error and blocks (eliminating variety) variances, $w = 1/E_e = 1/0.716 = 1.397$, $w' = (r-1)/(rE_b-E_e) = 2/[3(1.277)-0.716] = 0.642$. The weighting factor, μ, is obtained as

$$\mu = \frac{2(w-w')}{2w+w'} = \frac{q[E_b-E_e]}{k[2qE_b+(q-1)E_e]} = \frac{[1.277-0.716]}{3[2(1.277)]} = 0.073,$$

and μ times each of the quantities $(A)_i-3(X)_i$, $(B)_j-3(Y)_j$, and $(AB^2)_h-3(Z)_h$ yields the corrections for adjusting the variety totals. These are entered in the last column of both parts of Table XI-13 and in the last row of the first part of the table. The adjusted mean for any variety is obtained by adding three correction terms to the variety total, one for each of the three arrangements. (Only two corrections were added to each total in the double lattice, while four are required for the quadruple lattice.) The adjusted mean for variety 12 is

$$\frac{1}{3}[8+0.36+0.44-0.07] = 2.91.$$

The remaining adjusted means were obtained similarly and are given, together with the sum of the adjustments, in Table XI-15.

Table XI-12 Yields and field arrangement for triple lattice design with 3^2 varieties in three replicates. Variety numbers in parentheses.

Replicate I
(Y arrangement)

(00)	(20)	(10)	(02)	(12)	(22)	(21)	(11)	(01)
8	5	3	3	2	6	3	7	3

Block total - $(Y)_0=16$ $(Y)_2=11$ $(Y)_1=13$

Replicate total - 40

Replicate II
(Z arrangement)

(01)	(20)	(12)	(11)	(22)	(00)	(10)	(21)	(02)
3	3	3	5	7	8	3	2	4

$(Z)_2=9$ $(Z)_0=20$ $(Z)_1=9$

38

Replicate III
(X arrangement)

(21)	(20)	(22)	(10)	(11)	(12)	(01)	(02)	(00)
2	2	7	3	3	3	2	4	6

Block total - $(X)_2=11$ $(X)_1=9$ $(X)_o=12$

Replicate total - 32

Table XI-13. Total yields and other totals required for the analysis of the triple lattice experiment in Table XI-12

	Variety nos.		totals	$(A)_i$	$(X)_i$	$(A)_i-3(X)_i$	c'_x
	(00) 22	(01) 8	(02) 11	41	12	5	.36
	(10) 9	(11) 15	(12) 8	32	9	5	.36
	(20) 10	(21) 7	(22) 20	37	11	4	.29
$(B)_j$	41	30	39	110	32	14	
$(Y)_j$	16	13	11	40			
$(B)_j-3(Y)_j$	-7	-9	6	-10			
c'_y	-.51	-.66	.44				

	Variety nos.		totals	
	(00) 22	(01) 8	(02) 11	
	(11) 15	(12) 8	(10) 9	
	(22) 20	(20) 10	(21) 7	
$(AB^2)_h$	57	26	27	110
$(Z)_h$	20	9	9	38
$(AB^2)_h-3(Z)_h$	-3	-1	0	-4
c'_z	-.22	-.07	.00	

Table XI-14. Analysis of variance

Randomized Complete Blocks Analysis

Source of variation	d.f.	s.s.	m.s.
Replicates	2	3.85	1.925
Varieties	8	81.18	10.148
Residual	16	14.82	0.926
Total	26	99.85	

Triple Lattice Analysis

Source of variation	d.f.*	d.f.	m.s.	s.s
Replicates	$r-1$	2	3.85	1.925
Varieties(ignor.blocks)	k^2-1	8	81.18	10.148
Blocks(elim.varieties)	$r(k-1)$	6	7.66	$1.277 = E_b$
Component (a)	$(k-1)(r-3)$	0	0.00	
Component (b)	$3(k-1)$	6	7.66	
Intrablock error	rk^2-k^2-rk+1	10	7.16	$0.716 = E_e$
Total	rk^2-1	26		

* General case

Table XI-15. Adjusted totals and means for the triple lattice experiment in Table XI-12.

Variety number	Unadjusted totals	Sum of adjustments	Adjusted totals	Adjusted means
00	22	-.37	21.63	7.21
01	8	-.37	7.63	2.54
02	11	.80	11.80	3.93
10	9	-.15	8.85	2.95
11	15	-.52	14.48	4.83
12	8	.73	8.73	2.91
20	10	-.29	9.71	3.24
21	7	-.37	6.63	2.21
22	20	.51	20.51	6.64
Total	110	-.03	109.97	36.46

*

The sum of the adjustments must equal zero within rounding errors.

The average effective error variance for the triple lattice design is

$$\frac{3}{k+1}\left\{\frac{3}{2w+w'}+\frac{k-2}{3w}\right\} = E_e\left\{1+\frac{3k}{k+1}\mu\right\} = 0.716\left\{1+\frac{9}{4}(.073)\right\} = 0.833.$$

The efficiency of the triple lattice relative to a randomized complete blocks design is

$$\text{Efficiency} = \frac{0.926}{0.833} = 111 \text{ percent.}$$

The average standard error of a difference between any two adjusted variety means is

$$\sqrt{\frac{2}{k+1}\left\{\frac{3}{2w+w'}+\frac{k-2}{3w}\right\}} = \sqrt{\frac{2E_e}{r}\left\{1+\frac{3k\mu}{k+1}\right\}} = \sqrt{\frac{2}{3}(.716)[1+\frac{9}{4}(0.0730]} = 1.281.$$

The standard error of a mean difference between two varieties appearing together in an incomplete block is

$$\sqrt{\frac{2E_e(1+2\mu)}{r}} = \sqrt{\frac{2}{3}(.716)[1+2(.073)]} = 1.274.$$

The standard error of a mean difference between two varieties not appearing together in an incomplete block is

$$\sqrt{\frac{2E_e(1+3\mu)}{r}} = \sqrt{\frac{2(.716)}{3}(1+3(.073)} = .763.$$

For more than one set of the triple lattice, the reader is referred to references 2, 3, 12, and 16. However, the procedure follows that outlined for the double lattice with slight alterations.

<u>Example XI-4. Balanced Lattice</u> The balanced lattice design requires that all pseudo-effects be confounded equally in the set of replicates chosen. If one pseudo effect or interaction

is confounded in each replicate, it requires k+1 replicates for the balanced lattice. Balanced lattices are available for all prime numbers or powers of prime numbers, for example, 3,4,5,7,8,9,11,etc.

The illustrative example chosen was for $3^2=k^2$ varieties in 4 = k+1 replicates. Effect A was confounded with incomplete block differences in replicate III, B in Replicate I, AB^2 in replicate II, and AB in replicate IV. The first three replicates of Table XI-12 are used with one additional replicate added to the example presented in Table XI-16. The 3^2 balanced lattice with four replicates is also a quadruple lattice but the analysis may be somewhat shortened due to the balanced feature of the design. The randomization plan follows that for the triple lattice.

As with other lattices a table of totals (Table XI-17) is required. This table for the balanced lattice is different from those for the double and triple lattice. The second column contains the variety totals. The third column contains the block totals in which a variety appeared in the 4 = k+1 replicates. Thus, variety 20 appeared in the first incomplete block in replicate I, II,III, and IV and the corresponding total of the incomplete blocks is 16 + 9 + 11 + 13 = 49.

The fourth column is obtained by adding k times the variety total (=kV) to the grand total (=G) and subtracting k+1 times the sum of the block totals in which the variety appeared (=(k+1)ΣB). For variety 01 this quantity is

$$3(10) + 146 - (3+1)44 = 0.$$

Table XI-16. Yields and field arrangement for balanced lattice with 3^2 varieties in four replicates. Variety numbers in parentheses.

Replicate I
(Y arrangement)

(00)	(20)	(10)	(02)	(12)	(22)	(21)	(11)	(01)
8	5	3	3	2	6	3	7	3

Block totals - $(Y)_0=16$ $(Y)_2=11$ $(Y)_1=13$

Replicate totals- 40

Replicate II
(Z arrangement)

(01)	(20)	(12)	(11)	(22)	(00)	(10)	(21)	(02)
3	3	3	5	7	8	3	2	4

Block totals - $(Z)_2= 9$ $(Z)_0=20$ $(Z)_1= 9$

Replicate total - 38

Replicate III
(X arrangement)

(21)	(20)	(22)	(10)	(11)	(12)	(01)	(02)	(00)
2	2	7	3	3	3	2	4	6

Block totals - $(X)_2=11$ $(X)_1= 9$ $(X)_0=12$

Replicate total - 32

Replicate IV

(11)	(20)	(02)	(22)	(10)	(01)	(00)	(12)	(21)
5	4	4	5	3	2	7	4	2

Block totals - $(W)_2=13$ $(W)_1=10$ $(W)_0=13$

Replicate total - 36

Field arrangement

Rep. I	Rep. II
Rep. IV	Rep. III

Table XI-17. Total yields and other totals required for the analysis of the balanced lattice in Table XI-16, adjusted totals and adjusted means.

Variety number	Variety total=V	Sum of block totals containing variety = SB	kV+G-(k+1)SB=W	μW	Adjusted variety total	Adjusted variety mean
00	29	61	-11	-.0825	28.9175	7.229
01	10	44	0	.0000	10.0000	2.500
02	15	45	11	.0825	15.0825	3.771
10	12	44	6	.0450	12.0450	3.011
11	20	53	-6	-.0450	19.9550	4.989
12	12	42	14	.1050	12.1050	3.026
20	14	49	-8	-.0600	13.9400	3.485
21	9	46	-11	-.0825	8.9175	2.229
22	25	54	5	.0375	25.0375	6.259
Total	146	438	0	0.0000	146.0000	36.499

Table XI-18. Analyses of variance

Randomized Complete Blocks Analysis

Source of variation	d.f.	s.s.	m.s.
Replicates	3	3.89	1.297
Varieties	8	96.89	12.111
Residual	24	19.11	0.796
Total	35	119.89	

Balanced Lattice Analysis

Source of variation	d.f.*	d.f.	s.s.	m.s.
Replicates	k**	3	3.89	1.297
Varieties(ignor.blocks)	k^2-1	8	96.89	12.111
Blocks (elim.varieties)	k^2-1	8	6.67	$.8338=E_b$
Intrablock	k^3-k^2-k+1	16	12.44	$.7775=E_e$
Total	$(k+1)k^2-1$	35		

* General case
** Number of replicates r = k+1.

After completing the computations in columns 2, 3, and 4 of Table XI-17, the analysis of variance for the balanced lattice may be computed. The replicate, variety, and total sums of squares are computed in the usual manner. The sum of squares of the kV+G-(k+1)SB=W quantities, divided by $k^3(k+1)$,

$\frac{\Sigma W^2}{k^3(k+1)} = \frac{720}{108} = 6.67$, yields the sum of squares for blocks eliminating varieties with $r(k-1)=(k+1)(k-1)=k^2-1=8$ degrees of freedom.

The intrablock error sum of squares is obtained by subtracting the blocks (eliminating variety) sum of squares from the randomized complete blocks error sum of squares, $19.11 - 6.67 = 12.44$, with $(k^2-1)(r-1) - r(k-1) = k^3-k^2-k+1 = 16$ degrees of freedom.

The weighting factor μ is: $\mu = \frac{E_b-E_e}{k^2E_b} = \frac{0.8338-0.7775}{9(0.8338)} = .0075.$

The adjusted totals are obtained by adding μW to the unadjusted total. The adjusted mean for variety 21 is $1/4\ (9-0.0825) = 2.229.$

The average effective error mean square is

$$E_e(1+k\mu) = 0.775[1+3(.0075)] = 0.7950.$$

The efficiency of the balanced lattice relative to the randomized complete blocks design is the ratio in percent of the two average effective error variances, 0.796/0.7950 = 100.1 %.

The standard error of a mean difference between any two adjusted variety means is

$$\sqrt{\frac{2E_e}{k+1}[1+k\mu]} = \sqrt{\frac{2(0.7950)}{4}} = 0.629.$$

All variety comparisons are of equal accuracy since each variety is compared once and only once with every other variety in an incomplete block. Hence, only a single standard error of a mean difference is required. This is a feature common to all balanced lattice designs.

The coefficient of variation is computed similarly to that for all lattices, i.e. the square root of the average effective error variance divided by the experimental mean,

$$\frac{\sqrt{0.7950}}{146/36} = 21.98 \text{ percent.}$$

As with all lattices several checks are available in computing the totals of Table XI-17. The sum of column 2 equals the grand total. The sum of column 3 equals k times the grand total, 3(146) = 438. The sums of columns 4 and 5 equal zero. The sum of column 6 equals the grand total within rounding errors and the sum of column six equals the grand total divided by the number of replicates within rounding errors.

XI-4. Two-dimensional two-restrictional Lattice Designs

The two-dimensional two-restrictional lattices are designs for k^2 varieties in which the varieties are grouped into incomplete blocks in two ways, rows and columns, similar to the latin square. The comparison of two-restrictional with one-restrictional lattices is comparable to the contrast of latin squares with randomized complete block designs. The relative efficiencies are also comparable and the two-dimensional lattice generally is more efficient than the one-restrictional lattice design.

Illustrative examples of a 3^2 semi-balanced lattice square and a 3^2 balanced lattice square are given as examples XI-5 and XI-6. For a discussion of unbalanced lattice squares the reader is referred to other sources (1, 13, 14).

Example XI-5. Semi-Balanced Lattice Square

A $k^2=3^2$ lattice square example in $(k+1)/2 = 2$ replicates is given in Table XI-19. The A effect is confounded with row differences and B with column differences in Replicate I and AB with rows and AB^2 with columns in Replicate II.

The randomization plan is as follows:

(i) assign the numbers to the varieties at random

(ii) assign the arrangements to the replicates at random

(iii) assign the levels of the effects confounded with rows to the rows at random

(iv) assign the levels of the effects confounded with columns to the columns at random.

The above procedure of randomization preserves the arrangements or the confounding of the particular effects with row and column differences.

The data are systematically rearranged in Table XI-20 and the totals for the analysis of variance are computed. The variety totals are given in the last part of the table and from the totals of rows and columns the levels of the effects A, B, AB, and AB^2 are obtained. The totals for levels of the effects are placed opposite the corresponding row or column total in the top part of Table XI-20. The $(A)_i-r(X)_i$, $(B)_j-r(Y)_j$, $(AB)_g-r(W)_g$, and $(AB^2)_h-r(Z)_h$ quantities are computed in the same manner as for the double and triple lattices. The second $(AB)_g-2(W)_g$ value is $24 - 2(9) = 6$.

Table XI-19. Yields and field arrangement for a semi-balanced lattice square with 3^2 varieties in two replicates. Variety numbers in parentheses.

Replicate I

(12) 2	(10) 3	(11) 7	$12=(X)_1$
(02) 3	(00) 8	(01) 3	$14=(X)_0$
(22) 6	(20) 5	(21) 3	$14=(X)_2$
$(Y)_2=11$	$(Y)_0=16$	$(Y)_1=13$	40

Replicate II

(10) 3	(01) 2	(22) 6	$11=(W)_1$
(02) 4	(20) 2	(11) 3	$9=(W)_2$
(21) 2	(12) 3	(00) 7	$12=(W)_0$
$(Z)_1=9$	$(Z)_2=7$	$(Z)_0=16$	32

Table XI-20. Systematic arrangement of yields and totals required for the analysis of variance.

Replicate I

				$(X)_i$	$(A)_i$	$(A)_i-2(X)_i$	c'_x
	(00) 8	(01) 3	(02) 3	14	26	-2	--
	(10) 3	(11) 7	(12) 2	12	21	-3	--
	(20) 5	(21) 3	(22) 6	14	25	-3	--
$(Y)_j$	16	13	11				
$(B)_j$	27	20	25		72		
$(B)_j-2(Y)_j$	-5	-6	3			-8	
c'_y	--	--	--				

Replicate II

				$(W)_g$	$(AB)_g$	$(AB)_g-2(W)_g$	c'_w
	(00) 6	(12) 3	(21) 2	11	24	2	--
	(11) 3	(20) 2	(02) 4	9	24	6	--
	(22) 7	(01) 2	(10) 3	12	24	0	--
$(Z)_h$	16	7	9	32			
$(AB^2)_h$	37	17	18		72		
$(AB^2)_h-2(Z)_h$	5	3	0			8	
c'_z	--	--	--				

Variety totals

				$(A)_i$
	(00) 14	(01) 5	(02) 7	26
	(10) 6	(11) 10	(12) 5	21
	(20) 7	(21) 5	(22) 13	25
$(B)_j$ -	27	20	25	72

				$(AB)_g$
	(00) 14	(12) 5	(21) 5	24
	(11) 10	(20) 7	(02) 7	24
	(22) 13	(01) 5	(10) 6	24
$(AB^2)_h$	37	17	18	72

The sum of the $(A)_i - 2(X)_i = -8$ must equal the sum of the $(B)_j - 2(Y)_j = -8$ in Replicate I and similarly for Replicate II. The sum of the $(A)_i - 2(X)_i$ and the $(AB)_g - 2(W)_g$ must equal zero in the 3^2 semi-balanced lattice square, i.e., the row contrasts must equal zero. The same thing is true for the sum of all the column contrasts, i.e., 8 + (-8) = 0.

The last row and column of the tables headed "Replicate I" and "Replicate II" are obtained after performing the analysis of variance and the weighting factors obtained. (In this case no row and column corrections are obtainable since there are zero degrees of freedom for the intrablock error sum of squares.)

The sum of squares of the quantities

$$\frac{\Sigma[(A)_i - r(X)_i]^2}{\frac{k^2(k^2-1)}{4}} - \frac{\{\Sigma[(A)_i - r(X)_i]\}^2}{\frac{k^3(k^2-1)}{4}} + \frac{\Sigma[(AB)_g - r(W)_g]^2}{\frac{k^2(k^2-1)}{4}} - \frac{\{\Sigma[(AB)_g - r(W)_g]\}^2}{\frac{k^3(k^2-1)}{4}}$$

$$= \frac{(-2)^2+(-3)^2+(-3)^2}{18} - \frac{(-8)^2}{54} + \frac{2^2+6^2+0^2}{18} - \frac{8^2}{54} = 3.22$$

is the rows (eliminating variety) sum of squares with $r(k-1)$ $= \frac{k+1}{2}(k-1) = \frac{k^2-1}{2} = 4$ degrees of freedom.

The columns (eliminating variety) sum of squares is the sum sum of the quantities,

$$\frac{\Sigma[(B)_j - r(Y)_j]^2}{\frac{k^2(k^2-1)}{4}} - \frac{\{\Sigma[(B)_j - r(Y)_j]\}^2}{\frac{k^3(k^2-1)}{4}} + \frac{\Sigma[(AB^2)_h - r(Z)_h]^2}{\frac{k^2(k^2-1)}{4}} - \frac{\{\Sigma[(AB^2)_h - r(Z)_h]\}^2}{\frac{k^3(k^2-1)}{4}}$$

$$= \frac{(-5)^2+(-6)^2+3^2}{18} - \frac{(-8)^2}{54} + \frac{5^2+3^2+0^2}{18} - \frac{8^2}{54} = 10.22,$$

with $r(k-1)$ = four degrees of freedom.

Table XI-21. Analysis of variance

Randomized complete blocks analysis

Source of variation	d.f.	s.s.	m.s.
Replicates	1	3.56	3.560
Varieties	8	49.00	6.125
Residual	8	13.44	1.680
Total	17	66.00	

Semi-balanced lattice square analysis

Source of variation	Degrees of freedom General	$3^2=k^2$	s.s.	m.s.
Replicates	$\frac{k+1}{2}-1$	1	3.56	3.560
Varieties(ignor.rows&cols)	k^2-1	8	49.00	6.125
Columns (elim.varieties)	$(k^2-1)/2$	4	3.22	$0.805=E_r$
Rows (elim. varieties)	$(k^2-1)/2$	4	10.22	$2.555=E_c$
Intrablock	$(k^2-1)(k-3)/2$	0	0.00	$0.000=E_e$
Total	$\frac{k^2(k+1)}{2}-1$	17		

The intrablock error sum of squares with $(k^2-1)(k-3)/2=0$ degrees of freedom is obtained by subtracting the rows (eliminating variety) and columns (eliminating variety) sums of squares from the randomized complete blocks error sum of squares, 13.44 - 3.22 - 10.22 = 0.00, which it should since all variation is accounted for.

The weighting factors for columns,

$$\mu = \frac{E_c-E_e}{k(r-1)E_c} = \frac{2(E_c-E_e)}{k(k-1)E_c},$$

and for rows, $\lambda = \frac{E_r-E_e}{k(r-1)E_r} = \frac{2(E_r-E_e)}{k(k-1)E_r}$

were not computed since they are not valid for k less than five for the semi-blanced lattice design. In the event that k was five or greater the row corrections are computed as

$$c'_x = \lambda[(A)_i - r(X)_i], \quad c'_w = \lambda[(AB)_g - r(W)_g], \text{ etc.}$$

and the column corrections as

$$c'_y = \mu[(B)_j - r(Y)_j], \quad c'_z = \mu[(AB^2)_h - r(Z)_h], \text{ etc.}$$

The corresponding row and column corrections from each replicate are added to the variety totals to obtain the adjusted totals. Since every pseudo main effect or interaction is confounded either with a row or a column, or alternatively, since every variety is compared with every other variety <u>either in a row or a column</u>, there are k+1 adjustments added to each variety total.

The average effective error variance is $E_e[1+\frac{k(\lambda+\mu)}{2}]$, and the efficiency of the semi-balanced lattice square relative to the randomized complete blocks design is the ratio of the two average effective error variances,

$$\frac{\text{Randomized blocks error}}{E_e\left[1+\frac{k(\lambda+\mu)}{2}\right]} \times 100.$$

The average standard error of a difference of two adjusted variety means is $\sqrt{\frac{4E_e}{k+1}\left\{1+\frac{k(\lambda+\mu)}{2}\right\}}$

The standard error of a mean difference for two varieties appearing together in a row is $\sqrt{\frac{2E_e}{k+1}\left\{2+(k-1)\lambda+(k+1)\mu\right\}}$

and for two varieties appearing together in one of the columns is $\sqrt{\frac{2E_e}{k+1}\left\{2+(k+1)\lambda+(k-1)\mu\right\}}$

Example XI-6. Balanced Lattice Square

In the balanced lattice square design every effect and interaction is confounded with row differences in one of the k+1 replicates and with column differences in another of the replicates. Alternatively, each variety is compared with every other variety once in a row and once in a column. Balanced lattice square designs are available for k = 3,4,5,7,8,9,11,13, etc., which are prime numbers or powers of prime numbers.

The randomization plan follows that for the semi-balanced lattice square with (k+1)/2 replicates, i.e., the rows are randomized and then the columns with the arrangements being assigned to the replicates and the varieties being assigned to the variety numbers at random.

A $3^2=k^2$ balanced lattice square illustrative example in 4 = k+1 replicates with the row, column, and replicate totals is given in Table XI-22. Effect A is confounded with rows in replicate I and columns in replicate III, effect B with columns in replicate I and rows in replicate III, effect AB with rows in replicate II and columns in replicate IV, and effect AB^2 with columns in replicate II and rows in replicate IV.

Table XI-23 contains the totals necessary for computing the analysis of variance. The column headed variety total = V contains the totals of the varieties from the four replicates. The total yield for variety 01 is 3+2+2+3 = 10.

Column 3 with the heading SR represents the sum of the row totals in which the variety appeared. For example, the value SR for variety 02 is obtained as 14+9+13+9 = 45. The sum of the SR values should equal k times the grand total, kG = 3(146) = 438.

The column headed SC contains the total of column sums from the individual replicates for a variety. For variety 01 the SC value is 13+7+13+13 = 46. The sum of the SC's should equal k times the grand total, 3(146) = 438.

Column 5 is the difference of Columns 3 and 4, thus for variety 01: SR-SC = 44-46 = -2 = D.

The L' values in Column 6 are obtained as kV+G-(k+1)SR=L', which for variety 10 is 3(12)+146-(3+1)47 = -6.

The J values are the sum of the corresponding D and L' value. which for variety 21 is J = D + L' = -3 + 1 = -2.

K = J + (k-1)D which for variety 20 is K = J + (k-1)D = 4 + (3-1)(0) = 4.

M' = D + K which for variety 00 is- M' = D+K = 1+0 = 1.

The sum of any of the columns 5 to 9 should equal zero exactly. As a further check M' = kV+G-(k+1)SC which for variety 01 is M' = 3(10) + 146 - (3+1)46 = -8.

The replicate, variety ignoring rows and columns, and the total sums of squares (Table XI-24) are computed in the usual manner. The various row and column sums of squares are computed as the sums of squares of the L', J, K, and M' values.

The rows (eliminating varietal effect but ignoring column effects) sum of squares with $(k+1)(k-1) = k^2-1 = 8$ degrees of freedom is $\dfrac{\Sigma(L')^2}{k^3(k+1)} = \dfrac{(-3)^2+0^2+\ldots+1^2+15^2}{27(4)} = 4.148.$

The rows (eliminating varietal and column effects) sum of squares is $\dfrac{\Sigma J^2}{k^3(k-1)} = \dfrac{(-2)^2+(-2)^2+\ldots+(-2)^2+(-7)^2}{27(2)} = 5.630.$ with $r(k-1) = 8$ degrees of freedom.

Table XI-22. Yields and field arrangement for a balanced lattice square design with 3^2 varieties in four replicates. Variety numbers in parentheses.

Replicate I

(12) 2	(10) 3	(11) 7	12
(02) 3	(00) 8	(01) 3	14
(22) 6	(20) 5	(21) 3	14
11	16	13	40

Replicate II

(10) 3	(01) 2	(22) 6	11
(02) 4	(20) 2	(11) 3	9
(21) 2	(12) 3	(00) 7	12
9	7	16	32

Replicate IV

(21) 2	(10) 3	(02) 4	9
(12) 3	(01) 3	(20) 3	9
(00) 8	(22) 7	(11) 5	20
13	13	12	38

Replicate III

(20) 4	(00) 7	(10) 3	14
(22) 5	(02) 4	(12) 4	13
(21) 2	(01) 2	(11) 5	9
11	13	12	36

Table XI-23. Variety totals and other totals used in analysis. Adjusted means.

Variety No.	Variety total=V	SR	SC	SR-SC =D	kV+G - (k+1)SR =L'	D+L' =J	J+(k-1)D = K	D+K =M'	$\lambda L'+\mu M'$	Adj. means
00	29	59	58	1	-3	-2	0	1	-.0765	7.231
01	10	44	46	-2	0	-2	-6	-8	-.7416	2.315
02	15	45	45	0	11	11	11	11	1.6401	4.160
10	12	47	50	-3	-6	-9	-15	-18	-2.0070	2.498
11	20	50	53	-3	6	3	-3	-6	-.2178	4.946
12	12	45	43	2	2	4	8	10	1.0398	3.260
20	14	46	46	0	4	4	4	4	.5964	3.649
21	9	43	46	-3	1	-2	-8	-11	-.9633	2.009
22	25	59	51	8	-15	-7	9	17	.7299	6.432
	146	438	438	0	0	0	0	0	--	36.500

Table XI-24. Analyses of Variance

Randomized Complete Blocks Analysis

Source of Variation	d.f.	s.s.	m.s.
Replicates	3	3.89	1.297
Varieties	8	96.89	12.111
Residual	24	19.11	0.796
Total	35	119.89	

Balanced Lattice Square Analysis

Source of Variation	d.f.*	d.f.	s.s	m.s.
Replicates	k	3	3.89	1.297
Varieties(ignor.rows&cols)	k^2-1	8	96.89	12.111
Rows(ignor.col.,elim.var.)	k^2-1	8	4.148	
Columns(elim.rows & var.)	k^2-1	8	11.407	$1.426=E_c$
Columns(elim.var.,ign.rows)	k^2-1	8	9.926	
Rows (elim.var. and cols.)	k^2-1	8	5.630	$0.704=E_r$
Intrablock error	k^3-k-2k^2+2	8	3.554	$0.444=E_e$
Total	$k^2(k+1)-1$	35	119.89	

* General case

The columns (eliminating varietal effect but ignoring row effect) sum of squares with $r(k-1) = 8$ degrees of freedom is

$$\frac{\Sigma(M')^2}{k^3(k+1)} = \frac{1^2+(-8)^2+\ldots+(-11)^2+17^2}{27(4)} = 9.926.$$

The columns (eliminating both variety and row effects) sum of squares is $\frac{\Sigma K^2}{k^3(k-1)} = \frac{0^2+(-6)^2+\ldots+(-8)^2+9^2}{27(2)} = 11.407,$ with $(k+1)(k-1) = 8$ degrees of freedom.

The sum of the row (eliminating varieties but ignoring columns) and the column (eliminating variety and row) sums of squares should equal the sum of the column (eliminating variety but ignoring row) and the row (eliminating variety and column) sums of squares within rounding errors, thus

$4.148 + 11.407 = 15.555$
$9.926 + 5.630 = 15.556.$

Either of the above sums is subtracted from the randomized complete blocks error sum of squares to obtain the intrablock error sum of squares, $19.11 - 15.556 = 3.554$ with $k(k^2-1)-2(k^2-1) = k^3-k-2k^2+2 = 8$ degrees of freedom.

The row weighting factor is

$$\lambda = \frac{(E_r-E_e)(kE_c-E_e)}{(k-1)(k^2E_rE_c-E_e^2)} = \frac{(0.704-0.444)[3(1.426)-0.444]}{(3-1)[9(0.704)(1.426)-(0.444)^2]} = 0.0564$$

and the column weighting factor is

$$\mu = \frac{(E_c-E_e)(kE_r-E_e)}{(k-1)(k^2E_rE_c-E_e^2)} = \frac{(1.426-0.444)[3(0.704)-0.444]}{(3-1)[9(0.704)(1.426)-(0.444)^2]} = 0.0927.$$

The adjustments for the variety totals are $\mu M'+\lambda L'$, which for variety 00 is $(.0927)(1)+(.0564)(-3) = -0.0765$ and the adjusted mean is $1/4[29+(-.0765)] = 7.2309.$

The standard error of a mean difference between any two variety means is

$$\sqrt{\frac{2E_e}{k+1}[1+k(\lambda+\mu)]} = \sqrt{\frac{2(0.444)}{4}[1+3(0.1491)]} = 0.567.$$

There is a single standard error of a mean difference since all comparisons are of equal accuracy.

The average effective error variance is

$$E_e[1+k(\lambda+\mu)] = 0.444[1+3(0.1491)]$$

and the efficiency of the balanced lattice square relative to the randomized complete blocks design is

$$\frac{\text{Randomized complete blocks error}}{E_e[1+k(\lambda+\mu)]} \times 100 = \frac{0.756}{0.643} \times 100 = 117.6 \text{ percent.}$$

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