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# INCOMPLETE RANDOMIZED BLOCKS

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*Rothamsted Experimental Station*

## INTRODUCTION

MOST biological workers are probably by now familiar with the methods of experimental design known as randomized blocks and the Latin square. These were originally developed by Prof. R. A. Fisher, when Chief Statistician at Rothamsted Experimental Station, for use in agricultural field trials. The same principles of design are of general utility, wherever the basic material is at all variable, for comparing the effects of different treatments, or the growth of different strains of an organism, or other similar problems.

In a randomized block arrangement each treatment occurs equally frequently, usually once, in each block, the treatments being assigned at random to the experimental units within the block. The word block may be extended to denote any group containing the requisite number of experimental units, and by arranging the grouping so that similar experimental units occur in the same block the accuracy of the treatment comparisons is considerably enhanced, since differences due to dissimilarities between the different blocks are eliminated from these comparisons. The process of random arrangement within the blocks ensures that no treatment shall be unduly favoured, and, moreover, enables an unbiased estimate of experimental error to be obtained, which is itself the basis of valid tests of significance.

A Latin square arrangement is similar in principle to a randomized block arrangement, but in a Latin square two cross-groupings of the experimental units are made, corresponding to the rows and columns of a square, and the treatments are so arranged that each occurs once and once only in each row and in each column. Thus differences between rows and columns (which may represent any desired groupings) are eliminated from the treatment comparisons. The appropriate randomization process consists of taking any square arrangement which satisfies the conditions of a Latin square and rearranging either the rows or the columns, or both, at random, and then allotting the treatments at random.

In agricultural field trials the elimination of differences of soil fertility is of great importance, and for this purpose randomized block and Latin square arrangements have proved eminently suitable. In such experiments there is no definite natural limit to the number of experimental units, here plots, which may be included in a block, or in a row or column of a Latin square. There is thus no definite limit to the number of treatments which may be comprised by an experiment, though naturally the effectiveness of the blocks or rows and columns of a square in eliminating fertility differences becomes less as the number of treatments increases.

In some other experimental material the groupings which most effectively eliminate heterogeneity are more definitely limited in numbers. Pigs cannot be relied on to produce more than six or eight suitable young in a litter. Monozygotic twins form a natural experimental grouping in man. In the local lesion method of studying the virulence of different preparations of virus by inoculating the leaves of susceptible plants and counting the number of lesions produced there are only from two to five suitable leaves to a plant (depending on the species), and only two treatments can be applied to each leaf, one to each half.

In factorial experiments, that is in experiments with all combinations of two or more sets of treatment factors, a method, known as confounding, has been devised whereby the block size may be kept moderate. In confounded arrangements the treatments of each replication are allotted to two or more sub-blocks in such a manner that some or all of the information is sacrificed on unimportant comparisons, usually high-order interactions between the different factors.

The present paper describes another possible modification of the randomized block type of arrangement, analogous to confounding, which enables us to dispense with the restriction that the number of experimental units in a block shall equal the number of treatments. In this modified type of arrangement the number of experimental units per block is fixed, being less than the number of treatments, and the treatments are so allotted to the blocks that every two treatments occur together in a block equally frequently. With six treatments *a, b, c, d, e, f*, and blocks of three experimental units, for example, the following grouping of treatments into ten blocks satisfies the above conditions:

1	<i>a</i>	<i>b</i>	<i>c</i>	6	<i>b</i>	<i>c</i>	<i>f</i>
2	<i>a</i>	<i>b</i>	<i>d</i>	7	<i>b</i>	<i>d</i>	<i>e</i>
3	<i>a</i>	<i>c</i>	<i>e</i>	8	<i>b</i>	<i>e</i>	<i>f</i>
4	<i>a</i>	<i>d</i>	<i>f</i>	9	<i>c</i>	<i>d</i>	<i>e</i>
5	<i>a</i>	<i>e</i>	<i>f</i>	10	<i>c</i>	<i>d</i>	<i>f</i>

In this set of groupings every two treatments occur together in a block twice, there being five replications of each treatment.

It is proposed to call this type of arrangement a *symmetrical incomplete randomized block arrangement*, or more briefly, when the symmetry and randomization are understood, an *incomplete block arrangement*. Although groupings of the experimental treatments fulfilling the required condition are always possible, whatever the number of treatments and of units in a block, the number of groupings (and therefore of blocks and units) required is likely to be large where the number of treatments is at all large. In a later section we shall investigate the smallest number of groupings required.

A further advantage of symmetrical incomplete block arrangements, which may be of importance in certain lines of research, is that the differences between the various blocks can be properly estimated. Thus if the blocks represent litters, differences between different litters can be studied simultaneously with the investigation of the effects of treatments

on the individual animals. If the experiment were arranged in ordinary randomized blocks including animals from more than one litter no proper estimate of differences between litters would be readily available: the use of the residuals after eliminating treatment effects always tends to reduce the apparent differences between litters.

The simplest and most important example of a symmetrical incomplete randomized block arrangement is that in which each block contains only two units, and all possible combinations of the treatments, taken in pairs, are represented in the different blocks. Thus with six treatments there are  $\frac{1}{2}(6 \times 5)$  or 15 pairs, and the number of blocks must therefore be some multiple of 15. It will be shown that such an arrangement is decidedly more efficient than the customary method of arrangement in such cases, namely that of treating one treatment as a control and comparing each other treatment with it. In the proposed type of arrangement all treatment comparisons are of the same accuracy, and it is a remarkable fact that this accuracy is the same as that obtained on the comparisons between the control and the other treatments in an arrangement of the customary type using the same number of experimental units.

An experimental arrangement is not likely to be of much use in practice if it involves long and tedious calculations to analyse the results. Fortunately in the case of symmetrical incomplete blocks the procedure of the analysis of variance is very little more complicated than in the case of ordinary randomized block arrangements. The formulae required in the analysis of the results are given in the following sections. They may all be derived by a straightforward application of the method of least squares.

The chief disadvantage of symmetrical incomplete blocks is, as mentioned above, that the number of replications required is in most cases large when the number of treatments is at all large. This disadvantage can be overcome by dispensing with the condition of symmetry, but at the cost of some small loss of efficiency and the inconvenience of having slight variations in accuracy for different sets of treatment comparisons. The simplest arrangement of this type is that in which the treatments ( $p^2$  in number) are set out in the form of a square and two sets of  $p$  blocks are formed from the rows and the columns respectively of this square. With  $pq$  treatments the square may be replaced by a rectangle. Such arrangements, and analogous arrangements derived from a cube, are eminently suitable for variety trials involving large numbers of varieties. A description is given in (5).

A further analogous type of arrangement is available in the case of a Latin square. If in a Latin square arrangement one row, one column, or one treatment is missing, or one row and one column, or one row or column and one treatment, not only is the analysis of the results quite simple, but the estimate of error is unbiased, so that such arrangements give perfectly valid tests of significance. The analytical procedure is of considerable interest to agriculturalists, who are occasionally confronted with Latin square arrangements in which a row, column or treatment has failed, and a full account of this procedure has therefore been given in (4). The worker who wishes to lay down an incomplete Latin square, or to analyse one, is referred to that paper. No simple analysis appears to exist when two or

more rows, columns or treatments are missing, nor when a single row, column and treatment are all missing.

SYMMETRICAL PAIRS

The case in which the blocks contain two experimental units only is capable of specially simple treatment. In this case all combinations of the treatments two at a time are required for symmetry, so that if there are  $t$  treatments there must be some multiple of  $\frac{1}{2}t(t-1)$  blocks. With  $r$  replicates of each treatment each pair of treatments is replicated  $r/(t-1)$  or say  $r'$  times.

If the treatments are  $a, b, c$ , etc., and  $S[a-b]$  represents the total of the  $r'$  differences of the treatments  $a$  and  $b$  in the blocks in which they occur together, we may set out these totals as in Table I.

Table I

	—	$S[b-a]$	$S[c-a]$	$S[d-a]$	...
$S[a-b]$	—	$S[c-b]$	$S[d-b]$	...	...
$S[a-c]$	$S[b-c]$	—	$S[d-c]$	...	...
$S[a-d]$	$S[b-d]$	$S[c-d]$	—	...	...
...	...	...	...	...	...
Column totals	$T'_a$	$T'_b$	$T'_c$	$T'_d$	...

In this table each difference total is repeated twice, with opposite signs.

The differences of the quantities

$$\frac{1}{r't} T'_a, \frac{1}{r't} T'_b, \dots,$$

represent the treatment differences, in units of the effect on a single experimental unit. Since the sum of these quantities is zero they may conveniently be increased by the mean of the experimental values when presenting the final results.

If  $\sigma_d^2$  is the variance of the difference of two experimental values from the same block the standard error of the difference of any two of the above quantities is

$$\sqrt{2} \times \sigma_d \sqrt{\frac{1}{r't}}.$$

If one of the treatments had been used as a control and compared with each of the other treatments in turn there would have been  $rt/2(t-1)$  replicates of each pair. The standard error of the difference of the control and any other treatment would then be  $\sigma_d \sqrt{2(t-1)/rt}$  or  $\sigma_d \sqrt{2/r't}$ , as above, and the standard error of all other comparisons would be  $\sqrt{2}$  times this. When the number of treatments is large, therefore, the symmetrical arrangement is almost twice as efficient as the arrangement using one treatment as a control.

If the analysis of variance is performed in units of a single difference between two experimental units, giving an estimate of  $\sigma_d^2$  directly, the sum of squares due to treatments is

$$\frac{1}{r't} (T_a'^2 + T_b'^2 + \dots),$$

and the total error sum of squares, with  $\frac{1}{2}(t-1)(r't-2)$  degrees of freedom, is the difference of this and the total sum of squares of all differences (without any correction for the mean).

#### EXAMPLE OF SYMMETRICAL PAIRS

In an experiment to determine the degree of variability in the number of lesions produced when leaves of young plants of *Nicotiana glutinosa* were inoculated with a suspension containing tobacco mosaic virus, fifty plants were taken, each with five leaves, and the numbers of lesions produced on each half-leaf were counted. The numbers observed on the leaves of the first six plants are shown in Table II. Considerable association between halves of the same leaf is apparent.

Table II. *Number of lesions on half-leaves*

Plant	Leaf				
	1	2	3	4	5
1	<i>L</i> <i>e</i> 26	<i>d</i> 16	<i>c</i> 21	<i>b</i> 11	<i>e</i> 12
	<i>R</i> <i>b</i> 40	<i>b</i> 26	<i>e</i> 14	<i>c</i> 16	<i>a</i> 12
2	<i>L</i> <i>b</i> 34	<i>c</i> 69	<i>c</i> 42	<i>a</i> 22	<i>e</i> 19
	<i>R</i> <i>a</i> 49	<i>a</i> 68	<i>d</i> 35	<i>d</i> 31	<i>d</i> 25
3	<i>L</i> <i>b</i> 28	<i>c</i> 83	<i>a</i> 24	<i>c</i> 12	<i>c</i> 9
	<i>R</i> <i>e</i> 11	<i>d</i> 58	<i>e</i> 27	<i>a</i> 15	<i>b</i> 13
4	<i>L</i> <i>b</i> 12	<i>a</i> 23	<i>b</i> 28	<i>e</i> 15	<i>d</i> 5
	<i>R</i> <i>a</i> 26	<i>d</i> 20	<i>d</i> 34	<i>c</i> 13	<i>e</i> 11
5	<i>L</i> <i>a</i> 14	<i>b</i> 17	<i>b</i> 5	<i>b</i> 11	<i>d</i> 4
	<i>R</i> <i>e</i> 16	<i>d</i> 12	<i>e</i> 10	<i>c</i> 8	<i>e</i> 6
6	<i>L</i> <i>a</i> 5	<i>d</i> 17	<i>c</i> 16	<i>a</i> 12	<i>a</i> 7
	<i>R</i> <i>c</i> 15	<i>c</i> 15	<i>e</i> 18	<i>d</i> 15	<i>b</i> 10

An arrangement to compare five suspensions by the method of symmetrical pairs has been imposed on these values, the allocation of pairs of treatments being at random. The actual arrangement arrived at is shown in the table, the treatments being denoted by *a* ... *e*.

The possibility in experiments of this kind of using different dilutions of all suspensions to be compared should be borne in mind, but need not be discussed here. The best function of the numbers of lesions to use in the analysis also requires consideration. Here we have taken differences of the square roots.

These differences are shown in Table III, together with their totals. Table IV shows the calculation of the treatment effects, the estimated differences in the square roots per half-leaf being given by the differences of the quantities in the last line.

Table V shows the analysis of variance. The treatment sum of squares is given by the sum of the squares of the totals of Table IV divided by 15. The sums of squares between different pairs and between replicates of pairs are obtained from Table III in the ordinary manner. The component of error from Table IV is obtained by subtraction of the treatment sum of squares from the sum of squares between different pairs. There are no significant

Table III. *Differences of square roots of numbers of lesions*

Plants	$a-b$	$a-c$	$a-d$	$a-e$	$b-c$	$b-d$	$b-e$	$c-d$	$c-e$	$d-e$
1, 2	+1.2	-0.1	-0.9	0.0	-0.7	+1.1	+1.2	+0.6	+0.9	+0.6
3, 4	+1.6	+0.4	+0.3	-0.3	+0.6	-0.5	+2.0	+1.5	-0.3	-1.1
5, 6	-0.6	-1.7	-0.4	-0.3	+0.5	+0.6	-1.0	-0.2	-0.2	-0.4
Total	+2.2	-1.4	-1.0	-0.6	+0.4	+1.2	+2.2	+1.9	+0.4	-0.9

Table IV. *Estimation of treatment effects*

	$a$	$b$	$c$	$d$	$e$
	—	-2.2	+1.4	+1.0	+0.6
	+2.2	—	-0.4	-1.2	-2.2
	-1.4	+0.4	—	-1.9	-0.4
	-1.0	+1.2	+1.9	—	+0.9
	-0.6	+2.2	+0.4	-0.9	—
Total	-0.8	+1.6	+3.3	-3.0	-1.1
Total/15	-0.053	+0.107	+0.220	-0.200	-0.073

Table V. *Analysis of variance*

	Degrees of freedom	Sum of squares	Mean square
Between different pairs:			
Total	10	6.3933	0.6393
Treatments	4	1.6200	0.4050
Remainder	6	4.7733	0.7956
Between replicates	20	16.9467	0.8473
Total error	26	21.7200	0.8354
Total	30	23.3400	0.7780

differences between the mean squares, as is to be expected, since the treatments are dummy. The estimated standard error of the differences of the quantities in the last line of Table IV is  $\sqrt{2 \times 0.8354/15}$  or  $\pm 0.334$ .

In an example of this kind we are not normally interested in the comparisons of the numbers of lesions on different leaves, but if such a comparison is to be made the effects of treatments must clearly be allowed for. This can be done very simply by subtracting from the square roots of the actual counts the values given in the last line of Table IV for the appropriate treatments. Thus the first leaf of the first plant has treatments  $e$  and  $b$ , the sum of the square roots of the half-leaf counts is 11.4, and the adjusted value of this sum is therefore

$$11.4 - 0.107 + 0.073 = 11.366,$$

a practically identical value, as it should be since the treatments are without effect.

If the treatments are arranged in a  $5 \times 5$  Latin square on the whole leaves of the first

five plants, so that leaf positions as well as plants are equalized for each treatment, the accuracy will be very considerably less. The residual mean square of the square roots of the number of lesions on whole leaves after eliminating plants and leaf positions is found to be 1.6116. Dividing this by 5 will give the mean square error for the mean of five replicates, and dividing by a further 2 (instead of 4, since square roots of lesions on whole leaves have been taken) and multiplying by 5/6 to allow for the smaller number of plants used gives a mean square error of 0.1343, which is comparable with the mean square error 0.7780/15 or 0.0519 derived from the total mean square of Table V. The method of symmetrical pairs therefore gives 2.59 times the information given by the Latin square arrangement.

There are types of arrangement other than symmetrical pairs which make use of the association between halves of the same leaf, and which are consequently more efficient than the Latin square arrangement on whole leaves, but there is no need to discuss these here. A general discussion of the relative efficiency of symmetrical incomplete randomized blocks is given later in the paper.

#### GENERAL CASE: FORMULAE

The following symbols will be used:

Number of treatments:  $t$ .

Number of experimental units per block:  $k$ .

Number of replications of each treatment:  $r$ .

Number of blocks:  $b$ .

Total number of experimental units:  $N = tr = bk$ .

Number of times any two treatments occur together in a block:  $\lambda = r(k-1)/(t-1)$ .

Sum of all  $N$  experimental values (i.e. "grand total"):  $G$ .

Sum of all  $r$  experimental values for treatment 1:  $T_1$ .

Sum of all  $k$  experimental values for block 1:  $B_1$ .

Error variance of a single experimental value when arranged in blocks of  $k$  units:  $\sigma_k^2$ .

It is first necessary to calculate the  $t$  quantities

$$Q_1 = kT_1 - B_1 - B_2 - \dots - B_r,$$

etc., where the  $r$   $B$ 's for  $Q_1$  are the totals of the  $r$  blocks containing treatment 1, etc.

The treatment effects, in terms of the change produced in a single experimental unit, are represented by the differences of the quantities

$$v_1 = \frac{(t-1)}{N(k-1)} Q_1,$$

etc., the standard error of each difference being

$$\sqrt{2} \times \sqrt{\frac{k(t-1)}{N(k-1)} \sigma_k^2}.$$

It is best to add the general mean  $G/N$  to each of these quantities in order that their mean shall equal the general mean.

The sums of squares for blocks and treatments in the analysis of variance are as follows:

$$\text{Blocks: } \frac{1}{k} (B_1^2 + B_2^2 + \dots + B_b^2) - \frac{1}{N} G^2.$$

$$\text{Treatments: } \frac{t-1}{Nk(k-1)} (Q_1^2 + Q_2^2 + \dots + Q_t^2).$$

If the number of experimental units in a block is greater than one half the number of treatments the quantities  $Q$  are best replaced by

$$Q'_1 = kT_1 + B_{r+1} + \dots + B_b,$$

etc., the  $B$ 's for  $Q'_1$  being those in which treatment 1 does *not* occur.

The quantities representing the treatment effects must now be diminished by

$$G(t-k)/N(k-1)$$

if their mean is to equal the general mean, and the sum of the squares of  $Q$  must be replaced by the sum of the squares of the deviations of  $Q'$ .

It is worth noting that the numerical factors in the above expressions can be written

$$\frac{1}{kr} \cdot \frac{1-1/t}{1-1/k}, \quad \frac{1}{r} \cdot \frac{1-1/t}{1-1/k}, \quad \frac{1}{k^2r} \cdot \frac{1-1/t}{1-1/k}.$$

The first part of each of these factors is the fraction that would be obtained if the quantities  $kT_1$ , etc., were being analysed instead of  $Q$ .

Normally estimates of the differences between blocks (allowing for possible treatment effects) are of no interest. If, however, they are required, as in the example given later, they can be calculated by deducting from each block mean the mean of the  $v$ 's of the treatments in that block, or alternatively by adding the mean of the  $v$ 's of the treatments not in the block.

In the special case of the arrangements which are such that the same relations hold for blocks as for treatments, i.e. in which each pair of blocks has the same number of treatments in common, the estimates of the differences between blocks may be calculated in the same way as the treatment effects, and the standard error of each difference will be the same, and equal to the standard error of differences of the treatment effects. Only a few arrangements can possibly be of this nature.

In the more general case the standard error of the difference of two block means having  $\mu$  treatments in common is

$$\sqrt{2} \times \sqrt{\frac{1}{k} \left\{ 1 + \frac{(k-\mu)(t-1)}{rt(k-1)} \right\} \sigma_k^2}.$$

The average value of  $\mu$  is  $k(r-1)/(b-1)$ , and therefore the average value of the variance of the difference of the block means is

$$\bar{V}_b = \frac{2\sigma_k^2}{k} \left\{ 1 + \frac{(t-1)(t-k)}{t(k-1)(b-1)} \right\}.$$

## EFFICIENCY

The expression for the standard error of a treatment comparison enables us to make a comparison of the efficiencies of symmetrical incomplete blocks and the ordinary randomized block or Latin square arrangement, provided we know the ratio of the error variances of the experimental units when arranged in blocks of size  $k$  and in blocks of size  $t$ .

The variance of the difference of two treatment means in an ordinary randomized block experiment with  $r$  replicates will be  $2\sigma_t^2/r$ , and therefore the ratio of this variance to that obtained with an arrangement in incomplete blocks of size  $k$  will be

$$\frac{2\sigma_t^2/2k(t-1)}{r/N(k-1)} \sigma_k^2 = \frac{1-1/k}{1-1/t} \cdot \frac{\sigma_t^2}{\sigma_k^2},$$

since with the same number of units the number of replicates will be the same in both arrangements. This expression is therefore a measure of the relative efficiency of the two methods. The fraction

$$\frac{1-1/k}{1-1/t}$$

may be called the *efficiency factor* of the incomplete block arrangement, and measures the loss of information when there is no reduction in error variance per unit by reduction in block size. It may be looked on as a measure of the inevitable loss inherent in the arrangement.

In agricultural field trials, and other experimental material in which there is no definite limit to the number of experimental units in a block, the ratio  $\sigma_t^2/\sigma_k^2$  can only be properly determined by the examination of uniformity trial data, the error variances being calculated for arrangements in blocks both of  $t$  and of  $k$  units. In material in which the natural block size is definitely fixed, however, with no association between different blocks, a knowledge of the variance between and within blocks is all that is required. This can be determined from the results of actual experiments arranged in blocks of the natural size of  $k$  units.

In this latter case when treatments produce no effect any experimental value  $y$  can be regarded as made up of the sum of three parts, one constant over the whole experiment, a second  $\alpha$  varying from block to block of  $k$  units but constant for all values in a given block, and a third  $\beta$  varying independently from value to value. If the variance of  $\alpha$  is  $V(\alpha)$  and of  $\beta$  is  $V(\beta)$  it can be shown that the average variance within blocks of  $t$  units made up of numbers  $l, l', l'', \dots$  of units from different blocks of  $k$  units is equal to

$$\frac{2(l' + l'' + l''' + \dots)}{t(t-1)} V(\alpha) + V(\beta).$$

This expression, or its mean for the various sets of  $l, l', l'', \dots$  required, will be equal to  $\sigma_t^2$ , and  $V(\beta)$  is  $\sigma_k^2$ , so that  $\sigma_t^2/\sigma_k^2$  is calculable if  $V(\alpha)/V(\beta)$  (equal to  $\rho$  say) is known. The ratio of the mean square for blocks to the mean square for error in the ordinary analysis of variance of an experiment in blocks of size  $k$  will be equal to  $k\rho + 1$ , except for errors of

estimation. In order to remove bias when estimating the mean  $\rho$  from a group of experiments each such ratio should be multiplied by  $(n - 2)/n$ ,  $n$  being the number of degrees of freedom for error.

Table VI shows the values in terms of  $\rho$  of the ratio of the efficiencies for natural blocks of three, four and six, and treatments from four to ten; the first column of figures for each value of  $k$  will be recognized as the previously defined efficiency factor. The values  $\rho_0$  of  $\rho$  for which the ratio is equal to unity are also given. These latter range from about 0.4 for blocks of three down to about 0.12 for blocks of six. With blocks of six, therefore, even slight block heterogeneity will make the method of incomplete randomized blocks more efficient; with blocks of three, on the other hand, there must be considerable block heterogeneity.

Table VI. Ratio of the efficiencies of arrangements in incomplete and complete randomized blocks

$t$	$k=3$		$k=4$		$k=6$	
	Ratio	$\rho_0$	Ratio	$\rho_0$	Ratio	$\rho_0$
4	$0.889 + 0.444\rho$	0.25				
5	$0.833 + 0.528\rho$	0.32	$0.938 + 0.375\rho$	0.17		
6	$0.8 + 0.48\rho$	0.42	$0.9 + 0.48\rho$	0.21		
7	$0.778 + 0.556\rho$	0.40	$0.875 + 0.531\rho$	0.24	$0.972 + 0.278\rho$	0.10
8	$0.762 + 0.580\rho$	0.41	$0.857 + 0.490\rho$	0.29	$0.952 + 0.408\rho$	0.12
9	$0.75 + 0.562\rho$	0.44	$0.844 + 0.562\rho$	0.28	$0.938 + 0.469\rho$	0.13
10	$0.741 + 0.593\rho$	0.44	$0.833 + 0.593\rho$	0.28	$0.926 + 0.521\rho$	0.14

POSSIBLE ARRANGEMENTS

The condition that every two treatments occur together in a block an equal number of times is satisfied if the blocks are chosen so as to include every possible grouping of the  $t$  treatments  $k$  at a time. In this case

$$b = {}_tC_k, \quad r = {}_{t-1}C_{k-1}, \quad \lambda = {}_{t-2}C_{k-2},$$

where  ${}_tC_k$  is the number of combinations of  $t$  things  $k$  at a time, and equals

$$t(t-1) \dots (t-k+1)/k(k-1) \dots 2.1.$$

If  $f$  is the highest common factor of the above values of  $b$ ,  $r$  and  $\lambda$  lower limits of  $b$ ,  $r$  and  $\lambda$  are given by  $1/f$  of these values, since all must be whole numbers. It does not follow, however, that any arrangement exists for these lower limits.

Table VII gives these lower limits of  $r$  for all  $t$  up to twenty-five and all  $k$  not greater than  $\frac{1}{2}t$ . The investigation of whether any arrangement is possible for any given values of  $t$ ,  $k$  and  $r$  involves intricate and extensive combinatorial problems which have not in general been solved, but certain of the simpler cases have been investigated. For numbers of treatments not exceeding ten an arrangement has been found for every value of  $k$ . For numbers of treatments greater than ten only those cases in which the lower limit of

$r$  is not greater than six have been examined. Two of these,  $t = 16, k = 6, r = 3$ , and  $t = 21, k = 6, r = 4$  were found to be impossible, but the former yielded an arrangement, discussed below, for  $r = 6$ . Table VIII gives the various arrangements which have been found (other than those in which all possible groupings are necessary).

Table VII. Lower limits to the number of replications ( $r$ ) in symmetrical incomplete randomized block experiments of various sizes

Number of treatments ( $t$ )	Number of units per block ( $k$ )										
	2	3	4	5	6	7	8	9	10	11	12
25	24*	(12)	(8)	6	(24)	(28)	(24)	(9)	(8)	(132)	(24)
24	23*	(23)	(23)	(115)	(23)	(161)	(23)	(69)	(115)	(253)	(23)
23	22*	(33)	(44)	(55)	(66)	(77)	(88)	(99)	(110)	(11)	
22	21*	(21)	(14)	(105)	(21)	(7)	(12)	(63)	(35)	(21)	
21	20*	(10)	(20)	5	[4]	(10)	(40)	(15)	(20)		
20	19*	(57)	(19)	(19)	(57)	(133)	(38)	(171)	(19)		
19	18*	(9)	(12)	(45)	(18)	(21)	(72)	(9)			
18	17*	(17)	(34)	(85)	(17)	(119)	(68)	(17)			
17	16*	(24)	(16)	(20)	(48)	(56)	(16)				
16	15*	(15)	5	(15)	[3] 6	(35)	15				
15	14*	(7)	(28)	(7)	(14)	(7)					
14	13*	(39)	(26)	(65)	(39)	(13)					
13	12*	6	4	(15)	(12)						
12	11*	(11)	(11)	(55)	(11)						
11	10*	(15)	(20)	5							
10	9*	9	6	9							
9	8*	4	8								
8	7*	21*	7								
7	6*	3									
6	5*	5									
5	4*										
4	3*										

Curved brackets ( ) indicate that the possibility of an arrangement has not been investigated, square brackets [ ] that no arrangement exists.

An arrangement is shown in Table VIII for each of the unbracketed values, except those, marked by an asterisk (\*), which involve all possible combinations, and  $t = 16, k = 8, r = 15$ . The arrangements for 31, 49, etc., treatments, described in the text on p. 134, but not shown in Table VII or VIII, should also be noted.

When  $k$  is greater than  $\frac{1}{2}t$  lower limits and arrangements can be derived as indicated in the text.

Table VIII. Experimental arrangements

(a) Arrangements symmetrical for blocks as well as for treatments

Seven treatments: 7 blocks of 3

Eleven treatments: 11 blocks of 5

(3-fold)

(5-fold)

$a$	$b$	$c$	$d$	$e$	$f$	$g$	$a$	$b$	$c$	$d$	$e$	$f$	$g$	$h$	$i$	$j$	$k$
+	+	+	.	.	.	.	+	+	+	+	+	.	.	.	.	.	.
+	.	.	+	+	.	.	+	+	.	.	.	+	+	+	.	.	.
+	.	.	.	.	+	+	+	.	+	.	.	+	.	.	+	+	.
.	+	.	+	.	+	.	+	.	.	+	.	+	.	+	.	+	+
.	.	+	+	.	.	+	.	+	+	.	.	+	.	+	.	+	+
.	.	+	.	+	+	.	.	+	.	+	.	.	+	+	.	+	+
.	.	.	.	.	.	.	.	.	+	+	.	+	.	+	.	.	+
.	.	.	.	.	.	.	.	.	.	+	+	+	+	.	.	+	.



Table VIII (cont.)

## (b) Other arrangements

Six treatments: 10 blocks of 3 (5-fold). See Introduction

Eight treatments: 14 blocks of 4 (7-fold)

<i>a b c d</i>	<i>a b e f</i>	<i>a c e g</i>	<i>a b g h</i>	<i>a c f h</i>	<i>a d e h</i>	<i>a d f g</i>
<i>e f g h</i>	<i>c d g h</i>	<i>b d f h</i>	<i>c d e f</i>	<i>b d e g</i>	<i>b c f g</i>	<i>b c e h</i>

Nine treatments: 12 blocks of 3 (4-fold)

<i>a b c</i>	<i>a d g</i>	<i>a e i</i>	<i>a f h</i>
<i>d e f</i>	<i>b e h</i>	<i>b f g</i>	<i>b d i</i>
<i>g h i</i>	<i>c f i</i>	<i>c d h</i>	<i>c e g</i>

Nine treatments: 18 blocks of 4 (8-fold)

<i>a b c d</i>	<i>a c e g</i>	<i>a d h i</i>	<i>b d e i</i>	<i>b e f h</i>	<i>c d e f</i>
<i>a b e f</i>	<i>a d f h</i>	<i>a e g i</i>	<i>b f g i</i>	<i>c e h i</i>	<i>c f g h</i>
<i>a b g h</i>	<i>a c f i</i>	<i>b c h i</i>	<i>b c d g</i>	<i>d f g i</i>	<i>d e g h</i>

Ten treatments: 30 blocks of 3 (9-fold)

<i>a b c</i>	<i>a f h</i>	<i>b d j</i>	<i>b h j</i>	<i>c h i</i>	<i>d g h</i>
<i>a b d</i>	<i>a g i</i>	<i>b e h</i>	<i>c d g</i>	<i>c i j</i>	<i>e f j</i>
<i>a c e</i>	<i>a h j</i>	<i>b e i</i>	<i>c d h</i>	<i>d e i</i>	<i>e g h</i>
<i>a d f</i>	<i>a i j</i>	<i>b f g</i>	<i>c e f</i>	<i>d e j</i>	<i>f g j</i>
<i>a e g</i>	<i>b c f</i>	<i>b g i</i>	<i>c g j</i>	<i>d f i</i>	<i>f h i</i>

Ten treatments: 15 blocks of 4 (6-fold)

<i>a b c d</i>	<i>a d i j</i>	<i>b c f i</i>	<i>b g h i</i>	<i>c d e h</i>
<i>a b e f</i>	<i>a e g i</i>	<i>b d g j</i>	<i>c e i j</i>	<i>d e f g</i>
<i>a c g h</i>	<i>a f h j</i>	<i>b e h j</i>	<i>c f g j</i>	<i>d f h i</i>

Ten treatments: 18 blocks of 5 (9-fold)

<i>a b c d e</i>	<i>a c f h i</i>	<i>a e g i j</i>	<i>b e f h j</i>	<i>c e f h i</i>
<i>a b c f g</i>	<i>a c g h j</i>	<i>b c d h j</i>	<i>b f g i j</i>	<i>d e f g h</i>
<i>a b d f i</i>	<i>a d e f j</i>	<i>b c e i j</i>	<i>c d f g j</i>	
<i>a b e g h</i>	<i>a d h i j</i>	<i>b d g h i</i>	<i>c d e g i</i>	

Thirteen treatments: 26 blocks of 3 (6-fold)

<i>a b c</i>	<i>a j k</i>	<i>b f k</i>	<i>c e j</i>	<i>d f j</i>	<i>e g l</i>	<i>h j m</i>
<i>a d e</i>	<i>a l m</i>	<i>b g j</i>	<i>c f l</i>	<i>d g m</i>	<i>e i k</i>	<i>i j l</i>
<i>a f g</i>	<i>b d i</i>	<i>b h l</i>	<i>c g i</i>	<i>d k l</i>	<i>f i m</i>	
<i>a h i</i>	<i>b e m</i>	<i>c d h</i>	<i>c k m</i>	<i>e f h</i>	<i>g h k</i>	

Sixteen treatments: 20 blocks of 4 (5-fold)

<i>a b c d</i>	<i>a e i m</i>	<i>a f k p</i>	<i>a h j o</i>	<i>a g l n</i>
<i>e f g h</i>	<i>b f j n</i>	<i>b g l m</i>	<i>b e k p</i>	<i>b h i o</i>
<i>i j k l</i>	<i>c g k o</i>	<i>c h i n</i>	<i>c f l m</i>	<i>c e j p</i>
<i>m n o p</i>	<i>d h l p</i>	<i>d e j o</i>	<i>d g i n</i>	<i>d f k m</i>

Twenty-five treatments: 30 blocks of 5 (6-fold)

<i>a b c d e</i>	<i>a f k p u</i>	<i>a g m s y</i>	<i>a h o q x</i>	<i>a i l t w</i>	<i>a j n r v</i>
<i>f g h i j</i>	<i>b g l q v</i>	<i>b h n t u</i>	<i>b i k r y</i>	<i>b j m p x</i>	<i>b f o s w</i>
<i>k l m n o</i>	<i>c h m r w</i>	<i>c i o p v</i>	<i>c j l s u</i>	<i>c f n q y</i>	<i>c g k t x</i>
<i>p q r s t</i>	<i>d i n s x</i>	<i>d j k q w</i>	<i>d f m t v</i>	<i>d g o r u</i>	<i>d h l p y</i>
<i>u v w x y</i>	<i>e j o t y</i>	<i>e f l r x</i>	<i>e g n p w</i>	<i>e h k s v</i>	<i>e i m q u</i>

In the case of blocks of two, i.e. pairs of experimental units, all possible pairs of treatments must be taken; there are  $\frac{1}{2}t(t-1)$  such pairs, giving a minimum of  $t-1$  replications. Arrangements for blocks containing one less unit than the number of treatments are

formed by omitting every treatment an equal number of times, and arrangements for blocks containing two less units are formed by omitting every treatment pair an equal number of times.

In general the minimum number of replications of  $t$  treatments in blocks of  $t - k$  units is equal to the minimum number of replications in blocks of  $k$  units multiplied by  $(t - k)/k$ , an arrangement for blocks of  $k$  units being convertible into one for blocks of  $t - k$  units by replacing every block by its complement, i.e. by a block containing all the treatments missing from the original block.

The five arrangements in which  $t$  equals  $b$ , shown in the first part of Table VIII, have the additional property already referred to that every pair of blocks has the same number of treatments in common. No arrangement having this property is possible for the values of  $k$ ,  $t$  and  $r$  for which arrangements are shown in the second part of the table, since in no one of these cases has  $\bar{\mu}$  an integral value.

Three series of values from Table VII are worth comment. Arrangements for the series in which  $\lambda = 1$  and  $k = r - 1$ , and the series in which  $\lambda = 1$  and  $k = r$ , can both be derived from completely orthogonalized Latin squares (i.e. hyper-Graeco-Latin squares) of side  $r - 1$ . The values of  $t$  corresponding to the first few values of  $r$  for these series are:

$r$	3	4	5	6	7	8	9	10
$t$ $\left\{ \begin{array}{l} \lambda = 1, k = r - 1: \\ \lambda = 1, k = r: \end{array} \right.$	4	9	16	25	[36]	49	64	81
	7	13	21	31	[43]	57	73	91

It is known that completely orthogonalized squares exist when the side is a prime number and also for sides 4, 8 and 9(2). It is also known that no such square of side 6 exists(3). Higher non-primes have not been investigated.

The structure of the first series is easily seen. If the treatments are set out in the form of a square and on this a hyper-Graeco-Latin square is superimposed the first set of blocks is formed from the rows of this square, the second from the columns, the third from treatments having the same Latin letter, the fourth from those having the same Greek letter, and so on. (In the case of primes the selection for the third, fourth, fifth, etc., sets is made by moving one, two, three, etc., columns in proceeding from one row to the next.) The arrangements for nine, sixteen and twenty-five treatments are shown in Table VIII.

The structure of the second series is exemplified in Table VIII by the arrangements for seven, thirteen, and twenty-one treatments. The arrangement for thirteen treatments will serve as an example. The first four treatments  $a, b, c, d$  form the first block. The remaining nine may then be arranged in the form of a square:

$e$	$f$	$g$
$h$	$i$	$j$
$k$	$l$	$m$

The next set of three blocks  $a e f g$ , etc., is formed by taking  $a$  with each of the rows of this square. The third set is formed by taking  $b$  with each of the columns, the fourth and

fifth by taking  $c$  and  $d$  respectively with the two sets of three groups derived from the right and left diagonal groupings.

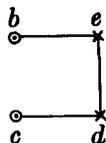
The series  $\lambda=2$  and  $k=r$  is also interesting, and I am indebted to Prof. Fisher for the elucidation of its structure. The first few values of this series are:

$r:$	3	4	5	6	7	8	9	10
$t:$	4	7	11	16	(22)	(29)	(37)	(46)

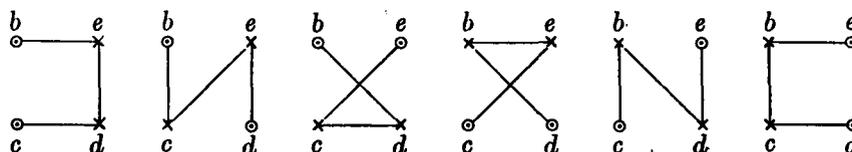
For the first two values  $k$  is greater than  $\frac{1}{2}t$ , the arrangement for  $t=7$  being the complement of that already considered for  $k=3$  and  $t=7$ . Arrangements for eleven and sixteen treatments are given in Table VIII.

We will first consider the arrangement for eleven treatments. If the first five rows and the first five columns are written as shown, any one of the treatments  $f$  to  $k$  is defined by its conjunction with two of the treatments  $b$  to  $e$  in blocks 2-5. Thus  $f$  has  $b$  and  $c$  as key letters. In blocks 6-11, therefore,  $f$  must occur once with  $b$ , once with  $c$ , and twice with  $d$  and  $e$ . It must also occur once with  $g, h, i,$  and  $j$ , all of which has  $b$  or  $c$  as one of their key letters, and twice with  $k$ , which has neither  $b$  nor  $c$  as a key letter. Moreover, each of the blocks 6-11 can itself be defined by two of the key letters  $b$  to  $e$ .

Consider now the following diagram for  $f(b, c)$ :

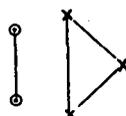


which is such that one line passes through  $b$  and  $c$ , and two lines through  $d$  and  $e$ . The lines  $be, cd,$  and  $de$  are to be taken to represent the three of the blocks 6-11 in which treatment  $f$  occurs. An arrangement will be possible if a set of diagrams of this type can be found such that every pair of diagrams having a key letter in common have one line in common and every other pair have two lines in common, for the treatments  $f$  to  $k$  will then each have two conjunctions. It is easy to see that



is such a set. This set corresponds to the arrangement set out in Table VIII.

The case of sixteen treatments involves five points, and a system of diagrams of the type



fulfils the required conditions. The higher members of this series have not been exhaustively

investigated; the possible alternatives here become numerous. It should also be noted that there are other ways of arranging the first  $r$  rows which might conceivably yield arrangements, though such possibilities can be immediately excluded for values of  $r$  up to 7.

#### EXAMPLE

In a preliminary report<sup>(1)</sup> on an experiment in which different generations of rats were being tested for ability to escape from a tank by a given route Prof. A. E. Crew publishes the scores of rats of various litters from five lines,  $A$ ,  $B$ ,  $C$ ,  $G$ ,  $K$ . The numbers of young in each litter are shown in Table IX.

Crew was concerned with the inheritance of ability in this one test, and tested every rat of every litter. Here we wish to consider the design of a suitable experiment for measuring the effects of variations in the test, or of different treatments of the rats prior to the test, e.g. the use of different drugs. We will assume, moreover, that the experimenter is interested also in the mean value for each litter of whatever ability he is measuring and that the governing factor in limiting the size of the experiment is the number of rats tested. This situation is very likely to arise when ample funds are available but sufficiently skilled assistance is hard to find; in any case the cost of rats destroyed at birth can hardly be of much consequence.

From Table IX we see that there are only four litters out of forty-three with more than seven rats and only nine with more than six rats. If, therefore, more than six variants are to be tested an ordinary randomized block arrangement with each block formed of a complete litter will not be of much use. Even if there are only six variants many litters will be rejected, and therefore no information on inheritance will be obtained from these litters.

Table IX

	$A$	$B$	$C$	$G$	$K$
$F_1$	6, (6), (5)	4, 1	5	3, 3, 4, 7	6, (7)
$F_2$	6, 6, 7, 5	3, 1, 2	5, 4, 4, 7, 9, 1	4, 6	7
$F_3$	5, 5, 10, 6, 9	3, 4, 5	5, 10, 5, 7*	5, 4	—
$F_4$	5	—	5*	4, 6	—

Brackets indicate litter died or was eaten before testing, an asterisk (\*) that testing was not complete when the report was written.

The effect of this rejection is even more serious than it appears at first sight, for the different lines exhibit very different levels of fertility, and line  $B$  would be entirely excluded from the tests if litters of six were demanded. In this test there is an association between fertility and success in the test which would remain unobserved if only rats of high fertility were used. Such differential fertility is usually of considerable interest: in this case it may account for part at least of the remarkable changes observed by Prof. McDougall.

We are therefore forced to consider the use of randomized blocks made up of rats from more than one litter, or alternatively incomplete randomized blocks. If four rats from

each litter are tested all litters except five of line *B* (which will require special treatment) will be included. We shall not, of course, obtain as much information on the large litters as we should if every rat were tested, but we shall be able to spread the tests over a larger number of litters (thus including, if we wish, more lines). In this connection it should be remembered that when comparing a litter of four rats with a litter of eight we only obtain one-third more information if all eight of the second litter are tested instead of only four.

In order to see the results that may be expected from material of this type, Crew's data were analysed as a uniformity trial, taking a random selection of four rats from each litter, and excluding those litters with less than four rats. Square roots of the test scores were analysed, the analysis of variance given in Table X being obtained.

Table X. *Analysis of variance, all litters, four rats from each litter*

	D.F.	Sum of squares	Mean square
Between lines	4	54.99	13.748
Between litters within lines	28	201.22	7.186
Within litters	99	309.71	3.128
Total	131	565.92	4.320

The additional variance due to litters within the same line, though quite appreciable, is not very large, the value for  $\rho$  being

$$\rho = \frac{\frac{1}{4}(7.186 - 3.128)}{3.128} = 0.324.$$

The values given in Table VI show that the use of ordinary randomized blocks, made up as far as necessary from two or more litters, will be about as efficient as incomplete blocks. With five treatments incomplete blocks are 6 per cent. more efficient, with ten treatments  $2\frac{1}{2}$  per cent. By the use of incomplete blocks we shall, however, avoid the necessity of using residuals to calculate the litter differences. The reduction in the apparent differences between litters by the use of residuals is quite appreciable when the number of replications  $r$  of each treatment is small, the differences of two litters having no treatment in common being reduced (on the average) by  $1/r$  times the true difference.

We will illustrate the computations by superimposing an arrangement of seven dummy variants on the first seven litters of line *C*, which give the four replications required for a symmetrical arrangement.

Table XI shows the square roots of these litters, and also the random arrangement obtained. The set of treatments assigned to each litter was determined (using the arrangement given in Table VIII) by random choice. The treatment totals and the quantities  $Q$  are shown in Table XII. It will be noted that the sum of all the  $Q$ 's is seven times the grand total of all the values. The quantities in the last line of Table XII are the adjusted treatment means, being equal to  $\frac{1}{14}Q - 4.318$ . The differences of these quantities represent the estimates of the effects of the treatments on a single rat.

Table XI. *Square roots of scores, line C*

	Litter						
	1	2	3	4	5	6	7
	(b) 0.0 (g) 2.2 (f) 3.3 (c) 5.0	(g) 2.4 (d) 2.6 (a) 6.2 (b) 1.4	(f) 2.4 (c) 2.4 (d) 3.0 (a) 4.0	(b) 3.6 (a) 5.0 (f) 5.1 (e) 6.5	(g) 4.7 (f) 5.3 (e) 5.5 (d) 6.6	(b) 5.1 (d) 4.7 (e) 7.6 (c) 9.6	(e) 7.5 (a) 2.2 (g) 2.6 (c) 4.4
Total	10.5	12.6	11.8	20.2	22.1	27.0	16.7
<i>Q'</i>	103.4	115.0	96.3	131.0	137.3	153.4	109.9
Adjusted litter means	3.07	3.90	2.56	5.04	5.49	6.64	3.53

Table XII. *Treatment totals and adjusted means*

	Treatment							Total or mean
	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>e</i>	<i>f</i>	<i>g</i>	
Total	17.4	10.1	21.4	16.9	27.1	16.1	11.9	120.9
<i>Q</i>	129.2	91.0	140.5	115.0	143.3	120.7	106.6	846.3
Adjusted mean	4.91	2.18	5.72	3.90	5.92	4.30	3.30	4.318

The analysis of variance is given in Table XIII. The sum of squares between litters is computed in the ordinary manner. The sum of squares for treatments is the sum of squares of the deviations of the *Q*'s multiplied by 6/28.4.3 or 1/56. The error sum of squares is obtained by subtraction.

Table XIII. *Analysis of variance*

	Degrees of freedom	Sum of squares	Mean square
Between litters	6	56.12	9.353
Treatments	6	37.28	6.213
Error	15	30.18	2.012
			} 3.212
Total	27	123.58	4.577

Fitting treatments before litters (test for litters):

	Degrees of freedom	Sum of squares	Mean square
Between treatments	6	48.86	8.143
Litters	6	44.54	7.423

The arrangement is notable in that the analysis shows a significant difference between the dummy treatments, for  $z=0.564$ , whereas the 5 per cent. point is 0.513. That this is

due to a genuine chance conjunction of one treatment with rats having good performances (low scores) relative to their litter mates, and not to any defect of the statistical analysis, is indicated by examination of Table XI. Treatment (*b*) has much the lowest score in three of the four litters in which it occurs, and a score nearly equal to the lowest in the fourth. The chance of any one of the seven treatments having either all four lowest or all four highest scores of the litters concerned is approximately  $2 \times 7/4^4$  or  $1/18$ .

The standard error of the difference of any two of the adjusted treatment means is equal to

$$\sqrt{2 \times \frac{4.6}{28.3} \times 2.012} = 1.07.$$

In the arrangement of seven treatments in blocks of four every pair of blocks has the same number of treatments in common, so that the differences between litters can here be estimated in precisely the same manner as the differences between treatments. Quantities  $Q'$ , similar to  $Q$ , and adjusted litter means, have been calculated and are shown in Table XI. The standard errors are the same as those already calculated.

Had an arrangement which was not reciprocally symmetrical been used the differences between litters could only have been computed by applying corrections for the treatment differences. The adjusted mean for litter 1, for instance, is given by

$$\frac{1}{4} (10.5 - 2.18 - 5.72 - 4.30 - 3.30) + 4.318 = 3.07,$$

or by

$$\frac{1}{4} (10.5 + 4.91 + 3.90 + 5.92 - 3 \times 4.318) = 3.07.$$

#### SUMMARY

The paper describes a general method of arranging replicated experiments in randomized blocks when the number of treatments to be compared is greater than the number of experimental units in a block. This new type of arrangement, for which the name of *symmetrical incomplete randomized blocks* is proposed, is such that every two treatments occur together in a block the same number of times. This restriction enables estimates of the treatment effects and of the experimental error to be obtained expeditiously by the ordinary procedure of the analysis of variance. Estimates of block differences can also be obtained if required. The special case in which the blocks are formed of pairs of experimental units is capable of specially simple treatment. The method of symmetrical incomplete randomized blocks is likely to be of most use in cases in which the experimental material naturally divides itself into groups, such as litters of experimental animals, containing numbers less than the number of treatments that it is desired to test, especially if the differences between these natural groups are of interest.

The necessary formulae are presented and their application illustrated by numerical examples, one based on the numbers of local lesions produced by a virus on half leaves of susceptible plants, the other on the scores of rats in a discrimination test. The minimum number of replications required for different numbers of treatments and block sizes is

discussed, and actual arrangements are given for the cases likely to be of general utility. A short discussion of the relative efficiency of an arrangement of this type and an arrangement in ordinary randomized blocks is also included.

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