Commonwealth Bureau of Plant Breeding and Genetics

Field Trials
Their Lay-out and Statistical Analysis

by

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INTRODUCTION

The object of all experiments whether of a field or laboratory character is to provide the research worker with an answer to a question he has put When a number of distinct varieties of barley for example have been produced the plant breeder may require to know which are the best yielders Suppose that he has five such varieties and lays out on an apparently uniform area of ground five plots alike in size and shape he may obtain yield results as follows

TABLE I

Variety	*A*	*B*	*C*	*D*	*E*	Total	Mean
Yield (cwt per acre)	22 5	24 7	28 3	23 1	25 4	124 0	24 8

Now is he to conclude from this experiment that variety *C* is certainly the best that *B* and *E* come next in order and that *A* and *D* are the worst yielders? Further will it be possible for him to distinguish between *B* and *E* or between *A* and *D* in yielding capacity since the difference is only 0 7 (or 0 6) cwt a difference which is small compared with that between *C* and *E* for example? The answer to these questions depends on a number of factors The experimenter wants to know not just what the result of a particular experiment such as this will be laid out as it is on particular plots in a particular season and subject to the hazards of that season and the errors attendant upon the operations of sowing harvesting etc but rather whether a particular variety can be recommended as generally superior in yielding capacity to another or if such superiority can only be claimed for particular districts with well defined soil characteristics or in particular conditions of climate or weather he wants to know what these conditions are He is therefore concerned to know how far the above figures can be relied on to repeat themselves when the experiment is carried out again He will know not to expect the figures to be *exactly* the same as before but will appreciate information as to the margin of *error* to be expected By *error* we do not mean mistakes in setting out equal areas or in leaving part of the area uncropped or losing a few sheaves in the process of threshing or making a wrong reading of the balance in determining the grain weight for we may assume that such errors will not occur with a careful experimenter But there will be irregularities in the sowing of the drill there will be losses due to bird or disease attack or lodging some loss will inevitably be occasioned by the procedure of harvesting and threshing and so on These and other incidental factors we shall call the *experimental error* Further he is unlikely to want to repeat the experiment on the same plots if his results are to give general information to farmers and he will soon find that different plots will give different results even when the variety is the same for all Such differences we may refer to as soil irregularity or heterogeneity or *soil error* Indeed if the experimenter is concerned with testing his varieties at different places in the same season he will soon find himself facing this particular difficulty If on the other hand he repeats the experiment in different seasons at the same place he will again find that the results change this time because of seasonal differences

A comprehensive system of experimentation would in the course of time provide for a large number of experiments to be carried out at different places over a large number of years and in the end no doubt reliable results would be reached concerning the yield characteristics of the varieties under test But this may be neither possible nor desirable in the case of all crossings likely to be made A limited amount of seed only may be available from first crossings and there may be a large number of these and it may be a waste of time and money to continue experimenting with more than a small number of the most promising varieties Because of

these and other considerations it is important that the experimenter should obtain a measure of the *reliability* of the results of a particular experiment at the same time as he obtains yield determinations This means to go back to our illustration that not only does he want to know the extent of the variation occurring were it possible to repeat the experiment on variety *A* for example over and over again on the *same* plot and in the *same* season but also what variation is to be expected if variety *A* were to be grown on the second plot instead of the first and variety *B* on the first instead of the second This is where an appeal is made to the theory of statistics which is a study of the variation inherent in natural phenomena as expressed in terms of numerical results of which varietal yield data are an example To proceed to abstract ideas we may consider that the figure 22 5 is a single experimental determination of the yield of the first plot for which there is a certain unknown true value which would be obtained were all sources of experimental error tending to lead to *different* experimental results eliminated We are then concerned to attach to this figure another expressing the amount of the variation to be expected through just those sources of experimental error that inevitably occur Experimentally such a figure for the variation cannot be found for we can carry out the experiment only once on a given plot in a particular year We can however carry out what is known as a uniformity trial that is we can set out a number of equal plots on a certain area and sow these with the same variety The resultant yield figures will however bring in a second source of error namely that due to soil for no two plots will be exactly alike Furthermore it is an elementary statistical fact that for a given fixed error over a certain area the greater the number of plots examined the more accurately are we able to determine not only the yield but also the reliability of the yield for plots of this size and character But every increase made in the number of plots tested brings an increase in the area covered and leads therefore to an increasing preponderance in the error of that portion of it ascribable to soil differences For it is known that soil fertility as it is called varies considerably over even small experimental areas There is a good deal of small scale patchiness irregular in character but superimposed on this there is generally observed a drift of fertility of a systematic character Uniformity trial data then have value in enabling us to calculate the amount of error to be expected over an area this error being a composite of the experimental error proper and the soil error to be expected in such an area Given a certain unit size of plot (and the best size and shape of plot to take is another thing to be determined) it is then seen that the unit which should be considered for the study of variation is not that of a single plot but rather of an area which will comprise as many plots as the number of varieties it is desired to compare Such an area is known as a *block* At the present stage we shall consider a compromise between the experimenter s desire to test as many varieties as possible and the fact that considerable soil fertility variations take place over even a small area to be arrived at by confining our attention to experiments involving only a moderate number of varieties say between five and ten and by having the unit plot size as small as is consistent with not raising the experimental error for that plot to too high a proportion of the yield This is consistent with what we have said above as to the aims of the experimenter for in the case illustrated if the block is chosen as the area covered by five plots we are allowing for the effect on yield of interchanging the positions of varieties *A* and *B* *C* and *E* *A* and *E* etc

Measurement of Experimental Error

If we choose as a block the area covered by the five hypothetical varieties whose yields are given in Table 1 and if we assume for the time being that the same variety was used on all five plots we can illustrate from this small sample how to calculate a measure of the experimental error Let the yields be denoted by x_1 x_2 x_3 x_4 and x_5 i e $x_1 = 22\ 5$ $x_2 = 24\ 7$ etc Table 1 already shows the calculation of the first important statistical quantity the *mean* which is obtained by adding together the five yields and dividing by 5 giving the result 24 8 We shall denote this value by $\bar{x}$ (read x bar) We can now say that 24 8 cwt per acre is an *estimate* of the true yield of the area covered by the experiment and it is an estimate which will be more precise than if we had taken any one of the five plot yields as an estimate of the true yield not only because we have cropped the whole area instead of a part only but also because it is the mean of five independently obtained yields and in taking the mean the experimental errors will

tend to cancel out e g 22 5 might have been a slight underestimate of the yield of the plot marked *A* and 24 7 a slight overestimate of the yield of that marked *B* and so on

Now the deviations of the plot yields from the estimated mean are obtained by subtracting 24 8 from 22 5 24 7 etc Let us square these deviations and add them together We get the result 20 8 (for details see Table 2) Divide this result by 4 a figure obtained by remembering that while there are five deviations only four of these are independent since the sum of all the deviations must be zero and if four are given the fifth is automatically determined This divisor is known as the number of *degrees of freedom* (D F) The result of the division is 5 2 This figure is known as the estimate of the *variance* of the yield of a single plot of the chosen size within the chosen block and is generally denoted by s^2 Its square root s which is in this case 2 28 is an estimate of the *standard deviation* (S D) or *standard error* (S E) of the yields and is in units of cwt per acre This figure we use as a measure of the experimental error The calculations involved are set out in Table 2 which shows at the same time two other methods of obtaining the same result useful when longer series involving more figures have to be examined In the second method the numbers are squared and added without the mean being first subtracted and from the result is subtracted one fifth of the square of the total In the third method any round number in this case 20 is mentally subtracted from each figure to give smaller numbers for squaring and method II is applied to the resulting figures In general when following methods II and III the square of the total is divided by the number of plots and with all methods the number of degrees of freedom used in the final division is one less than the number of plots

TABLE 2

	Method I		Method II	Method III	
x	$x-\bar{x}$	$(x-\bar{x})^2$	x^2	$x-20$	$(x-20)^2$
22 5	−2 3	5 29	506 25	2 5	6 25
24 7	−0 1	0 01	610 09	4 7	22 09
28 3	3 5	12 25	800 89	8 3	68 89
23 1	−1 7	2 89	533 61	3 1	9 61
25 4	0 6	0 36	645 16	5 4	29 16
Total 124 0	Total	20 80	Total 3096 00	Total 24 0	136 00
− 5		− 4	$\frac{1}{5}(124\ 0) = 3075\ 20$	$\frac{1}{5}(24\ 0^2) = 115\ 20$	
$\bar{x} = 24\ 8$	$s^2 = 5\ 20$		diff = 20 80	diff =	20 80
	square root 2 28				

Estimate of true yield = 24 8 cwt per acre
Estimate of standard deviation = 2 28 cwt per acre (9 19 per cent)

A study of the theory of statistics tells us that if our yield figures are *normal* in their variation the mean range in a sample of five determinations should be 2 326 times the true standard deviation (see Tippett *The Methods of Statistics* 1931 (Williams and Norgate) p 26) *Mean range* is defined as the average value of the difference between the extreme measures in the sample over a very large number of samples of the size chosen The range in a single sample will diverge somewhat from the mean range and the estimate obtained from that sample of the standard deviation will differ from the true value of this constant Nevertheless it is interesting to notice that if we multiply 2 28 by 2 326 we get 5 3 while the range in this sample is 5 8 (28 3 − 22 5) In fact we might have obtained an estimate of the standard deviation somewhat more easily by dividing 5 8 by 2 326 giving the result 2 49 but the method illustrated in Table 2 is known to be more accurate so that the extra labour involved in the calculation is worth while

Test of Significance

We can now see how our measurement of experimental error enables us to distinguish real differences between the yields of different varieties Still assuming that the figures of Table 1 represent the yields of the same standard variety then if on a plot of the same size within this area and at the same time a different variety had been grown and had given a yield of say 30 cwt per acre we should be inclined to the statement that its true yield was probably above that of the standard variety because 30 lies outside the range of the variation given by the five plots growing the standard But no certainty can attach to such a statement for since a single plot of the new variety has been grown we cannot say how near the figure 30 is to the true yield of the new variety Since the new variety is probably like the old in its variation of yield over this area we may expect that the figure 30 also has a standard deviation of the order 2 3 so that the true yield might easily be as low as or even lower than $30 - 2\,3 = 27\,7$ a figure which is lower than the highest figure (28 3) from the sample of plots of the standard variety Of course the true yield might also be as high as $30 + 2\,3 = 32\,3$ or higher but in the absence of additional information we cannot neglect the possible inference that the yield of the new variety has been overestimated A much more precise statement can be made if it is known that five plots of the new variety grown at the same time and on this area independently of the other five gave a mean yield of say 28 6 cwt per acre We can here use another result of the theory of statistics namely that the standard error of the mean of five yield figures may be estimated by taking the value 2 28 for the estimated standard error of any one plot yield and dividing it by the square root of 5 namely 2 236 The easiest way of doing this is to divide $s^2 = 5\,2$ by 5 and then take the square root of the quotient 1 04 giving the result 1 02 This expresses the fact that the means of different groups of five plot yields grown at the same time and place may vary to an extent which is expressed by a standard deviation of 1 02 If we assume that the new variety has the same degree of variation although determined independently of the first then we have two results 24 8 and 28 6 each the mean of five determinations which have an estimated standard deviation of 1 02 Yet a third theoretical result can now be used The standard deviation of the difference between 24 8 and 28 6 namely 3 8 (ignoring the sign) is obtained since the samples are of the same size by multiplying 1 02 by the square root of 2 namely 1 414 We get the answer 1 44 Now had the two varieties compared been identical the true difference in the yields would have been zero But in an actual experiment if we confined our testing to five plots of each we should not find the difference to be exactly zero The difference would sometimes have favoured the one set of plots and sometimes the other and the variation in the difference of the means would be expressed by its standard deviation of 1 44 A final test is now possible with the aid of tables Divide the difference 3 8 by its standard error 1 44 obtaining the result 2 64 Look up the table of the *distribution of t** in the line n (number of degrees of freedom) $= 8$ since there are 4 degrees of freedom for each of two samples of size 5 We find that $t = 2\,64$ lies between 2 306 and 2 896 corresponding to probabilities of 0 05 and 0 02 respectively The meaning of this is that were the true difference zero a value of t equal to or greater than 2 306 would only occur once in twenty times on the average if the experiment were repeated a very large number of times A value of t equal to or greater than 2 896 would on the other hand only occur once in fifty times on the average These probabilities are so small that we may take it as established that such a difference as 3 8 leading to a t of 2 64 would be unlikely to occur in a single trial and we therefore say that the hypothesis that the varieties compared were identical in yielding capacity is disproved If the varieties were in fact identical there would be a chance of being wrong in this inference of something between 1 in 20 and 1 in 50 There is nothing mysterious about t It is simply the difference between the means measured on a scale of which the unit is the standard error of this difference

It is customary to regard the probability level of 0 05 as a critical value dividing results in which we cannot make such an inference as the above from those in which we can Thus

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* Fisher R A and Yates F *Statistical Tables* 1938 (Oliver and Boyd) p 26 This volume contains all the tables that are required by the agricultural experimental worker The table of t is reproduced not quite so extensively in most of the modern works on Statistics

if t is greater than 2 306 for 8 degrees of freedom we say that the hypothesis is disproved and we express this fact by saying that the observed difference is *significant* It will be seen from the table of t that when n is 60 or greater the 0 05 or 5 per cent probability point is 2 or less and this is the basis of the usual statement that when samples are large a difference between means of twice *its* standard error or more is significant When the samples are not large enough for this statement to be true it is advisable to consult the table to ascertain the exact value of t corresponding to the 5 per cent *significance level*

Although this calculation is useful as showing the statistical methods involved in a test of significance we are to some extent begging the question for so far only an area covering five plots of one variety has been considered from the yields of which an estimate of experimental error *over this area* has been worked out It would be impossible to set out five plots of a *different* variety on the same area at the same time The comparison between varieties can be made however if we extend the experimental area to double the original size so as to allow for ten plots being set out although in doing so we shall tend to increase the experimental error since for a fair comparison both varieties will have to sample fairly the double area and the five plots of each variety will therefore be scattered to a greater extent than before Alternatively we may halve the size of the experimental plot provided that in doing so the experimental error is not thereby unduly increased in proportion to the plot yield In either case we now have ten plots of which five are allotted to each of two varieties Consideration must then be given to the question of arrangement not only to provide the fair comparison hinted at above but also to eliminate as much as possible of the soil error in order to increase the precision of the comparison A suitable method applicable to any number of varieties is that of *randomized blocks*

Method of Randomized Blocks

Suppose in order to fix our ideas that we have four varieties that we wish to compare and that we agree to have five fold *replication* of each i e there are to be five equal sized plots sown to each of the four varieties This means that we must allow for twenty experimental plots Having fixed on a convenient unit size of plot we must therefore choose a fairly compact area of twenty times this size on ground which is as uniform as possible This should then be divided into five *blocks* and each block further subdivided into four plots The result might be an arrangement like the following —

	Block 1	Block 2	Block 3	Block 4	Block 5	
1	*C* 68	*C* 70	*B* 83	*B* 93	*C* 80	17
2	*D* 87	*B* 76	*C* 76	*D* 92	*A* 81	18
3	*A* 57	*A* 59	*D* 82	*A* 79	*B* 86	19
4	*B* 72	*D* 71	*A* 59	*C* 96	*D* 93	20
	Plots 1–4	Plots 5–8	Plots 9–12	Plots 13–16	Plots 17–20	

Fig 1

Let the varieties be designated *A* *B* *C* and *D* We must now arrange that each block contains one *A* one *B* one *C* and one *D* There will be certain variations in soil fertility between the plots of a block though they are unlikely to be as great as those between plots from different

blocks We ought to allot a plot of block 1 to *A* which is as likely to measure the fertility of that block as any other plot and so on for varieties *B C* and *D* As in addition we wish to secure that the individual members of the sample of *A* for example shall be independent of one another and of the members of *B C* etc in the probability sense if our formula for the standard deviation of the mean is to be applicable we must allot the four varieties wholly at random There are various ways of doing this perhaps the simplest being to take a random set of two figure numbers prepared for such problems as this (e g choose any two figure column on any page of the set of random numbers given by Fisher and Yates *loc cit* pp 82–87) and run down the list mentally dividing by 4 writing down the remainders as they occur If a number is a multiple of 4 the remainder is to be considered as 4 while if a particular remainder has occurred before it should be ignored Since all remainders from 1 to 4 have an equal chance of occurring with a set of random two figure numbers we have by this means randomized these numbers For example if the numbers run 23 79 44 81 etc this gives us the order 3 4 1 and 2 the last number following from the fact that the others have been allocated Numbering the varieties *A B C* and *D* as 1 2 3 and 4 respectively we see that the order in the first block should be *C D A B* and so on for the other blocks Fig 1 shows the result of randomizing the numbers 1 to 4 five times This gives the field arrangement which should be adhered to strictly and a plan of which should accompany the yield figures which result from the experiment The figures in Fig 1 give the yields of grain in a barley experiment carried out on this plan in lb each plot being 1/40 acre Re arranging these figures we have the following table —

TABLE 3—YIELDS OF BARLEY IN LB ON 1/40 ACRE PLOTS

	Varieties					
Blocks	*A*	*B*	*C*	*D*	Block Total	Mean
1	57	72	68	87	284	71
2	59	76	70	71	276	69
3	59	83	76	82	300	75
4	79	93	96	92	360	90
5	81	86	80	93	340	85
Variety Total	335	410	390	425	1560 = Grand Total	
Mean	67	82	78	85		78 = General Mean

The figures in this table have been slightly adjusted from yields actually obtained in an experiment to make for easier arithmetical work in illustrating the processes involved In addition to the yields we have recorded the block and variety totals and their means also the grand total 1560 and the general mean 78 The block totals should of course add up to the same grand total as the variety totals otherwise the arithmetic is at fault

This body of data is treated as a whole for purposes of analysis but to illustrate the method of working and the principles on which it is based we shall begin by confining our attention to varieties *A* and *B* only We can regard the difference between the yields of *A* and *B* in the first block for example as a measure of the superiority of one variety over the other but it is a difference which is subject to a certain amount of experimental error including in this term such soil fertility differences as exist between individual plots of the block However there are five such parallel differences and we can treat them as a sample of five numerical determinations and find from them the mean difference together with its standard error The calculations are as follows —

TABLE 4

	Diff *B–A*	Devn from mean	Square
	15	0	0
	17	2	4
	24	9	81
	14	−1	1
	5	−10	100
Total	75		186
Mean	15		

Estimate of true difference $= 15$

Estimated variance of mean difference $= \frac{186}{4 \times 5} = 9\,3$

Estimated standard error of mean difference $= \sqrt{9\,3} = 3\,05$

$t = \frac{15}{3\,05} = 4\,9$ $n = 4$ (number of D F)

From table of t for $P = 0\,01$ $t = 4\,6$

Note that in this and the following examples methods II and III of Table 2 can alternatively be followed in computing the sums of squares

Since t is greater than the value given in the table at the 1 per cent significance level we may say that the difference in yield in favour of B is clearly significant, by which we mean that we have disproved at this level of probability the possible hypothesis that the varieties are identical in yielding capacity

In order to show the advantage of laying out the land in blocks let us perform the calculation in another way Assume that we have five values of A and five of B without the added knowledge that they are associated in pairs in respect to soil fertility then we may calculate the estimated standard error of the mean difference in the following way —

TABLE 5

A devn from 67	Square	*B* devn from 82	Square	Product of devns
−10	100	−10	100	100
− 8	64	− 6	36	48
− 8	64	1	1	−8
12	144	11	121	132
14	196	4	16	56
	568		274	328

Total sum of squares $= 568 + 274 = 842$

Divide by 8 (number of D F $= 4 + 4$) giving 105 25

Estimated variance of A or B mean $= \frac{105\,25}{5} = 21\,05$

Estimated variance of $B–A$ mean difference $= 21\,05 + 21\,05 = 42\,1$

Estimated standard error of mean difference $= \sqrt{42\,1} = 6\,49$

$t = \frac{15}{6\,49} = 2\,31$ $n = 8$ (number of D F)

From table of t for $P = 0\,05$ $t = 2\,31$

The calculation has been set out in a way which is applicable to two samples of different sizes except that in that case the estimated variances of the A and B means would be different the divisor being the size of the sample in each case In the case illustrated since the samples are equal in size we have precisely the same results as if we had proceeded as indicated on

p 6 except that the estimated standard deviations of the two series are no longer equal Thus we might have calculated the estimated standard error of the A mean 67 as

$$\sqrt{\left(\frac{568}{4 \times 5}\right)} = \sqrt{28\,4} = 5\,33$$

and likewise that for the B mean 82 as

$$\sqrt{\left(\frac{274}{4 \times 5}\right)} = \sqrt{13\,7} = 3\,70$$

Then instead of multiplying a common figure by $\sqrt{2}$ we should add the squares of the separate standard errors and take the square root of the result to obtain the estimated standard error of the difference 15 of the A and B means i e

$$\sqrt{(28\,4 + 13\,7)} = \sqrt{42\,1} = 6\,49$$

as before

We may first note that since with this method of working t is only at the 5 per cent significance level the significance of the difference in yield in favour of B is not so clearly demonstrated This leads us to the second point namely that the difference between the two methods of working lies in the higher standard error of the second case due to the variation between the yields of A and of B in the different blocks entering into our calculation It is in fact the neglect of the information available that the varieties are associated in pairs that has led to a new result different from the old If there are soil fertility differences between the blocks this will tend to increase the standard error in the second method but not in the first since it was only the intra block differences which were used in that calculation The method of layout in fact indicates that the first method is the correct one to adopt The A and B series are not independent but are correlated in the sense that they are associated in pairs and as can be seen from Table 3 low values in one series tend to be associated with low values in the other and high with high To relate the methods we need only add that the calculation of the estimated standard error of the difference between the means as $\sqrt{(28\,4+13\,7)}$ is *no longer correct when the series are correlated* The last column of Table 5 gives the products of deviations from the A and B means respectively together with their sum 328 The correct calculation for the estimated standard error of the difference between the means is now

$$\sqrt{\left(\frac{568 + 274 - 2(328)}{4 \times 5}\right)}$$

$$= \sqrt{\left(\frac{186}{20}\right)} = \sqrt{9\,3} = 3\,05$$

in agreement with the first method and it should be noted that the number of degrees of freedom must be reduced to 4 In words we subtract twice the sum of products of deviations from the total of the two sums of squares

Another way of bringing out the difference of method is to use the arithmetical processes of the *Analysis of Variance* As these are of universal application in our subject it would be well to explain them now in connexion with this simple example Begin with the following extract from Table 3 —

TABLE 6

A	*B*	Total	
57	72	129	
59	76	135	
59	83	142	
79	93	172	
81	86	167	
335	410	745	Common mean = 74 5

The calculation proceeds in stages —

(1) *Total sum of squares* —Treat the data as one sample of ten yield results and work out the sum of squares of deviations from the common mean 74 5 Adopting the routine method of summing the squares of 57 59 86 we obtain 56907 from which must be subtracted the correction for the mean which is $745^2 \div 10$ or alternatively 745 × 74 5 (the product of grand total and common mean) This correction is 55502 5 and the difference is the required total sum of squares 1404 5 with 9 degrees of freedom since the composite sample is of size 10

(2) *Sum of squares for varieties* —Treat the *A* and *B* totals 335 and 410 as a sample of size 2 and treat as in (1) except that we must divide the result by 5 since each figure is a total of five unit plots The easiest way to do this in general is to sum the squares of 335 and 410 obtaining 280325 divide by 5 giving the result 56065 then subtract the correction 55502 5 as in (1) leaving the required sum of squares for varieties 562 5 with 1 degree of freedom The same result may be obtained in the present case by dividing the square of the difference (410 − 335) by 10 (the total number of plots) but note that this particular simplification is only applicable to a sample of size 2 as in the present case since we have only considered a simplified experiment with two varieties

(3) *Sum of squares for blocks* —Assuming that this is the whole experiment the block totals are 129 135 167 as in Table 6 Treat these as in (1) but divide by 2 since each figure is a total of two unit plots Summing the squares of 129 135 167 we obtain 112503 divide by 2 giving 56251 5 subtract the correction 55502 5 as in (1) leaving the required sum of squares for blocks 749 0 with 4 degrees of freedom

(4) *Sum of squares for error* —This is what is left over on subtracting the sums of squares for varieties and blocks from the total sum of squares We have 1404 5 − 562 5 − 749 0 = 93 0 and the degrees of freedom are 9 − 1 − 4 = 4 which number it should be noted is the product of 1 and 4 the degrees of freedom for varieties and blocks respectively It will be noted that the total sum of squares is now analysed into three components those due to differences between variety totals and block totals respectively and a remainder and with each component is associated a certain number of degrees of freedom While in the straight forward case the degrees of freedom are obtained by subtracting 1 from the size of sample considered it should be noted that the concept of degrees of freedom is more general than this This is illustrated by (4) and we say here that it represents the number of yields which must be given so that *row and column totals being provided* the data of Table 6 can be reconstructed It is easy to see that the answer is in this case 4 because if the first four yields of *A* are given the fifth is determined since the total of *A* is given the *B* yields follow since in each case the total of *A* and *B* is given

This sum of squares for error represents the amount of variation present between plots of the same variety (i e variation *within* varieties) after allowance is made for the differences in fertility of the different blocks To show this let us perform the calculation in another way Replace the plot values by their deviations from the mean of the block in which they are situated thereby eliminating from any further calculation the block differences For example the mean of the first block is 129 − 2 or 64 5 and 57 − 64 5 = −7 5 We get the following figures —

	A	*B*
	− 7 5	7 5
	− 8 5	8 5
	−12 0	12 0
	− 7 0	7 0
	− 2 5	2 5
Total	−37 5	37 5

Now calculate the sum of squares of deviations of the numbers in column *A* from their mean −7 5 Ignoring the negative signs which do not matter we have 327 75 for the sum of squares

of 7 5 8 5 2 5 from which we subtract the correction $37\ 5^2 - 5$ or 281 25 leaving us with 46 5 The corresponding calculation for the *B* column must yield the same result and therefore for the total sum of squares *within varieties* we have 93 0 agreeing with the result obtained in (4) The only new point about this calculation is that we are dealing with a series of deviations from changing means instead of a series of primary yields This should be contrasted with Table 5

If each component sum of squares is now divided by its corresponding number of degrees of freedom we obtain three *mean squares* as they are called The analysis of variance table is set out as under —

TABLE 7 —ANALYSIS OF VARIANCE

Variation	D F	Sum of Squares	Mean Square	Variance Ratio	log (Mean square/10)
Between varieties	1	562 5	562 5	24 19	4 0298
Between blocks	4	749 0	187 25	8 05	2 9299
Error	4	93 0	23 25		0 8437
Total	9	1404 5			

The theory on which the method is founded is that had the data consisted of one homogeneous sample of ten yields with no differences between varieties and between blocks except those due to experimental error the above three mean squares would all be estimates of the variance of a single plot yield In such a case there would in fact be no need for sub division and we would use $1404\ 5 - 9 = 156\ 1$ as an estimate of the variance Even were this so however an arbitrary division into classes is always possible for example the blocks might be equal in soil fertility and the varieties similar in yield yet they can still be classified by blocks and by varieties because that is the layout adopted in the field Thus the above calculations will be possible but the three estimates of variance will not be equal because they are calculated from very small samples and chance differences will enter in Now we want a method of estimating how much variation is to be expected due to chance causes in order that we may determine whether the data are compatible or not with our hypothesis namely that there are no true variety and block differences It is clear that a real difference in yield between *A* and *B* will raise the mean square for varieties above the value that it would have were there a chance difference only and real differences in soil fertility between blocks will increase the mean square for blocks In the present example we see that 562 5 and 187 25 are both considerably greater than 23 25 This latter figure can be taken as an estimate of the variance of a single plot yield due to experimental error since it has been calculated by a process of eliminating variety and block differences from the original variation of the ten plots We require a test as to how much greater than 23 25 a given mean square must be before we can say that there is evidence of real difference Again the test is based on probability and can be carried out with the aid of tables Divide 562 5 by 23 25 giving a ratio 24 19 Look up the table of *Variance Ratio* (Fisher and Yates Table V pp 28–35) selecting the 1 per cent table (p 33) and reading off the value in the column headed $n_1 = 1$ (the number of degrees of freedom corresponding to the larger of the two numbers whose ratio is being taken) and in the row marked $n_2 = 4$ (the number of degrees of freedom for the smaller of the two numbers) We find the entry to be 21 20 This means that the ratio would exceed the value 21 20 once in a hundred times on the average were there no real varietal yield difference between *A* and *B* According to the usual rule we therefore say that the observed ratio of 24 19 is quite significant and our inference is that variety *B* is significantly better in yield than variety *A*

An alternative way of making the test is to use logarithms which incidentally avoids a division There is a table of *natural* logarithms of the numbers from 1 to 100 in Fisher and Yates (Table XXVI pp 64–67) and to use these since it is only ratios of mean squares with which we are concerned we can divide all the mean squares by 10 The last column of Table 7 gives the corresponding natural logarithms Subtract 0 8437 from 4 0298 giving 3 1861

Dividing by 2 we get 1 5930 which is the quantity known as z The corresponding table to that of the variance ratio is to be found on p 32 of Fisher and Yates (facing the other) The 1 per cent point for $n_1 = 1$ $n_2 = 4$ is found to be 1 5270 and significance is established as before The calculation of z shows incidentally that the method of analysis of variance gives exactly the same results as working out a value of t from the B–A differences We found that t was 4 9 The natural logarithm of 4 9 is 1 59 which is the value of the corresponding This is a mathematical relation connecting t with z when the n_1 of the z distribution is 1 and n is the n of the t distribution The significance is the same in both cases P being less than 0 01

The mean square due to blocks may be tested in the same way and we find a variance ratio of 8 05 or a z of 1 0431 The 5 per cent points are 6 39 and 0 9272 respectively and the 1 per cent points 15 98 and 1 3856 Thus block differences are significant at the 5 per cent significance level justifying the arrangement of the experiment in blocks because by doing so we have succeeded in eliminating a large part of the soil fertility differences As a rule there is no need to make a precise test of the point We may note however that had the ten plots been allotted at random five to A and five to B without any attempt to group corresponding pairs of A and B into blocks the error mean square (see Table 7) would have been expected to be of the order of $(749\,0 + 93\,0) - (4 + 4)$ i e $842 - 8$ or 105 25 instead of 23 25 This would give a value more than four times greater so that the precision of the experiment has been quadrupled by the block arrangement The reason why we would expect a figure of the order of $842 - 8$ is because with no association in blocks the total variation using the methods of analysis of variance would now be divided into two parts only (1) variation *between* varieties as in previous case and (2) variation *within* varieties as worked out in Table 5 With a different field arrangement the yield results would not of course be the same as before but we infer that the above figure would be the *order* of the error mean square This argument throws light on the earlier calculation of Table 5 which ignored the correlation between A and B for this 842 is just the total sum of squares of deviations from variety means for which the number of degrees of freedom was 8 In a similar fashion we can identify the sum of squares for error of Table 7 namely 93 0 with one half of the 186 from Table 4 the half coming in because the estimated variance is doubled in Table 4 since it is the variance of a difference B–A The calculations of the analysis of variance in the case of two varieties with its elimination of blocks are identical in their results with those used for testing the significance of the difference of the A and B means after allowing for correlation

We have stated that 23 25 is the mean square for error This means that we take this figure as the estimated variance of the yield of a single plot i e yields of plots of this size all of the same variety and not subject to fertility differences of the kind eliminated between blocks may be expected to vary to an extent indicated by a figure for variance of 23 25 Its square root 4 82 gives the estimated standard deviation in lb for one plot and a comparative measure of the accuracy with which the experiment has been conducted may be obtained by expressing this figure as a percentage of 74 5 the common mean of A and B (Table 6) We have

$$\text{Standard deviation of a single plot yield} = \frac{4\,82}{74\,5} \times 100 = 6\,47 \text{ per cent}$$

We may evidently now summarize the results of the trial by writing down the mean yields of the two varieties and attaching to them a standard error The standard deviation of the mean of five yield determinations is $4\,82 - \sqrt{5}$ or $\sqrt{(23\,25 - 5)}$ and this is 2 16 lb A table such as the following should be a part of every experimental record —

TABLE 8 —SUMMARY OF RESULTS

Mean yield	Variety *A*	Variety *B*	Mean	Standard Error
Lb per plot	67	82	74 5	2 16
Cwt per acre	23 9	29 3	26 6	0 77
Per cent	89 9	110 1	100 0	2 90

The mean yields in lb per plot are obtained from the column totals of Table 6 on dividing by 5 The plots being 1/40 acre each we obtain the figures of the second line of Table 8 by multiplying those of the first line by the factor 40/112 The figures of the third line are obtained by expressing those of the first as percentages of the common mean 74 5 It is usual to give the standard error to one more decimal place than the means

The standard error of the difference 82 — 67 = 15 of the variety means is 2 16 multiplied by $\sqrt{2}$ giving 3 05 This is the denominator of t in Table 4 t being in fact 15 divided by 3 05 A conclusion should follow such a table as Table 8 In the present case this would read simply yield of variety B is significantly greater than yield of variety A this being the result of a test of significance as illustrated below Table 4 In the general case with more than two treatments we should divide the difference between the means of any two treatments by the corresponding figure to 3 05 and see whether the t so obtained reached a significant value for n equal to the number of degrees of freedom for error For $n = 4$ t is 2 776 at the 5 per cent significance level Thus a difference would have to be 2 776 × $\sqrt{2}$ or 3 9 times the standard error given in Table 8 to be significant Inspection of the table of the distribution of t shows that this factor will be less than 3 for $n = 16$ or more (2 120 × $\sqrt{2}$ = 2 998) As the number of degrees of freedom for error is usually of this order or greater this has led to the convenient rule that a difference is to be adjudged significant if it is at least three times the standard error given in the summary table though this conclusion should be qualified by the statement that treatment differences should first be shown to be significant in the analysis of variance table

General Method

Returning to the full data of Table 3 we now see that a possible method would be to examine all possible pairs of varieties in each case testing for the significance of the mean difference With four varieties there are six such differences however and the work would be tedious In addition we should never have more than 4 degrees of freedom for error (with 5 replicates) and relatively large differences would be needed to show significance The factor just mentioned would have to be 4 instead of 3 The great advantage of the analysis of variance method lies in its general applicability to any number of treatments and we shall now give the results of applying it to the full data of Table 3 (*q v*) The method is exactly that outlined except that there are now four treatments and therefore four columns of figures instead of two

(1) *Total sum of squares*

$57^2 + 59^2 + 59^2 +$ $+ 92^2 + 93^2$	=	124270	
Subtract correction 1560 × 78		121680	
leaving		2590	(19 D F)

(2) *Sum of squares for varieties*

$335^2 + 410^2 + 390^2 + 425^2$	=	613050	
Divide by 5 (number of blocks)		122610	
Subtract correction		121680	
leaving		930	(3 D F)

(3) *Sum of squares for blocks*

$284^2 + 276^2 + 300^2 + 360^2 + 340^2$	=	492032	
Divide by 4 (number of varieties)		123008	
Subtract correction		121680	
leaving		1328	(4 D F)

(4) *Sum of squares for error*

2590 — 930 — 1328	=	332	(12 D F)

TABLE 9 —ANALYSIS OF VARIANCE

Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio = V R
Varieties	3	930	310 00	11 20
Blocks	4	1328	332 00	12 00
Error	12	332	27 67	
Total	19	2590		

For $n_1 = 3$ $n_2 = 12$ V R $(P = 0\ 001) = 10\ 80$
For $n_1 = 4$ $n_2 = 12$ V R $(P = 0\ 001) = 9\ 63$

Differences between varieties and between blocks are highly significant
Standard error of single plot yield $= \sqrt{27\ 67} = 5\ 26$ lb which is 6 74 per cent of the mean yield 78 lb
Standard error of mean of five plot yields $= \sqrt{(27\ 67 - 5)} = 2\ 35$ lb or 3 02 per cent

TABLE 10 —SUMMARY OF RESULTS

Mean yield	*A*	*B*	*C*	*D*	Mean	Standard Error
Lb per plot	67	82	78	85	78	2 35
Cwt per acre	23 9	29 3	27 9	30 4	27 9	0 84
Per cent	85 9	105 1	100 0	109 0	100 0	3 02

Conclusion —Varieties *B C* and *D* have given significantly greater yields than the control variety *A* but do not differ significantly among themselves

On the rough rule of three times the standard error the difference between the yields of varieties *C* and *D* appears to approach significance and it is worth while making a more exact test at any rate for purposes of illustration The standard error of a difference between two means of five plots each is $2\ 35\sqrt{2}$ or

$$\sqrt{(27\ 67 \times 2/5)} = 3\ 33$$

$$t = \frac{7}{3\ 33} = 2\ 10 \quad n = 12 \text{ (D F for } \textit{error}\text{)}$$

For $P = 0\ 05$ $t = 2\ 18$

Therefore the difference is to be adjudged not significant

It is sometimes convenient in such a table as Table 9 to mark those variance ratios which are significant and so distinguish them from non significant ratios One system has been to use * for significance at the 5 per cent level and ** for 1 per cent significance another system uses *S* and *SS* The new 0 1 per cent level included in the latest tables may suggest the use of a triple asterisk or triple *S* but this is possibly an overelaboration

NOTES ON CALCULATION

The first step is to copy out the yields or other numerical data to be subjected to analysis from the original field records in the form of Table 3 1 e in columns corresponding to varieties and in rows representing the different blocks (or *vice versa*) The copying should be carefully checked as should every stage of the resulting calculation The column and row totals and means are then worked out The means are less important than the totals The latter can be depended on to the last figure since the last figure of each plot record is presumably accurate so far as measurement goes to within half a unit If the means are recorded the number of decimal places should bear some relation to the extent to which the result can be depended upon to be accurate For example if ten plots total 736 the mean is 73 6 and therefore if nine plots (e g) total 691 their mean would be recorded as 76 8 and not 76 77778 Only if the mean is to be used in a subsequent calculation e g to be squared and possibly multiplied by a large number should additional decimal places be recorded for the time being Thus if we had a total of 2317 for 26 plots the correction for the mean which is illustrated in the above

example (shown there as total × mean) is 206480 35 correct to two decimal places (the usual number retained in analysis of variance calculations for two or three figure yields) This can be shown by squaring 2317 and dividing the result by 26 If the mean were written down as 89 1 the total multiplied by mean would be 206444 7 which would be in error by 35 65 To get the correct result by the (total × mean) method the mean would have to be worked out to six decimal places as 89 115385 This difficulty can however be in all cases avoided by using (total ^{2}— number of plots) and of course the accuracy of the above mean cannot be depended on to more than one decimal place

The squares of the yields and of the column and row totals can be read off from any table of squares (e g Barlow) and added either by hand or by means of an adding machine Alternatively the numbers can be squared and added as a continuous process and the correction made if a calculating machine is available Square roots may be read off correct to four significant figures and without any calculation by reading Barlow s tables inversely i e finding in the table of squares opposite a four figure number the square which is nearest to the number the extraction of whose square root is desired

The sum of squares for error has been obtained by subtraction of the sums of squares for varieties and blocks from the total sum of squares and the accuracy of the result will therefore depend on the previous calculations being correctly performed As a check on accuracy the procedure indicated on pp 11–12 may be followed by constructing a new table in which deviations of plot yields from the block mean are given and working out the sum of squares *within varieties* Alternatively we may proceed as follows In Table 3 the first figure 57 has a column mean of 67 and a row mean of 71 while the general mean is 78 Add 78 to 57 and subtract the sum of 71 and 67 We obtain the result —3 Proceeding through the table in this way using the appropriate column and row means in each case we get 20 residuals some negative and some positive which should add up to zero taking account of their signs The sum of squares of the residuals is the sum of squares for error

Method of the Latin Square

The method of randomized blocks permits the incorporation of any number of varieties in an experiment with any desired degree of replication Differences in fertility between blocks as wholes are eliminated and the standard error of a single plot yield is therefore one appropriate to an area of the size of one block Certain differences due to soil fertility are therefore bound to enter in to disturb the varietal comparisons and to lessen their precision Blocks may be arranged in one long line or in two dimensions In the latter case some soil fertility is evidently being eliminated in two directions at right angles to one another Within the blocks it is usually advisable to have the plots long and narrow with their long sides adjacent but sometimes especially with a considerable number of varieties these may be arranged in a two dimensional pattern to form a compact block

A special arrangement suitable when the number of varieties is not too small and not too large is that of the Latin square and will be illustrated in the case of four varieties Consider the following two dimensional arrangement —

(1)

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

Here each line of letters is related to the one above by a simple shift of one place to the left the plots pushed out to the left of the diagram coming in again at the extreme right It will be noticed that all varieties are present in all rows and all column of the square so that no variety occurs more than once in each row or once in each column This is an example of a *Latin square* By analogy with the randomized blocks arrangement it should be possible to eliminate soil fertility differences between the rows of the square and equally between columns

so that there are no two plots between which some degree of elimination of soil fertility differences is not possible Save in the case of a somewhat exceptional distribution of fertility this arrangement is likely to lead to more precise results than the randomized block plan It should therefore be applied whenever it is possible to have as many replications of the varieties as there are varieties The square adopted for any particular experiment should be chosen at random out of all the squares that exist for a given size If we keep the letters *A B C* and *D* in this order along the first row and down the first column then the example shown is one of the three self conjugate standard squares of the first transformation set the other two being

(2)

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

(3)

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

There is in addition one self conjugate standard square of a second transformation set namely the following —

(4)

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

The terms self conjugate and transformation set come from the nomenclature adopted in the theoretical process of classifying and enumerating the Latin squares that can be con structed from a given number of elements For a discussion of this point see Fisher and Yates *Statistical Tables* Introduction p 8 and the references there cited To obtain an experimental arrangement select one of these squares at random by choosing in the way already indicated for randomized blocks one of the numbers (1) (2) (3) or (4) at random Then re arrange all the rows of the chosen square other than the first in random order by assigning to them the numbers 1 2 and 3 and re arranging these with the aid of the table of random numbers Finally either re arrange at random all four columns and then allot variety 1 to the plots marked *A* variety 2 to the plots marked *B* and so on or alternatively assign the letters *A B C* and *D* at random to the four varieties 1 2 3 and 4 In the case of larger squares use should be made of Tables XV and XVI of *Statistical Tables* and the introduction to these tables (pp 8–10) should be consulted for the necessary instructions

EXAMPLE

In a variety experiment comparing control *A* with three other varieties *B C* and *D* of sugar beet square (2) was first selected at random then the second third and fourth rows were re arranged by putting *B C D* into the random order *C B D* finally the columns were re arranged by putting *A B C D* into the random order *B C D A* The resulting square is shown below together with the yields of roots in lb for each plot of 1/50 acre taken to the nearest 10 lb for convenience of illustration

B 65	*C* 66	*D* 67	*A* 60
D 64	*B* 62	*A* 57	*C* 72
A 50	*D* 58	*C* 64	*B* 65
C 56	*A* 48	*B* 59	*D* 67

FIG 2

The calculations proceed as in the case of randomized blocks remembering that one extra component of variation has to be calculated and subtracted from the total

Row totals	258	255	237	230
Column totals	235	234	247	264
Variety totals	215	251	258	256

Grand total = 980
General mean = 61 25

(1) *Total sum of squares*

$65^2 + 64^2 + 50^2 + \quad + 65^2 + 67^2$ = 60638
Subtract correction 980 × 61 25 60025

leaving 613 (15 D F)

(2) *Sum of squares for rows*

$258^2 + 255^2 + 237^2 + 230^2$ = 240658
Divide by 4 (number of plots in row) 60164 5
Subtract correction 60025

leaving 139 5 (3 D F)

(3) *Sum of squares for columns*

$235^2 + 234^2 + 247 + 264^2$ = 240686
Divide by 4 (number of plots in column) 60171 5
Subtract correction 60025

leaving 146 5 (3 D F)

(4) *Sum of squares for varieties*

$215^2 + 251^2 + 258^2 + 256^2$ = 241326
Divide by 4 (number of plots of each variety) 60331 5
Subtract correction 60025

leaving 306 5 (3 D F)

(5) *Sum of squares for error*

613 − 139 5 − 146 5 − 306 5 = 20 5 (6 D F)

TABLE II —ANALYSIS OF VARIANCE

Variation	D F	Sum of Squares	Mean Square	Variance Ratio
Rows	3	139 5	46 50	13 61**
Columns	3	146 5	48 83	14 29**
Varieties	3	306 5	102 17	29 90**
Error	6	20 5	3 42	
Total	15	613 0		

For $n_1 = 3$ $n_2 = 6$ V R $(P = 0\,01) = 9\,78$
$(P = 0\,001) = 23\,70$

Differences between rows between columns and between varieties are significant
Standard error of single plot yield = $\sqrt{3\,42} = 1\,85$ (in units of 10 lb) which is 3 02 per cent of the mean yield 61 25
Standard error of mean of four plot yields = $\sqrt{(3\,42 - 4)}$
= 0 92 or 1 51 per cent

TABLE 12 —SUMMARY OF RESULTS

Mean yield	*A*	*B*	*C*	*D*	Mean	Standard Error
Lb per plot	537 5	627 5	645 0	640 0	612 5	9 2
Tons per acre	12 00	14 01	14 40	14 29	13 67	0 21
Per cent	87 8	102 4	105 3	104 5	100 0	1 51

Conclusion —It is obvious that varieties *B C* and *D* have each given a significantly higher yield than variety *A* the control but the differences in yield between *B C* and *D* are not significant

The degrees of freedom for rows columns varieties and total are in each case one less than the number of rows columns varieties and total number of plots and by virtue of the arrangement the first three are equal The degrees of freedom for error can therefore be obtained by difference (in the example $15 - 3 - 3 - 3 = 6$) In general with a square of side p the degrees of freedom are $p-1$ for each of rows columns and varieties and $(p-1)(p-2)$ for error

NOTES ON CALCULATION

The notes given in connexion with the randomized block experiment already illustrated hold here also except that the subsidiary calculation to obtain the sum of squares for error independently of the others requires modification In the case of the Latin square the residuals are obtained by adding to any plot value *twice* the general mean and subtracting the sum of the row column and variety means appropriate to the plot in question Then the sum of squares of the residuals is the sum of squares for error

CONSIDERATIONS AFFECTING CHOICE OF LATIN SQUARE LAYOUT

The measure of the precision of an experiment depends upon the number of degrees of freedom available for the estimation of error since this figure plus one gives the size of an ordinary sample of independent items that would yield an estimate of variance with the same precision as the experiment It is advisable that this number of degrees of freedom should be at least equal to 10 or 12 While therefore the randomized block experiment may be considered satisfactory in this respect the Latin square illustrated is not and we see that we are forced to rule out single 3×3 and 4×4 Latin squares This applies in spite of the fact that a lower standard error for the comparisons may be possible by the elimination of components of soil fertility in two directions The level of the standard deviation due to experimental error is one thing the degree of variability to which it may be subject is another A very satisfactory experiment could however be set up in the form of two Latin squares side by side and as this will furnish a suitable introduction to more elaborate arrangements the details of the analysis may be given here We shall suppose that two separate Latin squares have been chosen by the randomization procedure involving the same four varieties *A B C* and *D* Looked at first as an arrangement of 32 plots in two blocks of 16 each we may analyse the variation into two parts as shown in the following skeleton analysis of variance —

	D F	
Between squares	1	
Within squares	30	(15 + 15)
Total	31	

The first part calculated as the square of the difference between the Latin square totals divided by 32 (the total number of plots) may evidently be looked on as elimination of soil fertility

differences between the two squares The variation within squares is composed of two parts the variation between the unit plots within each square separately with 15 degrees of freedom each In each case the calculation is as described under (1) in the above analysis of the Latin square but as this can be further subdivided into rows columns treatments and error we can now re write our skeleton analysis of variance as follows —

TABLE 13

			D F		
	Between squares		1		
Within square 1	Rows	3	Within square 2	Rows	3
	Columns	3		Columns	3
	Varieties	3		Varieties	3
	Error	6		Error	6
	Total	15		Total	15
	Total		31		

The calculations for each square separately are exactly as indicated previously We must now attempt to put the two analyses together First note that the parts due to rows and columns eliminate much of the soil fertility differences apart from that which has been eliminated between the squares There is no need to do anything more about these components But there are two sums of squares due to varietal differences one from each square On the average of both squares or eight replicates of each variety there are however only 3 degrees of freedom for varietal differences How are these related to the above two sets of 3? Let us assume fictitious variety totals for the second square so obtaining the following figures —

TABLE 14 —TREATMENT TOTALS (OF FOUR PLOTS EACH)

	A	*B*	*C*	*D*	Total
Square 1	215	251	258	256	980
2	230	245	236	249	960
Total	445	496	494	505	1940
				General mean	60 625

The sum of squares for varieties in square 1 is 306 5 A similar calculation for the second square gives 55 5 Total 362 (6 D F) A similar calculation for all the eight totals (of four plots each) gives 374 5 (7 D F) while the single degree of freedom for the difference between the square totals yields $(980 - 960)^2 - 32 = 12\ 5$ It will be noticed that 362 is equal to 374 5 — 12 5 since it is the total sum of squares between varieties *within* squares (6 D F) If we now turn to the variety totals for the two squares together the sum of squares comes to 275 25 (3 D F) This is evidently a measure of the average effect of varieties and if we subtract this result from 362 we get 86 75 the remaining sum of squares (3 D F) and this we take as a measure of the variation in response to varieties in the two squares It is commonly spoken of as the interaction between varieties and squares and has the same significance as the error term in a randomized block experiment for example for that is really a measure of the variation in response to the varieties in the different blocks i e the interaction between varieties and blocks We should therefore take 86 75 as a contribution to error with 3 D F Notice that it may be directly calculated from the following table of the *differences* between the variety totals of the square

	A	*B*	*C*	*D*	Total
Square 1–Square 2	−15	6	22	7	20

$15^2 + 6^2 + 22^2 + 7^2 = 794$
Divide by 8 99 25
Subtract $20^2 - 32$ 12 5

86 75

The divisor is 8 because we are dealing with the difference between two totals of four plots each just as it is 8 when we are dealing with the sum of two totals of four plots each The correction is the square of the total divided by the total number of plots a calculation which is equally applicable to sums and differences of pairs of plot yields

We have two other components of error namely the sums of squares for error in squares 1 and 2 respectively The complete skeleton analysis of variance is therefore as follows —

TABLE 15

Elimination of soil heterogeneity	Between squares		1	
	Square 1	Rows	3	
		Columns	3	
	Square 2	Rows	3	
		Columns	3	
			—	13
	Variety			3
Error	Variety–square interaction		3	
	Error square 1		6	
	Error square 2		6	
			—	15
Total				31

The introduction of two Latin squares instead of one has increased the number of degrees of freedom for error from 6 to 15 To see if there are significant differences between varieties in the complete experiment we should work out the variance ratio of the mean squares for variety and total error and enter the table of the variance ratio with $n_1 = 3$ $n_2 = 15$

There is an upper limit to the size of Latin square that can usefully be laid out Quite apart from the unwillingness because of labour or expense of the experimenter to allow for nine ten or more replications of that number of varieties there is a disadvantage in having the rows or columns of the square too long It is only the average differences between rows and between columns that are eliminated and with long series of plots there may be irregular variations in fertility which cannot be dealt with An extreme case is illustrated in the following diagram —

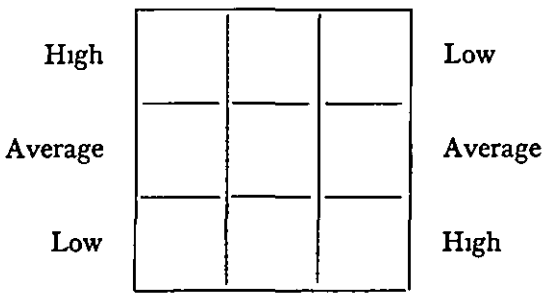

FIG 3

Let us assume that the fertility at the top of the square is falling off to the right from a high to a low value towards the middle it is average all the way across and at the bottom it is increasing

towards the right from a low value Taken as a whole the rows and columns will all be of about average fertility and so little in the way of fertility differences will be eliminated by the Latin square analysis resulting in a high error because of the extreme fertility differences If this was a 9 × 9 square it would be better to arrange it in nine randomized blocks of nine plots each as shown by the dotted lines for some of these being of high fertility some low and some about average a good deal in the way of fertility differences would be eliminated in blocks An approximation to this state of affairs may occur in practice and it is better to avoid if possible Latin squares of more than about eight treatments

Multiple Factor Experiments

The basic framework of an analysis of variance has now been presented for the two common forms of layout namely randomized blocks and the Latin square Within such a framework it is however possible to accommodate two or more sets of treatments Thus suppose it was desired to combine a manurial with a varietal trial and that there were three varieties *A B* and *C* and three levels of fertilizer e g 0 1 and 2 dressings of sulphate of ammonia Two separate experiments could only test (*a*) which was the best of the three varieties and (*b*) how one of these varieties or some other responded to the application of nitrogeneous fertilizer Even so these two experiments would take up 36 plots since there would have to be at least six replications in each case If we try out 0 1 and 2 dressings of nitrogen on each of the varieties we have nine combinations of treatment in all and if these are regarded as nine distinct treatments we can evidently arrange them in one experiment of the randomized block pattern with a minimum of four replications or 36 plots in all The advantage of such an arrangement is that we can get not only all the information that the separate experiments would provide but also additional information For example we have a possible manurial effect on all three varieties and also varietal differences at different levels of manuring Should we find out and the experiment will provide an answer on the point that all varieties respond equally to manure or what is complementary to what we have just said that varietal differences are the same at all manuring levels then not only do we have a satisfactory number of degrees of freedom for estimating the standard errors with some approach to precision but also we can compare averages for varieties or for manure levels as means of 3 × 4 = 12 instead of means of 6 If we find that the varieties do not respond equally to manure then that is additiona information that could not possibly have been provided by the separate experiments

The analysis of the variation is a combination of two analyses of the randomized block kind Thus with nine treatment combinations and four replications a first skeleton analysis of variance would be as follows —

Table 16

Variation	D F		D F
Blocks	3		
Treatment combinations	8	Varieties	2
		Nitrogen	2 (see below)
		Interaction	4
Error	24		
Total	35		

Now we may set out the treatment totals (of four plots each) as in the following scheme —

Table 17

		Varieties			
		A	*B*	*C*	Total
Levels of *N*	0				T_0
	1				T_1
	2				T_2
	Total	T_A	T_B	T_C	T = grand total

The sum of squares for treatment combinations is obtained by adding the squares of the nine treatment totals of four plots each dividing by 4 and then subtracting the correction 1/36 of the square of grand total T This is now divided up according to the following scheme as indicated on the right of Table 16 —

	D F
Between varieties	2
Between N levels	2
Interaction	4
Total treatment combinations	8

Let T_A T_B and T_C represent the variety totals (of 12 plots each) and T_0 T_1 and T_2 the nitrogen level totals (again of 12 plots each) while T represents the grand total Then the sum of squares between varieties is

$$(T_A^2 + T_B^2 + T_C^2)/12 - T^2/36 \quad (2 \text{ D F})$$

while that between nitrogen levels is

$$(T_0^2 + T_1^2 + T_2^2)/12 - T^2/36 \quad (2 \text{ D F})$$

These two components should add up to less than the total sum of squares for treatment combinations and the difference represents the interaction between varieties and nitrogenous manuring ($2 \times 2 = 4$ D F) This last analysis in fact is exactly like what it would be in a small experiment of three treatments in three fold replication in a randomized block trial except that each yield that occurs is to be regarded as a total of four ultimate unit plot yields What was called the error term before being as was indicated an interaction of varieties and blocks is now called the interaction of varieties and levels of nitrogenous manuring As a check this interaction term may be calculated independently by the method outlined under Method of Randomized Blocks in the section Notes on Calculation

Three distinct tests of significance are now possible By working out the variance ratio for varieties (2 D F) and error (24 D F) we are able to say whether there are significant varietal differences on the average of all manuring levels and if there are we can compare the three variety means x_A x_B and x_C (each of 12 plots) with a standard $s/\sqrt{12}$ where s^2 is the error mean square This should normally be a fairly accurate comparison because it is based on means of 12 plots In the same way the variance ratio for manure levels ($n_1 = 2$ $n_2 = 24$) will tell us whether there are significant differences between the three manurial averages (of 12 plots each) averaging over all varieties Here again the comparison of x_0 x_1 and x_2 is made by means of a standard error $s/\sqrt{12}$

The interaction is a measure of whether the responses to nitrogen are the same or different for the three varieties If the same apart from chance variation the variance ratio for interaction against error ($n_1 = 4$ $n_2 = 24$) will not be significant and the above two comparisons on the treatment means are all that we need in a summary table of results though we shall naturally put in the extra information that the interaction was insignificant while the experimenter will be interested in having presented to him a full table showing all nine treatment means But if the responses are different that state of affairs will be indicated by the variance ratio for interaction against error being significant Inspection of the nine treatment means x_{A0} x_{A1} x_{A2} x_{B0} etc (set out for convenience in the form of Table 17) in the light of their standard error $s/\sqrt{4}$ will show what is taking place and this full table should be given in the summary of results though it is convenient as well to add the marginal means together with their standard error $s/\sqrt{12}$ to show the average effects If the numbers of treatments in the two cases are not equal the standard errors of the two margins will not be the same Thus for four levels of nitrogen on three varieties with four fold replication the standard error of the manurial means will be $s/\sqrt{12}$ but that of the varietal means will be $s/\sqrt{16}$

This example of two interacting factors will serve to indicate the procedure that should be adopted in more complicated cases Since the only change from the ordinary case of simple treatments studied in the section Method of Randomized Blocks has been to subdivide the sum of squares for treatment combinations into its component parts we shall restrict ourselves

in what follows to this treatment sum of squares on the assumption that the other sums of squares due to blocks and error are worked out in the ordinary way The obvious extension is to more than two factors Thus let us suppose that five varieties are being tested at four levels of one fertilizer treatment e g nitrogen and at three levels of another fertilizer treatment e g phosphate Altogether there are $5 \times 4 \times 3$ or 60 variety treatment combinations and the unit block will consist of 60 plots The sum of squares for treatment combinations will contain in the first place the sums of squares for the average effects of varieties nitrogen and phosphate having 4 3 and 2 degrees of freedom respectively and the calculations will be as just described In the second place there will be three interaction terms of the first order namely the interactions of variety with nitrogen of variety with phosphate and of nitrogen with phosphate and having 12 8 and 6 degrees of freedom respectively ($12 = 4 \times 3$ $8 = 4 \times 2$ and $6 = 3 \times 2$) The first will be obtained by writing down the 20 total yields when all phosphate treatments have been amalgamated in five columns corresponding to varieties and in four rows corresponding to levels of nitrogen From this table can be obtained its total sum of squares with 19 degrees of freedom from which is to be subtracted the two sums of squares for the direct effects of variety and nitrogen leaving the required interaction sum of squares with 12 degrees of freedom The other two first order interactions are obtained in similar fashion But this has only accounted for $4 + 3 + 2 + 12 + 8 + 6$ or a total of 35 degrees of freedom out of 59 The sum of squares left over with 24 ($= 4 \times 3 \times 2$) degrees of freedom and obtainable by subtracting all the sums of squares so far obtained from the total treatment combinations sum of squares represents what is called the second order interaction of the three factors To see what this measures we may imagine that instead of working out the variety nitrogen first order interaction by amalgamating all phosphate treatments we could construct three two way tables showing the variation of variety nitrogen totals for each of the three phosphate levels separately It would then be possible to calculate three first order interactions of variety with nitrogen If the three mean squares derived from this calculation are all equal within the limits of experimental error i e if variety interacts with nitrogen in the same way at all levels of phosphate then the second order interaction will not be significant when compared with error If it is significant this will be evidence without carrying out the above detailed calculations that variety and nitrogen interact in different ways at the three phosphate levels and a comparison of all 60 treatment means in the light of their standard error will bring out the nature of the effect The skeleton analysis of variance for the treatment part of the whole analysis will be as follows —

TABLE 18

Variation	D F
Variety	4
Nitrogen	3
Phosphate	2
First order interactions	
Variety nitrogen	12
Variety phosphate	8
Nitrogen phosphate	6
Second order interaction	
Variety nitrogen phosphate	24
Total	59

EXAMPLE

A form of experiment which is of frequent occurrence in manurial trials consists in testing for the presence or absence of fixed quantities of the three main fertilizers used on the common crops namely nitrogen phosphate and potash These are therefore each introduced into the experiment at two levels each namely none and some and the total number of treatment combinations is therefore eight and may be symbolically referred to as *O N P K NP NK PK* and *NPK* Thus *O* represents the untreated or control plot *N* that having a dressing of nitrogen *NP* that having both nitrogen and phosphate and so on These constitute a block

which should be replicated a suitable number of times This is evidently a particular case of a multiple factor experiment and will be used for the purpose of illustrating the method of analysis which has just been described

In an experiment with four replications on the effect of these three fertilizer treatments on the yield of asparagus the layout was as in Fig 4 the figures referring to the yield in lb per plot Complete rows in this diagram constitute the four blocks

NPK 12 0	*K* 16 2	*P* 14 6	*N* 12 7	*PK* 13 0	*0* 12 7	*NK* 11 3	*NP* 10 3
NK 10 2	*NP* 12 8	*PK* 13 8	*0* 13 9	*K* 13 3	*P* 15 2	*N* 12 1	*NPK* 11 5
0 14 7	*NP* 9 3	*NK* 8 8	*PK* 9 0	*NPK* 11 4	*K* 10 4	*P* 11 7	*N* 9 3
N 10 8	*P* 8 9	*K* 8 3	*NPK* 10 3	*NP* 9 1	*0* 10 5	*NK* 8 2	*PK* 13 5

FIG 4

It will be noticed that the treatments are not completely randomized in blocks but are associated in that the group *N P K* and *NPK* remain together in one half of the block while the remainder occur in the other half This restriction was adopted deliberately for a reason which will be made clear in a later section dealing with what is known as confounding In the meantime let us analyse the yield figures as if the treatments had been completely randomized Arranging by blocks and treatments we have the results shown in Table 19 in which column and row totals have been inserted

TABLE 19

Treatment	Block 1	Block 2	Block 3	Block 4	Total
0	12 7	13 9	14 7	10 5	51 8
N	12 7	12 1	9 3	10 8	44 9
P	14 6	15 2	11 7	8 9	50 4
K	16 2	13 3	10 4	8 3	48 2
NP	10 3	12 8	9 3	9 1	41 5
NK	11 3	10 2	8 8	8 2	38 5
PK	13 0	13 8	9 0	13 5	49 3
NPK	12 0	11 5	11 4	10 3	45 2
Total	102 8	102 8	84 6	79 6	369 8
				General mean	11 556

It will be left as an exercise for the reader to show that a first analysis of variance into blocks treatments and error gives the following results —

TABLE 20 —ANALYSIS OF VARIANCE

Variation	D F	Sum of Squares	Mean Square	V R
Blocks	3	55 124	18 375	7 19**
Treatments	7	36 669	5 238	2 05
Error	21	53 686	2 556	
Total	31	145 479		

Treatments as a whole are not significant but we now proceed to analyse the sum of squares due to treatments into its separate components For this purpose the following three two way tables are prepared the first consisting of the totals of the plots with and without potash the second of the totals with and without phosphate and the third of the totals with and without nitrogen

TABLE 21

	No N	N	
No P	100 0	83 4	183 4
P	99 7	86 7	186 4
	199 7	170 1	369 8

	No N	N	
No K	102 2	86 4	188 6
K	97 5	83 7	181 2
	199 7	170 1	369 8

	No P	P	
No K	96 7	91 9	188 6
K	86 7	94 5	181 2
	183 4	186 4	369 8

The sum of squares (3 D F) in the first table is obtained by summing the squares of 100 0 83 4 99 7 and 86 7 dividing by 8 and subtracting the square of the grand total 369 8 divided by 32 The result is 28 066 (3 D F) from which must be subtracted 27 380 (1 D F) for the effect of nitrogen (obtained in this special case as the square of 199 7 — 170 1 divided by 32) and similarly 0 281 (1 D F) for the effect of phosphate leaving for the nitrogen phosphate interaction a sum of squares of 0 405 (1 D F) In the special case of a 2 × 2 table as here this interaction sum of squares can be obtained directly as

$$[(100\ 0 + 86\ 7) - (83\ 4 + 99\ 7)]^2 - 32 = 0\ 405$$

Similar calculations are made on the other two tables and as we now have the sums of squares for the three direct effects and the three first order interactions we can obtain the second order interaction by subtracting the total of the sums already calculated from the total sum of squares for treatments (7 D F) which was 36 669 The difference is 1 805 (1 D F) In this special case the second order interaction may be calculated directly by finding the difference of the two first-order interaction effects in the following tables which show the N and P effects with and without K

	No K			K	
	No N	N		No N	N
No P	51 8	44 9	No P	48 2	38 5
P	50 4	41 5	P	49 3	45 2

$$(51\ 8 + 41\ 5) - (44\ 9 + 50\ 4) = -2\ 0$$
$$(48\ 2 + 45\ 2) - (38\ 5 + 49\ 3) = +5\ 6$$
$$5\ 6 - (-2\ 0) = 7\ 6 \quad \text{and} \quad 7\ 6^2 - 32 = 1\ 805$$

Altogether we have the following decomposition of the treatment sum of squares* —

TABLE 22 —ANALYSIS OF VARIANCE (TREATMENTS)

Variation	D F	Sum of Squares
N	1	27 380
P	1	0 281
K	1	1 711
NP	1	0 405
NK	1	0 125
PK	1	4 962
NPK	1	1 805
Total	7	36 669

The symbols N NP etc. in the column headed Variation refer to the various effects and should not be confused with the same symbols used earlier to indicate combinations of treatments. Alternative expressions for the interaction NP for example are $N \times P$ or $N\ P$

This table when taken in conjunction with Table 20 shows that the only significant effect is that of nitrogen for which the variance ratio is 27 380/2 556 or 10 7 significant at the 1 per cent level Reference to Table 19 shows that nitrogen has depressed the yield the figures being (Table 21) —

Total without N	Total with N	Standard Error
199 7	170 1	6 40

The standard error is that of a total of 16 plots and is therefore $\sqrt{(16 \times 2\,556)} = 6\,40$ The ratio of the difference of the totals 29 6 to its standard error is

$$t = \frac{29\,6}{6\,40\sqrt{2}} = \frac{29\,6}{9\,04} = 3\,27 \quad n = 21$$

The value of t is the square root of the variance ratio 10 7 calculated above because this is a single degree of freedom effect

As this is an illustration of the general procedure too much is not made of the simplified method of calculation appropriate to the $2 \times 2 \times 2$ layout Since it is a common form of experimentation however the reader is referred for details to F Yates Technical Communication No 35 (Imperial Bureau of Soil Science) 1937 where a full treatment of the whole subject of multiple factor experiments is given

The Split Plot Experiment

It is sometimes convenient so to arrange the plots of a multiple factor experiment that all combinations are not distributed at random over the block As an example it may be impossible or at any rate difficult to have adjacent plots as small as might be necessary in the layout described at the beginning of the last section put down to different varieties We might however start with plots three times the unit size and have an experiment on the randomized block layout three varieties in a block and four blocks Each plot may now be subdivided for the purpose of the manurial comparison alone i e one third is to be chosen at random to be left unmanured one third to have a single nitrogenous dressing and the remaining one third a double dressing Let these parts of the main plot be called sub plots Then we end up with nine sub plots per block and all variety treatment combinations are represented But there is an essential difference from the previous layout in that the randomization pattern is different and this is reflected in the form taken by the analysis of variance Obviously the manurial comparisons for a single variety will be made with greater precision than inter varietal comparisons for they involve comparing sub plots within a main plot and will be less affected by soil differences To see how the analysis is carried out consider the data first as arranged in 12 main plots each consisting of three sub plots We have the skeleton analysis of variance —

	D F
Between main plots	11
Within main plots	24 (2 × 12)
Total	35

The analysis of the variation between main plots follows exactly the lines of a three treatment four block randomized block experiment except that all sums of squares are divided by an additional 3 since the unit is a sub plot That is we have components due to blocks (3 D F) varieties (2 D F) and the interaction of blocks and varieties (6 D F) which is the error term with which to compare varieties for significance Of the variation within sub plots there is evidently a component with 2 D F for the manurial comparisons on the average of all varieties and there will be the further component of interaction between varieties and manurial levels (4 D F) In practice the two direct effects and the interaction are calculated from a table of treatment totals exactly like Table 17 The remainder of the variation within main plots with 18 D F gives the sub plot error with which the manurial and interaction comparisons are made The skeleton analysis of variance is given below in full —

		D F	
Between main plots	Blocks	3	
	Varieties	2	
	Main plot error	6	
		—	11
Within main plots	Manurial levels	2	
	Interaction	4	
	Sub-plot error	18	
		—	24
Total			35

Let the estimated standard error per single sub-plot (square root of error mean square) be s_1 from the main plot error (6 D F) and s_2 from the sub plot error (18 D F) Then if the interaction is not significant we compare the three variety means of 12 sub plots each with a standard error $s_1/\sqrt{12}$ using the t corresponding to 6 D F in making the comparisons Likewise the three manurial means again of 12 sub plots each have a standard error $s_2/\sqrt{12}$ and the t will here be that for 18 D F Thus in the split plot experiment there are two different errors respectively applicable to the means of columns and rows of Table 17 Should the interaction be significant then as before it is necessary to examine individual means of four sub plots each In doing so we take $s_2/\sqrt{4}$ as the standard error of means compared in the same column using the t for 18 D F For means not in the same column the appropriate standard error is estimated as

$$\sqrt{\left(\frac{1}{3}\,\frac{s_1^2}{4}+\frac{2}{3}\,\frac{s_2^2}{4}\right)}$$

using for safety the t appropriate to 6 D F

The method is likely to be useful to the plant breeder who wishes to introduce a subsidiary manurial comparison into a variety experiment of the ordinary type One often finds that the main plots are split into two parts one half unmanured and the other half receiving a dressing of a fertilizer whose value in increasing yield it is desired to study The analysis takes on an especially simple form in such a case for if the sums and differences of adjoining sub plot yields are found a calculation on the sums yields the main plot part of the analysis and a parallel calculation on the differences gives the rest except that in the latter case there is no component for blocks Thus let us denote by x the yield of the unmanured half of a main plot and by y the yield of the manured half Let $x+y=t$ and $x-y=d$ Subscripts may be attached to designate the number of the main plot Suppose there are five varieties and four blocks Let T denote the sum of the t s over all plots T_A T_B T_C T_D and T_E the variety totals and T_1 T_2 T_3 and T_4 the block totals With a similar notation let D denote the sum of the d s Parallel calculations are made on the t s and d s as follows —

(1) Total sum of squares between main plots
$= \frac{1}{2}(t_1^2 + t_2^2 + \quad + t_{20}) - T^2/40$ (19 D F)

(2) Total sum of squares within main plots
$= \frac{1}{2}(d_1^2 + d_2^2 + \quad + d_{20}^2)$ (20 D F)

(3) Sum of squares between blocks
$= (T_1^2 + T_2^2 + T_3^2 + T_4^2)/10 - T^2/40$ (3 D F)

(4) Sum of squares between varieties
$= (T_A^2 + T_B^2 + T_C^2 + T_D^2 + T_E^2)/8 - T^2/40$ (4 D F)

(5) Interaction sum of squares
$= (D_A^2 + D_B^2 + D_C^2 + D_D^2 + D_E^2)/8 - D^2/40$ (4 D F)

(6) Sum of squares for fertilizer effect
$= D^2/40$ (1 D F)

Given the results of these calculations the error sums of squares are readily obtained by difference e g the main plot error is (1) — (3) — (4) while the sub plot error is (2) — (5) — (6)

Some of the examples illustrated by having a large number of treatment combinations have necessitated large blocks and this conflicts with the requirement stated earlier that blocks should be kept small It is obvious that unless special steps are taken the variation in soil fertility within the block may be large and thus the experimental error may be too large to show up as significant moderate differences between varieties or treatments A way of dealing with this situation is to introduce confounding which may be briefly described as a method of introducing as a unit block an area which is only a part of the area required for all treatment combinations to be represented and so arranging the treatments within sets of such partial blocks that on calculating the sum of squares between blocks we remove from the total sum of squares the differences in soil fertility between these partial blocks together with the least important part of the variation due to treatment combinations Some sacrifice is inevitable and the experiment is usually so arranged that it is the information on the highest order interactions which is sacrificed for experience shows that such interactions are seldom or never of any importance There are various ways of doing this and it would take up too much space in an elementary exposition to go fully into details but the method can be illustrated by means of the worked out example of the $2 \times 2 \times 2$ layout given in the section on Multiple Factor Experiments It was stated there that the treatments were not completely randomized in four blocks of eight plots each Each block was divided into a left half and a right half and the treatment combinations were arranged in two groups one consisting of the set N P K and NPK and the other consisting of the set 0 NP NK and PK In each block it is decided at random which set shall go into the left half and which into the right and within each half block the treatments of the allocated set are arranged at random Reference to Fig 4 will show the details of the resulting layout The experiment is now regarded as one of eight blocks although there are still eight treatment combinations and only 32 plots in all The assumption is that the second order interaction is unimportant i e that the sum of squares due to this component of treatment is of the same order as the error sum of squares Now this sum of squares is obtained as indicated in the analysis (p 26) by calculating the eight treatment totals from all four blocks and subtracting the sum of the four totals for 0 NP NK and PK from the sum of the totals for N P K and NPK For

$$\{(48\,2 + 45\,2) - (38\,5 + 49\,3)\} - \{(51\,8 + 41\,5) - (44\,9 + 50\,4)\}$$
$$= (44\,9 + 50\,4 + 48\,2 + 45\,2) - (51\,8 + 41\,5 + 38\,5 + 49\,3) = 7\,6$$

This figure is then squared and divided by 32 Now it will be noticed that this difference of 7 6 is just the difference between the total of a selected four of the half blocks and the total of the other four If we call the left half of each block A and the right half B 7 6 is the difference between the sum of 1 4 $2B$ $3B$ $4A$ and the sum of $1B$ $2A$ $3A$ $4B$ A difference in yield may therefore be expected here due to soil fertility differences between the two sets of half blocks and therefore it is impossible to measure the second order interaction This effect has been confounded with the soil differences It should next be noted that this is the only component of the treatment sum of squares which is so confounded If the reader examines from Table 21 how the differences leading to the calculation of sums of squares for the direct effects of N P and K and for the three first order interactions NP NK and PK are made up he will find that in all cases they resolve themselves into the sum of eight differences each being the difference between the total of two plots in a half block and the total of the other two *in the same* half block Thus the difference for the effect of N is (Tables 19 and 21) —

$$(51\,8 + 48\,2 + 50\,4 + 49\,3) - (44\,9 + 38\,5 + 41\,5 + 45\,2)$$
$$= \{(16\,2 + 14\,6) - (12\,0 + 12\,7)\} + \{(13\,0 + 12\,7) - (11\,3 + 10\,3)\}$$
(see first block in Fig 4)
$$+ \text{ three other pairs of differences from the remaining three blocks}$$

Each of these eight differences is therefore unaffected by soil fertility differences between the half blocks and the result of the calculation is to measure the unconfounded effect of N The same will be found to be true of the other direct effects and of the first order interactions

A good check on the procedure of confounding may be had by adding a constant number say 10 to the yield of each plot in a selected half block Any treatment effect for which this makes no difference to the sum of the squares is unconfounded if there is a change in the sum of squares the effect is confounded

We can now see how the modified layout is reflected in the analysis of variance The second order interaction term (1 D F) goes into blocks together with the interaction of this effect with the original four blocks (3 D F) which previously was part of the error term The rest of the analysis remains unaltered In practice we calculate the sums of squares for the unconfounded treatment effects as previously described (totalling 6 D F) then calculate the variation between the eight half blocks (7 D F) from the following eight totals (of four plots each) —

55 5 47 3 50 7 52 1 41 8 42 8 38 3 41 3

The error (18 D F) is obtained by difference of these two sums of squares from the total sum of squares Finally the analysis of variance takes the following form —

TABLE 23 —ANALYSIS OF VARIANCE

Variation	D F	Sum of Squares	Mean Square
Blocks	7	65 024	9 289
N	1	27 380	
P	1	0 281	
K	1	1 711	
NP	1	0 405	
NK	1	0 125	
PK	1	4 962	
	— 6		
Error	18	45 591	2 533
Total	31	145 479	

The calculations are completed in the usual way It happens that in this example most of the soil variation occurs between the four main blocks so that little is added to the blocks sum of squares by using eight blocks instead of four On dividing by 7 the mean square for blocks is only half its former figure The fertility changes might very easily however have been the other way i e along the line of the blocks instead of across them This would have increased the error of the comparisons had the eight treatments been completely randomized over the whole width of the block

Each case of confounding presents its own special problems and the reader who has mastered the details of the simple experimental layouts described in these pages will be well able to tackle the descriptions of the more complicated cases described by Yates (*loc cit* Tech Comm No 35)

EXPERIMENTS WITH LARGE NUMBERS OF VARIETIES

To return to varietal trials a problem frequently facing the plant breeder is that he may have a large number of lines which he wishes to put to test in order to select the most promising varieties for further work If an accurate experiment is laid out to test these for yield performance or for quality characteristics this will involve a large number of plots a thing he may be prepared to face provided that some method is found for getting over the difficulty of the large block The case is somewhat different from that in which the introduction of a number of different treatments leads to a large number of treatment combinations for in the latter case it is possible by studying the higher order interactions to arrange a layout on the confounded pattern which will reduce the size of the block to manageable dimensions A number of varieties can only be arbitrarily arranged into sets and there will be no interaction in the ordinary sense of the term Nevertheless it is possible to make use of the idea of confounding to produce a workable experiment in a series of blocks of moderate size and with a degree of replication

that is not prohibitive The subject is scarcely one for the beginner and requires to be studied in some detail for all its ramifications to be appreciated This has been done and references for more advanced reading will be appended Suffice to say here that for selected numbers of varieties workable arrangements may be devised and the use of Tables XVII–XIX in *Statistical Tables* by Fisher and Yates will assist the experimenter in any given case to see what is possible in the way of an experiment and what is not The introduction to these tables provides a guide to their use Once a layout has been decided the analysis of variance can be carried out without undue difficulty In this section we shall describe one such experiment carried out by the Plant Breeding Institute of the University of Cambridge in the season 1935–39 and for the data of which we are indebted to Dr G D H Bell

In general such layouts are all of the incomplete block kind and the designs have been termed quasi factorial (Yates *loc cit* Tech Comm No 35 and references therein cited) They consist in associating the varieties with one another in *groups* of *sets* and the degree of complexity increases with the number of groups But there comes a point where all possible groups of sets are included and this case which is then symmetrical is that considered by Fisher and Yates (*loc cit*) under the name balanced incomplete blocks The arrangement consists in constructing a block of a given number of varieties less than the total number and having a number of blocks each made up of this number of varieties some containing the varieties already used and some not until in the end there is the same degree of replication for all varieties Every two varieties should occur together in the same number of blocks (one only in the cases to be considered here) To illustrate how such an arrangement can be worked out let us take the case of 16 varieties Represent these by a notation in which the numbers 1 2 3 and 4 are associated in pairs as follows i e the first variety is 11 the second 12 and so on —

(1)

11	12	13	14
21	22	23	24
31	32	33	34
41	42	43	44

In this case the number of varieties to be included in a block is four and the rows of the above diagrammatic representation show four such blocks constituting the first group A second group of four blocks consists of the rows of the following diagram obtained by interchanging rows and columns in (1) thus —

(2)

11	21	31	41
12	22	32	42
13	23	33	43
14	24	34	44

There are three other groups which can be obtained from the set of three orthogonal Latin squares of size 4×4 listed in Table XVI of *Statistical Tables* If we interchange rows and columns these are shown below —

1 2 3 4	1 3 4 2	1 4 2 3
2 1 4 3	2 4 3 1	2 3 1 4
3 4 1 2	3 1 2 4	3 2 4 1
4 3 2 1	4 2 1 3	4 1 3 2

The numbers 1 2 3 4 in these squares have the same significance as the letters *A B C D* in the diagrams given in the section Method of the Latin Square The first square is that

marked (4) in the earlier section and the others are obtained from it by re arranging the last three columns

To obtain the third group take the numbers of the first square and write in front of each the figure 1 for all numbers in the first column 2 for the second column and so on thus obtaining —

(3)

11	22	33	44
12	21	34	43
13	24	31	42
14	23	32	41

The rows of this diagram form the third group of four blocks

The remaining two groups are got by adopting the same procedure with the second and third of the orthogonal squares Thus —

(4)

11	23	34	42
12	24	33	41
13	21	32	44
14	22	31	43

(5)

11	24	32	43
12	23	31	44
13	22	34	41
14	21	33	42

The experiment consists of the 20 blocks of four plots each shown above as the rows in groups (1) (2) (3) (4) and (5) and we see that we have provided five fold replication of each of the 16 varieties In setting out the experiment we should first divide the area into 20 blocks of four plots each The rows of the above five groups may then be numbered 1–20 and assigned at random to the blocks as we proceed across the experimental area thus determining which varieties shall go into the different blocks The varieties to be used are each of course first given at random one of the key numbers 11 12 13 43 44 Within each block the varieties to be placed within it are then allotted at random to the four plots into which it is divided and the experiment proceeds as usual

Associated with this particular layout there is another which deals with 21 varieties in five fold replication Let the five additional varieties be designated by the symbols 01 02 03 04 and 05 It is very easy to derive the set up from the foregoing one Add variety 01 to each of the four sets in group (1) making in each case a block of five plots Add variety 02 to each of the sets in group (2) variety 03 to group (3) variety 04 to group (4) and variety 05 to group (5) Finally add a twenty first set consisting of the varieties 01 02 03 04 and 05 The same considerations as to randomization apply as in the former case and the result is an experiment of 21 blocks of five plots each which tests all 21 varieties in five fold replication

The analysis appropriate to a layout in balanced incomplete blocks will be illustrated by the yield data of an experiment on 21 barley varieties The layout is given in Fig 5 the upper number denoting the variety (numbered 1–21) and the lower figure being the yield of the plot

Block No											Block No
21	5 796	4 1165	20 737	16 800	18 829						
19	6 824	10 666	18 781	1 700	7 579	14 622	11 543	6 641	5 805	8 944	20
17	9 773	13 736	21 824	18 920	11 698	8 782	10 511	20 766	19 764	21 565	18
15	19 677	13 892	6 866	16 615	12 627	13 593	14 607	20 624	17 518	1 549	16
13	5 645	7 512	17 501	9 628	19 622	2 578	14 632	15 707	19 813	18 880	14
11	4 746	17 641	6 867	21 731	15 481	8 868	12 658	17 652	3 1029	18 757	12
9	8 522	1 510	9 563	16 465	15 379	1 453	3 686	4 922	11 670	19 775	10
7	4 743	12 758	14 499	10 453	9 473	7 532	14 653	3 872	21 765	16 596	8
5	11 628	10 565	2 579	16 476	17 402	1 458	5 337	21 642	12 945	2 748	6
3	10 488	15 473	5 322	13 242	3 534	9 455	6 493	3 649	20 614	2 457	4
1	15 491	11 566	7 612	20 655	12 720	2 472	8 501	4 488	13 480	7 580	2

FIG 5

The grand total is 67648 and the general mean is got by dividing this by 105 yielding 644 27 The total sum of squares is got by adding the squares of all 105 yields giving 46 279 974 and subtracting from it the correction 43 583 352 obtained by squaring 67648 and dividing by 105 The result is 2 696 622 (104 D F) The block totals are given below —

TABLE 24

Block No	Total	Block No	Total
1	3044	12	3964
2	2521	13	2908
3	2059	14	3610
4	2668	15	3677
5	2650	16	2891
6	3130	17	3951
7	2926	18	3388
8	3418	19	3550
9	2439	20	3555
10	3506	21	4327
11	3466		
		Total	67648

The sum of squares due to blocks is obtained by squaring and adding these 21 block totals giving 224 429 064 then dividing by 5 (the number of plots in a block) yielding 44 885 813 and finally subtracting the same correction as for the total sum of squares We are left with 1 302 461 (20 D F)

The calculation of the varietal sum of squares requires special consideration Each variety is only present in five blocks and thus block differences due to fertility will come in to disturb the varietal comparisons We require to correct for this and so obtain adjusted varietal means which are directly comparable one with another at the same time obtaining the neces ary data for calculating the sum of squares This is done in stages as illustrated in Table 25 We

TABLE 25

Variety No	(*a*) Variety total	(*b*) Sum of block totals containing variety	(*c*) $5(a)-(b)$	Adjusted mean $644\,3 + (c) - 21$
1	2670	15516	−2166	541 1
2	2834	14579	− 409	624 8
3	3770	15615	+3235	798 3
4	4064	16746	+3574	814 5
5	2905	15979	−1454	575 0
6	3691	16916	+1539	717 6
7	2815	15441	−1366	579 2
8	3617	15867	+2218	749 9
9	2892	14892	− 432	623 7
10	2683	14573	−1158	589 1
11	3105	16706	−1181	588 0
12	3708	16741	+1799	729 9
13	2943	15099	− 384	626 0
14	3013	16400	−1335	580 7
15	2531	14618	−1963	550 8
16	2952	16511	−1751	560 9
17	2714	15879	−2309	534 3
18	4167	19402	+1433	712 5
19	3651	17089	+1166	699 8
20	3396	16318	+ 662	675 8
21	3527	17353	+ 282	657 7
Total	67648	338240	0	13529 6
Mean	644 3			644 3

first record the variety totals in the usual way (col (*a*)) Opposite each is put the total yield of the blocks containing this variety (col (*b*)) For example 15516 is the sum of 3130 2439 3506 2891 and 3550 for variety 1 occurs in blocks 6 9 10 16 and 19 The total of this column should be five times the total of all plots i e 5×67648 We then subtract the numbers in column (*b*) from five times those in column (*a*) obtaining a series of positive and negative numbers which should add up to zero (col (*c*)) The sum of squares of these numbers is 63 635 750 which on dividing by 105 yields 606 055 which is the required sum of squares due to varieties (20 D F) To obtain the divisor 105 we first note that the efficiency factor E which gives the ratio of the amount of information provided by this layout to that provided by an ordinary randomized block lay out if there is no reduction in error variance per plot due to arranging the experiment in blocks of 5 instead of blocks of 21 plots is got by dividing $1-1/5$ by $1-1/21$ This gives $E = 21/25 = 0\,84$ Then the divisor of the sum of squares is E multiplied by the product of the square of the number of plots in the block (5) and the number of replications (5 also) Thus the divisor is $21 \times 125/25 = 105$

Having obtained the sums of squares for total blocks and varieties the sum of squares for error is got by difference and the analysis of variance is as follows —

TABLE 26 —ANALYSIS OF VARIANCE

Variation	D F	Sum of Squares	Mean Square	Variance Ratio
Blocks	20	1 302 461	65 123 1	5 29**
Varieties	20	606 055	30 302 8	2 46**
Error	64	788 106	12 314 2	
Total	104	2 696 622		

Blocks and varieties are both significant at the 1 per cent level The standard error per plot is 110 97 which is 17 22 per cent of the mean yield 644 27 *

The calculation of the adjusted varietal means (last column of Table 25) consists first in dividing the quantities in column (*c*) by the efficiency factor E multiplied by the product of the number of plots in a block (5) and the number of replications (5 also) Thus the divisor is $21 \times 25/25 = 21$ We then add to each the general mean 644 3 As a check the mean of this final column should be 644 3 To look for significant differences we must now find the standard error appropriate to the difference between any two of these adjusted varietal means Divide the error variance per plot namely 12 314 2 by the product of E and the number of replications (5) i e by 21/5 The result is 2931 95 Double this to obtain the variance of the difference between the means of any two varieties and we get 5863 9 the square root 76 58 is the standard error of the difference With 64 degrees of freedom we may take t as 2 for $P = 0\ 05$ and thus a difference to be significant must exceed 153 2 Using this figure as a scale of measurement we can then set out the varieties in order of yield and indicate those that are significantly higher than certain others The order is 4 3 8 12 6 18 19 20 21 13 2 9 10 11 14 7 5 16 15 1 17 and for example 4 yields significantly greater than 21 and those following after it in this list the varieties up to and including 19 yield significantly greater than 17 and so on

CONCLUDING REMARKS

Apart from further details on the more elaborate forms of layout in connexion with which references have been given for further reading there are other problems on which it has not been possible to touch in an elementary account For example there is the question of dealing with a co ordinated series of experiments carried out in a number of places during the same season or covering a number of seasons or both There are devices for calculating missing experimental values when for some reason or other the experiment as originally set out suffers through the failure to obtain numerical results from one or more plots There is the question of taking into simultaneous consideration two or more observational variables from the same plot with a view either to the elaboration of methods for taking account of soil fertility variations in a more complete fashion than is possible merely by eliminating block or row and column differences or to the further elucidation of the nature of the facts sought to be learnt from the experiment This brings in the calculations involved in the analysis of co variance procedure One or two further references for reading may be given The detailed manual of *Methods of Statistical Analysis* by C H Goulden 1939 (Chapman and Hall) covers the analysis of variance and co variance and the field plot test and is profusely illustrated with examples See also *The Design of Experiments* by R A Fisher 2nd Edn 1937 (Oliver and Boyd) Another recent book is *Statistical Technique in Agricultural Research* by D D Paterson 1939 (McGraw Hill) For a discussion of the analysis of co variance and also of the practical considerations governing

* This example has been chosen for illustrative purposes but it ought to be pointed out that as a trial it was not considered satisfactory since 9 out of the 21 winter varieties were killed off by unusually severe weather conditions and were then replaced by varieties sown in the spring Later a good deal of damage was done by summer drought This accounts for the high standard error

field experimental procedure see *Principles and Practice of Field Experimentation* by J Wishart and H G Sanders 1935 (Empire Cotton Growing Corporation) The serious student will also be well repaid by studying the original papers on the subject full references to which are provided up to quite recent times by Goulden Finally although the reference has already been given it should be emphasized that the methods discussed in the present bulletin which is designed for the beginner lead up naturally to those which are dealt with more systematically than has been possible here in *The Design and Analysis of Factorial Experiments* by F Yates 1937 (Imperial Bureau of Soil Science—Technical Communication No 35 Harpenden)

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