THE LATIN SQUARE AS A REPEATED MEASUREMENTS DESIGN

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1. Introduction

By a repeated measurements design we shall mean that type of arrangement where each experimental unit (an individual or an animal) receives all of the treatments under investigation. The simplest illustration of this type of design is m individuals each of which are subjected to k treatments. If the individuals are considered to be drawn at random from a multivariate normal population, the analysis for treatment effects depends on the structure of the variance-covariance matrix. If $k = 2$ the analysis of variance (mixed model) will be appropriate, for $m > k > 2$ an exact analysis, in the general variance-covariance case, can be made by the use of Hotelling's T^2 (see Scheffé [10]) and an approximate analysis by adjusting the degrees of freedom of the usual mixed model F ratio (see Box [2], Geisser and Greenhouse [3]). In particular, if the variance-covariance structure is uniform (equal variances and equal covariances) the usual F test ratio is exact.

Now repeated measurements designs may have certain disadvantages depending on the nature of the treatments, the response variable, and the population under study, for assessing treatment differences. The three main disadvantages are: (a) carry-over effect; (b) latent effect; (c) order or learning effect. When a treatment has been administered before a previous treatment's effect on the response variable has worn off, the assessment of the treatment differences are obscured by what we shall call carry-over effect. Sometimes when the apparent effect of a treatment has worn off the administration of another treatment may activate the effect of the previous treatment which has been dormant (or alter the effect of the new treatment). This we shall call a latent effect. Another effect may be the order or practice effect on the response variable itself, for example, the variable is the response to a performance test on which individuals may tend to improve merely by repetition of the task, independent of any treatment.

The carry-over effect is usually controlled by the administration of the treatments far enough apart in time so as to eliminate this effect. If a latency effect is suspected, the repeated measurements design probably should not be used unless this effect is itself of interest rather than a pure treatment comparison. The order effect, when carry-over is eliminated and latency is not present, is primarily a function of practice or learning (or even tolerance if very similar drugs are used). A method that has been used to eliminate this order effect from treatment

comparisons is the single latin square design. In the case of only two treatments the replicated cross-over design is also often used. Also replicated latin square arrangements are employed (see Lindquist [8], Geisser [4]).

The purpose of this paper is to discuss the analysis of these designs.

2. The single latin square

Let n individuals be given n treatments on n different days or in n different orders in a latin square arrangement. A particular latin square arrangement is presented in table I, where T_j, D_k represent the jth treatment and the kth day

TABLE I

Individuals	T_1	$T_2 \cdots T_n$
1	D_1	$D_2 \cdots D_n$
2	D_2	$D_3 \cdots D_1$
3	.	
.	.	
.	.	
.	.	
n	D_n	$D_1 \cdots D_{n-1}$

or order. The n^2 observations may be written in the form of x_{ijk}, where i represents the ith individual. If $n = 3$ and the latin square of table I were used, then we would have the array of x_{ijk} as shown in table II.

TABLE II

x_{111}	x_{122}	x_{133}
x_{212}	x_{223}	x_{231}
x_{313}	x_{321}	x_{332}

Now let $x' = (x_1', x_2', \cdots, x_n')$ be the vector variable of the n^2 variables where x_i' is the ith row vector of an nth order latin square. Further let x' be multivariate normal with mean μ' and variance-covariance matrix Λ where

$$\mu' = (\mu_1', \cdots, \mu_n') \tag{1}$$

and

$$\begin{aligned}
\mu_1' &= (t_1 + d_1, t_2 + d_2, \cdots, t_{n-1} + d_{n-1}, t_n + d_n) \\
\mu_2' &= (t_1 + d_2, t_2 + d_3, \cdots, t_{n-1} + d_n, t_n + d_1) \qquad (2) \\
&\ \ \vdots \\
\mu_n' &= (t_1 + d_n, t_2 + d_1, \cdots, t_{n-1} + d_{n-2}, t_n + d_{n-1})
\end{aligned}$$

where t_j is the jth treatment effect and d_k is the kth day or order effect, and

$$\Lambda = \begin{pmatrix} V & 0 & \cdots & 0 \\ 0 & \cdot & \cdots & \cdot \\ \cdot & \cdot & \cdots & 0 \\ 0 & \cdots & 0 & V \end{pmatrix} \tag{3}$$

Now suppose we partitioned the sums of squares in the usual latin square analysis of variance method presented in table III.

TABLE III

Source	Sum of Squares	D.F.	F
Individuals	Q_1	$n-1$	
Treatments	Q_2	$n-1$	$(n-2)Q_2/Q_4 = F_1$
Days	Q_3	$n-1$	$(n-2)Q_3/Q_4 = F_2$
Residual	Q_4	$(n-1)(n-2)$	

We shall use the quadratic form notation for the sums of squares, that is, the Q. Hence

$$Q_1 = x'A_1x,\ Q_2 = x'A_2x,\ Q_3 = x'A_3x,\ Q_4 = x'A_4x, \tag{4}$$

where the A are the n^2 by n^2 matrices partitioned into n by n submatrices so that

$$A_1 = n^{-1}\begin{pmatrix} (n-1)E & -E & \cdots & \cdot & -E \\ -E & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & -E \\ -E & \cdot & \cdots & -E & (n-1)E \end{pmatrix} \tag{5}$$

$$A_2 = n^{-1}\begin{pmatrix} I-E & \cdots & I-E \\ \cdot & & \cdot \\ \cdot & & \cdot \\ \cdot & & \cdot \\ I-E & \cdots & I-E \end{pmatrix} \tag{6}$$

$$A_3 = n^{-1}\begin{pmatrix} I-E & L_1L_2'-E & \cdots & \cdot & L_1L_n'-E \\ L_2L_1'-E & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & L_{n-1}L_n'-E \\ L_nL_1'-E & \cdot & \cdots & L_nL_{n-1}'-E & I-E \end{pmatrix} \tag{7}$$

$$A_4 = n^{-1}\begin{pmatrix} (n-2)(I-E) & 2E-(I+L_1L_2') & \cdots & \cdot & 2E-(I+L_1L_n') \\ 2E-(I+L_2L_1') & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & \cdot \\ \cdot & & & & 2E-(I+L_{n-1}L_n') \\ 2E-(I+L_nL_1') & \cdot & \cdots & 2E-(I+L_nL_{n-1}') & (n-2)(I-E) \end{pmatrix} \tag{8}$$

where I is the n by n identity matrix; $E = n^{-1}1_n1_n'$ and $1_n' = (1, \cdots, 1)$; L_i is the n by n matrix which transforms the ith individual vector into a vector which is in the natural order as regards time and hence must be a matrix which has a single 1 in every column and row and zeros elsewhere. Further $L_iL_i' = I$ and $L_i \sum_j L_j' = nL_iE = nE$. These relationships are true in general for any nth order latin square although we have illustrated this by a particular latin square.

Further manipulation of these matrices show that Q_2 and Q_3 are each independent of Q_4,since $A_2\Lambda A_4 = A_3\Lambda A_4 = 0$, which is the condition for independence of two quadratic forms in multivariate normal variables. The expected values of these quadratic forms are found to be

$$EQ_1 = (n - 1)\mathrm{Tr}EV, \tag{9}$$

$$EQ_2 = \mathrm{Tr}(V - EV) + n \sum_{j=1}^{n} (t_j - \bar{t})^2, \tag{10}$$

$$EQ_3 = \mathrm{Tr}(V - EV) + n \sum_{k=1}^{n} (d_k - \bar{d})^2, \tag{11}$$

$$EQ_4 = (n - 2)\mathrm{Tr}(V - EV). \tag{12}$$

These results imply that even in the correlated case the numerator of F_1 and of F_2 in table III is independent of the denominator in each case. Further since both the numerator and denominator are each linear sums of independent chi-square variates we may use the methods of Box [2], and Geisser and Greenhouse [3] to get analogous approximate distributions of F_1 and F_2. These distributions will depend on the population variances and covariances. In [2] and [3] it was possible to approximate a function of these parameters which appeared as an adjustment to the original degrees of freedom. However, in the unreplicated latin square case we cannot estimate these parameters so that we can only use the lower bound developed in [3] on the adjustment which in this case is $(n - 1)^{-1}$.

Hence in the general correlated case while the numerator and denominator both estimate the same quantity under the null hypothesis and are independent, thus making the F ratio a reasonable statistic to use, one can only use the conservative test which would be $F(1, n - 2)$. Only in the case where the variances and covariances are equal would the original F test be valid.

It is worth noting and well known that if we had postulated a treatment by day (order) interaction the expectations of individuals and of the residual would be altered to include the interactive variance and hence bias the test for treatment effects (for details see Wilk and Kempthorne [7]). This implies that the conservative test suggested above is conservative in two ways when an interaction is present. Firstly, from the point of view of the distribution of the ratio and, secondly, from the fact that the denominator is inflated.

Note added in proof. The introduction of an interaction effect, in the above paragraph and subsequently is primarily for mathematical completeness. It is not to be inferred that the test for treatment differences necessarily retains its

usual interpretation in the presence of interaction. See Scheffé ([11], p. 94) for a more detailed discussion.

3. The replicated latin square

Suppose we had replicated the previous latin square m times, that is, we started out with nm individuals from the same population and then at random we split them up into n groups of m individuals and subjected each group to the same latin square arrangement, then a particular design would be as shown in table IV,

TABLE IV

	T_1	$T_2 \cdots T_n$
S_1	D_1	$D_2 \cdots D_n$
S_2	D_2	$D_3 \cdots D_1$
	.	
	.	
	.	
S_n	D_n	$D_1 \cdots D_{n-1}$

where S_i stands for the ith subgroup, T_j stands for the jth treatment and D_k stands for the kth day. Such a setup can be analyzed by exact methods even if we postulate an interaction effect between treatments and days. The method has been given in [4] and will be extended here so as to include an interaction effect and several different populations. Here we will assume an interactive model and develop the methods on this basis.

Let $x'_{i\alpha} = (x_{i\alpha 1}, \cdots, x_{i\alpha n})$, where $\alpha = 1, \cdots, m$, and $i = 1, \cdots, n$ be the vectorial representation of the αth individual in the ith subgroup, and where the indices $1, \cdots, n$ represent the n treatments. Assume $x'_{i\alpha}$ has a multivariate normal distribution with mean $\mu'_i = (\mu_{i1}, \cdots, \mu_{in})$ and arbitrary variance-covariance matrix V for all i. Hence for the particular arrangement given,

(13)

$$\mu'_1 = (t_1 + d_1 + a_{11}, t_2 + d_2 + a_{22}, \cdots, t_{n-1} + d_{n-1} + a_{n-1,n-1}, t_n + d_n + a_{nn})$$

$$\mu'_2 = (t_1 + d_2 + a_{12}, t_2 + d_3 + a_{23}, \cdots, t_{n-1} + d_n + a_{n-1,n}, t_n + d_1 + a_{n1})$$

$$\vdots$$

$$\mu'_n = (t_1 + d_n + a_{1n}, t_2 + d_1 + a_{21}, \cdots, t_{n-1} + d_{n-2} + a_{n-1,n-2}, t_n + d_{n-1} + a_{n,n-1}),$$

where t_j is the effect of the jth treatment and d_k is the effect of the kth day and a_{jk} is the day by treatment interaction effect. Although we are treating a particular latin square the results are general enough to include any latin square arrangement. Further, since the treatments and days are fixed effects we will assume that

(14)
$$\sum_j a_{jk} = \sum_k a_{jk} = 0.$$

Before we give the exact method it will be of interest to give the analysis of variance approach which has been commonly applied to this design.

An analysis of this data by the analysis of variance procedure is given in table V. The methods for finding $E(Q_i)$ are the same for the Q of table III.

TABLE V

Source	D.F.	Sum of Squares Q_i	$E(Q_i)$
Treatments	$n-1$	Q_1	$\mathrm{Tr}(V-EV)+nm\sum(t_j-\bar{t})^2$
Days	$n-1$	Q_2	$\mathrm{Tr}(V-EV)+nm\sum(d_k-\bar{d})^2$
Subgroups	$n-1$	Q_3	$(n-1)\mathrm{Tr}EV+mn\sum a_i^2$
Individuals within Subgroups	$n(m-1)$	Q_4	$n(m-1)\mathrm{Tr}EV$
Latin Square Residual	$(n-1)(n-2)$	Q_5	$(n-2)\mathrm{Tr}(V-EV)+m\sum\sum(a_jk-a_i)^2$
Discrepance	$n(n-1)(m-1)$	Q_6	$n(m-1)\mathrm{Tr}(V-EV)$

There a_i represents the average of the a_{jk} in the ith mean vector. Now using methods similar to the previous section, we can make approximate tests on the treatment effects and the day effects using the discrepance as the error term. For the interaction of days and treatments there are two tests available each testing a different interaction of day by treatment. One tests the row averages of the day by treatment interactions, that is, $n(m-1)Q_3/(n-1)Q_4$ and the other possible test is the within rows day by treatment interaction, that is, $n(m-1)Q_5/(n-2)Q_6$. If we define the total interaction of days by treatments to be

$$\sigma_{TD}^2 = (n-1)^{-2}\sum\sum a_{jk}^2 \tag{15}$$

then

$$\sigma_{TD}^2 = n(n-1)^{-1}\sigma_{TD(B)}^2 + (n-2)(n-1)^{-1}\sigma_{TD(W)}^2, \tag{16}$$

where

$$\sigma_{TD(B)}^2 = (n-1)^{-1}\sum a_i^2 \tag{17}$$

$$\sigma_{TD(W)}^2 = (n-1)^{-1}(n-2)^{-1}\sum\sum(a_{jk}-a_i)^2. \tag{18}$$

An exact analysis of the treatment effects in the above setup can be made without resorting to the approximate test indicated by the analysis of variance.

Apply a transformation to $x_{i\alpha}$ so that $y_{i\alpha} = Cx_{i\alpha}$, where C is any $n-1$ by n matrix such that every row sums to zero and C is of rank $n-1$ (for details see Geisser [4]). Hence we get a new vector for each individual $y'_{i\alpha} = (y_{i\alpha 1}, \cdots, y_{i\alpha,n-1})$ and $y_{i\alpha}$ has variance-covariance matrix CVC' and mean $C\mu_j$. Now $y'_{i\cdot} = m^{-1}\sum_\alpha y'_{i\alpha}$, the mean vector of the ith subgroup, has variance-covariance matrix $n^{-1}CVC'$ and mean $C\mu_j$. Further $y'_{\cdot\cdot} = n^{-1}\sum_i y'_{i\cdot}$ has variance-covariance matrix $(mn)^{-1}CVC'$ and mean $n^{-1}C\sum_j \mu_j = C\mu$ where $\mu' = (t_1 + \bar{d}, \cdots, t_{n-1} + \bar{d})$ and $\bar{d} = n^{-1}\sum_k d_k$. It can also be shown that if the matrix C has the element $n-1$ in the (i, i) position $i = 1, \cdots, n-1$ and -1 in every other position, then

$$(C\mu)' = (t_1 - \bar{t}, t_2 - \bar{t}, \cdots, t_{n-1} - \bar{t}). \tag{19}$$

Hence testing the hypothesis that $t_1 = t_2 = \cdots = t_n$ is equivalent to testing the hypothesis that $t_1 - \bar{t} = t_2 - \bar{t} = \cdots = t_{n-1} - \bar{t} = 0$. We then estimate CVC' by computing the sample variance-covariance matrix for each of the n subgroups on the transformed variables and then pooling the n matrices. The statistic

$$T^2 = mny'..S^{-1}y.., \tag{20}$$

where S is the pooled variance-covariance matrix, has the T^2 distribution with $n(m-1)$ degrees of freedom and

$$\frac{T^2[(m-2)n+2]}{n(n-1)(m-1)} = F[n-1, (m-2)n+2], \tag{21}$$

where F is the usual F distribution with $n-1$ and $(m-2)n+2$ degrees of freedom. This statistic then provides an exact test for the equality of the treatment effects.

Now an extension of this type of design to several groups, that is, different types of individuals can be given, multivariate tests for treatments, groups, treatment by group interaction will be indicated based on the usual assumption that the variance-covariance matrix for each group is the same.

The design setup for this case would be to repeat the same latin square arrangement as was done for the single group previously to say g groups. Now to test whether there is a group difference over all treatments simultaneously one would first compute the treatment vector mean over all subgroups within a group. Letting an individual vector observation be represented now by $x'_{i\gamma\alpha} = (x_{i\gamma\alpha 1}, x_{i\gamma\alpha 2}, \cdots, x_{i\gamma\alpha n})$, that is, the ith individual in the αth subgroup in the γth group and indices $1, \cdots, n$ represent the n treatments, the treatment vector mean for the γth group would be

$$x'_{\cdot\gamma\cdot} = (x_{\cdot\gamma\cdot 1}, x_{\cdot\gamma\cdot 2}, \cdots, x_{\cdot\gamma\cdot n}). \tag{22}$$

One can then compute a between variance-covariance matrix based on these group vector means. Call this matrix B. Further if we pool the variance-covariance matrix from each subgroup within a group and then over all groups we get a within variance-covariance matrix W. The test statistic then used is either based on the product of the roots of BW^{-1}, Wilks [12], or on the sum of the roots of BW^{-1}, Hotelling [6], or on the maximum of the characteristic roots of BW^{-1}, Roy [9]. The detail of this test may be found in Anderson [1] and is essentially the multivariate analysis of variance.

Now to test whether there are treatment differences, one applies the transformation given earlier, and computes on the $n-1$ adjusted treatment variables the vector mean over all groups and the pooled variance-covariance matrix over all subgroups and groups, and applies Hotelling's T^2 test. If one now wishes to test for a group by treatment interaction he computes on the adjusted data the vector mean for each group and from this he gets a between variance-covariance matrix and uses the same adjusted within pooled variance-covariance

matrix and following Greenhouse and Geisser [5] tests for the group treatment interaction.

4. The treatment covariance assumption

It is important to point out that the assumption of a covariance matrix between the treatments which leads to the equality of covariance matrices among the subgroups has interesting ramifications if we wish to study the day effect or the effect of the order of administration of the treatments. It is easier to discuss this point by considering the case of a 3 by 3 latin square and using a slightly different notation. For notational convenience we will also omit the day by treatment interaction. We then have m observations on each of the variables

TABLE VI

Subgroup	Treatment 1	Treatment 2	Treatment 3
1	$X_1 = x_1 + t_1 + d_1$	$Y_1 = y_1 + t_2 + d_2$	$Z_1 = z_1 + t_3 + d_3$
2	$X_2 = x_2 + t_1 + d_2$	$Y_2 = y_2 + t_2 + d_3$	$Z_2 = z_2 + t_3 + d_1$
3	$X_3 = x_3 + t_1 + d_3$	$Y_3 = y_3 + t_2 + d_1$	$Z_3 = z_3 + t_3 + d_2$

appearing in table VI. Let $Ex_i = Ey_i = Ez_i = \mu$ and $V(x_i) = \sigma_x^2$, $V(y_i) = \sigma_y^2$, $V(z_i) = \sigma_z^2$ for $i = 1, 2, 3$. Further let

$$\text{Cov}\,(x_i x_j) = \text{Cov}\,(y_i y_j) = \text{Cov}\,(z_i z_j) = 0 \tag{23}$$

for $i \neq j$ and $i, j = 1, 2, 3$ and

$$\text{Cov}\,(x_i y_j) = \begin{cases} 0, & i \neq j, \\ \rho_{xy}\sigma_x\sigma_y, & i = j, \end{cases} \tag{24}$$

$$\text{Cov}\,(x_i z_j) = \begin{cases} 0, & i \neq j, \\ \rho_{xz}\sigma_x\sigma_z, & i = j, \end{cases} \tag{25}$$

$$\text{Cov}\,(y_i z_j) = \begin{cases} 0, & i \neq j, \\ \rho_{yz}\sigma_y\sigma_z, & i = j. \end{cases} \tag{26}$$

In table VII the data are rearranged by days, under the same assumptions. The means we wish to compare are

$$\bar{w}_1 = \overline{X}_1 + \overline{Z}_2 + \overline{Y}_3;\ \bar{w}_2 = \overline{Y}_1 + \overline{X}_2 + \overline{Z}_3;\ \bar{w}_3 = \overline{Z}_1 + \overline{Y}_2 + \overline{X}_3, \tag{27}$$

since $E\bar{w}_i = \mu + \bar{t} + d_i$. However, it is obvious that the covariance structure in each subgroup is different so if we proceeded as before (making the transforma-

TABLE VII

Subgroup	Day 1	Day 2	Day 3
1	$X_1 = x_1 + t_1 + d_1$	$Y_1 = y_1 + t_2 + d_2$	$Z_1 = z_1 + t_3 + d_3$
2	$Z_2 = z_2 + t_3 + d_1$	$X_2 = x_2 + t_1 + d_2$	$Y_2 = y_2 + t_2 + d_3$
3	$Y_3 = y_3 + t_2 + d_1$	$Z_3 = z_3 + t_3 + d_2$	$X_3 = x_3 + t_1 + d_3$

tion, and so forth) our statistic would no longer have Hotelling's T^2 distribution. However, an inspection of the covariance structure of $\bar{w}_1, \bar{w}_2, \bar{w}_3$ reveals that

$$\text{(28)} \qquad \text{Cov } \bar{w}_i\bar{w}_j = \begin{cases} \frac{1}{3m}(\sigma_x^2 + \sigma_y^2 + \sigma_z^2), & i = j, \\ \frac{1}{3m}(\rho_{xy}\sigma_x\sigma_y + \rho_{xz}\sigma_x\sigma_z + \rho_{yz}\sigma_y\sigma_z), & i \neq j, \end{cases}$$

for all i and j.

Having three independent estimates of each parameter of the covariance matrix, we may use these to estimate this uniform covariance matrix. We may then transform the $\bar{w}_i$ as before to $\bar{w}_1 - \bar{w}_2 = \bar{u}_1$ and $\bar{w}_1 - \bar{w}_3 = \bar{u}_2$. The covariance matrix of the $\bar{u}_i$ is also uniform and the T^2 statistic which can be computed will be asymptotically $k\chi^2_{n-1}$ (where k is a constant depending on the uniform variance and covariance) for large m. This method would take advantage of the uniform covariance structure underlying the day effects and would be preferable to merely pooling and then using a test which took into account the fact that the covariance matrix was different for each subgroup.

However, the real point of importance is the fact that postulating an arbitrary covariance structure (other than a uniform structure) for treatments which leads to equality of covariance matrices for the subgroups leads simultaneously to inequality of the covariance matrices for days. In an experiment, if an investigator has good reason to suspect the postulation of a covariance structure on the treatments, he should test the equality of the subgroup covariance matrices for both treatments and days and then act accordingly in making his tests on treatment effects. If he finds the day covariance structure is really the underlying model he may use as a test for the treatments that test which was outlined above for days.

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