Plot	*1*		*2*		*3*	
Sample	*1*	*2*	*3*	*4*	*5*	*6*
Machine A	3.833	3.819	3.756	3.882	3.720	3.729
	3.866	3.853	3.757	3.871	3.720	3.768
Machine B	3.932	3.884	3.832	3.917	3.776	3.833
	3.943	3.888	3.829	3.915	3.777	3.827

Source: D. Robinson (1987), Estimation and use of variance components. *The Statistician* 36, 3–14.

a. Write a linear model for the experiment, assuming machines with fixed effects and plots and samples with random effects; explain the terms; and compute the analysis of variance for the data.
b. Show the expected mean squares.
c. Test the null hypothesis of no difference between the means for the two machines.

6. Use the rules given in Section 7.6 to derive the expected mean squares for the following studies or models:
a. the cholesterol study in Exercise 7.1
b. the cattle-feeding trial in Exercise 7.2
c. the alloy casting experiment in Exercise 7.3
d. the traffic study in Exercise 7.4
e. the soils study in Exercise 7.5
f. a four-stage nested design with the model

$$y_{ijkl} = \mu + a_i + b_{j(i)} + c_{k(ij)} + d_{l(ijk)}$$

$$i = 1,2,3,4 \quad j = 1,2,3 \quad k = 1,2 \quad l = 1,2,3$$

g. a model with nested and crossed factors written as

$$y_{ijklm} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + c_{k(ij)} + \delta_l + (\alpha\delta)_{il} + (\beta\delta)_{jl}$$

$$+ (\alpha\beta\delta)_{ijl} + (cd)_{kl(ij)} + e_{m(ijkl)}$$

$$i = 1, 2, \ldots, a \quad j = 1,22, \ldots, b \quad k = 1, 2, \ldots, c \quad l = 1, 2, \ldots, d \quad m = 1, 2, \ldots, r$$

where α_i, β_j, δ_k, and their interactions are fixed effects and $c_{k(ij)}$, $(cd)_{kl(ij)}$, and $e_{m(ijkl)}$ are random effects

7. How would your statistical inference change if the model with restrictions on the interactions had been used for Example 7.5?

8 Complete Block Designs

Experiment designs to improve the precision of results from research studies are the topics of discussion in this chapter and others to follow. Blocking was introduced in Chapter 1 as a method to reduce experimental error variation. Blocking groups the experimental units into homogeneous blocks to compare treatments within a more uniform environment. The designs in this chapter use either one grouping criterion in a randomized complete block design or two grouping criteria in Latin square arrangements. The features, randomization, analysis, and evaluation of these designs are discussed. Extensions of the designs include factorial treatment designs, multiple experimental units per treatment in each block, and subsampling. Conducting analysis when some observations are missing is discussed. The topic for the final section in this chapter is combining the results from several repetitions of the same experiment at several places or several times.

8.1 Blocking to Increase Precision

Our objective is to have precise comparisons among treatments in our research studies. Blocking is a means to reduce and control experimental error variance to achieve more precision.

Previous chapters concentrated on treatment designs and the associated statistical methods for efficient analysis of research hypotheses. All of the illustrations utilized completely randomized designs. However, outside of appropriate experimental unit selection and good research techniques, the completely randomized designs provide no control over experimental error variance. The experimental units are assumed to be relatively homogeneous with respect to the measured response variable in completely randomized designs. However, sometimes sufficient numbers of homogeneous units do not exist for a complete experiment with these designs.

Any factor that affects the response variable and that varies among the experimental units will increase the experimental error variance and decrease the precision of the experimental results. Factors such as age or weight of animals, different batches of reagents or manufactured material, gender of human subjects, and physical separation of field plot locations are examples of variables external to the treatments that can increase the variation among observations on the response variable.

Blocking stratifies experimental units into homogeneous groups, or like units. A successful choice of blocking criteria results in less variation among the units within the blocks than that among units from different blocks. General categories of successful blocking criteria are (1) proximity (neighboring field plots), (2) physical characteristics (age or weight), (3) time, and (4) management of tasks in the experiment.

A group of neighboring field plots forms a block in agronomic field experiments. Animals grouped by weight, stage of lactation, or litter form blocks of homogeneous experimental units. The engineer uses a single batch of manufactured material to form a block or homogeneous group of experimental units for the treatments. Laboratory experiments use technicians as a blocking factor to eliminate variation among technicians. Each technician performs one replication of the treatments as a block.

8.2 Randomized Complete Block Designs Use One Blocking Criterion

The randomized complete block design is the simplest of the blocking designs used to control and reduce experimental error. The experimental units are stratified into blocks of homogeneous units. Each treatment is assigned randomly to an equal number (usually one) of experimental units in each block. More precise comparisons are possible among treatments within the homogeneous set of experimental units in a block. Blocking turned out to be very beneficial in the following study.

Example 8.1 Timing of Nitrogen Fertilization for Wheat

Current nitrogen fertilization recommendations for wheat included applications of specified amounts at specified stages of plant growth. The recommendations were developed through the use of periodic stem tissue analysis for nitrate content of the plant. Stem tissue analysis was thought to be an effective means to monitor the nitrogen status of the crop and provide a basis for predicting required nitrogen for optimum production.

Research Objective: In certain situations, however, the stem nitrate tests were found to overpredict the required nitrogen amounts. Consequently, the researcher wanted to evaluate the effect of several different fertilization timing schedules on the stem tissue nitrate amounts and wheat production to refine the recommendation procedure.

Treatment Design: The treatment design included six different nitrogen application timing and rate schedules that were thought to provide the range of conditions necessary to evaluate the process. For comparison, a control treatment of no nitrogen was included as was the current standard recommendation.

Experiment Design: The experiment was conducted in an irrigated field with a water gradient along one direction of the experimental plot area as a result of irrigation. Since plant responses are affected by variability in the amount of available moisture, the field plots were grouped into blocks of six plots such that each block occurred in the same part of the water gradient. Thus, any differences in plant responses caused by the water gradient could be associated with the blocks. The resulting experiment design was a randomized complete block design with four blocks of six field plots to which the nitrogen treatments were randomly allocated.

The layout of the experimental plots in the field is shown in Display 8.1. The observed nitrate nitrogen content (ppm $\times 10^{-2}$) from a sample of wheat stems is shown for each plot along with the treatment numbers, which appear in the small box of each plot.

Display 8.1 Arrangement of Experimental Plots for the Wheat Experiment in a Randomized Complete Block Design

Irrigation Gradient ↓

Block 1	2	5	4	1	6	3
	40.89	37.99	37.18	34.98	34.89	42.07
Block 2	1	3	4	6	5	2
	41.22	49.42	45.85	50.15	41.99	46.69
Block 3	6	3	5	1	2	4
	44.57	52.68	37.61	36.94	46.65	40.23
Block 4	2	4	6	5	3	1
	41.90	39.20	43.29	40.45	42.91	39.97

Source: Dr. T. Doerge, Department of Soil and Water Science, University of Arizona.

How to Randomize the Design

The random allocation of treatments to experimental units is restricted in the randomized complete block design such that each treatment must occur an equal number of times (one or more) within each block. Randomization is illustrated with the wheat experiment of Example 8.1.

A random permutation of the order in which the treatments are placed with the units in each block provides a random allocation of treatments to units. There are $6! = 720$ possible permutations of the six treatments. One permutation is randomly selected for each block since a separate randomization is required for each block.

Assign the treatment label, such as A, B, C, D, E, F, to the respective integer values 1, 2, 3, 4, 5, 6. Obtain a random permutation of the integer values from a computer program or a table of permutations found in Appendix Table XIII. One such permutation is shown in Display 8.2. Given the permutation 2, 5, 4, 1, 6, 3, assign treatment B to unit 1, treatment E to unit 2, treatment D to unit 3, and so forth in block 1.

Display 8.2 Assignment of Treatments to Experimental Units in a Complete Block

Permutation	2	5	4	1	6	3
Treatment	B	E	D	A	F	C
Experimental unit	1	2	3	4	5	6

Separate random permutations are required for each of the remaining three blocks. Given additional random permutations, say $(1,3,4,6,5,2)$, $(6,3,5,1,2,4)$, and $(2,4,6,5,3,1)$, the final assignment of treatments to units within each block is shown in Figure 8.1.

Statistical Model and Analysis for Randomized Complete Block Design

The linear model for an experiment in a randomized complete block design requires a term to represent the variation identifiable in the observations as a consequence of blocking. The response of the unit with the ith treatment in the jth block is written as

$$y_{ij} = \mu + \tau_i + \rho_j + e_{ij} \tag{8.1}$$

$$i = 1, 2, \ldots, t \qquad j = 1, 2, \ldots, r$$

where μ is the general mean, τ_i is the treatment effect, and e_{ij} is the experimental error. The block effect ρ_j represents the average deviation of the units in block j from the general mean. The treatment and block effects are assumed to be additive. *Additivity* means there is no interaction between treatments and blocks. The experimental errors are assumed independent with zero means and common variance σ^2. The independence assumption is justified through random assignment of treatments to the experimental units.

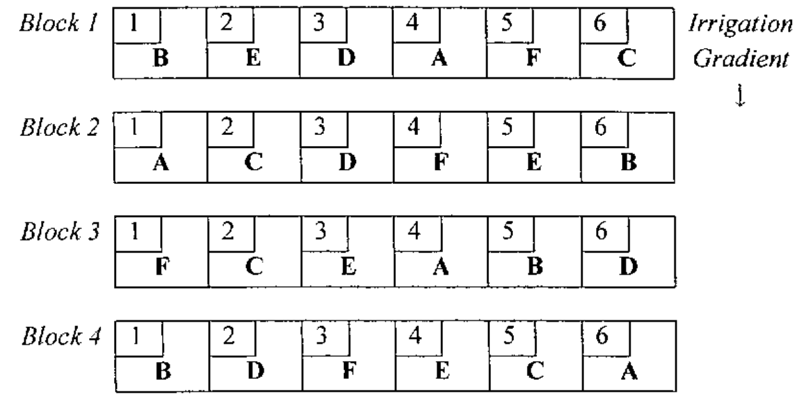


Figure 8.1 Randomized assignment of treatments in a randomized complete block design

Blocks Add a Sum of Squares Partition to the Analysis of Variance

The basic data table for a randomized complete block design is shown in Table 8.1. The deviation of any observation from the grand mean in Table 8.1, $y_{ij} - \bar{y}_{..}$, may be written as the algebraic identity

$$y_{ij} - \bar{y}_{..} = (\bar{y}_{i.} - \bar{y}_{..}) + (\bar{y}_{.j} - \bar{y}_{..}) + (y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..}) \tag{8.2}$$

Table 8.1 Data table for a randomized complete block design

	Block				Treatment
Treatment	1	2	...	r	*Means*
1	y_{11}	y_{12}	$\cdots$	y_{1r}	$\bar{y}_{1.}$
2	y_{21}	y_{22}	$\cdots$	y_{2r}	$\bar{y}_{2.}$
$\vdots$	$\vdots$	$\vdots$		$\vdots$	$\vdots$
t	y_{t1}	y_{t2}	$\cdots$	y_{tr}	$\bar{y}_{t.}$
Block means	$\bar{y}_{.1}$	$\bar{y}_{.2}$	$\cdots$	$\bar{y}_{.r}$	$\bar{y}_{..}$

The terms on the right-hand side of Equation (8.2) are

- a treatment deviation $(\bar{y}_{i.} - \bar{y}_{..})$
- a block deviation $(\bar{y}_{.j} - \bar{y}_{..})$
- experimental error $(y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..})$

For example, the means for the wheat experiment in Example 8.1 (shown in Table 8.3) are used to illustrate the deviations for treatment 1 in block 2, y_{12}, as

- the treatment 1 deviation: $\overline{y}_{1.} - \overline{y}_{..} = 38.28 - 42.07 = -3.79$
- the block 2 deviation: $\overline{y}_{.2} - \overline{y}_{..} = 45.89 - 42.07 = 3.82$
- the experimental error for y_{12}:

$$y_{12} - \overline{y}_{1.} - \overline{y}_{.2} + \overline{y}_{..} = 41.22 - 38.28 - 45.89 + 42.07 = -0.88$$

and the sum of the three deviations, $-3.79 + 3.82 - 0.88 = -0.85$, is the same as the total deviation $y_{12} - \overline{y}_{..} = 41.22 - 42.07 = -0.85$, as it should be.

On closer inspection the last two terms of Equation (8.2) compose an algebraic identity for the deviation of the observation from the treatment mean

$$y_{ij} - \overline{y}_{i.} = (\overline{y}_{.j} - \overline{y}_{..}) + (y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..}) \tag{8.3}$$

The experimental error deviation from the completely randomized design, $y_{ij} - \overline{y}_{i.}$, is partitioned into two components. The first term is identified with the blocking criteria as $\overline{y}_{.j} - \overline{y}_{..}$. The second term is identified only as a residual or experimental error, $y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..}$.

Squaring and summing both sides of Equation (8.2) results in

$$\sum_{i=1}^{t}\sum_{j=1}^{r}(y_{ij} - \overline{y}_{..})^2 = r\sum_{i=1}^{t}(\overline{y}_{i.} - \overline{y}_{..})^2 + t\sum_{j=1}^{r}(\overline{y}_{.j} - \overline{y}_{..})^2$$

$$+\sum_{i=1}^{t}\sum_{j=1}^{r}(y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..})^2 \tag{8.4}$$

or

$$SS \text{ Total} = SS \text{ Treatment} + SS \text{ Blocks} + SS \text{ Error}$$

The sum of any crossproducts from the right-hand side is zero. Table 8.2 summarizes the sum of squares partition.

Table 8.2 Analysis of variance for an experiment in a randomized complete block design

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Expected Mean Square*
Total	$rt - 1$	$\sum_i\sum_j(y_{ij} - \overline{y}_{..})^2$		
Blocks	$r - 1$	$t\sum_j(\overline{y}_{.j} - \overline{y}_{..})^2$	MSB	
Treatments	$t - 1$	$r\sum_i(\overline{y}_{i.} - \overline{y}_{..})^2$	MST	$\sigma^2 + r\theta_t^2$
Error	$(r-1)(t-1)$	SS Error	MSE	σ^2

The observations on stem nitrate nitrogen along with treatment and block means for the wheat fertilization study are shown in Table 8.3. The analysis of variance for the stem nitrate data is shown in Table 8.4.

Table 8.3 Nitrate nitrogen in wheat plant stems (ppm $\times$ 10^{-2}) for six nitrogen-timing treatments in each of four blocks of a randomized complete block design

Nitrogen Timing Schedule	*Block* 1	2	3	4	*Treatment Means* ($\overline{y}_{i.}$)
Control	34.98	41.22	36.94	39.97	38.28
2	40.89	46.69	46.65	41.90	44.03
3	42.07	49.42	52.68	42.91	46.77
4	37.18	45.85	40.23	39.20	40.62
5	37.99	41.99	37.61	40.45	39.51
6	34.89	50.15	44.57	43.29	43.23
Block Means ($\overline{y}_{.j}$)	38.00	45.89	43.11	41.29	$\overline{y}_{..} = 42.07$

Table 8.4 Analysis of variance for wheat stem nitrate nitrogen

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Total	23	506.33			
Blocks	3	197.00	65.67	9.12	
Nitrogen	5	201.32	40.26	5.59	.004
Error	15	108.01	7.20		

As a consequence of blocking, a sum of squares for blocks is partitioned out of what would have been the sum of squares for experimental error with the completely randomized design. The blocked design will markedly improve the precision on the estimates of the treatment means if the reduction in SS Error with blocking is substantial. The reduction in SS Error can be offset by a reduction in degrees of freedom, since $r - 1$ of the degrees of freedom must be allocated to SS Blocks. A measure of relative efficiency, shown later in this section, is necessary to evaluate the full benefit of blocking.

Standard Errors for Treatment Means

The standard error estimate for a treatment mean is

$$s_{\overline{y}_{i.}} = \sqrt{\frac{MSE}{r}} = \sqrt{\frac{7.20}{4}} = 1.34 \tag{8.5}$$

and a 95% confidence interval estimate of any treatment mean in Table 8.3 is $\overline{y}_{i.} \pm t_{.025,15}(s_{\overline{y}_{i.}})$, where $t_{.025,15} = 2.131$. The standard error of a difference between any two treatment means is estimated by

$$s_{(\overline{y}_{i.} - \overline{y}_{j.})} = \sqrt{\frac{2MSE}{r}} = \sqrt{\frac{2(7.20)}{4}} = 1.90 \tag{8.6}$$

Tests of Hypothesis About Treatment Means

The F_0 statistic to test the null hypothesis of no differences among the treatment means is

$$F_0 = \frac{MST}{MSE} = \frac{40.26}{7.20} = 5.59 \tag{8.7}$$

which exceeds the critical value of $F_{.05,5,15} = 2.90$. The observed significance level is $Pr > F = .004$ (Table 8.4). There are significant differences among the nitrogen treatments with respect to stem nitrate nitrogen at this stage of plant development.

There is little interest in formal inferences about block effects, and the F_0 statistic generally is not computed for this purpose even though it may appear in the output of a computer program. The extent to which blocking increased the efficiency of the design to utilize existing resources is discussed later in this section.

Interpretations with Multiple Comparisons

Schedule 4 was the standard fertilizer recommendation for wheat. The nitrate nitrogen in the stem of the wheat plant measured throughout the growing season is used to assess nitrogen requirements for optimum wheat yields. The investigator would be interested in differences between any of the individual nitrogen-timing treatments and the current recommendation at each stage of growth. The Dunnett method (Chapter 3) can be used to compare the standard recommendation with each of the other timing treatments, including the no-nitrogen control treatment. The no-nitrogen control provides a means of evaluating the nitrogen available without fertilization in these particular plots. (See Chapter 1 on the use of control treatments.)

The Dunnett 95% simultaneous confidence intervals require the standard error of the difference, $s(\overline{y}_{i.} - \overline{y}_{j.}) = 1.90$, and the Dunnett statistic from Appendix Table VI, $d_{.05,5,15} = 2.82$, for a two-sided comparison.

The 95% SCI for the difference between the mean of any other schedule for nitrogen application and schedule 4 requires $D(5, .05) = d_{.05,5,15}[s(\overline{y}_{i.} - \overline{y}_{4.})] = 2.82(1.90) = 5.36$ and are computed as $\overline{y}_{i.} - \overline{y}_{4.} \pm 5.36$. The intervals are given in Table 8.5, along with the results of the confident inequalities test in which the absolute difference is declared significantly different from 0 if the difference exceeds $D(5, .05) = 5.36$. Schedule 3 is the only treatment that has a mean nitrate nitrogen level significantly different from the current recommended schedule 4. It has a nitrate content greater than that of schedule 4 since the lower bound of the SCI is

greater than 0. The SCI for all other treatment comparisons include 0 and have upper and lower bounds considerably removed from 0.

Table 8.5 Results of the Dunnett method for treatments vs. control (Example 8.1)

Schedule	*Mean*	$\overline{y}_i - \overline{y}_c$	*95% SCI* (*L*, *U*)
4	$\overline{y}_c = 40.62$	—	–
1	38.28	− 2.34	(− 7.70, 3.02)
2	44.03	3.41	(− 1.95, 8.77)
3	46.77	6.15*	(0.79, 11.51)
5	39.51	− 1.11	(− 6.47, 4.25)
6	43.23	2.61	(− 2.75, 7.97)

* $\mid \overline{y}_i - \overline{y}_c \mid$ exceeds $D(5, .05) = 5.36$ and is significantly different from schedule 4.

If Treatments Interact with Blocks

The assumption of no treatment × block interaction implies that the treatment and block effects are additive. The differences among the treatments are assumed to be relatively constant from block to block as a consequence of additive block and treatment effects even though the use of blocking may result in large differences in responses between units from different blocks. The nonadditivity test, introduced in Chapter 6 (Tukey, 1949b), can be conducted to detect nonadditivity of the multiplicative form $\lambda\tau_i\rho_j$. If the environmental conditions are sufficiently different in one or more of the blocks the relative performance of the treatments may be affected. For example, if the residual nutrient base in the soil is quite different from block to block in a field crops fertility trial there can be little or no response to fertilizers in some blocks, whereas the responses can be quite substantial in other blocks.

Multiple Experimental Units per Treatment in Each Block

A more general nonadditivity is represented by the general interaction term $(\tau\rho)_{ij}$. To test the existence of interaction the experiment must have more than one experimental unit for each treatment within each of the blocks. The linear model for an experiment with u experimental units for each treatment in each of r blocks is

$$y_{ijk} = \mu + \tau_i + \rho_j + (\tau\rho)_{ij} + e_{ijk} \tag{8.8}$$

$$i = 1, 2, \ldots, t \quad j = 1, 2, \ldots, r \quad k = 1, 2, \ldots, u$$

where e_{ijk} are the random, independent experimental errors with means 0 and variance σ^2. The variance σ^2 is the variability among experimental units within a block that have received the same treatment. The computations for sum of squares partitions and the test for interaction are the same as for a two-factor factorial arrangement shown in Chapter 6.

Subsamples of Experimental Units

There are occasions wherein two or more samples from the experimental units are required for data collection. Situations requiring subsampling of the units were discussed in Chapter 5. The linear model for an experiment in a randomized complete block design with n subsamples of the experimental units is

$$y_{ijk} = \mu + \tau_i + \rho_j + e_{ij} + d_{ijk} \tag{8.9}$$

$$i = 1, 2, \ldots, t \qquad j = 1, 2, \ldots, r \qquad k = 1, 2, \ldots, n$$

where the d_{ijk} are the random effects for subsamples with mean 0 and variance σ_d^2. The other terms are as described in Equation (8.1) for the randomized complete block design. The adjustments to the analysis are those shown in Chapter 5.

Gates (1995) provided a detailed discussion on experimental error estimation in block designs with different configurations of experimental units and sampling units.

Analysis of Residuals to Evaluate Assumptions

The assumptions regarding the experimental errors of the linear model for the randomized complete block design can be evaluated with an analysis of the residuals (discussed in Chapter 4). The residuals are computed from the experimental error deviation component shown in Equation (8.2) as $\widehat{e}_{ij} = y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} + \overline{y}_{..}$. For example, the residual for the control treatment in block 1 of Example 8.1 is

$$\begin{aligned}\widehat{e}_{11} &= y_{11} - \overline{y}_{1.} - \overline{y}_{.1} + \overline{y}_{..} \\ &= 34.98 - 38.28 - 38.00 + 42.07 = 0.77\end{aligned}$$

The plot of residuals versus the estimated values and the normal probability plot of the residuals are shown in Figures 8.2a and 8.2b. The assumptions of homogeneous variance (Figure 8.2a) and normal distribution (Figure 8.2b) appear to hold for these data.

Did Blocking Increase Precision?

The expectation of increased precision in the estimates of treatment means motivates us to use the randomized complete block design. Planning and conducting an experiment with the randomized complete block design requires extra effort relative to the completely randomized design. The relative efficiency measure (discussed in Chapter 1) evaluates the benefits of blocking for a particular experiment.

The efficiency of a randomized complete block design is compared with that of a completely randomized design. The estimate of σ^2, say s_{rcb}^2, is the mean square

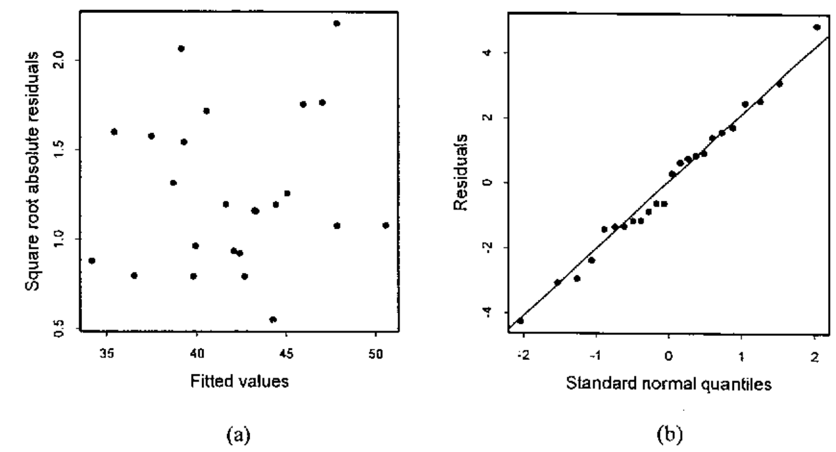


Figure 8.2 Residual plots from the analysis of variance for data on nitrate nitrogen in Example 8.1: (a) square root of the absolute residuals vs estimated values and (b) normal probability plot of residuals

for experimental error from the analysis of variance for the current experiment in a randomized complete block design. An estimate of σ^2 from the completely randomized design not used, say s_{cr}^2, is required for the relative efficiency measure. The computation of s_{cr}^2 from the information in the randomized complete block analysis of variance is (Cochran & Cox, 1957; Kempthorne, 1952)

$$s_{cr}^2 = \frac{SS\ \text{Blocks} + r(t-1)MSE}{rt - 1} \tag{8.10}$$

The estimate of σ_{cr}^2 for the wheat fertilization study is

$$s_{cr}^2 = \frac{197.0 + 20(7.2)}{23} = 14.8$$

and the estimate of σ_{rcb}^2 is $s_{rcb}^2 = MSE = 7.2$.

The relative efficiency estimate, without degrees of freedom correction for estimates of σ^2, is

$$RE = \frac{s_{cr}^2}{s_{rcb}^2} = \frac{14.8}{7.2} = 2.06 \tag{8.11}$$

The correction for estimation of σ^2 by s^2 is

$$\frac{(f_{rcb}+1)(f_{cr}+3)}{(f_{rcb}+3)(f_{cr}+1)} = \frac{16 \cdot 21}{18 \cdot 19} = 0.98$$

where $f_{rcb} = 15$ and $f_{cr} = 18$ are the error degrees of freedom for the randomized complete block and completely randomized design, respectively. The correction reduces the RE to $0.98(2.06) = 2.02$. The correction has little effect with moderately sized degrees of freedom for experimental error variance estimates.

The randomized complete block design for the wheat experiment is estimated to be slightly more than twice as efficient as a completely randomized design. The completely randomized design would require twice as many replications of the treatments as the randomized complete block design. Eight replications are required by the completely randomized design to have equivalent variances for the treatment means under the same experimental conditions in that field. Blocking on the irrigation gradient was effective as a measure to control and reduce the experimental error variance estimate in this instance. Future experiments of this same nature would likely benefit from the blocking practice.

A Quick Check for Effective Blocking

Lentner, Arnold, and Hinkelmann (1989) discuss a relationship between relative efficiency and the ratio $H = MSB/MSE$. They point out that, although the ratio H is equivalent to an F_0 statistic, a valid test for block effects does not exist. From Equations (8.10) and (8.11), the relative efficiency measure can be expressed as

$$RE = \frac{s^2_{cr}}{s^2_{rcb}} = \frac{(r-1)MSB + r(t-1)MSE}{(rt-1)MSE} \qquad \textbf{(8.12)}$$

With some rewriting, the expression becomes

$$RE = k + (1-k)H \qquad \textbf{(8.13)}$$

where $H = MSB/MSE$ and $k = r(t-1)/(rt-1)$. The following relationships for RE and H can be determined from Equation (8.13):

$RE < 1$ if and only if $H < 1$

$RE = 1$ if and only if $H = 1$

$RE > 1$ if and only if $H > 1$

H can be used to evaluate the effectiveness of blocking even though it is not a valid F statistic for testing block effects. For example, if $RE > 1$, then $H > 1$; blocking has been effective in reducing experimental error. Fewer replications are required for the randomized complete block design relative to the completely randomized design. The value of H does not provide the complete information about relative efficiency but only the implication of greater, lesser, or equal efficiency. H is a quick check for whether blocking was effective.

Random Blocks

The blocks of units often constitute a random sample of blocks available to the investigator. Sites used as blocks in ecological, forestry, or wildlife studies can be random samples of many sites available for the study. Plots may be established in each of the sites for the treatments. Manufactured batches of material used as blocks for experimental treatments are random batches. The batch of material (such as fabric, asphalt, or chemical product) is divided into smaller experimental unit batches to which the experimental treatments are administered. Schools used as blocking criteria in education studies can be random representatives of available schools in the school district. Classrooms within the schools serve as the experimental units for treatments.

The base of inference for treatments in a study with random blocks extends to a population of blocks from which the blocks in the study are a random sample. As a consequence of random blocks, the standard errors for treatment means will be different from those for an experiment with fixed blocks. The linear model with random block effects is

$$y_{ij} = \mu + \tau_i + b_j + e_{ij} \qquad \textbf{(8.14)}$$

$$i = 1, 2, \ldots, t \qquad j = 1, 2, \ldots, r$$

where μ is the general mean, τ_i is the fixed treatment effect, b_j is the random block effect with mean 0 and variance σ_b^2, and e_{ij} is the random experimental error with mean 0 and variance σ^2. Under the mixed model an observation has expected value $E(y_{ij}) = \mu + \tau_i$ and variance $\sigma^2 + \sigma_b^2$. There is also a covariance of σ_b^2 between any two observations in the same block with random blocks.

The variance of a treatment mean with random blocks in the randomized complete block design is

$$\sigma^2_{\bar{y}_{i.}} = \frac{1}{r}(\sigma^2 + \sigma_b^2) \qquad \textbf{(8.15)}$$

The variance of a treatment mean with random blocks includes the component of variance for blocks, σ_b^2, and will be larger than the variance with fixed block effects. The variance of the difference between two treatment means is not affected by the random block effects and will be the same as that shown for fixed effects in Equation (8.6).

8.3 Latin Square Designs Use Two Blocking Criteria

Two Blocking Factors May Be Necessary

Recognition of a factor, other than planned treatments, that influences the response variable was important in the experiment on wheat fertilization in Example 8.1. Blocking the experimental plots on the basis of the irrigation gradient doubled the efficiency of the experiment.

In some experimental settings two factors, other than treatments, may influence the response variable. Even more precision can be achieved if we can block the units on the basis of the two factors. If a second factor is a candidate for a blocking criterion, the Latin square arrangement can be used for the experiment design.

The Latin square arrangement derives from an arrangement of the Latin letters A, B, C, ... into a square array such that each letter appears once in each column and once in each row of the square. In applications to experiments, the rows and columns of the array are identified with the two blocking criteria and the Latin letters are identified with the treatments. One such application occurred in the following example.

Example 8.2 Relationship Between Wheat Yield and Seeding Rate

Wheat cultivation practices such as seeding rate, row spacing, and date of planting have direct effects on the crop yield. Cultivation practices to optimize production are established with experiments on newly introduced wheat cultivars.

Research Objective: In one such instance, a researcher wanted to determine the optimum seeding rate for a newly introduced durum wheat with a high semolina extract important to the making of pasta.

Treatment Design: Five seeding rates (30, 80, 130, 180, and 230 lb/acre) were used for the treatment design. Based on other cultivars common to the area, these seeding rates should have bracketed the rate for optimum production.

Experiment Design: The experiment was conducted in an irrigated field with the water gradient along one direction of the experimental area. In addition, the experimental fields on this farm were known to have soil gradients created by the grading required to make the land suitable for irrigation. These soil differences were generally perpendicular to the irrigation runs.

The researcher blocked the field plots in a row and column arrangement to control for soil and water gradients in two directions on the experimental field. The seeding rate treatments were randomized to the field plots in a 5×5 Latin square arrangement.

The layout of the experimental plots in a Latin square design after randomization is shown in Display 8.3. The grain yield for each plot in hundredweight (100 lb) per acre is shown in each plot along with a treatment letter.

The field row blocks coincided with the irrigation gradient, and the column row blocks coincided with the soil gradient perpendicular to the irrigation gradient. The treatments are arrayed in a Latin square arrangement with each of the treatments appearing once in each row block and once in each column block.

Display 8.3 Arrangement of Experimental Plots for the Wheat Experiment in a Latin Square Design

Field Row	*Column 1*	*Column 2*	*Column 3*	*Column 4*	*Column 5*	*Irrigation Gradient* ↓
1	59.45(**E**)	47.28(**A**)	54.44(**C**)	50.14(**B**)	59.45(**D**)	
2	55.16(**C**)	60.89(**D**)	56.59(**B**)	60.17(**E**)	48.71(**A**)	
3	44.41(**B**)	53.72(**C**)	55.87(**D**)	47.99(**A**)	59.45(**E**)	
4	42.26(**A**)	50.14(**B**)	55.87(**E**)	58.74(**D**)	55.87(**C**)	
5	60.89(**D**)	59.45(**E**)	49.43(**A**)	59.45(**C**)	57.31(**B**)	

Soil Gradient ⟶

Source: Dr. M. Ottman, Department of Plant Sciences, University of Arizona.

Other Applications of Latin Squares

An experiment to test automobile tire treatments is a classic example used to illustrate the Latin square design. The experiment tests four automobile tire treatments (A, B, C, D) on four automobiles. Each tire treatment appears on one of the four tire positions of each automobile. The row and column blocking criteria are the automobiles and tire positions, respectively, for the design in Display 8.4.

Display 8.4 Latin Square Arrangement for Automobile Tire Treatments

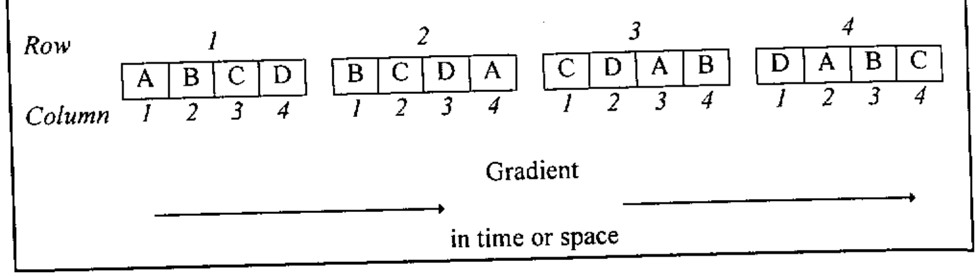
	Tire Position			
Auto	*1*	*2*	*3*	*4*
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C

Each treatment (A, B, C, or D) appears once in each row (auto) and once in each column (tire position). The rationale for the blocking criteria is that the wear on the tires may differ among the automobiles and the positions in which the tires are mounted on the automobile.

The blocking arrangement does not have to be rectangular for a Latin square arrangement to be useful for error variance reduction. A linear arrangement of treatments in a greenhouse experiment or a linear arrangement of treatments processed over time may be ordered according to the Latin square assignment. Display

8.5 illustrates a linear arrangement of four treatments with a Latin square assignment.

Display 8.5 Latin Square Assignment with a Linear Arrangement of Experimental Units



Use Standard Latin Squares to Generate the Designs

All Latin squares of a specified size can be generated from the *standard squares.* A standard square has the treatment symbols (A, B, C, ...) written in alphabetical order in the first row and in the first column of the array. Each treatment symbol occurs once in each column and once in each row of the array. Only one standard square exists for $t = 2$ or 3 treatments. There are 4 standard squares with $t = 4$ treatments and 56 standard squares with $t = 5$. The number of standard squares increases dramatically with the number of treatments since there are 9408 standard squares with 6 treatments.

The standard squares for $t = 2$, 3, and 4 treatments and samples of squares up to $t = 10$ treatments are shown in Appendix 8A. Fisher and Yates (1963) published the complete set of Latin squares for $t = 4$ through $t = 6$ treatments along with samples of squares for up to $t = 12$ treatments.

A standard square of any size can be generated by writing the first row of letters in alphabetical order. The second row is obtained from the first by shifting the first row one letter to the left, moving the letter A to the extreme right-hand position of row 2. The third row is obtained by shifting the second row one letter to the left, placing the letter B in the extreme right-hand position of row 3. This process is continued for the remaining rows. A standard 6×6 Latin square constructed in this manner is

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

The Latin square is a restrictive design because it requires the number of treatments, rows, and columns to be equal values. The requirement can be difficult to satisfy in some experimental settings that require two blocking criteria. Latin squares with $t = 4$ or fewer treatments have few degrees of freedom for estimating experimental error variance; thus, their value can be limited with small experiments unless two or more repetitions of the design are possible. With treatment numbers in excess of $t = 8$ to 10 the required number of experimental units can become prohibitive, depending on the circumstances of the contemplated experiments. The suitable size for many experiments in the Latin square arrangement includes those with $t = 5$ to 7 treatments.

Preece (1983) provides a historical background of the Latin square and a general discussion of variations on the Latin square design suggested for experimental work with row–column blocking design.

How to Randomize the Design

If all standard Latin squares of size $t \times t$ are available, randomization is accomplished with the following steps:

Step 1. Randomly select one of the standard squares.

Step 2. Randomly order all but the first row.

Step 3. Randomly order all columns.

Step 4. Randomly assign treatments to the letters.

All possible randomizations can be generated without including the first row in Step 2 if a standard square is randomly selected. If all standard squares are not available for selection, then it is recommended in Step 2 that all rows be included in the randomization. Not all possible Latin squares are generated in this way but the number of possibilities is increased considerably. Suppose the standard square selected at Step 1 for the 4×4 Latin square experiment with automobile tires is

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

Step 2. Obtain a random permutation of numbers to order the last three rows:

Permutation	*Original Row*
3	2
1	3
2	4

The placement of the rows for the standard square with row 1 in its original position is

Original Row				
1	A	B	C	D
3	C	D	A	B
4	D	A	B	C
2	B	C	D	A

Step 3. Obtain a random permutation of numbers to order the four columns from Step 2.

Permutation	*Original Column*
1	1
4	2
3	3
2	4

The placement of the columns for the standard square is

Original Column			
1	*4*	*3*	*2*
A	D	C	B
C	B	A	D
D	C	B	A
B	A	D	C

Step 4. Obtain a random permutation to assign treatments to the letters. This assignment is not necessary if the standard square has been selected at random from all possible standard squares. The method of assignment is shown here for illustration. Suppose the treatment labels are W, X, Y, and Z:

Permutation	*Treatment*
4 = D	W
2 = B	X
3 = C	Y
1 = A	Z

The treatment labels W, X, Y, Z replace the Latin square letters in the order D, B, C, A in the randomized arrangement. The final placement of the tire treatments on the automobiles and tire positions is

	Tire Position			
Auto	*1*	*2*	*3*	*4*
1	Z	W	Y	X
2	Y	X	Z	W
3	W	Y	X	Z
4	X	Z	W	Y

Statistical Model and Analysis for Latin Square Designs

The linear statistical model for an experiment with t treatments in a $t \times t$ Latin square design is

$$y_{ij} = \mu + \rho_i + \gamma_j + \tau_k + e_{ij} \qquad (8.16)$$
$$i, j, k = 1, 2, \ldots, t$$

where y_{ij} is the observation on the experimental unit in the ith row and jth column of the design. The row and column effects are ρ_i and γ_j, respectively; τ_k is the effect of the kth treatment; and the e_{ij} are random, independent experimental errors with mean 0 and variance σ^2. It is assumed there is no interaction between the treatments and the rows and columns.

The notation for totals and means of observations for rows and columns follows the usual convention with $y_{i.} = \sum_j^t y_{ij}$ for a row total and $y_{.j} = \sum_i^t y_{ij}$ for a column total. The treatment total will be represented simply as y_k, implying a sum of the observations over the t experimental units receiving treatment k. Likewise, $\overline{y}_k$ will represent the mean of the observations on the kth treatment.

Two Sum of Squares Partitions for Blocking

The sum of squares partitions can be derived from the algebraic identity

$$y_{ij} - \overline{y}_{..} = (\overline{y}_{i.} - \overline{y}_{..}) + (\overline{y}_{.j} - \overline{y}_{..}) + (\overline{y}_k - \overline{y}_{..})$$
$$+ (y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} - \overline{y}_k + 2\overline{y}_{..}) \qquad (8.17)$$

The deviation of an observation from the grand mean $y_{ij} - \overline{y}_{..}$ is expressed as an additive sum of:

- a row deviation $(\overline{y}_{i.} - \overline{y}_{..})$
- a column deviation $(\overline{y}_{.j} - \overline{y}_{..})$
- a treatment deviation $(\overline{y}_k - \overline{y}_{..})$
- experimental error $(y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} - \overline{y}_k + 2\overline{y}_{..})$

For example, the means for the wheat experiment in Example 8.2 (shown in Table 8.7) are used to illustrate deviations for the observation in row 1 and column 1 with treatment 5 = E, as

- the row 1 deviation: $\overline{y}_{1.} - \overline{y}_{..} = 54.15 - 54.53 = -0.38$
- the column 1 deviation: $\overline{y}_{.1} - \overline{y}_{..} = 52.43 - 54.53 = -2.10$
- the treatment E deviation: $\overline{y}_5 - \overline{y}_{..} = 58.88 - 54.53 = 4.35$
- the experimental error for y_{11}:

$$y_{11} - \overline{y}_{1.} - \overline{y}_{.1} - \overline{y}_5 + 2\overline{y}_{..} = 59.45 - 54.15 - 52.43 - 58.88 + 2(54.53) = 3.05$$

The sum of the four deviations is $-0.38 - 2.10 + 4.35 + 3.05 = 4.92$, which is the same as the total deviation $y_{11} - \overline{y}_{..} = 59.45 - 54.53 = 4.92$.

Squaring both sides of Equation (8.17) and summing the terms leads to an additive partition of

$$SS \text{ Total} = SS \text{ Rows} + SS \text{ Columns} + SS \text{ Treatment} + SS \text{ Error}$$

Table 8.6 summarizes the sums of squares in an analysis of variance with expected mean squares for fixed treatment effects.

Table 8.6 Analysis of variance for experiments in a Latin square design

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Expected Mean Square*
Total	$t^2 - 1$	$\sum_i \sum_j (y_{ij} - \overline{y}_{..})^2$		
Rows	$t - 1$	$t \sum_i (\overline{y}_{i.} - \overline{y}_{..})^2$	MSR	
Columns	$t - 1$	$t \sum_j (\overline{y}_{.j} - \overline{y}_{..})^2$	MSC	
Treatments	$t - 1$	$t \sum_k (\overline{y}_k - \overline{y}_{..})^2$	MST	$\sigma^2 + t\theta_t^2$
Error	$(t-1)(t-2)$	SS Error	MSE	σ^2

The sum of squares for experimental error has been reduced from that of the randomized complete block design by an amount equal to SS Rows or SS Columns at a cost of $t - 1$ degrees of freedom. The mean square for experimental error as an estimate of σ^2 has very few degrees of freedom with a small number of treatments. Considerable power is lost in the tests of hypotheses for treatment comparisons unless the reduction in error sum of squares due to blocking by both row and column criteria is substantial.

The effectiveness of blocking by either criterion evaluated with the relative efficiency measure is demonstrated with the analysis of grain yield from the wheat seeding rate experiment in Example 8.2.

The observations along with row, column, and treatment means are shown in Table 8.7 in the Latin square arrangement. The data are the grain yield for each plot in hundredweight (100 lb) per acre. The analysis of variance is shown in Table 8.8.

Table 8.7 Grain yield of a wheat variety for five different seeding rates in a Latin square design [Treatment label (**A**, **B**, **C**, **D**, or **E**) in parentheses following yield value]

Field Row	*Field Column 1*	*2*	*3*	*4*	*5*	*Row Means* ($\overline{y}_{i.}$)
1	59.45(**E**)	47.28(**A**)	54.44(**C**)	50.14(**B**)	59.45(**D**)	54.15
2	55.16(**C**)	60.89(**D**)	56.59(**B**)	60.17(**E**)	48.71(**A**)	56.30
3	44.41(**B**)	53.72(**C**)	55.87(**D**)	47.99(**A**)	59.45(**E**)	52.29
4	42.26(**A**)	50.14(**B**)	55.87(**E**)	58.74(**D**)	55.87(**C**)	52.58
5	60.89(**D**)	59.45(**E**)	49.43(**A**)	59.45(**C**)	57.31(**B**)	57.31
Column Means ($\overline{y}_{.j}$)	52.43	54.30	54.44	55.30	56.16	$\overline{y}_{..} =$ 54.53
Treatment		**A**	**B**	**C**	**D**	**E**
Seed rate		30	80	130	180	230
Mean ($\overline{y}_k$)		47.13	51.72	55.73	59.17	58.88

Table 8.8 Analysis of variance for grain yield of a wheat variety at five seeding rates in a 5 × 5 Latin square design

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*F*	*Pr > F*
Total	24	716.61			
Row	4	99.20	24.80		
Column	4	38.48	9.62		
Seed Rate	4	522.30	130.57	27.67	.000
Error	12	56.63	4.72		

Standard Errors for Treatment Means

The standard error estimate for a treatment mean is

$$s_{\overline{y}_k} = \sqrt{\frac{MSE}{t}} = \sqrt{\frac{4.72}{5}} = 0.97 \tag{8.18}$$

and the standard error estimate for a difference between two treatment means is

$$s_{(\overline{y}_k - \overline{y}_m)} = \sqrt{\frac{2MSE}{t}} = \sqrt{\frac{2(4.72)}{5}} = 1.37 \qquad \textbf{(8.19)}$$

Tests of Hypotheses About Treatment Means

The F_0 statistic to test the null hypothesis of no differences among treatment means,

$$F_0 = \frac{MST}{MSE} = \frac{130.57}{4.72} = 27.67 \qquad \textbf{(8.20)}$$

exceeds the critical value $F_{.05,4,12} = 3.26$ with observed significance level $Pr > F = .000$ (Table 8.8).

Interpretations of the Quantitative Treatment Factor with Regression Contrasts

The treatment factor for this experiment is a quantitative factor with five levels. An analysis for the regression of grain yield on seeding rate with orthogonal polynomial contrasts for seeding rate will provide a good description of the effect of seeding rate on grain yield. The coefficients for the orthogonal polynomial contrasts are found in Appendix Table XI. Orthogonal coefficients and the sums of squares for the linear and quadratic polynomial regression contrasts are shown in Table 8.9.

Table 8.9 Linear and quadratic polynomial regression sums of squares partitions for seeding rate (Example 8.2)

Seeding Rate	*30*	*80*	*130*	*180*	*230*	*SS**
Mean ($\overline{y}_k$)	47.13	51.72	55.73	59.17	58.88	
Linear (P_{1k})	−2	−1	0	1	2	478.95
Quadratic (P_{2k})	2	−1	−2	−1	2	38.11

$^*SS = r[\Sigma P_{ck}\overline{y}_k]^2/\Sigma P_{ck}^2$

The respective F_0 statistics to test the null hypotheses for the linear and quadratic contrasts are $F_0 = 478.95/4.72 = 101.47$ and $F_0 = 38.11/4.72 = 8.07$. Both ratios exceed the critical value $F_{.05,1,12} = 4.75$. The sum of squares for deviations from linear and quadratic regression is

$$SS\ \text{Seed(deviations)} = SS\ \text{Seed} - SS\ \text{Seed(linear)} - SS\ \text{Seed(quadratic)}$$
$$= 522.30 - 478.95 - 38.11 = 5.24$$

with 2 degrees of freedom; a test of hypothesis would indicate no significant deviations from the quadratic equation.

The computed quadratic polynomial regression equation to estimate grain yield ($\widehat{y}$) from seeding rate (R), using the techniques described in Chapter 3, is

$$\widehat{y} = 43 + 0.14R - 0.0003R^2$$

A plot of the equation is shown in Figure 8.3. The maximum estimated grain yield occurs for a seeding rate of $R = 233$ pounds per acre, which is an extrapolation beyond the highest rate used in the experiment. Another experiment with a seeding rate above 230 pounds per acre would be required to safely estimate the maximum seeding rate.

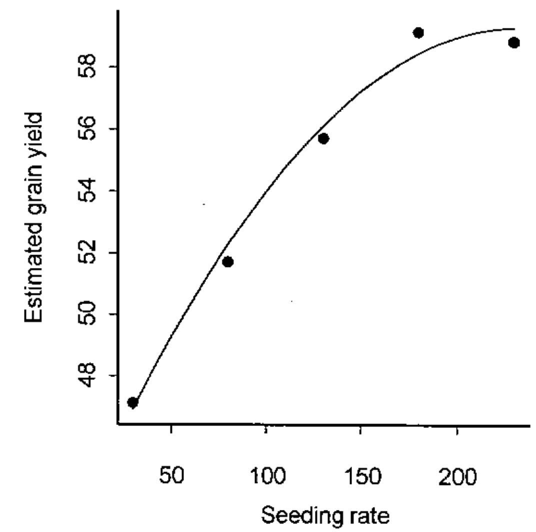


Figure 8.3 Estimated response between grain yield and seeding rate

Analysis of Residuals to Evaluate Assumptions

Residuals can be used to evaluate the assumptions of the model (as discussed in Chapter 4). From Equation (8.17), the residual for the observations on the kth treatment in the ith row and jth column is

$$\widehat{e}_{ij} = y_{ij} - \overline{y}_{i.} - \overline{y}_{.j} - \overline{y}_k + 2\overline{y}_{..}$$

The residual plots are left as an exercise for the reader.

If Treatments Interact with Blocks

Tukey (1955) devised a test for the assumption of additive treatment, row, and column effects. From Equation (8.17), the additivity of treatment, row, and column effects results in the estimated value of an observation as

$$\widehat{y}_{ij} = \overline{y}_{..} + (\overline{y}_{i.} - \overline{y}_{..}) + (\overline{y}_{.j} - \overline{y}_{..}) + (\overline{y}_{k} - \overline{y}_{..}) = \overline{y}_{i.} + \overline{y}_{.j} + \overline{y}_{k} - 2\overline{y}_{..}$$

The computation of a 1 degree of freedom sum of squares for nonadditivity requires the values of $\widehat{y}_{ij}$ for each experimental unit and the residual for each experimental unit, $\widehat{e}_{ij} = y_{ij} - \widehat{y}_{ij}$. The sum of squares for nonadditivity is

$$SS(\text{nonadditivity}) = \frac{\left[\sum_{i=1}^{t}\sum_{j=1}^{t} \widehat{y}_{ij}^2 \widehat{e}_{ij}\right]^2}{SS} \tag{8.21}$$

where SS is the sum of squares for error from the Latin square analysis of variance on $\widehat{y}_{ij}^2$. The F_0 statistic to test the hypothesis for nonadditivity is

$$F_0 = \frac{SS(\text{nonadditivity})}{MS\ \text{Residual}} \tag{8.22}$$

where

$$MS\ \text{Residual} = \frac{[SSE - SS(\text{nonadditivity})]}{\nu}$$

has $\nu = (t-1)(t-2) - 1$ degrees of freedom and SSE is the sum of squares for error from the Latin square analysis of variance for the original observations y_{ij}. It is recommended that the values for $\widehat{y}_{ij}$ be coded as $k(\widehat{y}_{ij} - \overline{y}_{..})$, where k is a constant to scale the size of the values for computational convenience.

Did Both Blocking Factors Increase Precision?

The efficiency of the Latin square design with two blocking criteria is determined relative to the randomized complete block design with only one blocking criterion. Relative efficiency measures can be computed separately for the row and column blocking criteria of the Latin square.

Relative Efficiency of Column Blocking

If only the row blocking criterion is used for blocking in a randomized complete block design, the estimated mean square for error is

$$s_{rcb}^2 = \frac{MS\ \text{Columns} + (t-1)MSE}{t} \tag{8.23}$$

where MSE is the mean square for error from the current Latin square analysis of variance.

The estimated value for Example 8.2 is $s_{rcb}^2 = [9.62 + 4(4.72)]/5 = 5.70$ and $s_{ls}^2 = MSE = 4.72$. The relative efficiency of column blocking for the experiment is

$$RE_{col} = \frac{s_{rcb}^2}{s_{ls}^2} = \frac{5.70}{4.72} = 1.21$$

There is a 21% gain in efficiency over the randomized complete block design in which only the row criterion of the Latin square design is used for blocking. Thus, the column blocks for soil gradients across the field effectively reduced the error variance by 21%. The randomized block design without the column blocks for soil gradients would require $1.21(5) = 6$ replications to have an estimated variance of the treatment mean equal to that from the Latin square design.

Relative Efficiency of Row Blocking

If only the column criterion is used for blocking in a randomized complete block design, the estimated mean square for error is

$$s_{rcb}^2 = \frac{MS\ \text{Rows} + (t-1)MSE}{t} \tag{8.24}$$

For Example 8.2, $s_{rcb}^2 = [24.80 + 4(4.72)]/5 = 8.74$ and $RE_{row} = 8.74/4.72 = 1.85$. There is an 85% gain in efficiency with row blocking for the irrigation gradient in the experiment. Without row blocking for irrigation gradient the experiment would require $1.85(5) = 9.25$ or 10 replications of each treatment in the randomized complete block design to have an estimated variance of the treatment equal to that from the current Latin square design.

Correction for Estimating σ^2

The correction for estimating σ^2 by s^2 is

$$\frac{(f_{ls}+1)(f_{rcb}+3)}{(f_{ls}+3)(f_{rcb}+1)} = \frac{13 \cdot 19}{15 \cdot 17} = 0.97$$

where $f_{ls} = 12$ and $f_{rcb} = 16$ are the error degrees of freedom for the Latin square and randomized complete block design, respectively. The correction reduces the RE from 1.21 to $0.97(1.21) = 1.17$ for column blocking and from 1.85 to $0.97(1.85) = 1.79$ for row blocking. The correction has a small effect on the efficiency estimates.

Multiple Latin Squares and Latin Rectangles

The Latin square design with two blocking criteria and four or less treatments is very restrictive and provides too few degrees of freedom for an effective estimate of experimental error variance. It is common to repeat the experiment with more than one square under these circumstances.

There are two distinct forms of the design with multiple squares. The first form has distinct row and column identification for each square in the experiment. The second form has either the row or the column identification common to all squares. An example of the first form occurs in agricultural field trials in which two Latin squares are utilized in separated areas of the research farm. The second form is exemplified with the automobile tire experiment in which two groups of four automobiles are used for two Latin square arrangements, with the eight automobiles representing column blocking and the four tire positions common to both squares representing row blocking. This latter form of the design is known as the *Latin rectangle.* The two forms are illustrated in Figure 8.4.

Figure 8.4a is representative of the agricultural field experiment with multiple unique Latin squares and Figure 8.4b is representative of the automobile tire experiment with positions as rows and automobiles as columns in a Latin rectangle.

(a)

				Column				
Row	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	A	B	C	D				
2	B	C	D	A				
3	C	D	A	B				
4	D	A	B	C				
5					A	B	C	D
6					B	A	D	C
7					C	D	B	A
8					D	C	A	B

(b)

				Column				
Row	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
1	A	B	C	D	A	B	C	D
2	B	C	D	A	B	A	D	C
3	C	D	A	B	C	D	B	A
4	D	A	B	C	D	C	A	B

Figure 8.4 Multiple Latin squares with (a) rows and columns unique for each square and (b) the Latin rectangle with rows common to both squares and columns unique to each square

Randomization is performed separately for each square when row and column blocking is unique for each of the s squares. With the Latin rectangle it is also possible to consider each square unique and randomize accordingly. When the row criterion is consistent across columns it is only necessary to require that each treatment occur s times in each row and one time in each column. The randomization then consists of one randomization of the t rows and a separate randomization of the entire set of st columns.

The linear model for the Latin rectangle with unique row and column blocking criteria in each Latin square is

$$y_{ijl} = \mu + \kappa_l + \rho_{i(l)} + \gamma_{j(l)} + \tau_k + e_{ijl} \qquad (8.25)$$

$$l = 1, 2, \ldots, s \quad i, j, k = 1, 2, \ldots, t$$

where κ_l is the square effect, and $\rho_{i(l)}$ and $\gamma_{j(l)}$ are the row and column effects nested in the squares, respectively. It may be necessary to consider a square $\times$ treatment interaction component in the model if it is suspected that the treatment comparisons may differ from one square to another. A discussion of an experiment by treatment interaction is found in Section 8.6. An outline of the analysis of variance with unique row and column blocking is shown in Table 8.10.

Table 8.10 Analysis of variance for an experiment repeated with s unique Latin square arrangements

Source of Variation	*Degrees of Freedom*	*Sum of Squares*
Total	$st^2 - 1$	$\sum_l \sum_i \sum_j (y_{ijl} - \bar{y}_{...})^2$
Squares	$s - 1$	$t^2 \sum_l (\bar{y}_{..l} - \bar{y}_{...})^2$
Rows within squares	$s(t - 1)$	$t \sum_l \sum_i (\bar{y}_{i.l} - \bar{y}_{..l})^2$
Columns within squares	$s(t - 1)$	$t \sum_l \sum_j (\bar{y}_{.jl} - \bar{y}_{..l})^2$
Treatments	$t - 1$	$st \sum_k (\bar{y}_k - \bar{y}_{...})^2$
Error	$(st - s - 1)(t - 1)$	By subtraction

The linear model for the Latin rectangle that has common row blocking criteria for s complete Latin squares and unique column blocking is

$$y_{ij} = \mu + \rho_i + \gamma_j + \tau_k + e_{ij} \qquad (8.26)$$

$$j = 1, 2, \ldots, st \quad i, k = 1, 2, \ldots, t.$$

An outline of the analysis of variance for common row and unique column blocking is shown in Table 8.11. The standard error estimates for treatment means and differences between two treatment means, respectively, are $\sqrt{MSE/st}$ and $\sqrt{2MSE/st}$.

8.4 Factorial Experiments in Complete Block Designs

The treatment design used to address the research hypothesis can be placed in any compatible experiment design. The appropriate sum of squares partitions can be computed for the analysis of variance as long as the randomization restrictions are followed for the experiment design in question.

Table 8.11 Analysis of variance for an experiment with t treatments in a Latin rectangle with t rows and st columns

Source of Variation	*Degrees of Freedom*	*Sum of Squares*
Total	$st^2 - 1$	$\sum_i \sum_j (y_{ijl} - \overline{y}_{..})^2$
Rows	$t - 1$	$st \sum_i (\overline{y}_{i.} - \overline{y}_{..})^2$
Columns	$st - 1$	$t \sum_j (\overline{y}_{.j} - \overline{y}_{..})^2$
Treatments	$t - 1$	$st \sum_k (\overline{y}_k - \overline{y}_{..})^2$
Error	$(st - 2)(t - 1)$	By subtraction

The two-factor factorial in a randomized complete block design has all ab treatment combinations an equal number of times in each block. With the Latin square design each of the ab treatment combinations appears one time in each row and one time in each column. A Latin rectangle design with a common row criterion for s squares requires each treatment combination to appear one time in each column and s times in each row.

The linear model for a two-factor factorial, factor A with a levels and factor B with b levels, in a randomized complete block design with r blocks is

$$y_{ijk} = \mu + \rho_k + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk} \tag{8.27}$$

$$i = 1, 2, \ldots, a \qquad j = 1, 2, \ldots, b \qquad k = 1, 2, \ldots, r$$

The sum of squares partitions for a two-factor factorial in a randomized complete block design are illustrated in Table 8.12. The treatment sum of squares with $(t - 1) = (ab - 1)$ degrees of freedom is partitioned into sums of squares for the main effects of factors A and B and interaction effects as described in Table 6.5. The sum of squares for blocks and experimental error are analogous to those shown for the randomized complete block design in Table 8.2. Standard errors are computed according to the conventions outlined in Chapter 6 for the factorial experiment in a completely randomized design.

A similar pattern of analysis is followed for the Latin square and Latin rectangle in which the square, row, and column sums of squares are partitioned according to the analyses in Tables 8.6, 8.10, or 8.11 with $t = ab$. The sum of squares partitions for factorial main effects and interactions are computed as described in Chapter 6.

Table 8.12 Analysis of variance for a two-factor treatment design in a randomized complete block experiment design

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Expected Mean Square*
Total	$rab - 1$	*SS* Total		
Blocks	$r - 1$	*SS* Blocks		
A	$a - 1$	*SSA*	MSA	$\sigma^2 + rb\theta_a^2$
B	$b - 1$	*SSB*	MSB	$\sigma^2 + ra\theta_b^2$
AB	$(a - 1)(b - 1)$	*SS(AB)*	$MS(AB)$	$\sigma^2 + r\theta_{ab}^2$
Error	$(ab - 1)(r - 1)$	*SSE*	MSE	σ^2

[a]See Chapter 6 for computational formulae of SSA, SSB, and $SS(AB)$. SSE is obtained by subtraction. SS Blocks $= ab\Sigma(\overline{y}_{..k} - \overline{y}_{...})^2$.

8.5 Missing Data in Blocked Designs

Missing data in research studies were discussed with factorial treatment designs in Chapter 6. Missing observations affect the relationships between blocks and treatments just as they affected the relationships between factors in the factorials.

The effects of treatments and blocks are nonorthogonal when data are missing. A contrast for one set of effects conveys some information about the other set of effects. Thus, the effects for blocks must be considered when computing the sum of squares partition for treatments.

Full and reduced alternative models are used for the complete block design to (1) compute unbiased sum of squares partitions for treatments and experimental error and (2) compute unbiased least squares estimates of treatment means and their standard errors from the available data. The procedures for least squares solutions to the normal equations and the sums of squares for the full and reduced models were outlined in Chapter 6. Many of the present-day computer programs for the analysis of variance are capable of performing this analysis.

Analysis with Missing Data in Complete Block Designs

The analysis with missing data for the randomized complete block design will differ slightly from that for the factorial design described in Chapter 6 because the randomized complete block model assumes no interaction effects. Thus, the analysis omits any test for interaction prior to estimating the treatment effects.

Solutions to the normal equations for the full model, $y_{ij} = \mu + \tau_i + \rho_j + e_{ij}$, are used to compute the estimates, $\widehat{y}_{ij} = \widehat{\mu} + \widehat{\tau}_i + \widehat{\rho}_j$, and the experimental error sum of squares for the full model,

$$SSE_f = \Sigma(y_{ij} - \widehat{\mu} - \widehat{\tau}_i - \widehat{\rho}_j)^2$$

Solutions to the normal equations for the reduced model, $y_{ij} = \mu + \rho_j + e_{ij}$, are used to compute the estimates, $\widehat{y}_{ij} = \widehat{\mu} + \widehat{\rho}_j$, and the experimental error sum of squares for the reduced model,

$$SSE_r = \Sigma(y_{ij} - \widehat{\mu} - \widehat{\rho}_j)^2$$

The treatment sum of squares is computed as

$$SS\text{ Treatment (adjusted)} = SSE_r - SSE_f \tag{8.28}$$

and it represents the reduction in sum of squares as a result of including τ_i in the full model. It is referred to as the adjusted treatment sum of squares, implying that block effects are also considered when estimating the treatment effects in the full model.

The orthogonal sum of squares partitions with m missing observations are shown in Table 8.13. The reduced model ignores the treatment classification, and SS Blocks (unadjusted) from the reduced model is the sum of squares due to differences among block means ignoring treatments.

The application of an analysis with missing observations is reserved for an exercise at the end of the chapter.

Table 8.13 Analysis of variance for a randomized complete block design with m missing observations

Source of Variation	*Degrees of Freedom*	*Sum of Squares*
Total	$tr - m - 1$	$\sum_i\sum_j (y_{ij} - \overline{y}_{..})^2$
Blocks (unadjusted)*	$r - 1$	$\sum_j n_j(\overline{y}_{.j} - \overline{y}_{..})^2$
Treatment (adjusted)	$t - 1$	$SSE_r - SSE_f$
Error	$(r-1)(t-1) - m$	SSE_f

*SS Blocks (unadjusted) $= SS$ Total $- SSE_r$; n_j = number of observations in jth block

8.6 Experiments Performed Several Times

Experiments are repeated at several places or on several different occasions for various reasons. Repetition over time or space provides a form of replication to increase the precision of treatment mean estimates or increase the degrees of freedom for estimates of experimental error. Repeated experiments can provide an expanded inference base to evaluate treatments over a broader range of conditions. In other cases, the magnitude of treatment comparisons is expected to differ among places or times. The series of experiments are used to examine the variation in treatment differences relative to the environmental changes.

Whatever the reason for conducting the same experiment over a series of times or places, a certain amount of discretion must be exercised before we automatically

combine the data from the series to conduct a single overall analysis. The following example will help clarify some caveats with a series of experiments.

Example 8.3 Efficiency of Water Use by Bermuda Grass

Bermuda grass is used extensively for lawns, parks, and golf courses in warm and dry climates. In dry climate areas maintenance of the Bermuda grass requires regular irrigation. Turf managers want species of plants that efficiently utilize water to reduce maintenance costs and conserve water. Considerable variation in water use efficiency exists among species and within species.

Research Objective: A plant breeder wanted to determine the amount of variability in water use efficiency of Bermuda grass that he could attribute to genetic differences. Given sufficient genetic variability he could initiate a breeding program to develop a water-use efficient hybrid Bermuda grass.

Treatment Design: The plant breeder made all possible (30) hybrid crosses between six Bermuda grass cultivars in what is known as a *diallel mating design*. This particular mating design allows the plant breeder to evaluate the genetic potential of specific cultivars or the population represented by the cultivars in the designs.

Experiment Design: He grew the progeny and the six parental cultivars in a randomized complete block design with two blocks at each of four separate locations in a field plot experiment. Two of the crosses did not produce progeny, so there were 34 plots in each block of each experiment.

The plant breeder measured the total dry matter produced by the plants in each plot and the amount of water utilized on the plot to produce the plant material. The measurement he utilized for analysis was the ratio of water used to total dry matter production on each plot. The analysis of variance with source of variation, degrees of freedom, and mean square for each of the four experiments is shown in Table 8.14.

Table 8.14 Analysis of variance for water use in each of four experiments from a diallel mating design with six Bermuda grass cultivars

Source of Variation	*Degrees of Freedom*	*Mean Squares by Location*			
		1	*2*	*3*	*4*
Block	1	2.80	20.17	2.53	1.81
Genotypes	33	1.08	17.85	1.92	1.39
Error	33	1.61	10.56	1.07	0.74

Source: Dr. W. Kneebone, Department of Plant Sciences, University of Arizona.

Location 2 Is Different

The most noticeable feature of the analyses in Table 8.14 is that the mean squares for all sources of variation at location 2 are considerably larger than those for the other three locations. In particular, the error variance at location 2, $MSE = 10.56$, is considerably larger than the error variance at the other locations. The greater variability at location 2 is an indication that the experimental conditions there may have been quite different from other locations.

Homogeneous Variances Required to Combine Results of Several Experiments

There were four experiments, each with two replications, for a total of eight replications. If the four experiments were combined into one analysis the estimates of the genotype means would be much more precise.

Homogeneity of error variances is required for a combined analysis of variance for the four experiments. A homogeneity of variance test for the error variances with the *F Max* test (Chapter 4) rejects the hypothesis of equal error variances for the four locations. One possible solution is a transformation to a logarithmic scale to achieve homogeneous variances prior to a combined analysis of variance. The analyses of variance for each location after the logarithmic transformation are shown in Table 8.15.

Table 8.15 Analysis of variance for $10[\log_{10}(\text{water use})]$ in each of four experiments from a diallel mating design with six Bermuda grass cultivars

Source of Variation	*Degrees of Freedom*	*Mean Squares by Location* 1	2	3	4
Block	1	1.68	3.28	1.42	1.01
Genotypes	33	0.56	2.51	1.09	1.11
Error	33	0.82	1.54	0.56	0.51

The logarithmic transformation considerably reduced the disparity in the error variances. However, the observed mean squares for all sources of variation are still somewhat larger at location 2. The combined analysis still should be approached with caution, and an interpretation of the results initially should be made from each of the separate experiments. The F_0 statistics at each location to test the hypothesis of no differences among the genotypes, $F_0 = MS\,\text{Genotypes}/MS\,\text{Error}$, result in the rejection of the null hypothesis at the .05 level of significance for locations 3 and 4. The null hypothesis is not rejected at locations 1 and 2. The same conclusions are reached from the F_0 statistics with the untransformed data in Table 8.14.

Statistical Model and Analysis of Variance for Combined Analysis of Experiments

The results of the F tests indicate a differential performance among the genotypes at the four locations. The combined analysis should include the possibility of a genotype $\times$ location interaction. The linear statistical model for the combined analysis with random genotype and location effects is

$$y_{ijk} = \mu + p_i + b_{j(i)} + g_k + (gp)_{ik} + e_{ijk} \tag{8.29}$$

where μ is the general mean, p_i is the random location effect, $b_{j(i)}$ is the random block within location effect, g_k is the random genotype effect, $(gp)_{ik}$ is the random effect for genotype $\times$ location interaction, and e_{ijk} is the random error effect. The combined analysis of variance for the transformed data from the four experiments is shown in Table 8.16 with expected mean squares for random genotype effects and either fixed or random location effects.

Table 8.16 Analysis of variance for $10[\log_{10}(\text{water use})]$ for combined experiments from a diallel mating design of six Bermuda grass cultivars

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*	*Expected Mean Square*
Locations (L)	3	433.63	144.54	
Blocks/L	4	7.39	1.85	
Genotypes (G)	33	81.11	2.46	$\sigma^2 + r\sigma_{gl}^2 + rl\sigma_g^2$
$G \times L$	99	93.01	0.94	$\sigma^2 + r\sigma_{gl}^2$
Pooled error	132	113.19	0.86	σ^2

The mean square for error in the combined analysis is the pooled error from the four experiments (average of the mean squares for error from the four experiments). The blocks are unique to each experiment and they are a nested-factor effect for the analysis of variance. The sum of squares for blocks within locations is the sum of the block sum of squares from the individual analyses of variance.

The expected mean squares for the random or mixed model were derived using guidelines given in Chapter 7. If genotypes (treatments) are fixed effects, then σ_g^2 is replaced by the equivalent component for fixed effects, θ_g^2. When both locations and genotypes (or treatments) are fixed, then σ_{gl}^2 is also replaced by the equivalent component for fixed effects, θ_{gl}^2 in Table 8.16.

Whether the repetitions of experiments over time and places are random or fixed effects depends upon the objective of the repetition. If the repetitions are chosen to investigate the treatment responses to deliberate changes in environment, then fixed effects models for places or time, seem appropriate. If the repetitions can be justified as legitimate random representatives of places or time, then the random effects model can be used for places or time. It is perhaps most difficult to consider the repetitions random if only a limited number of places are available for experiments or a sequence of successive weeks, months, or years represent the time repetition.

Tests of Hypotheses in Combined Analysis

The hypothesis of no genotype $\times$ location interaction is tested with the F_0 statistic in Table 8.16 as $F_0 = MS(G \times L)/MSE = 0.94/0.86 = 1.09$, and critical value

$F_{.05,99,132} = 1.36$. The null hypothesis of no genotype × location is not rejected. The hypothesis of no genotype effects is tested with the F_0 statistic $F_0 = MSG/MSE = 2.46/0.94 = 2.62$ with critical value $F_{.05,33,99} = 1.55$. The null hypothesis of no genotype effects is rejected.

Dissecting Treatment × Experiment Interaction

The nonsignificance of genotype × location interaction should not be taken lightly since an analysis of the separate experiments revealed significant genotype differences at the .05 level of significance at only two of the locations. It is entirely possible that certain treatment comparisons interact with the environment while other comparisons are relatively constant across environments. Three sets of comparisons important in the Bermuda grass diallel mating design are

- comparisons among the six parent cultivars
- comparisons among the 28 crosses
- a contrast between the mean of the six parent cultivars and that of the 28 crosses

A separate analysis of variance for the parent cultivars provides the sums of squares for the first set of comparisons. A separate analysis of variance for the crosses provides the sums of squares for the second set of comparisons. The linear model in Equation (8.29) is used for the analysis. It is the same model used when all genotypes were included in the analysis. Only those sums of squares required for the comparisons of interest are shown in Table 8.17. The sums of squares for locations and blocks within location are not shown.

Table 8.17 Separate analyses of variance for parents and crosses from a diallel mating design of six Bermuda grass cultivars

(1) *Parents Analysis*			
Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*
Parents	5	3.45	0.69
Parents × locations	15	4.87	0.32
Error (*P*)	20	10.04	0.50
(2) *Crosses Analysis*			
Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*
Crosses	27	46.06	1.71
Crosses × locations	81	79.56	0.98
Error (*C*)	108	101.22	0.94

The sum of squares for the contrast between the means of the parents and crosses and the sum of squares for the interaction between the contrast and locations can be computed by subtraction. Utilizing the sums of squares from Tables 8.16 and 8.17, they are

(1) Parents versus Crosses

$$\begin{aligned} SS(\boldsymbol{P} \text{ versus } \boldsymbol{C}) &= SSG - SS\boldsymbol{P} - SS\boldsymbol{C} = 81.11 - 3.45 - 46.06 \\ &= 31.60 \end{aligned}$$

(2) (Parents versus Crosses) × Locations

$$\begin{aligned} SS[(\boldsymbol{P} \text{ versus } \boldsymbol{C}) \times \boldsymbol{L}] &= SS(\boldsymbol{G} \times \boldsymbol{L}) - SS(\boldsymbol{P} \times \boldsymbol{L}) - SS(\boldsymbol{C} \times \boldsymbol{L}) \\ &= 93.01 - 4.87 - 79.56 \\ &= 8.58 \end{aligned}$$

The separate analyses of variance for parent cultivars and the crosses also provide separate sums of squares for experimental error that can be identified with each set of comparisons. A partition of the error sum of squares can be useful because the experimental errors can differ considerably among the several comparisons. The experimental error mean squares for parents and crosses, Error (*P*) and Error (*C*), from the separate analyses are found in Table 8.17.

The sum of squares for experimental error associated with the contrast between parents and crosses is found from the error sums of squares in Tables 8.16 and 8.17 as

$$\begin{aligned} SS \text{ Error}(\boldsymbol{P} \text{ versus } \boldsymbol{C}) &= SS \text{ Error} - SS \text{ Error}(\boldsymbol{P}) - SS \text{ Error}(\boldsymbol{C}) \\ &= 113.19 - 10.04 - 101.22 \\ &= 1.93 \end{aligned}$$

A summary of the analysis of variance with all of the sum of squares partitions is shown in Table 8.18. The three mean squares for the partition of experimental error shown at the bottom of Table 8.18 have similar values. The pooled error with 132 degrees of freedom probably can be used with some confidence that the error variances of the three groups of comparisons are relatively homogeneous.

To Pool or Not to Pool Variances

The decision to use the partitioned error term or the pooled error can have an effect on the tests of hypotheses. Tests with the pooled error have larger degrees of freedom and, therefore, greater ability (power) to detect differences than the partitioned mean square error. This effect can be seen in Table 8.18 with a test of the ($\boldsymbol{P}$ vs. $\boldsymbol{C}$) × $\boldsymbol{L}$ interaction. The test statistic with the pooled error is $F_0 = 2.86/0.86 = 3.33$ with critical value $F_{.05,3,132} = 2.67$. The test statistic with the partitioned mean square error is $F_0 = 2.86/0.48 = 5.96$ with critical value $F_{.05,3,4} = 6.59$. In the latter test with fewer degrees of freedom for mean square error the null hypothesis is not rejected. The hypothesis is rejected with the pooled

Table 8.18 Analysis of variance for $10[\log_{10}(\text{water use})]$ for combined experiments from a diallel mating design of six Bermuda grass cultivars

Source of Variation	*Degrees of Freedom*	*Sum of Squares*	*Mean Square*
Locations (L)	3	433.63	144.54
Blocks/L	4	7.39	1.85
Genotypes (G)	33	81.11	2.46
Parents (P)	5	3.45	0.69
Crosses (C)	27	46.06	1.71
P vs C	1	31.60	31.60
$G \times L$	99	93.01	0.94
$P \times L$	15	4.87	0.32
$C \times L$	81	79.56	0.98
(P vs. C) $\times L$	3	8.58	2.86
Pooled Error	132	113.19	0.86
Error (P)	20	10.04	0.50
Error (C)	108	101.22	0.94
Error (P vs. C)	4	1.93	0.48

mean square error. It should be recognized that the null hypothesis would not be rejected for either of the tests at the 0.01 significance level. The difference between the two tests is not dramatic in this particular case, but it is necessary to be aware of the two possibilities for the test.

In this particular example the results of other F tests are not affected by which error term is used, partitioned or pooled. Neither of the other $G \times L$ interaction partitions is significant. Although the overall genotype $\times$ location interaction was not significant, there appears to be some evidence that a component of the interaction was significant. This result may explain the differences in significance levels of the tests for genotypes from the separate location analyses in Table 8.15.

More Details to be Found

McIntosh (1983) provided analysis of variance tables with sources of variation, degrees of freedom, and appropriate F_0 statistics to test hypotheses for an extensive array of experiments with fixed, random, and mixed models combined over time, places, or a combination of time and places. Carmer, Nyquist, and Walker (1989) provided formulae for the estimation of variances for pairwise mean differences from combined experiments with two- and three-factor treatment designs. The experiments had randomized complete block designs with fixed treatment effects and random time and place effects.

EXERCISES FOR CHAPTER 8

1. An irrigation experiment was conducted in a randomized complete block design in a Valencia orange grove. Six irrigation treatments were used in eight blocks of trees. The data that follow are the weight in pounds of harvested fruit from each plot.

	Block							
Method	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Trickle	450	469	249	125	280	352	221	251
Basin	358	512	281	58	352	293	283	186
Spray	331	402	183	70	258	281	219	46
Sprinkler	317	423	379	63	289	239	269	357
Sprinkler + Spray	479	341	404	115	182	349	276	182
Flood	245	380	263	62	336	282	171	98

Source: Dr. R. Roth and Dr. B. Gardner, Department of Soil and Water Science, University of Arizona.

a. Write a linear model for the experiment, explain the terms, and compute the analysis of variance.

b. What are the assumptions necessary for the analysis of variance to be valid? How do they relate to the experiment?

c. Compute the standard error estimate for an irrigation treatment mean and the difference between two irrigation treatment means.

d. Consider the flood irrigation method to be the standard practice. Use the Dunnett method to test the difference between the flood irrigation and each of the other methods.

e. Compute the relative efficiency of this design relative to a completely randomized design. What are your conclusions?

f. Obtain the residual plots from the analysis, and interpret them.

2. A fertilizer trial on a range grass, blue grama, was conducted in a randomized complete block design by a management scientist. Five fertilizer treatments were randomly assigned to plots in each of five blocks. The data are 100 $\times$ (percent phosphorus) in a plant tissue sample from each plot.

	Block				
Treatment	*1*	*2*	*3*	*4*	*5*
No fertilizer	7.6	8.1	7.3	7.9	9.4
50 lb. nitrogen	7.3	7.7	7.7	7.7	8.2
100 lb. nitrogen	6.9	6.0	5.6	7.4	7.0
50 lb nitrogen + 75 lb P_2O_5	10.8	11.2	9.0	12.9	11.6
100 lb nitrogen + 75 lb P_2O_5	9.6	9.3	12.0	10.6	10.4

Source: Dr. P. Ogden, Range Management, University of Arizona.

a. Write a linear model for this experiment, explain the terms, and compute the analysis of variance.
b. Compute the 1 degree of freedom sum of squares for each contrast that follows, and test the null hypothesis for each. The four orthogonal contrasts among the five treatments are (1) no fertilizer versus the four fertilizer treatments, (2) the main effect of nitrogen, (3) the main effect of P_2O_5, and (4) the interaction between nitrogen and P_2O_5.
c. Compute the standard error for each of the contrasts in part (b).
d. Compute the relative efficiency of the randomized complete block design.
e. Obtain the residual plots from the analysis, and interpret them.

3. The self-inductance of coils with iron-oxide cores was measured under different temperature conditions of the measuring bridge. The coil temperature was held constant. Five coils were used for the experiment. The self-inductance of each coil was measured for each of four temperatures (22°, 23°, 24°, and 25°) for the measuring bridge. The temperatures were utilized in a random order for each coil. The data are percentage deviations from a standard.

	Coil				
Temperature	*1*	*2*	*3*	*4*	*5*
22	1.400	0.264	0.478	1.010	0.629
23	1.400	0.235	0.467	0.990	0.620
24	1.375	0.212	0.444	0.968	0.495
25	1.370	0.208	0.440	0.967	0.495

Source: H. Hamaker (1955), Experimental design in industry. *Biometrics* 11, 257–286.

a. Write a linear model for this experiment, explain the terms, and compute the analysis of variance.
b. What are the assumptions necessary for the analysis of variance to be valid? How do they relate to the experiment?
c. Compute the orthogonal polynomial regression contrasts for temperature and their sums of squares. Determine the best-fitting equation for the data.
d. Compute the relative efficiency of using coils as blocks.
e. Obtain the residual plots from the analysis, and interpret them.

4. A traffic engineer conducted a study to compare the total unused red light time for five different traffic light signal sequences. The experiment was conducted with a Latin square design in which the two blocking factors were (1) five randomly selected intersections and (2) five time periods. In the data table the five signal sequence treatments are shown in parentheses as A, B, C, D, E, and the numerical values are the unused red light times in minutes.

	Time Period				
Intersection	*1*	*2*	*3*	*4*	*5*
1	15.2(**A**)	33.8(**B**)	13.5(**C**)	27.4(**D**)	29.1(**E**)
2	16.5(**B**)	26.5(**C**)	19.2(**D**)	25.8(**E**)	22.7(**A**)
3	12.1(**C**)	31.4(**D**)	17.0(**E**)	31.5(**A**)	30.2(**B**)
4	10.7(**D**)	34.2(**E**)	19.5(**A**)	27.2(**B**)	21.6(**C**)
5	14.6(**E**)	31.7(**A**)	16.7(**B**)	26.3(**C**)	23.8(**D**)

Source: Mason, Gunst, and Hess (1989), 393.

a. Write a linear model for this experiment, explain the terms, and compute the analysis of variance.
b. Compute the standard error for a signal sequence treatment mean and for the difference between two signal sequence treatment means.
c. Use the Multiple Comparisons with the Best procedure to select the set of signal sequences with the shortest unused red light time.
d. What is the relative efficiency of blocking by time periods?
e. Obtain the residual plots from the analysis, and interpret them.

5. A research engineer studied the time efficiency of four construction methods (A, B, C, D) for an electronic component. Four technicians were selected for the study. The construction process produces fatigue such that the required construction time by the technicians increases as they change from one method to another regardless of the order of construction methods. The engineer used a Latin square design with columns as "technician" and rows as "time period." The construction methods were randomized to the technicians and time periods according to the Latin square arrangement. The values are construction times in minutes required for the component with the construction method indicated in parentheses.

	Technician			
Time Period	*1*	*2*	*3*	*4*
1	90(**C**)	96(**D**)	84(**A**)	88(**B**)
2	90(**B**)	91(**C**)	96(**D**)	88(**A**)
3	89(**A**)	97(**B**)	98(**C**)	98(**D**)
4	104(**D**)	100(**A**)	104(**B**)	106(**C**)

a. Write a linear model for this experiment, explain the terms, and compute the analysis of variance.
b. Compute the standard error for a construction method mean and the difference between two construction method means.
c. Use the Tukey method to make all pairwise comparisons between the means of the construction method times.
d. Use the relative efficiency measure to determine whether the time period was a critical blocking factor to reduce experimental error variance.

6. The experiment in Example 8.2 on the relationship between wheat yield and seeding rate was conducted in a 5 × 5 Latin square design. A second replication of the experiment was conducted in an adjacent field of the experimental farm. Consequently, there are two repetitions of the experiment with unique row and column blockings for each of the two experiments. The data for the second experiment follow. The seeding rates were 30, 80, 130, 180, and 230 for A through E, respectively. The grain yield for each plot is expressed in hundredweight (100 pounds) per acre.

Field Row	*Field Column* 1	2	3	4	5
1	26.88(**A**)	38.40(**D**)	35.33(**E**)	34.56(**C**)	24.57(**B**)
2	37.63(**E**)	24.57(**A**)	36.09(**C**)	23.81(**B**)	32.25(**D**)
3	29.95(**C**)	29.18(**B**)	33.02(**D**)	22.27(**A**)	33.02(**E**)
4	32.25(**D**)	31.49(**C**)	21.50(**B**)	33.02(**E**)	18.43(**A**)
5	26.11(**B**)	36.09(**E**)	23.81(**A**)	29.95(**D**)	29.95(**C**)

Source: Dr. M. Ottman, Department of Plant Sciences, University of Arizona.

a. Write the linear model for the second experiment, explain the terms, and compute the analysis of variance.
b. Compute the standard error estimates for a seeding rate mean and the difference between two seeding rate means.
c. Compute the relative efficiency of row blocking for this experiment. Would more replications be required for a randomized complete block design using only the columns as blocks? If so, how many more would you recommend?
d. Compute the linear and quadratic orthogonal polynomial regression sum of squares partitions for seeding rate, and test their null hypotheses. Are the deviations from a linear or a quadratic relationship significant?
e. Do you think it is reasonable to perform an analysis of variance for the two experiments combined? Explain.
f. Compute the analysis of variance for the two experiments combined.
g. Compute the standard error estimates for a seeding rate mean and the difference between two seeding rates from the combined experiments.
h. Is there an interaction between seeding rate linear or quadratic contrasts and experiments?

7. A horticulturalist conducted a nitrogen fertility experiment for lettuce in a randomized complete block design. Five rates of ammonium nitrate treatments (0, 50, 100, 150, and 250 lb/acre) were randomly assigned to each of two plots in each of two blocks for a total of four plots for each level of nitrogen. Each block consisted of ten plots, two plots for each treatment in each block. The data are the number of lettuce heads from each plot.

Nitrogen	*Block 1*		*Block 2*	
0	104	114	109	124
50	134	130	154	164
100	146	142	152	156
150	147	160	160	163
200	133	146	156	161

Source: Dr. W.D. Pew, Department of Plant Sciences, University of Arizona.

a. Write the linear model for the experiment, explain the terms, and compute the analysis of variance. Note that there are multiple plots for each treatment in each block. How does this affect your estimates of experimental error from the analysis of variance?
b. Test the assumption of no block × treatment interaction.
c. Compute the linear and quadratic polynomial regression contrasts sum of squares partitions for nitrogen, and test the null hypotheses. Interpret the results.
d. Are cubic deviations significant?

8. A field plot experiment was conducted to evaluate the interaction between timing of nitrogen application to the soil (early, optimum, late) and two levels of a nitrification inhibitor (none, .5 lb/acre). The inhibitor delays conversion of ammonium forms of nitrogen into a more mobile nitrate form to reduce leaching losses of fertilizer-derived nitrates. The nitrogen was supplied as pulse-labeled ^{15}N through a drip irrigation system at an early, an optimum, and a late date of application. The data are percent of ^{15}N taken up by sweet corn plants grown on the plots.

	Nitrogen Inhibitor					
	None			*.5 lb/acre*		
Block	*Early*	*Optimum*	*Late*	*Early*	*Optimum*	*Late*
1	21.4	50.8	53.2	54.8	56.9	57.7
2	11.3	42.7	44.8	47.9	46.8	54.0
3	34.9	61.8	57.8	40.1	57.9	62.0

Source: Dr. T. Doerge, Department of Soil and Water Science, University of Arizona.

a. Write the linear model for the experiment, explain the terms, and compute the analysis of variance.
b. Compute the standard error estimates for the marginal means of nitrogen inhibitor and timing of nitrogen application and the cell means.
c. Test the null hypotheses of no interaction effects and no main effects for the two factors.
d. Compute the relative efficiency of the randomized complete block design.
e. Obtain the residual plots from the analysis, and interpret them.

9. Use the data from Exercise 8.1 for the Valencia orange grove irrigation experiment. Assume the plots for trickle irrigation in block 1 (450) and flood irrigation in block 5 (336) were lost from the experiment.
a. Use an appropriate computer program to compute the orthogonal analysis of variance according to the following partitions:

Source of Variation: *SS* Blocks (unadjusted) = 432,384

SS Irrigation Treatments (adjusted for blocks) = 51,923

SS Error = 130,402

b. Test the hypothesis of no treatment differences.
c. Show the least squares estimates of the treatment means and their standard error estimates if the computer program is capable of producing the estimates. For treatment means with no

missing observations, the standard errors should be the same as those computed in the usual manner.

d. How have the standard errors for the trickle and flood irrigation treatment means been affected by the lost plots?
e. If the computer program is capable, compute the standard error of the difference between
   (i) the trickle and flood irrigation means
   (ii) the trickle and basin irrigation means
   (iii) the flood and sprinkler irrigation means
   (iv) the basin and sprinkler irrigation means
f. How were the standard errors in part (e) affected by the lost plots? What effect do the missing plots have on associated tests of differences between pairs of treatment means?

10. An animal scientist conducted a beef animal-feeding trial with four treatments composed of different qualities of drinking water for the animals in a completely randomized design with two replications. The experiment was conducted in the spring months and the winter months on two successive years. Each of the four feeding trials lasted 112 days. The data that follow are the average daily gains for each pen of animals in each of the trials.

	Year 1		*Year 2*	
Treatment	*Spring*	*Winter*	*Spring*	*Winter*
1	1.81	2.14	2.06	2.17
	1.88	2.32	1.91	2.55
2	1.77	2.27	1.57	2.06
	1.60	2.02	1.32	2.20
3	1.85	2.13	1.51	2.25
	1.59	1.93	1.49	1.94
4	1.51	1.85	1.31	1.83
	1.56	1.95	1.20	2.15

Source: Dr. D. Ray, Department of Animal Sciences, University of Arizona.

a. Compute the analysis of variance for each of the four trials as a completely randomized design.
b. Determine whether the experimental error variances are homogeneous among the experiments.
c. Compute the combined analysis of variance for the four trials with year, season, treatment, and all interaction effects in the model. The experimental error is the pooled experimental error from the four analyses of variance from the separate trials.
d. Assume years are random while seasons and treatments are fixed effects. Test the null hypotheses for seasons, treatments, and interaction effects. What are your conclusions?

11. Use the data from Exercise 8.4, the study on traffic signals. Assume the observations at intersection 1 during time period 2 (33.8) and at intersection 4 during time period 5 (21.6) are missing.

a. Use an appropriate computer program to compute the orthogonal analysis of variance according to the following partitions:

Source of Variation
Intersections (unadjusted)
Time periods (adjusted for intersections)
Signal sequence (adjusted for intersections and periods)
Error

b. Test the hypothesis of no signal sequence differences.
c. Show the least squares estimates of the signal sequence means and their standard error estimates if the computer program is capable of producing the estimates.
d. How do the lost data affect the standard errors for the sequence **B** and **C** treatment means?
e. If the computer program is capable, compute the standard error of the difference between
   (i) the sequence **B** and **C** means
   (ii) the sequence **B** and **A** means
   (iii) the sequence **C** and **D** means
   (iv) the sequence **A** and **D** means
f. How were the standard errors in part (e) affected by the lost data? What effect do the missing data have on associated tests of differences between pairs of treatment means?

12. An experiment is to be conducted in a randomized complete block design with $t = 6$ treatments in $r = 4$ blocks.

a. Randomize the six treatments to the experimental units in a randomized complete block design. Show details of your randomization procedure.
b. How many different arrangements of the treatments are possible in each of the blocks?
c. How many different arrangements are possible for the entire experiment?

13. An experiment is to be conducted in a Latin square arrangement with $t = 5$ treatments. Choose one of the standard Latin squares from Appendix 8A, and randomize the five treatments to the experimental units in a Latin square arrangement. Show details of your randomization procedure.

14. Construct a standard square for a 7×7 Latin square arrangement.

a. Randomize the seven treatments to the experimental units in a Latin square arrangement. Show details of your design construction and randomization.
b. How many ways can the Latin square columns be arranged on the column blocks?
c. How many ways can the Latin square rows be arranged on the row blocks?
d. How many ways can the Latin square letters be arranged on the treatment labels?
e. How many arrangements of columns, rows, and treatments are possible for the entire experiment?

15. An experiment is to be conducted in a 4×8 Latin rectangle arrangement with four treatments. Choose two standard Latin squares from Appendix 8A, and randomize the four treatments to the experimental units in the Latin rectangle arrangement. Show details of your randomization procedure.

16. An experiment is to be conducted on accelerated failure tests with small electric motors at five different temperatures. A maximum of five tests can be conducted in one day. There are 20 motors

available for testing. Design an experiment with a randomized assignment of temperatures to the motors. Show a sketch of the final set of tests that are to be conducted.

17. You are designing a study to investigate soil–plant relationships in oak–pine mixed forests. The factor under study is the percent of oak in the forest mix. You have identified three suitable replicate sites for each of the following oak percentages in the forest mix: (1) 0%, (2) 20%–30%, (3) 45%–55%, (4) 70%–80%, and (5) 100%. You must collect soil and forest floor litter samples from each site and conduct laboratory chemical analyses of the samples. The labor is to be divided equally among three people because of the amount of work involved in the field and the laboratory.
 a. Design the study to control the experimental error variation with the 15 chosen sites using yourself and two other people as workers.
 b. Sketch the analysis of variance for data from the study. Include the source of variation and degrees of freedom for each sum of squares partition.
 c. Suppose you take two samples from each site. Repeat part (b).

18. You are conducting an in-vitro feedstuff digestion trial in flasks that must be inoculated with CO_2 and rumen microorganisms obtained from a steer just prior to inoculating the flasks in the lab. Oxygen and temperatures less than 37°C adversely affect the microorganisms. Given the time required to add the CO_2 and microorganisms to the flasks even under the best of controlled conditions the first flasks receive warm healthy microorganisms, but the later flasks receive microorganisms with reduced activity.
 a. Suppose you have five treatments and 25 flasks to inoculate in sequence by yourself. Set up a complete block design for the study to control variation caused by reduced microorganism activity due to exposure.
 b. Sketch the analysis of variance for data from the study. Include the source of variation and degrees of freedom for each sum of squares partition.
 c. Suppose you have 50 flasks available for the five treatments. How could you design the study? Repeat part (b).

19. You are going to conduct a study to determine the contamination of stream water by human activity in a national forest. You have located four streams, each of which has a small permanent community located near the stream. The communities each have a waste disposal plant in the watershed of the stream. Also, each stream has a large recreational camp site located five to ten miles downstream from the community.

You want to take a water sample at each of four locations on each stream: a sample upstream from the community, a sample one mile below the community, and one sample each from immediately above and below the recreational campsite.

You are also required to take a sample on each of four days of the week: Friday, Sunday, Monday, and Wednesday. Your resources are limited such that you can only take 4 water samples from each stream for a total of 16 water samples for the entire study.
 a. Set up a complete block design to acquire the water samples with "stream location" as the treatment factor.
 b. Sketch the analysis of variance for the data showing source of variation and degrees of freedom for each sum of squares partition.
 c. Suppose you take two water samples each time you sample a stream location. Repeat part (b).

8A Appendix: Selected Latin Squares

4 × 4

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

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

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

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

5 × 5

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

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

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

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

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

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

6 × 6

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

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

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

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

7 × 7

A	B	C	D	E	F	G
B	E	A	G	F	D	C
C	F	G	B	D	A	E
D	G	E	F	C	B	A
E	D	B	C	A	G	F
F	C	D	A	G	E	B
G	A	F	E	B	C	D

A	B	C	D	E	F	G
B	E	A	G	F	D	C
C	F	G	B	D	A	E
D	G	E	F	B	C	A
E	D	B	C	A	G	F
F	C	D	A	G	E	B
G	A	F	E	C	B	D

8 × 8

A	B	C	D	E	F	G	H
B	C	D	E	F	G	H	A
C	D	E	F	G	H	A	B
D	E	F	G	H	A	B	C
E	F	G	H	A	B	C	D
F	G	H	A	B	C	D	E
G	H	A	B	C	D	E	F
H	A	B	C	D	E	F	G

A	B	C	D	E	F	G	H
B	C	A	E	F	D	H	G
C	A	D	G	H	E	F	B
D	F	G	C	A	H	B	E
E	H	B	F	G	C	A	D
F	D	H	A	B	G	E	C
G	E	F	H	C	B	D	A
H	G	E	B	D	A	C	F

9 × 9

A	B	C	D	E	F	G	H	I
B	C	D	E	F	G	H	I	A
C	D	E	F	G	H	I	A	B
D	E	F	G	H	I	A	B	C
E	F	G	H	I	A	B	C	D
F	G	H	I	A	B	C	D	E
G	H	I	A	B	C	D	E	F
H	I	A	B	C	D	E	F	G
I	A	B	C	D	E	F	G	H

10 × 10

A	B	C	D	E	F	G	H	I	J
B	C	D	E	F	G	H	I	J	A
C	D	E	F	G	H	I	J	A	B
D	E	F	G	H	I	J	A	B	C
E	F	G	H	I	J	A	B	C	D
F	G	H	I	J	A	B	C	D	E
G	H	I	J	A	B	C	D	E	F
H	I	J	A	B	C	D	E	F	G
I	J	A	B	C	D	E	F	G	H
J	A	B	C	D	E	F	G	H	I