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Latin Square

A latin square is a design in which each treatment is assigned to each time period the same number of times and to each subject the same number of times (see Dean and Voss 1999, chap.

From: International Encyclopedia of the Social & Behavioral Sciences, 2001

Related terms:

Ondansetron, Pharmacokinetics, Cefdinir, Cefpodoxime, G Protein, Methylphenidate, Cefaclor, Thioridazine, Antibiotics, Alcoholism

Design and Statistical Analysis of Mass-Spectrometry-Based Quantitative Proteomics Data

F. Yu, ... J. Meza, in Proteomic Profiling and Analytical Chemistry (Second Edition), 2016

12.3.5.2 Latin Square Design

The Latin square design is a general version of the dye-swapping design for samples from more than two biological conditions. The Latin square design requires that the number of experimental conditions equals the number of different labels. The same number of experimental runs as the number of treatment conditions is also used. The treatment conditions are labeled once using each label and sampled once under each experimental run. Fig. 12.1B shows one way of experiment layout when Latin square design is used for three-label experiments studying the protein expressions under three biological conditions. The advantage of the Latin square design is to control the variation from different labels and different experimental runs. The Latin square also provides better efficiency than the RCBD [5].

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URL: https://www.sciencedirect.com/science/article/pii/B9780444636881000124

Experimental Design: Overview

A.M. Dean, in International Encyclopedia of the Social & Behavioral Sciences, 2001

8.3 Latin Squares

A latin square design is ideal for any experiment in which it is possible to measure each subject under every treatment and, in addition, it is necessary to control for changing conditions over the course of the experiment. A latin square is a design in which each treatment is assigned to each time period the same number of times

and to each subject the same number of times (see Dean and Voss 1999, chap. 12). If there are *t* treatments, *t* time periods, and *mt* subjects then *m* latin squares (each with *t* treatment sequences) would be used.

Carry-over effects are controlled by using latin squares that are 'counterbalanced' (Cotton 1993). This means that, looking at the sequences of treatments assigned to all the subjects taken together, every treatment is preceded by every other treatment for the same number of subjects. Counterbalanced latin squares exist for any even number of treatments and for some odd numbers of treatments (e.g., *t*=9, 15, 21, 27; see Jones and Kenward 1989 Sect. 5.2.2, for references). For other odd numbers, a pair of latin squares can be used which between them give a set of 2*t* counterbalanced sequences.

If a carry-over effect is expected to persist for more than one time period, then the counterbalancing must be extended to treatements occurring more than one time period prior to the current treatment.

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Periods, Sequences, and Trial Design

Richard Chin, Bruce Y. Lee, in Principles and Practice of Clinical Trial Medicine, 2008

6.3.3 Latin Square

A Latin square design is a variation of a crossover study design. In a Latin square, each patient receives each intervention once. So, if there are *n* types of interventions or treatments (including placebo), the study will last *n* periods. Figure 6.5 shows a schematic representation of a three-period Latin square design. The rows represent different groups. The columns are different periods. Each group undergoes a sequence of three treatments over three periods (e.g., Group 1 receives Intervention A in Period 1, Intervention B in Period 2, and Intervention C in Period 3). As you can see, each group receives a different treatment each period. Patients in Group 2 start with Intervention B, and patients in Group 3 start with Intervention C. It is called a square because schematically the number of rows (i.e., groups) equals the number of columns (i.e., periods).

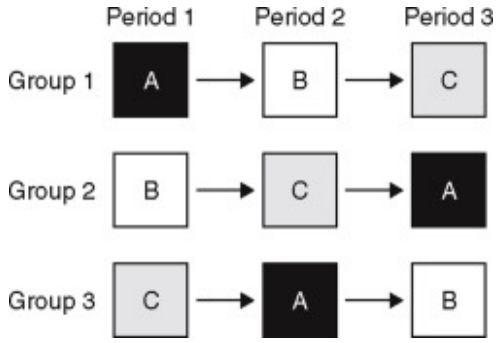

FIGURE 6.5. Latin square design.

In the example in Figure 6.6, the treatment sequence is fixed. When Intervention A occurs in one period, B always occurs the next period, C the period after, and A the period after, and so forth. In other words, the sequence is never ACB, BAC, or CBA. This is an example of *circular permutation*, that is, the order of the treatments is

always the same (A→B→C). In general, a circular permutation will magnify any potential carryover effects and, as a result, may introduce a systematic bias. If Intervention A has a carryover effect on Intervention B, every group will suffer since Intervention B always follows Intervention A. Noncircular permutations, that is, shuffling the order in which interventions follow (Figure 6.7), may help elucidate the presence and magnitude of this problem.

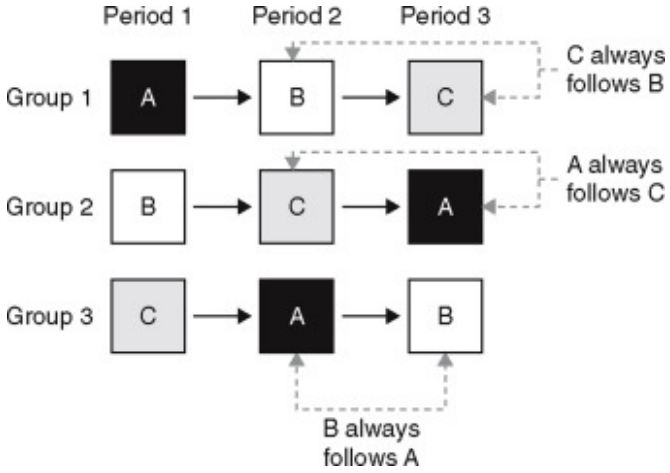

FIGURE 6.6. Circular permutation.

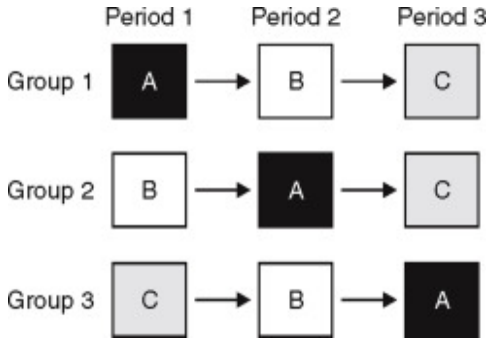

FIGURE 6.7. Latin square with non-circular permutation.

For a study with even number of treatments, you can easily modify the sequence to ensure that each treatment is followed by each other treatment (Figure 6.8a, b). This allows you to estimate the presence and magnitude of any carryover effect. As you can see, in Group 1 Intervention C follows B, Group 2 D follows B, and Group 4 A follows B. Such a design helps you compare the effects of B on A, C, and D.

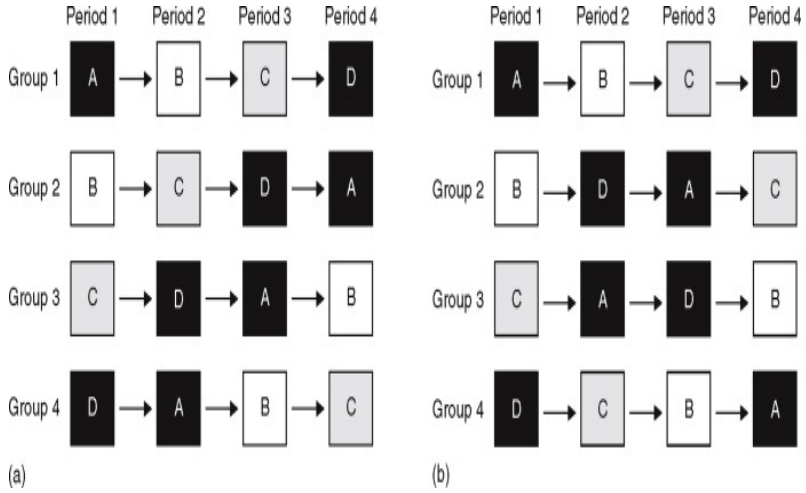

FIGURE 6.8. Study with an even number of treatments: (a) Circular permutation does not allow all treatment sequences (e.g., Treatment B never follows Treatment C) (b) Modified design ensures that each treatment is followed by each other treatment (e.g., Treatment B now follows Treatment C.)

Adequate sequence modifications are not possible for studies with odd numbers of groups. Instead, there are several alternatives:

- *Use two or more complementary sequences (preferred)*: The two sets in Figure 6.9 are complementary. A goes to C in the first set, and A goes to B in the second set.

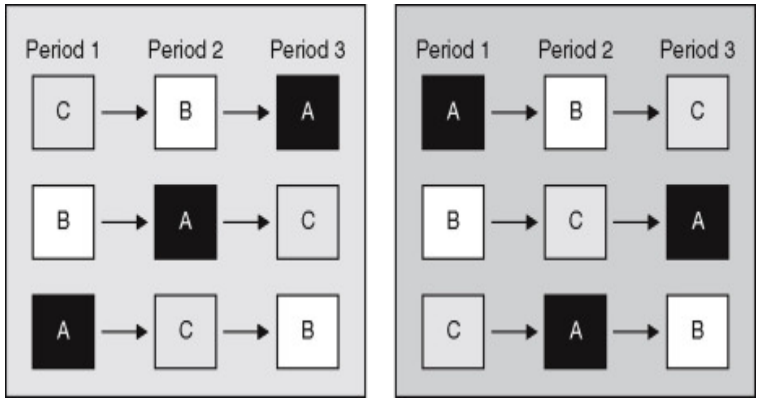

FIGURE 6.9. Complementary sequences.

- *Add an extra group to make it an even number of groups*: As Figure 6.10 demonstrates, adding an extra group allows you to have A follow B (last row).

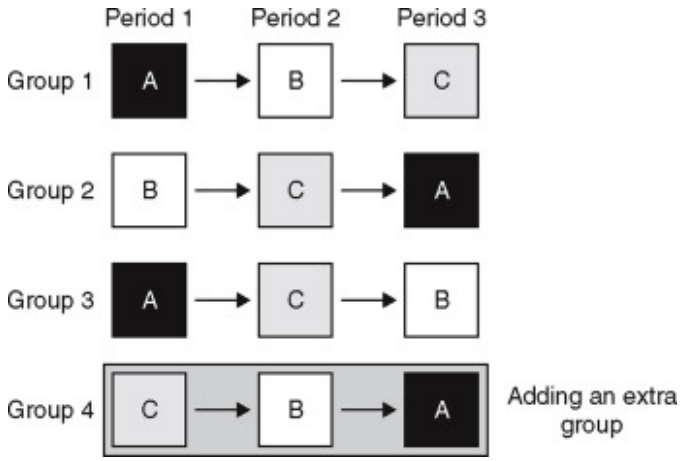

FIGURE 6.10. Adding an extra group.

- *Add an extra period*: In a true Latin square, each group occurs only once in each row or column. So, as Figure 6.11 shows, this is no longer a true Latin square (i.e., the BCA column appears twice).

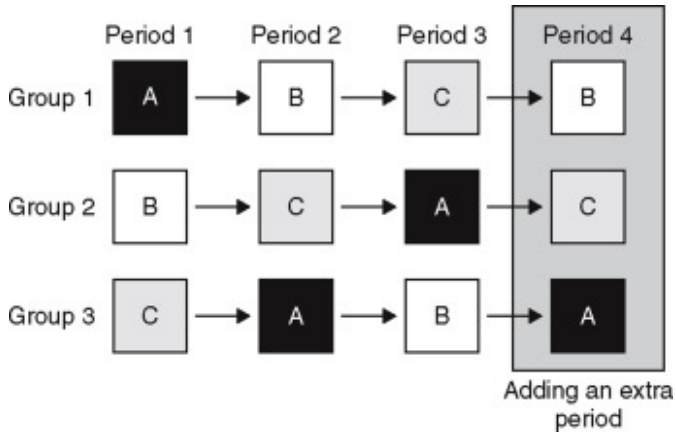

FIGURE 6.11. Adding an extra period.

If desired, you may repeat a treatment (e.g., in Figure 6.12, the CAB column appears twice in subsequent periods) to determine if two sequential treatment periods have a clinical effect.

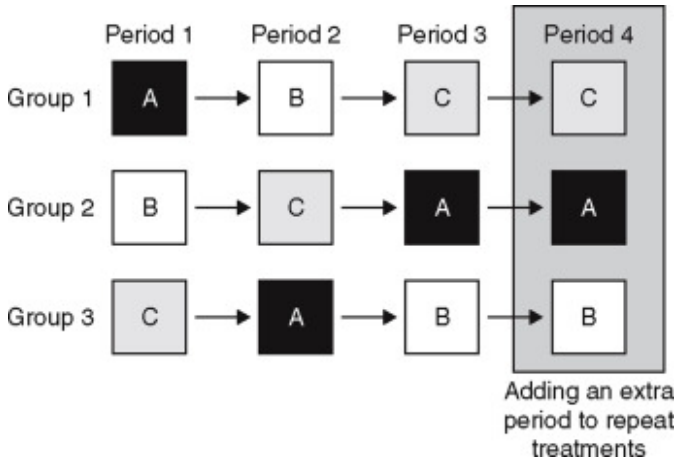

FIGURE 6.12. Adding an extra period to repeat treatment.

If the full Latin square design is not feasible because multiple periods are not practical, you may use incomplete Latin square designs (i.e., the number of rows does not equal the number of columns) (Figures 6.13a-c). Conversely, if the availability of the patients is an issue (e.g., with orphan indications), intensive design may be used.

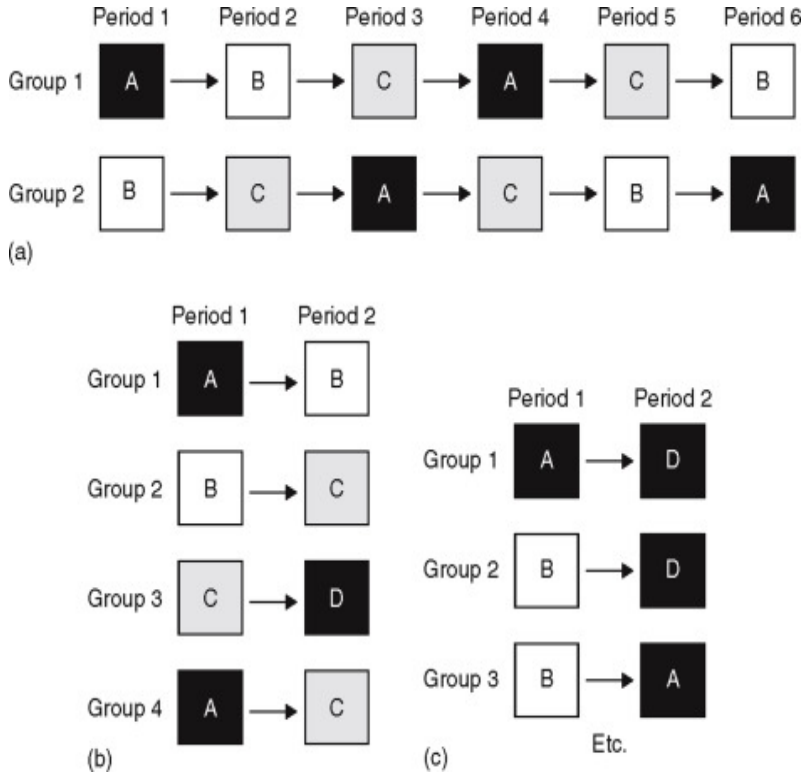

FIGURE 6.13. Incomplete Latin squares.

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Analysis of Variance II. More Complex Forms

Julien I.E. Hoffman, in Biostatistics for Medical and Biomedical Practitioners, 2015

Multiple Factors

More than two factors can be analyzed by using Latin squares. The name (based on the name of an early mathematical puzzle) was introduced into statistical analysis for use in agricultural experiments. To allow for possible underlying differences in soil fertility, the plot of land is divided into smaller blocks to which seeds and treatments (fertilizers) are allocated at random, but in such a way that each treatment occurs once in each row and each column, and each seed occurs once in each row and each column. This principle can be applied to biological experiments as well. In an experiment with testicular spreading factor (now known to be hyaluronidase), Bacharach et al. (1940) injected it subcutaneously in six different sites on each of six rabbits. This example appears in the book by Finney (Finney, 1955). The study was done so that each site and each rabbit had the injection in a different order. As a result, each site (symbolized by A to F) occurred once with each rabbit and once with each order of injection, each rabbit occurred once with each order and each site; and each order occurred once with each rabbit and each site.

(Individual data are in the original publication.) Then a three-factor ANOVA was performed (Table 26.7).

Table 26.7. Three-factor ANOVA

Source of variation	SS	DF	MS	F	P
Total	30.36	35			
Between animals	12.83	5	2.566	3.91	0.0123
Between order	0.56	5	0.112	0.17	0.9707
Between sites	3.83	5	0.766	1.17	0.3582
Within (residual)	13.14	20	0.657		

There are three factors that contribute to the total sums of squares: order, animal, and site. Adding up their contributions and subtracting that sum from the total produces a residual error independent of site, order, or animal. The contribution of each factor is compared with the residual mean square to provide an F ratio. In Table 26.7, there are significant differences among animals, with rabbit number 2 having the largest bleb and thus the slowest fluid absorption. Neither site nor order had any major effect, so that in planning future experiments site and order can be ignored, but precautions are needed to allow for interanimal variability.

It is possible to do an ANOVA with four factors, by allocating the fourth factor (symbolized by Greek letters) once to each of the other three factors. This is known as a Graeco-Latin square, and is analyzed in similar fashion. Latin squares must have the same number of cells for each factor. The requirements for ratio numbers, normality, and homogeneity of variance apply equally to these more complex ANOVA.

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Acute Efficacy of 20 Psychotropic Drugs on Human EEG Power Spectrum Variables Shown by Multivariate and Univariate Statistics

W.M. HERRMANN, ... St. KUBICKI, in Neuro-Psychopharmacology, 1979

Parametrisation of EEG Time Series

The treatment-unit of each subject and study day in the Latin square is called a subject-treatment-unit. The total number of subject-treatment-units is 375.

Due to missing data, a total of 364 subject-treatment-units were available. Each unit is represented by three (pre, 1 hr. and 3 hr. post) 5 min. EEG-recordings under RR conditions. In the following symbolic vector (x) representing a subject-treatment-unit

$$\underline{x} = \begin{matrix} \mathbf{EEG - pre} \\ \mathbf{EEG - 1\ hr.\ post} \\ \mathbf{EEG - 3\ hr.\ post} \end{matrix}$$

each component is a vector itself, the five-minute EEG-recording being represented by 7 parameters. Therefore each subject-treatment-unit is represented by a 3 × 7 component vector. While epochs with artefacts were excluded, 30 epochs of 10 sec. were included. Each epoch is represented by 1000 values, which give the time-serie in $\Delta = \frac{1}{100}\ \mathbf{sec.}$ intervals. The spectrum is estimated by Fourier-transformation of the modified autocovariance function using hamming-window with lags of up to 1 sec. (= 1/10 of the epoch).

For each power spectrum the following variables were calculated:

relative power in

δF	=	1.5 – 5.5 Hz	α_1F	=	8.5 – 10.5 Hz	β_1F	=	12.0 – 18.0 Hz
ϑF	=	5.5 – 8.5 Hz	α_2F	=	10.5 – 12.0 Hz	β_3F	=	21.0 – 30.0 Hz

and the total power (1.5 – 30.0 Hz).

Selection of variables was based on factor analysis by Herrmann/Fichte/Kubicki (1978).

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Logistics

Douglas Wahlsten, in Mouse Behavioral Testing, 2011

Latin square

When six objects are to be arranged in different orders and the objects cannot communicate with each other or exert an influence on those adjacent in the order, the Latin square can be used (Table 7.1). Objects can be shifted to the right on each step or to the left on each step, but left and right should not be mixed in the same balancing operation. In the square for six objects, objects are shifted to the right by one position for each order. For six objects, there are six orders, and each object occurs only once in any one place in the sequence. For the example with six sex–treatment combinations, the squad numbers can be shifted to the left by one step, as shown in the spreadsheet (**4 × 2 × 3 Balanced**, **Sheet 2**). This yields three unique sequences. Then append these three end to end in three different sequences and assign them to the first replication. The fourth strain must get a duplicate sequence, perhaps 123123, the same as strain 129. This method yields two duplicate sex–treatment combinations in every squad, a 129-DBA duplication in each case. Now, across replications, assign the sequences so that different pairs of strains are duplicated in a replication. In the spreadsheet the duplicated strains are highlighted in yellow. The nine replications have five duplications for two strains and four for the other two. It cannot be perfectly balanced when there are four strains and nine replications.

Table 7.1. Latin Square to Determine Order of Six Tests

	1st	2nd	3rd	4th	5th	6th
Order 1	A	B	C	D	E	F
Order 2	B	C	D	E	F	A
Order 3	C	D	E	F	A	B
Order 4	D	E	F	A	B	C
Order 5	E	F	A	B	C	D
Order 6	F	A	B	C	D	E

There are probably many other ways to balance the sequences. An expert in combinatorics or someone who enjoys working with puzzles may devise a more elegant solution. A criterion for an acceptable level of balancing can be proposed: if there are no biases in the end result and no human could possibly determine how one made the sequences just by looking at the end product, it is good enough for use with lab animals. It is not necessary to adopt extreme measures to achieve perfection.

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Facets of Dyslexia and its Remediation

M. Brysbaert, C. Meyers, in Studies in Visual Information Processing, 1993

Stimuli

The stimuli consisted of 700 five-letter words randomly divided over seven groups. Care was taken to incorporate words with which young children were likely to be familiar. Subjects were distributed over seven latin-square groups with each group seeing the words at a different fixation location (see below). All subjects processed the whole sample of stimuli, but in a different order (for the randomization procedure used, see Brysbaert, 1991a). Stimulus presentation was controlled by an IBM XT microcomputer and displayed on a Philips monochrome CRT screen.

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URL: https://www.sciencedirect.com/science/article/pii/B9780444899491500146

A worldwide yearly survey of new data in adverse drug reactions

Dominik Schrey, ... Andreas H. Groll, in Side Effects of Drugs Annual, 2011

Halofantrine

The effect of fluconazole 50 mg on the pharmacokinetics of a single 500-mg oral dose of halofantrine, which is mainly metabolized by CYP3A4 to the active metabolite N-desbutylhalofantrine, has been evaluated in 15 healthy volunteers in a Latin square crossover design [36C]. Co-administration of fluconazole did not alter the pharmacokinetics of halofantrine, but significantly altered the pharmacokinetics of its active metabolite, reducing C_{max}, AUC, and the ratio of N-desbutylhalofantrine to halofantrine by 35–41% and increasing the t_{max} by 50%. Although the therapeutic consequences of this interaction are not clear caution should be taken during co-administration to avoid accumulation and subsequent cardiotoxic effects of halofantrine, particularly if higher doses of fluconazole are used.

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URL: https://www.sciencedirect.com/science/article/pii/B9780444537416000271

Experimental Design

Derek Fry, in Laboratory Animal Welfare, 2014

Latin Square Designs

If in the weight gain experiment with diets A, B, C, and D, the lighting was at the side of the room and there was concern that, as well as a possible effect of temperature at the different levels, there were differences in light intensity across the room that could influence the results, then a design blocking in two directions could be used. Each treatment would occur at each level and in each column of four cages. This is a "Latin square" design. A suitable arrangement could be that as shown in Figure 8.5.

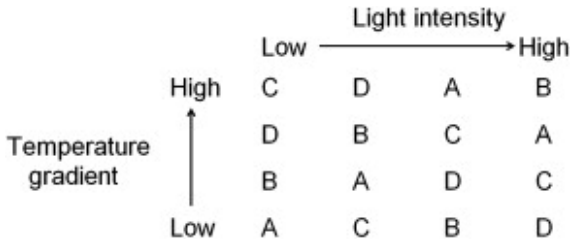

FIGURE 8.5. An arrangement for a Latin square design for cage positioning where temperature and light intensity are factors that might have an important influence on the findings. Treatments A, B, C, and D are arranged so there is one of each in every row (representing in this cage height in the rack) and every column (representing light intensity).

A Latin square design could also be used when the experimental unit is an individual or cage for a period of time. Say the objective was to test potential short-acting diuretics. Four rats could be housed in metabolism cages and two test substances, a vehicle control and a known diuretic administered to each in sequence, with urine collected for 24 h after each administration and a gap in between each administration and collection period. The sequence of the four treatments (control, known, test X, and test Y) could be as A, B, C, and D in the Latin square shown, with the columns the different time periods and the rows the different animals. In this particular (modified Latin square) arrangement, no treatment follows another more than once, so it would be very suitable for this type of study as any unexpected carry-over effects between a pair of treatments would

not be repeated. A Latin square like this, with only four animals and time periods, should normally be replicated to increase power.

Latin square cross-over designs can be very efficient in use of animals, and show the effects of treatment and two other factors, but the levels of each factor must be the same, so it is not suitable if an animal is likely to have to be taken off study because of adverse effects. As with other cross-over designs, there should be no carry-over effects expected. So treatments need to be reversible, and “wash-out” periods between treatment periods need to be long enough for the animals to return to their preexperiment condition. In the previous cross-over example, the animal's response to treatment should not change with time. This might be a consideration in the example given if female rats and longer time periods were used and the effect changed during the estrus cycle.

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Experiment, Observation, and Modeling in the Lab and Field

K. Yasukawa, in Encyclopedia of Animal Behavior, 2010

Experimental Designs

The design of treatments is basic but crucial because it defines our hypothesis tests. Treatments can be broadly divided into unstructured (random differences) and structured (fixed differences) designs of which there are many. The design of layout, or how we assign treatments to experimental subjects, is a complementary consideration to treatment design. Five commonly used designs in studies of behavior are completely randomized one factor, randomized block, nested, Latin square, and completely randomized two factor (factorial) designs. Analysis of variance (ANOVA) can be used to analyze data from these designs.

In contrast to the previous designs, which use specific levels of each factor (e.g., low-, medium-, and high-hormone treatments), in gradients we attempt to assess behavioral response to a continuous range of treatments (e.g., hormone concentration). Analysis of gradients uses statistical testing such as correlation or regression because both the measurement variable and the treatment variable are numeric.

When two (or more) treatment variables are categorical and our measurement variable is a count (number of occurrences), enumeration methods such as goodness-of-fit tests and tests of independence are appropriate.

Once the experimental methods are set, it is time to do the experiment and analyze the results with the proper statistical test. It is also important to remember once again that your purpose remains testing hypotheses to answer the four principal questions of animal behavior.

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