14.6: Some other ANOVA designs

Introduction

There are several additional ANOVA models in common use. The crossed, balanced design is but one example of the two-way ANOVA. And, from a consideration of two factors, it logically follows that there can be more than two factors as part of the design of an experiment. As the number of factors increase, the number of two-way, three-way, and even higher-order interactions are possible and at least in principle may be estimated.

Our purpose here is to highlight several, but certainly not all, possible experimental designs from the perspective of ANOVA. Examples are provided. Keep in mind that the **general linear model** approach unifies these designs.

Some of the classical experimental ANOVA designs one sees include:

- Two-way randomized complete block design
- Two-way factorial with no replication design
- Repeat-measures ANOVA with one factor
- Nested ANOVA
- Three-way ANOVA
- Split-plot ANOVA
- Latin squares ANOVA

Put simply, these designs differ in how the groups are arranged and how members of the groups are included.

Two-way randomized complete block design

This design refers to the “textbook” design. For each, factor A and factor B, there are multiple levels, in this example three levels of Factor A and three levels of Factor B, and subjects (sampling units) are randomly assigned to each level. However, one of the factors is, perhaps, of less interest, yet certainly accounts for variation in the response variable.

		Factor A		
		1	2	3
Factor B	1	$n_{1,1}$	$n_{1,2}$	$n_{1,3}$
	2	$n_{2,1}$	$n_{2,2}$	$n_{2,3}$
	3	$n_{3,1}$	$n_{3,2}$	$n_{3,3}$

where $n_{1,1}$, $n_{2,1}$, etc. represents the number of subjects in each cell. Thus, in this design there are nine groups. Typically, minimum replication would be three subjects per group.

Two-way factorial with no replication design

While it may seem obvious that a good experiment should have replication, there are situations in which replication is impossible. While this seems rather odd, this scenario very much describes a typical microarray, gene expression project.

		Factor A		
		1	2	3
Factor B	1	$n_{1,1}$	$n_{1,2}$	$n_{1,3}$
	2	$n_{2,1}$	$n_{2,2}$	$n_{2,3}$
	3	$n_{3,1}$	$n_{3,2}$	$n_{3,3}$

where, again, $n_{1,1}$, $n_{2,1}$, etc., represents the number of subjects in each cell and there are nine groups in this study. With no replication, then there is no more than one subject per group.

Repeated-measures ANOVA

When subjects in the study are measured multiple times for the dependent variable, this is called a repeated-measures design. We introduced the design for the simple case of before and after measures on the same individuals in Chapter 12.3. It's straight-forward to extend the design concept to more than two measures on the subjects. The **blocking effect** is the individual (see Chapter 14.4), and, therefore, a random effect (see Chapters 12.3 and 14.3) in this type of experimental design.

Although straightforward in concept, repeated measure designs have many complications in practice. For example, long-term studies can expect for subjects to drop out of the study, resulting in **censored data**. Another complication affects the assumption is that there is no **carry over effect** — it doesn't matter the order different treatments are applied to the subjects. Think of this assumption as akin to the **equal variances assumption** in ANOVA; just like unequal variances effects Type I error rates in ANOVA, deviations from **sphericity** inflate Type I error rates in repeated-measures designs.

Sphericity assumption is described in two ways:

> **Assumption of sphericity** — the ranking of individuals remains the same across treatment levels — no interaction between individual and treatment. Sphericity assumption is always met if there are just two levels of the repeated measure, e.g., before and after.
>
> **Compound symmetry assumption** — the variances and covariances are equal across the study: the changes experienced by the subjects are the same across the study regardless of the order of treatments.

Tests for sphericity include:

Mauchly test: `mauchly.test(object)`

If results of tests for violations of sphericity warrant, corrections are available. One recommended correction is called **Greenhouse-Giesser correction**, which adjusts the degrees of freedom and so results in a better p-value estimate. A second correction is called **Huyhn-Feldt correction**; this correction, too, adjusts the degrees of freedom to improve the p-value estimate.

Three-way ANOVA

It is relatively straightforward to imagine an experiment that involves three or more factors. The analysis and interpretation of such designs, while feasible, becomes somewhat complicated, especially for the mixed models (Model III).

Consider just the case of a fixed-effects 3-way ANOVA. How many tests of null hypotheses are there?

1. Three tests for main effects.
2. Three tests of two-way interactions.
3. A test for a three-way interaction.

Thus, there are seven separate null hypotheses from a three-way ANOVA with fixed effects! As you can imagine, large sample sizes are needed for such designs, and the "higher-order" interactions (e.g., three-way interaction) can be difficult to interpret and may lack biological significance.

ANOVA designs without random assignment to treatment levels

Latin square design

We have introduced you to several ANOVA experimental designs that employed randomization for assignment of subjects to treatment groups. The purpose of randomization is even out differences due to confounding variables. However, if we know in advance something about the direction of the influence of these confounding variables, strictly random assignment is not in fact the best design. For example, the **Latin square** design is common in agriculture research and is very useful for situations in which two gradients are present (e.g., soil moisture levels, soil nutrient levels).

	Dry soil ← → Wet soil			
Soil Nutrients	T1	T4	T3	T2
	T3	T2	T1	T4
low ↑ ↓	T2	T3	T4	T1

high	T4	T1	T2	T3

Split-Plot Design

Another design from agriculture research is especially useful to ecotoxicology research. We mentioned the repeated measures design in which individuals are measured more than once and each individual receives all levels of the treatment in a random order (**cross-over design**). However, this design assumes that there are no **carry-over effects** (see Hills and Armitage 1979; For ecology/evolution definition see O'Connor et al 2014). While this assumption may hold for many experiments, we can also imagine many more situations in which this is undoubtedly false. For example, if we wish to measure the effects of ozone and relative humidity on frog behavior, we might consider using the individual as its own control. But we also wish to compare frog behavior following ozone exposure against behavior exhibited in clean air. But we are likely to violate the carry-over assumption. If a frog receives ozone then air, the effects of ozone may inhibit activity for several days after the initial exposure, which would then influence subsequent measures. The solution to this dilemma is to use what's called a **split plot** design. The design combines elements of nesting.

Consider our frog experiment. There would be three factors:

Factor 1 = Exposure (air or ozone),

Factor 2 = Saturation (dry, intermediate, wet),

Factor 3 = Individual (each frog is measured 3 times).

The design table would look like

		Exposure					
		Air			**Ozone**		
Humidity	**Dry**	Frog1	Frog2	Frog3	Frog4	Frog5	Frog6
	Intermediate	Frog1	Frog2	Frog3	Frog4	Frog5	Frog6
	Wet	Frog1	Frog2	Frog3	Frog4	Frog5	Frog6

Thus, the design is crossed for one factor (saturation), but nested for another factor (individuals are nested within Exposure factor).

Questions

1. Which of the study designs mentioned so far are sensitive to carry-over effects?
2. With respect to how levels of Factors are assigned, distinguish the split-plot design from the Latin square design.