Other forms of ANOVA

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1 - Introduction: different forms of analysis of variance

1. *One-way* or *single classification* ANOVA (previous lecture)

Equal or unequal numbers of observations in the groups.

Non-parametric analysis:
Kruskal-Wallis test.

Factor

1	2	3	4	5
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•
•	•	•	•	•

2. *Nested* or *hierarchic* ANOVA

Factor 1

	1		2		3	
Fact. 2	1.1	1.2	2.1	2.2	3.1	3.2
	•	•	•	•	•	•
	•	•	•	•	•	•
	•	•	•	•	•	•
	•	•	•	•	•	•
	•	•	•	•	•	•

3. *Two-way* ANOVA

Factor 1

		1	2	3	4
Factor 2	1	• • • • •	• • • • •	• • • • •	• • • • •
	2	• • • • •	• • • • •	• • • • •	• • • • •

4. More than two factors: *multiway* ANOVA.

5. More than one response variable: *multivariate* ANOVA, MANOVA.
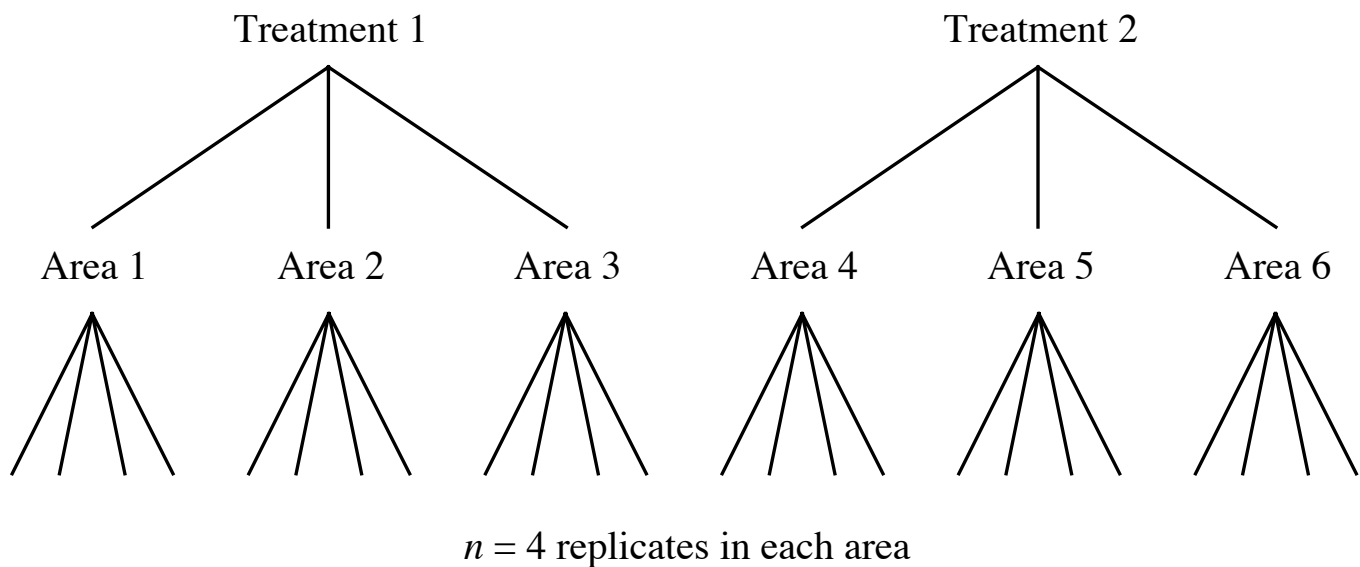
2 - Nested ANOVA

Motivation example 1 — Researchers are studying the effect of 7 growth media on the wing length of the house fly *Musca domestica*. One may be tempted to use a single batch of each culture medium, and raise fly larvae from the eggs in a jar; the individual flies would be the replicates in such a study. But what if variation (mistakes in ingredient dosage, contamination, etc.) occurs in the preparation of the growth media? This could bias the results of the study. In order to account for that type of variation, the researchers separately prepare 5 different batches of each culture medium. The 'batches' factor is not *crossed* with the 'culture media' factor since each batch is prepared according to the recipe of the type of culture medium to which it corresponds. The 'batches' factor is said to be *nested* within the 'culture media' factor. The analysis will involve a main fixed factor 'culture media' (the researchers are interested in the effect of that factor on wing length), a random factor 'batches' for the different types of media, and replicate flies within the batches. One could add another nested level to the experiment by dividing each batch of culture medium into several jars in which fly larvae are grown.

Motivation example 2 — A botanist is studying the sources of morphological variation in an alpine plant, the spring gentian *Gentiana verna*. He plans to collect the plant in several mountain chains. In each one, several localities will be visited. And in each locality, several sites will be visited, in which several plants will be collected and measured. With that survey design, he will be able to study the variation (1) among mountain chains, (2) among localities within the mountain chains, and (3) among the sites within each locality. This is thus a sampling design with three nested levels, all of which are random.

Nested analysis of variance is used when the classes of an inner factor are unrelated among the classes of the outer factor. For instance, terrestrial or subaquatic transects are unrelated to each other among sites, even though they may be called transects 1, 2, 3 ... within each site. Nested factors are always random factors. The main factor may be either fixed or random. This form of anova is discussed in all textbooks of statistics.

Consider the following experiment with a main factor A "Treatment" (fixed) with $a = 2$ levels, a nested factor B "Area" (random) with $b = 3$ levels within each level of A, and $n = 4$ replicates per area in each treatment:

Treatment 1   Treatment 2

Area 1   Area 2   Area 3   Area 4   Area 5   Area 6

$n = 4$ replicates in each area

The nested ANOVA statistical model to explain the variation in a response variable **y** is the following for this example:

$$y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk}$$

where $i = \{1,...,a\}$ treatments, $j = \{1,...,b\}$ areas within each treatment, and $k = \{1,...,n\}$ replicate observations within each area i,j.

Contrary to crossed 2-way or multiway fixed-factor ANOVA, in nested ANOVA the F-statistics do not all use the residual mean square as their denominator. The rules for construction of the F-statistics in a balanced 3-way nested anova are described in the following table, where a is the number of levels (groups) in factor A, b the number of levels in factor B, c the number of levels in factor C, and n the number of replicates in each level of factor C.

Sources of variation	Degrees of freedom (df)	Sums of Squares	Mean squares	F-statistics
Main factor (A)	$a - 1$	SS_A	$MS_A = SS_A/(a-1)$	MS_A / MS_B
1st nested factor (B)	$a(b - 1)$	SS_B	$MS_B = SS_B/a(b-1)$	MS_B / MS_C
2nd nested factor (C)	$ab(c - 1)$	SS_C	$MS_C = SS_C/ab(c-1)$	MS_C/ MS_{Res}
Within subgroups	$abc(n - 1)$	SS_{Res}	$MS_{Res} = SS_{Res}/abc(n-1)$	
Total variation	$abcn - 1$	SS_{Total}	$s_y^2 = SS_{Total}/(abcn - 1)$	

Because they use fewer degrees of freedom in the tests of the factors, crossed anova designs have more power to detect small effects than nested designs. So, if they have a choice, users should plan their experiments in such a way that they can be analyzed by crossed instead of nested anova. Many experiments require, however, the use of nested factors.

Example 3 — Nested anova with one main factor and one nested factor. The following example is presented by Sokal and Rohlf (1995, Box

10.1)[*] to illustrate the calculations involved in nested ANOVA. Two independent measurements (replication level) of the length of the left wing have been made on <mark>4 female mosquitoes *Aedes intrudens* (nested factor)</mark> reared in each of <mark>3 cages (main factor)</mark>. The total number of observations is thus 3 x 4 x 2 = 24. The data are the following:

Wing length	Cage	Female #
58.5	1	1
59.5	1	1
77.8	1	2
80.9	1	2
84.0	1	3
83.6	1	3
70.1	1	4
68.3	1	4
69.8	2	5
69.8	2	5
56.0	2	6
54.5	2	6
50.7	2	7
49.3	2	7
63.8	2	8
65.8	2	8
56.6	3	9
57.5	3	9
77.8	3	10

[*] Sokal, R. R. and F. J. Rohlf. 1995. Biometry — The principles and practice of statistics in biological research. 3rd edition. W. H. Freeman, New York.

Wing length	Cage	Female #
79.2	3	10
69.9	3	11
69.2	3	11
62.1	3	12
64.5	3	12

The analysis of variance table is:

	df	Sums of squares	Mean squares	*F* value	P-values
Cages	2	665.67583	332.83792	1.74091	0.2295
Females:cages	9	1720.67750	191.18639	146.87815	< 0.0001
Residuals (within females)	12	15.62000	1.30167		
Total	23	2401.97333	$s_y^2 = 104.4336$		

Conclusion: The variation among females is very highly significant, but the cage factor did not have a significant effect on the response variable.

R language: The function *nest.anova.perm.R* (Borcard and Legendre) computes nested ANOVA with parametric and permutation tests.

3 - Two-way factorial ANOVA with replication: Model I

Crossed or *factorial* experimental designs are used when there are more than one factor of interest and the levels of each factor are the same in the levels of the other factor(s).

Motivation example 1 — Researchers are interested in studying the effect of different types of culture media (fixed factor A) on the growth

of individuals (replicates) pertaining to three species of *Drosophila* (fixed factor B) and at two temperatures (fixed factor C: low, high).

This is a *crossed* or *factorial* design because, for each level of factor A, the experiment is conducted for all levels of factor B (the 3 species) and all levels of factor C (the 2 temperatures). Compare with the examples for nested ANOVA in section 2. Factorial designs offer the great advantage of allowing us to test the interaction (described below), in addition to the tests of significance of the main factors.

The data resulting from a two-way factorial design can be represented in a two-way table, as shown in section 1.

In this section, we will only consider two-way factorial designs with fixed factors (Model I ANOVA), with *replication* and *balanced designs* (i.e. equal number of replicates in each subgroup). Random factors (Model II ANOVA) and mixed models (Model III ANOVA) will be studied in later sections.

The statistical model to explain the variation in a response variable **y** is the following in two-way Model I ANOVA with replication:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the value of the response variable for the k-th replicate in the i-th level of factor A and the j-th level of factor B, μ is the overall mean, α_i is the effect of the i-th level of A, β_j is the effect of the j-th level of B, $\alpha\beta_{ij}$ is the effect of the interaction between α_i and β_j, and ε_{ijk} is the error term. There are $i = \{1,...,a\}$ levels in factor A, $j = \{1,...,b\}$ levels in factor B, and, in a *balanced design*, $k = \{1,...,n\}$ replicate observations in each combination i,j.

The following ANOVA table shows the construction of the F-statistics:

Sources of variation	Degrees of freedom (*df*)	Sums of Squares	Mean squares	*F*-statistics
Fixed factor A	$a - 1$	SS_A	$MS_A = SS_A/(a-1)$	MS_A / MS_{Res}
Fixed factor B	$b - 1$	SS_B	$MS_B = SS_B/(b-1)$	MS_B / MS_{Res}
Interaction (A x B)	$(a - 1)(b - 1)$	SS_{Int}	$MS_{Int} = SS_{Int}/(a - 1)(b - 1)$	MS_{Int}/ MS_{Res}
Residuals	$ab(n - 1)$	SS_{Res}	$MS_{Res} = SS_{Res}/ab(n-1)$	
Total variation	$abn - 1$	SS_{Total}	s_y^2	

The degrees of freedom are shown for the balanced case, where there are exactly *n* replicates in each subgroup *i,j*. Details for the calculation of the sums-of-squares are shown in textbooks. The table shows that in Model I ANOVA, the denominator of all *F*-statistics is the residual mean square.

For testing, each computed F-statistic is compared with $F_{(\alpha,\ df1,\ df2)}$ from the F-distribution for significance level α (e.g. 0.05), with degrees of freedom $df1$ from the numerator MS and $df2$ from the denominator MS. The F-statistic can also be tested by permutation.

Why is it that $\mathrm{MS_{Res}}$ is used as the denominator of all F-statistics? It is because the expected mean square (EMS) of each term in the ANOVA table, under H_0, is the error variance (σ_ε^2) plus the effect to be tested:

Sources of variation	Degrees of freedom (df)	Expected mean squares (EMS)
Fixed factor A	$a-1$	$\sigma_\varepsilon^2 + bn\sum_i \alpha_i^2/(a-1)$
Fixed factor B	$b-1$	$\sigma_\varepsilon^2 + an\sum_j \beta_j^2/(b-1)$
Interaction (A x B)	$(a-1)(b-1)$	$\sigma_\varepsilon^2 + n\sum_i \sum_j \alpha\beta_{ij}^2/((a-1)(b-1))$
Residuals	$ab(n-1)$	σ_ε^2

Consider the EMS for factor A: $\mathrm{EMS_A} = \sigma_\varepsilon^2 + bn\sum_i \alpha_i^2/(a-1)$. Under H_0, the portion $bn\sum_i \alpha_i^2/(a-1)$ of this term is zero and $\mathrm{EMS_A} \approx \sigma_\varepsilon^2$.

So, if H_0 is true, the statistic $F = \dfrac{\mathrm{MS_A}}{\mathrm{MS_{Res}}}$ should be ≈ 1 and distributed like F. Likewise for the F-statistics for B and the interaction: the appropriate denominator mean square in each case is $\mathrm{MS_{Res}}$.
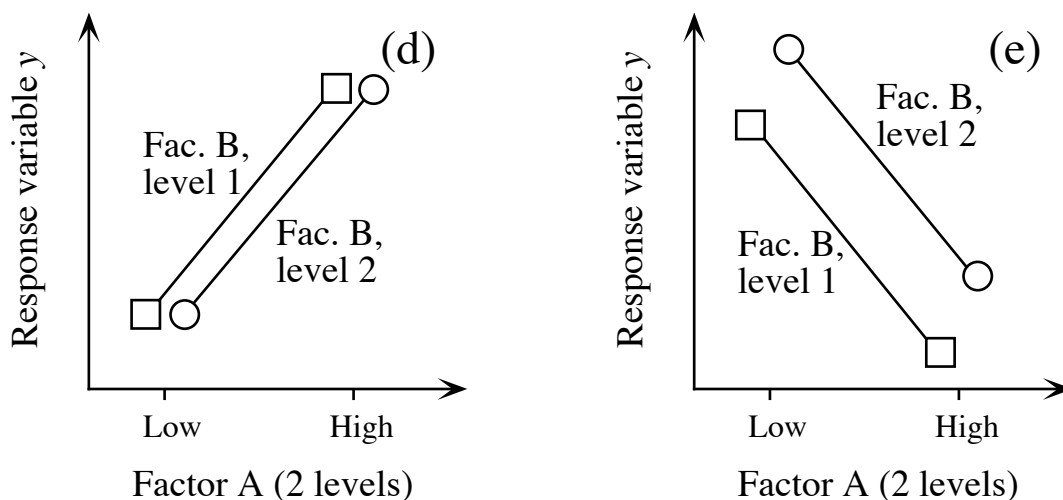
Interaction

The interaction sum-of-squares measures to what extent the effect of a factor depends on the level of the other factor. We are interested to recognize the different situations illustrated by the following examples:
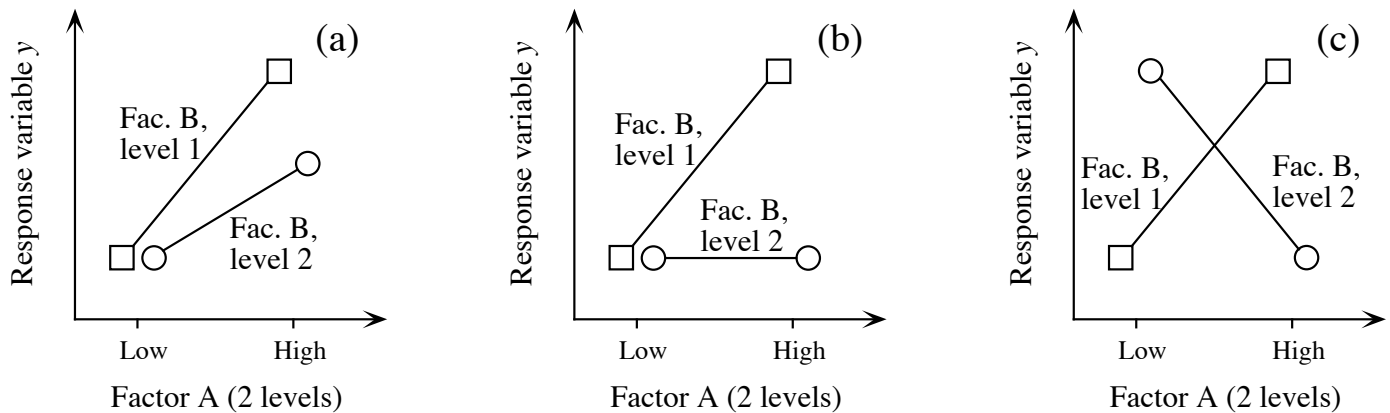
• In (a), the effect of factor A on **y**, which increases the response from A-1 to A-2, is reduced in magnitude in level 2 of factor B.

• In (b), there is no effect of A in the highest level of B while there is an effect of A in the lower level of B.

• In (c), the effect of A is reversed in B-1 compared to B-2.

The situation without interaction would correspond to one of the following diagrams:

where the level of A does not influence the response of **y** to the levels of B, and vice versa.

• In (d), A has a significant effect on the response **y** but B has not and there is no A**x**B interaction.

• In (e), both factors have significant effects on **y**, without interaction. This means that the difference in effect (on **y**) between the two levels of A remains the same in the different levels of B.

The figures could have been drawn to illustrate the effect of factor B on the response **y** for the different levels of A.

A significant interaction between factors A and B reflects interesting and interpretable ecological properties or processes. From the methodological point of view, a significant interaction means that the effect of each factor has to be analyzed separately in each level of the other factor, since there is no effect of a factor that is common to all levels of the other factor. In other words, a significant interaction renders the tests for the main effects meaningless.

4 - Two-way factorial ANOVA without replication

Sometimes, we have to analyze data from experiments in which there was no replication. A common example in ecology is the analysis of surveys through space repeated at two or several times: there usually are no replicates for specific points in space and time.

The main drawback in that situation is that it is not possible to estimate a mean square for the interaction separately from the residual mean square

because there is a single observation in each group. This can be easily seen from the ANOVA table when the number of replicates $n = 1$:

Sources of variation	Degrees of freedom (df)	Sums of Squares	Mean squares	F-statistics
Fixed factor A	$a - 1$	SS_A	$MS_A = SS_A/(a-1)$	$MS_A / ?$
Fixed factor B	$b - 1$	SS_B	$MS_B = SS_B/(b-1)$	$MS_B / ?$
Interaction (A x B)	$(a - 1)(b - 1)$	SS_{Int}	$MS_{Int} = SS_{Int}/(a - 1)(b - 1)$	$MS_{Int}/ ?$
Residuals	$ab(1 - 1) = 0$	SS_{Res}	$MS_{Res} = SS_{Res}/0$	
Total variation	$ab - 1$	SS_{Total}	s_y^2	

If we estimate the interaction mean square, there is <u>no degree of freedom</u> left to compute the residual mean square, which forms the denominator of the interaction F-statistic.

In order to be able to test the main factors (fixed or random), we must assume that there is no interaction, and use the interaction mean square as an estimate of the residual mean square:

Sources of variation	Degrees of freedom (*df*)	Sums of Squares	Mean squares	*F*-statistics
Fixed factor A	$a - 1$	SS_A	$MS_A = $ $SS_A/(a{-}1)$	MS_A / MS_{Res}
Fixed factor B	$b - 1$	SS_B	$MS_B = $ $SS_B/(b{-}1)$	MS_B / MS_{Res}
Residuals	$(a - 1)(b - 1)$	SS_{Res}	$MS_{Res} = $ $SS_{Res}/(a - 1)(b - 1)$	
Total variation	$ab - 1$	SS_{Total}	s_y^2	

The two-way ANOVA model without replication is:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk}$$

If an interaction really exists in the data, the residual mean square will be overestimating the population variance. As a consequence, $F_A = MS_A / MS_{Res}$ and $F_B = MS_B / MS_{Res}$ will be smaller than they should and the tests for significant effects of the factors A and B will lose power, meaning that they may not detect effects of A and/or B that are really present in the data. Numerical example: see *Practicals*.

A solution to the problem of testing the interaction in space-time ecological surveys is described by Legendre et al. (2010).[*] That method only applies to spatially or temporally-structured response variables.

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[*] Legendre, P., M. De Cáceres, and D. Borcard. 2010. Community surveys through space and time: testing the space-time interaction in the absence of replication. *Ecology* 91: 262-272.

Special case — In experimental designs, the *randomized complete block design* is used to study the effect of a treatment in experimental units that are dispersed throughout a landscape which is, for example, varying in soil conditions. The design consists in finding portions of the study area that are fairly homogeneous in soil conditions, and attributing at random *all levels* of the treatment to experimental units located *within each block*. For example, we could run an experiment about the effect of 3 types of fertilizer (fixed factor of interest) and conduct it in 4 fairly homogeneous fields (blocks: random factor): each fertilizer would be applied to an experimental unit chosen at random within each of the blocks. In this example, we would end up with 12 experimental units. The results of this type of experiment are analyzed using two-way ANOVA without replication.

5 - Two-way ANOVA with replication: Model II

Model II ANOVA is used for the analysis of two-way factorial experiments with replication in which the two factors are random. This type of analysis differs in some details from Model I ANOVA where the two factors were fixed.

Motivation example — A field (mensurative) experiment examines the spatial and temporal variability in abundance of a bird species along the course of a river.

• Factor A: 5 randomly chosen localities along the river.

• Factor B: 6 randomly chosen days during the summer.

• Replication: 5 listening stations located along the shore, 100 m from the previous station.

The statistical model to explain the variation in a response variable **y** is the same as in two-way Model I ANOVA with replication:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

The sums-of-squares and mean squares are computed in the same way as in the fixed-effect Model I ANOVA, but here we have different expected mean squares (EMS):

Sources of variation	Degrees of freedom (*df*)	Expected mean squares (EMS)
Random factor A	$a - 1$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\beta}^2 + bn\sigma_\alpha^2$
Random factor B	$b - 1$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\beta}^2 + an\sigma_\beta^2$
Interaction (A x B)	$(a - 1)(b - 1)$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\beta}^2$
Residuals	$ab(n - 1)$	σ_ε^2

So, the denominator mean square will change depending on the term to be tested. The *F*-statistics are computed as follows:

Sources of variation	Degrees of freedom (*df*)	Sums of Squares	Mean squares	*F*-statistics
Random factor A	$a - 1$	SS_A	$MS_A = SS_A/(a-1)$	MS_A / MS_{Int}
Random factor B	$b - 1$	SS_B	$MS_B = SS_B/(b-1)$	MS_B / MS_{Int}
Interaction (A x B)	$(a - 1)(b - 1)$	SS_{Int}	$MS_{Int} = SS_{Int}/(a - 1)(b - 1)$	MS_{Int}/ MS_{Res}

Sources of variation	Degrees of freedom (*df*)	Sums of Squares	Mean squares	*F*-statistics
Residuals	$ab(n-1)$	SS_{Res}	$MS_{Res} = SS_{Res}/ab(n–1)$	
Total variation	$abn-1$	SS_{Total}	s_y^2	

6 - Two-way ANOVA with replication: Mixed model (Model III)

Model III ANOVA is used for the analysis of two-way factorial experiments with replication in which one of the factors is fixed and the other is random. This type of analysis differs in some details from Models I and II.

Motivation example — A field experiment examines the effects of fertilizers on the growth of individuals of a plant species of interest, at different sites.

• Factor A: 3 fertilizers (fixed factor).

• Factor B: 5 randomly chosen sites in the area where the species occurs (random factor).

• Replication: 50 individual plants.

The statistical model to explain the variation in a response variable **y** is still the same as in two-way Model I ANOVA with replication:

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$$

The sums-of-squares and mean squares are computed in the same way as in the fixed-effect Model I ANOVA, but here we have different expected mean squares (EMS):

Sources of variation	Degrees of freedom (*df*)	Expected mean squares (EMS)
Fixed factor A	$a - 1$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\beta}^2 + bn \sum_i \alpha_i^2 / (a - 1)$
Random factor B	$b - 1$	$\sigma_\varepsilon^2 + an\sigma_\beta^2$
Interaction (A x B)	$(a - 1)(b - 1)$	$\sigma_\varepsilon^2 + n\sigma_{\alpha\beta}^2$
Residuals	$ab(n - 1)$	σ_ε^2

So, the denominator mean square will change depending on the term to be tested. The *F*-statistics are computed as follows:

Sources of variation	Degrees of freedom (*df*)	Sums of Squares	Mean squares	*F*-statistics
Fixed factor A	$a - 1$	SS_A	$MS_A = SS_A/(a{-}1)$	MS_A / MS_{Int}
Random factor B	$b - 1$	SS_B	$MS_B = SS_B/(b{-}1)$	MS_B / MS_{Res}
Interaction (A x B)	$(a - 1)(b - 1)$	SS_{Int}	$MS_{Int} = SS_{Int}/(a - 1)(b - 1)$	MS_{Int}/ MS_{Res}
Residuals	$ab(n - 1)$	SS_{Res}	$MS_{Res} = SS_{Res}/ab(n{-}1)$	
Total variation	$abn - 1$	SS_{Total}	s_y^2	

R language: The function *anova.2way.R* (P. Legendre) computes Models I, II or III ANOVA for 2-way ANOVA (balanced designs) with parametric and permutation tests.

7 - Discussion: crossed *versus* nested ANOVA

In some studies, several kinds of hypotheses can be tested with the same data. An example in community ecology is a two-factor crossed ANOVA where one of the factors is geographically nested in the other.

Consider a study in which fish have been counted along underwater transects on the windward and leeward sides (factor B) of two bays (factor A). Transects are the replicates in this analysis. A two-way crossed design should be used to test the hypothesis that factor B has an identifiable crossed effect (with respect to A) on the response variables. Crossed ANOVA has more power to detect small effects than a nested analysis. If, however, the interaction between the two factors is significant, one has two options: (1) carry out separate analyses of the two bays, which poses the problem of assembling the results, a posteriori, in a coherent global interpretation. Or (2), consider that the "sides" (factor B) do not actually behave like a crossed factor. One may then approach the problem in a different way by considering that B is nested in the "bays" (factor A) and proceeding with a nested ANOVA or MANOVA. The second option presents the advantage of providing a global analysis of the data, capable of showing if the geographically outer factor (bays) and/or the inner factor (sides within bay) are significant.