Multifactor Analysis of Variance

Recall: Previously, you have seen single-factor ANOVA experiments, in which the values of a quantitative response were compared for different levels of a FACTOR. In many experiments, two or more factors influence the response and will be studied simultaneously. Suppose the first factor (A) has I factor levels and the second factor (B) has J factor levels, then there are IJ factor level combinations which we will call TREATMENTS. For each treatment, one or more observations on the quantitative response may be available. Let K_{ij} be the number of observations available for factor level i of A and factor level j of B.

Example:

- A florist measures the time (in days) until freshly cut roses start to wither for roses of different stem length (15, 20, 25cm) kept under different temperatures (50°F, 60°F, 70°F)
- A group of avid movie watchers studies the proportion of popcorn kernels popped during a fixed time in pots of different size (small, medium, large) using different fats (oil, margarine).
- Some folklore blames the erratic behavior of people ("lunatics") on the phase of the moon. To study this phenomenon, admission rates to a mental health facility were recorded for twelve months where each month was separated into three phases (before, during and after full moon).

Two-Factor ANOVA with $K_{ij} = 1$

Factor A:	I levels ($i = 1, \ldots, I$)
Factor B:	J levels ($j = 1, \ldots, J$)
Treatments:	IJ combinations of factor levels
Observations:	at each factor level combination, $K_{ij} = 1$ observation is taken

Notation: X_{ij} denotes the random variable representing the measurement when factor A is held at level i and factor B is held at level j. x_{ij} will denote the corresponding observed value of X_{ij}.

Similarly to the single-factor ANOVA case, we will use the dot-notation to denote averages of measurements:

$$\bar{X}_{i\cdot} = \begin{array}{c}\text{the average of all measurements obtained}\\ \text{when factor } A \text{ is held at level } i\end{array} = \frac{1}{J}\sum_{j=1}^{J} X_{ij}$$

$$\bar{X}_{\cdot j} = \begin{array}{c}\text{the average of all measurements obtained}\\ \text{when factor } B \text{ is held at level } j\end{array} = \frac{1}{I}\sum_{i=1}^{I} X_{ij}$$

$$\bar{X}_{\cdot\cdot} = \text{ the grand mean } = \frac{1}{IJ}\sum_{i=1}^{I}\sum_{j=1}^{J} X_{ij}$$

The ANOVA Model

The ADDITIVE ANOVA model:

$$X_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

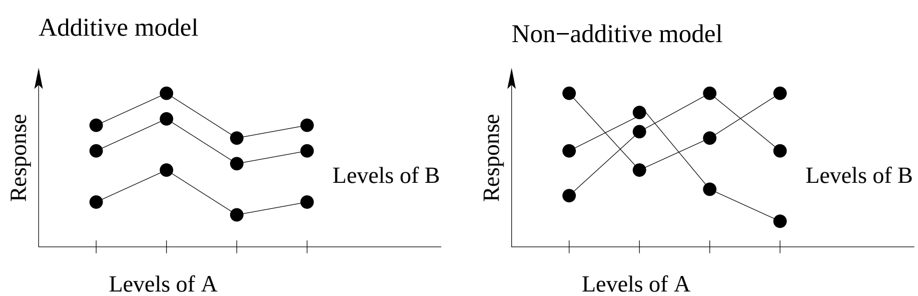
assumes that each observation can be modeled as a sum of factor effects, where

Factor A contributes α_i, $\quad i = 1, \ldots, I$ with $\sum_{i=1}^{I} \alpha_i = 0$, and

Factor B contributes β_j, $\quad j = 1, \ldots, J$ with $\sum_{j=1}^{J} \beta_j = 0$.

μ is the overall mean of all observations, and
the ϵ_{ij} are independent, normally distributed error terms with mean zero and common variance σ^2.

The additive assumption in this model is quite restrictive, but it is a necessity if only one observation is available at each treatment. Non-additive models can only be fitted in experiments where two or more observations are available at each factor level combination.



Estimating the parameters:

Estimating the parameters in an additive ANOVA model is easily done by averaging over appropriate observations:

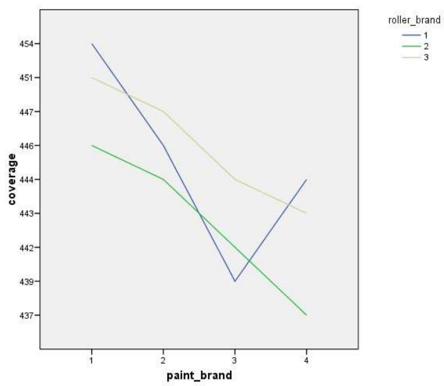
$$\hat{\mu} = \bar{x}_{..}, \quad \hat{\alpha}_i = \bar{x}_{i\cdot} - \bar{x}_{..}, \quad \hat{\beta}_j = \bar{x}_{\cdot j} - \bar{x}_{..}$$

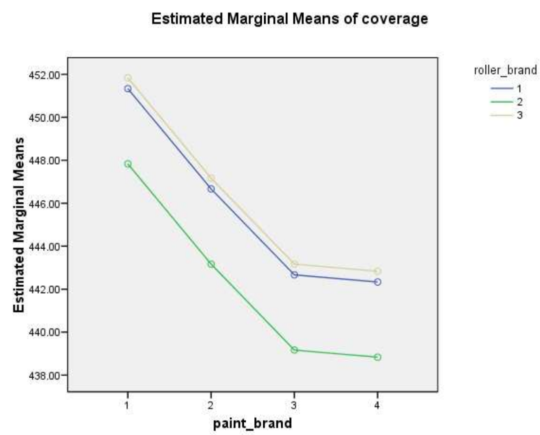
Example: In an experiment to see whether the amount of coverage of light-blue interior latex paint depends either on the brand of paint or on the brand of roller used, 1 gallon each of four brands of paint was applied using each of three brands of rollers, resulting in the following data (number of square feet covered).

		Roller Brand		
		1	2	3
	1	454	446	451
Paint	2	446	444	447
Brand	3	439	442	444
	4	444	437	443

(a) Do you think it is appropriate to fit an additive model in this experiment? Why or why not?

(b) Let the paint brand be factor A, and the roller brand be factor B. Identify I, J, $\hat{\mu}$, $\hat{\alpha}_2$, $\hat{\beta}_3$.





Hypotheses for the additive model:

$H_{0A} : \alpha_1 = \alpha_2 = \cdots = \alpha_I = 0$ or "no factor A effect",
vs. H_{aA}: at least one $\alpha_i \neq 0$.

$H_{0B} : \beta_1 = \beta_2 = \cdots = \beta_J = 0$ or "no factor B effect",
vs. H_{aB}: at least one $\beta_j \neq 0$.

Example: (cont.)

Formulate the null hypotheses and alternatives in words for the Paint Example.

Test Procedure

The test procedure for additive multifactor ANOVA is very similar to that of a single-factor ANOVA. Sums of squares are computed which represent the variation within the factors (separately for factors A (SSA) and B (SSB)), as well as error variation (SSE) and total variation (SST).

$$\text{SST} = \sum_{i=1}^{I}\sum_{j=1}^{J}(X_{ij} - \bar{X}_{..})^2, \qquad df = IJ - 1$$

$$\text{SSA} = \sum_{i=1}^{I}\sum_{j=1}^{J}(X_{i.} - \bar{X}_{..})^2, \qquad df = I - 1$$

$$\text{SSB} = \sum_{i=1}^{I}\sum_{j=1}^{J}(X_{.j} - \bar{X}_{..})^2, \qquad df = J - 1$$

$$\text{SSE} = \sum_{i=1}^{I}\sum_{j=1}^{J}(X_{ij} - \bar{X}_{i.} - \bar{X}_{.j} + \bar{X}_{..})^2, \qquad df = (I-1)(J-1)$$

To test the two hypotheses for the two-factor ANOVA model, F-tests statistics are formed as fractions of between and within factor variances. The null hypotheses are rejected if their value computed from the data is larger than the critical value of the corresponding F distribution.

Hypotheses	Test Statistic	Rejection region
H_{0A} vs. H_{aA}	$f_A = \frac{\text{MSA}}{\text{MSE}}$	$f_A \geq F_{\alpha,I-1,(I-1)(J-1)}$
H_{0B} vs. H_{aB}	$f_B = \frac{\text{MSB}}{\text{MSE}}$	$f_B \geq F_{\alpha,J-1,(I-1)(J-1)}$

Example: (cont.)

Below find an ANOVA table for the additive two-factor ANOVA design in the paint example. Ignore the “Corrected Model”, “Intercept” and “Total”’ rows which are artifacts of the additive model in SPSS. The “Corrected Total” is our SS_T row.

Tests of Between-Subjects Effects

Dependent Variable:Coverage

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	197.583[a]	5	39.517	5.830	.027
Intercept	2373630.75	1	2373630.75	350207.816	.000
Paint_Brand	159.583	3	53.194	7.848	.017
Roller_Brand	38.000	2	19.000	2.803	.138
Error	40.667	6	6.778		
Total	2373869.00	12			
Corrected Total	238.250	11			

a. R Squared = .829 (Adjusted R Squared = .687)

Draw a conclusion for the two hypotheses you previously formulated for this example.

Multiple Comparisons: Tukey's procedure can be used in a multifactor ANOVA analysis. Take care to use the appropriate number of factor levels I or J depending on which factor you are studying the mean differences for.

Randomized Block Experiment

Suppose you want to study the effect of different treatments on a population that is heterogeneous in a characteristic that may also influence the response, but that the experimenter cannot influence. This characteristic is called a BLOCK.

Examples:

- You want to study the effect of fertilizer on a field that has different types of soil in the front, middle and back.
- You want to study the effect of a cardiovascular drug on patients, but you suspect that the drug will effect people of different weights in different ways.

In a RANDOMIZED BLOCK DESIGN the block variable (e.g., soil quality, weight) is treated as a separate factor in a two-way ANOVA analysis. If possible, experimental subjects are chosen from each block level and then randomly assigned to the different treatments.

Two-Factor ANOVA Model with Interaction

Consider an experiment in which two categorical factors influence a quantitative response (2-way ANOVA). Other than before, we now assume that more than one ($K_{ij} > 1$) observation was taken at at least one factor level combination. Actually, for simplicity, we will assume that the number of observations taken at *every* factor level combination is the same ($K > 1$).

Having more observations allows us to expand the stringent assumptions of the additive ANOVA model. The additive model assumed that factor A affects the response *at every level* of factor B in the same way (parallel curves in the means plot).

Recall: Additive ANOVA model

$$X_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

New: Fixed Effects ANOVA Model with Interactions

The new ANOVA model will include additional terms γ_{ij} which represent the interaction of factors A and B.

$$X_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \epsilon_{ijk}$$

$$i = 1, \ldots, I, \qquad j = 1, \ldots, J, \qquad k = 1, \ldots, K$$

As before,we assume the ϵ_{ijk} to be independent and normally distributed with mean zero and common variance σ^2.

Example: Consider the following experiments and formulate what an interaction effect of the two factors would mean in practice.

- You perform a taste test for different types of bread (Cinnamon Raisin Bread, Pugliese, and Kalamata Olive Loaf) with different types of spreads (Butter, Margarine, Garlic Oil).

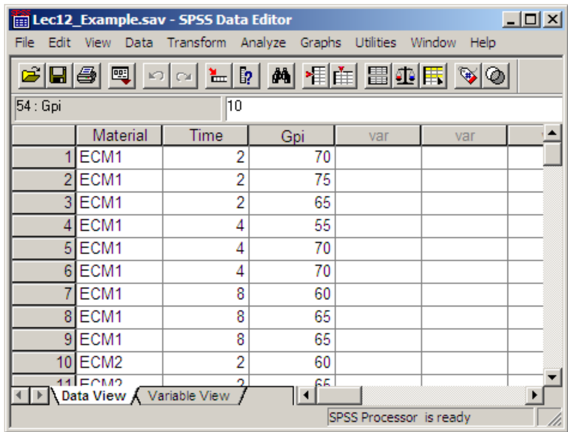
- You study the yield of corn plants of different seed types which were treated with different fertilizers.

Example: One way to repair serious wounds is to insert some material as a scaffold for the body's repair cells to use as a template for new tissue. Scaffolds made from extracellular material (ECM) are particularly promising for this purpose. Because they are made from biological material, they serve as an effective scaffold and are then absorbed. Unlike biological material that includes cells, however, they do not trigger tissue rejection reactions in the body. One study compared 6 types of scaffold material. Three of these were ECMs and the other three were made of inert materials. There were three mice used per scaffold type. The response measure was the percent of glucose phosphate isomerase (Gpi) cells in the region of the wound. A large value is good, indicating that there are many bone marrow cells sent by the body to repair the tissue. Here are the data on the mice 2 weeks, 4 weeks, and 8 weeks, after the repair:

	Gpi(%)								
Material	2 weeks			4 weeks			8 weeks		
ECM1	70	75	65	55	70	70	60	65	65
ECM2	60	65	70	60	65	65	60	70	60
ECM3	80	60	75	75	70	75	70	80	70
MAT1	50	45	50	20	25	25	15	25	25
MAT2	5	10	15	5	10	5	10	5	5
MAT3	30	25	25	10	15	10	5	15	10

(a) Identify the response, the factors and their levels in this example. What are the values of I, J, and K?

(b) You can find the data on the course website in the file “ScaffoldingMaterial.txt”. To enter the data into SPSS we have to make one column per variable. Below, the columns are labeled Material, Time and Gpi:



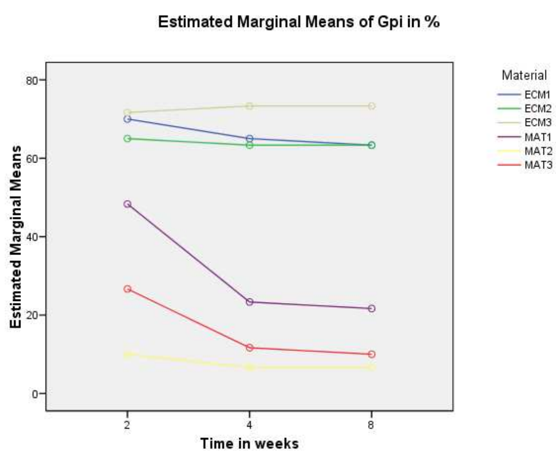
	Material	Time	Gpi	var	var
1	ECM1	2	70		
2	ECM1	2	75		
3	ECM1	2	65		
4	ECM1	4	55		
5	ECM1	4	70		
6	ECM1	4	70		
7	ECM1	8	60		
8	ECM1	8	65		
9	ECM1	8	65		
10	ECM2	2	60		

To do a two-way ANOVA in SPSS, click *Analyze* → *General Linear Model* → *Univariate*. Move “Gpi” into the “Dependent Variable” box and “Material” and “Time” into the “Fixed Factor(s)” box.
The buttons on the right in the “Univariate General Linear Model” menu allow you to choose different plots or statistics to be computed.

(c) Consider the following plot of the mean “Gpi” measurements for the different combinations of “Material” and “Time”. Comment on the main features of the plot. Can you see effects of the factors “Material” and “Time”? Does there appear to be an interaction effect?

To do this in SPSS, click on “Plots” in the “Univariate General Linear Model” menu. The above plot has “Time” on the horizontal axis and “Material” in the “Separate Lines” box.

Which material seems to work best (worst)?



Hypotheses: There are now three null hypotheses (and corresponding alternatives) that we can consider in a two-way ANOVA model with interaction.

Factor A effect:	$H_{0A} : \alpha_1 = \cdots = \alpha_I = 0$	"there is no factor A effect"
	H_{aA}: at least one $\alpha_i \neq 0$	"there is a factor A effect"
Factor B effect:	$H_{0B} : \beta_1 = \cdots = \beta_J = 0$	"there is no factor B effect"
	H_{aB}: at least one $\beta_j \neq 0$	"there is a factor B effect"
Interaction of A and B:	$H_{0AB} : \gamma_{ij} = 0$ for all i, j	"there is no interaction effect"
	H_{aAB}: at least one $\gamma_{ij} \neq 0$	"there is an interaction effect"

Commonly, the third hypothesis concerning the interaction effect is tested first. If no interaction effect is found then the main effects are tested next. On the other hand, if an interaction effect between the factors exist, then factor effects of both factors should also be included in the model.

Example: (cont.)

(d) Formulate the three null hypotheses in words in the context of the Scaffolding Material example.

Constructing the ANOVA table: The construction of the sums of squares and mean squares for the ANOVA table proceeds very similarly to the cases we have previously seen:

$$\text{SST} = \sum_i \sum_j \sum_k (X_{ijk} - \bar{X}_{...})^2, \qquad df = IJK - 1$$

$$\text{SSE} = \sum_i \sum_j \sum_k (X_{ijk} - \bar{X}_{ij.})^2, \qquad df = IJ(K-1)$$

$$\text{SSA} = \sum_i \sum_j \sum_k (\bar{X}_{i..} - \bar{X}_{...})^2, \qquad df = I - 1$$

$$\text{SSB} = \sum_i \sum_j \sum_k (\bar{X}_{.j.} - \bar{X}_{...})^2, \qquad df = J - 1$$

$$\text{SSAB} = \sum_i \sum_j \sum_k (\bar{X}_{ij.} - \bar{X}_{i..} - \bar{X}_{.j.} + \bar{X}_{...})^2, \qquad df = (I-1)(J-1)$$

Example: (cont.)

(e) Look at the table that contains a summary on the factor "Material". Why are the estimates for the standard errors of the six materials all the same? Coincidence?

Material

Dependent Variable: Gpi in %

Material	Mean	Std. Error	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
ECM1	66.111	1.682	62.700	69.522
ECM2	63.889	1.682	60.478	67.300
ECM3	72.778	1.682	69.366	76.189
MAT1	31.111	1.682	27.700	34.522
MAT2	7.778	1.682	4.366	11.189
MAT3	16.111	1.682	12.700	19.522

To obtain this table, check "Descriptive Statistics" for MATERIAL under "Options" in the "Univariate General Linear Model" menu.

(f) SPSS will provide you with an ANOVA table for the analysis of this experiment:

Tests of Between-Subjects Effects

Dependent Variable: Gpi in %

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	37609.259[a]	17	2212.309	86.883	.000
Intercept	99674.074	1	99674.074	3914.473	.000
Material	35659.259	5	7131.852	280.087	.000
Time	867.593	2	433.796	17.036	.000
Material * Time	1082.407	10	108.241	4.251	.001
Error	916.667	36	25.463		
Total	138200.000	54			
Corrected Total	38525.926	53			

a. R Squared = .976 (Adjusted R Squared = .965)

Refer to the ANOVA table in the output of the "Univariate General Linear Model" in SPSS. What is being reported in the different rows and columns? Find the estimates of MSE, MSA, MSB, and MSAB.

(g) Use the ANOVA table to draw conclusions for the hypotheses you formulated in part (d).

Multiple Comparisons: If the ANOVA F-test for the interaction term concludes that no interaction effect exists (H_{0AB} is accepted), and at least one of the two main effect null hypotheses (H_{0A} or H_{0B}) is rejected, then Tukey's method can be used to decide which factor levels differ.

Question: Is it possible to use two separate single-factor ANOVA models to replace a two-way ANOVA model? Why or why not?