Lecture 2
Analysis of Variance

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What's expected to be understood

- **Multiple regression in Response Surface Methodology. Dependent and independent variables are numeric. The predicted response is studied.**
- **Specific designs for RSM were discussed.**
- **In all approaches Completely Randomised Designs are assumed.**
- **RSM in constrained experimental regions.**

What's next?

What if experimental factors are categorical?

In many practical problems this is the case: fi gender, different drugs, different genes, insertions, varieties, bacterial strains, …

Analysis of variance (ANOVA) models

Experimental Study

A study was performed to examine the effect of a new sleep inducing drug on a population of insomniacs. Three treatment levels were used:

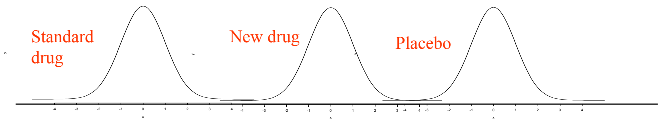
Standard Drug
New Drug
Placebo (as a control)

	Standard drug	New drug	Placebo	
Average number of hours of sleep per night	y_{11} y_{12} $\vdots$ y_{1n_1}	y_{21} y_{22} $\vdots$ y_{2n_2}	y_{31} y_{32} $\vdots$ y_{3n_3}	
Means per drug $\bar{y}_i$	$\bar{y}_1$	$\bar{y}_2$	$\bar{y}_3$	$\bar{y}$

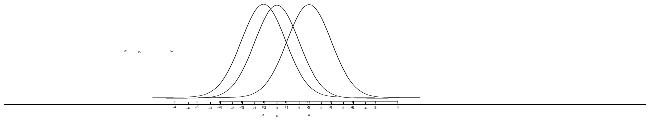
Overal mean $\bar{y}_{..}$

Testing Approaches - Analysis of Variance

The term "analysis of variance" comes from the fact that this approach compares the variability observed among sample means to a pooled estimate of the variability among observations within each group.



Within group variance is small compared to variability among means. Clear separation of means.



Within group variance is large compared to variability among means. Unclear separation of means.

Pooled Variance

From two-sample t-test with assumed equal variance, σ², we produced a pooled (within-group) sample variance estimate.

$$t = \frac{\bar{y}_1 - \bar{y}_2}{s_p\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad s_p = \sqrt{\frac{(n_1-1)s_1^2 + (n_2-1)s_2^2}{n_1+n_2-2}}$$

Extend the concept of a pooled variance to t groups as follows:

$$s_w^2 = \frac{(n_1-1)s_1^2 + (n_2-1)s_2^2 + \cdots + (n_t-1)s_t^2}{(n_1-1)+(n_2-1)+\cdots+(n_t-1)} = \frac{SSW}{n_T - t} \qquad n_T = \sum_{i=1}^{t} n_i$$

If all the n_i are equal to n then this reduces to an average variance.

$$s_w^2 = \frac{1}{t}\sum_{i=1}^{t} s_i^2$$

Variance among Group Means

Consider the variance among the t group means computed as:

$$\bar{y}_{\bullet\bullet} = \frac{\sum_{i=1}^{t} \bar{y}_i}{t}$$

$$s^2 = \frac{\sum_{i=1}^{t} (\bar{y}_i - \bar{y}_{\bullet\bullet})^2}{t-1}$$

If we assume each group is of the same size, say n, then, s is an estimate of σ²/n. Hence, n times s is an estimate of σ². When the sample sizes are unequal, the estimate is given by.

$$s_B^2 = \frac{\sum_{i=1}^{t} n_i(\bar{y}_{i\bullet} - \bar{y}_{\bullet\bullet})^2}{t-1} = \frac{SSB}{t-1}$$

$$y_{i\bullet} = \sum_{j=1}^{n_i} y_{ij}$$

$$\bar{y}_{\bullet\bullet} = \frac{1}{n_T}\sum_{i=1}^{t}\sum_{j=1}^{n_i} y_{ij}$$

F-test

Now we have two estimates of s². An F-test can be used to determine if the two statistics are equal. Note that if the groups truly have different means, s_b^2 will be greater than s_w^2. Hence the F-statistics is written as:

$$F = \frac{s_B^2}{s_W^2} \sim F_{(t-1),(n_T-t)}$$

If H_0 holds, the computed F-statistics should be close to 1.
If H_a holds, the computed F-statistic should be much greater than 1.
We use the appropriate critical value from the F - table to help make this decision.

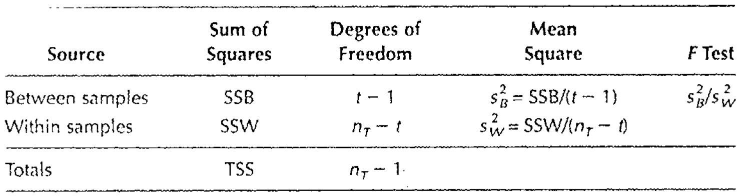
Hence,the F-test is really a test of equality of means under the assumption of normal populations and homogeneous variances.

Partition of Sums of Squares and the AOV Table

$$TSS = \sum_{i=1}^{t}\sum_{j=1}^{n_i}(y_{ij} - \bar{y}_{\bullet\bullet})^2 = (n_T-1)s^2 = \sum_{i=1}^{t}\sum_{j=1}^{n_i} y_{ij}^2 - \frac{y_{\bullet\bullet}^2}{n_T}$$

$$SSB = \sum_{i=1}^{t} n_i(\bar{y}_{i\bullet} - \bar{y}_{\bullet\bullet})^2 = \sum_{i=1}^{t}\frac{y_{i\bullet}^2}{n_i} - \frac{y_{\bullet\bullet}^2}{n_T}$$

$$SSW = TSS - SSB$$



Source	Sum of Squares	Degrees of Freedom	Mean Square	F Test
Between samples	SSB	$t-1$	$s_B^2 = SSB/(t-1)$	s_B^2/s_W^2
Within samples	SSW	$n_T - t$	$s_W^2 = SSW/(n_T - t)$	
Totals	TSS	$n_T - 1$		

The Linear Model

We have developed the one-way analysis of variance as an extension of the two-sample t-test with pooled variance. More complicated research designs require that we take a more formal, model-based approach to the analysis.

Much of statistical analysis is based on the general linear (regression) model structure. For the response y_{ij} for the i^{th} group and j^{th} individual or unit, we have.

$$y_{ij} = \mu_i + \varepsilon_{ij}$$

Where μ_i is the mean of the i^{th} group and ε_{ij} is the deviations of the response from the mean of the group.

Usual assumption: $\varepsilon_{ij} \sim N(0, s^2)$

residual or experimental error

Completely Randomized Design

Assumptions:

- Independent random samples (results of one sample do not effect other samples).
- Samples from normal population(s).
- Mean and variance for population i are respectively, μ_i and σ^2.

Model: $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$ $\quad E(y_{ij}) = \mu + \alpha_i$

overall mean

effect due to population i

random error ~ $N(0,\sigma^2)$

AOV model

Requirement for μ to be the overall mean:

$$\sum_{i=1}^{t} \alpha_i = 0$$

$H_0: \ \alpha_1 = \alpha_2 = \cdots = \alpha_t = 0$

$H_a:$ At least one of the α differ from 0

Reference Group Model

Model: $y_{tj} = \mu_t + \varepsilon_{tj} \qquad i = t$

$y_{ij} = \mu_t + \beta_i + \varepsilon_{ij} \qquad i = 1,2,\cdots,t-1$

reference group mean

effect due to population i

random error ~ $N(0,\sigma^2)$

Mean for the last group (i=t) is μ_t.

Mean for the first group (i=1) is $\mu_t + \beta_1$

Thus, β_1 is the difference between the mean of the reference group (cell) and the target group mean. Any group can be the reference group.

$H_0: \ \beta_1 = \beta_2 = \cdots = \beta_{t-1} = 0$

$H_a:$ At least one of the β differ from 0

One-Way ANOVA example

A study was performed to examine the effect of a new sleep inducing drug on a population of insommiacs. Three treatments were used:

Standard Drug
New Drug
Placebo (as a control)

What is the role of the placebo in this study?
What is a control in an experimental study?

18 individuals were drawn (at random) from a list of know insomniacs maintained by local physicians. Each individual was randomly assigned to one of three groups. Each group was assigned a treatment. Neither the patient nor the physician knew, until the end of the study, which treatment they were on (double blind).

Why double blind?

ANOVA

Response: Average number of hours of sleep per night.

```
Placebo:              5.6, 5.7, 5.1, 3.8, 4.6, 5.1
Standard Drug:        8.4, 8.2, 8.8, 7.1, 7.2, 8.0
New Drug:            10.6, 6.6, 8.0, 8.0, 6.8, 6.6
```

y_{ij} = response for the j-th individual on the i-th treatment.

	Placebo	Standard Drug	New Drug
	5.60	8.40	10.60
	5.70	8.20	6.60
	5.10	8.80	8.00
	3.80	7.10	8.00
	4.60	7.20	6.80
	5.10	8.00	6.60
sum	29.900	47.700	46.600
mean	4.983	7.950	7.767
variance	0.494	0.455	2.359
pooled variance			**1.102**
SSW			**16.537**
variance of the means			2.764
Between mean SSQ (SSB)			**16.582**

Source	Sums of Squares	Degrees of Freedom	Mean Square	F statistic	P-value
Between Groups	33.16	2	16.582	15.04	0.00026
Within Groups	16.54	15	1.102		
Total	49.70	17			

$$\begin{aligned} &TSS &&= SSB &&+ SSW \\ &\sum_{i,j}(y_{ij}-\bar{y}_{\bullet\bullet})^2 &&= \sum_{i,j}(y_{ij}-\bar{y}_{i\bullet})^2 &&+ \sum_{i} n_i(\bar{y}_{i\bullet}-\bar{y}_{\bullet\bullet})^2 \end{aligned}$$

$$MSB = s_b^2 = \frac{SSB}{t-1}$$

$$MSW = s_w^2 = \frac{SSW}{n_T - t}$$

$$F = \frac{MSB}{MSW} \sim F_{df_b, df_w}$$

Linear Model Approach

Response	Treatment	Dummy 1	Dummy 2
5.6	**Placebo**	0	0
5.7	**Placebo**	0	0
5.1	**Placebo**	0	0
3.8	**Placebo**	0	0
4.6	**Placebo**	0	0
5.1	**Placebo**	0	0
8.4	**Standard Drug**	1	0
8.2	**Standard Drug**	1	0
8.8	**Standard Drug**	1	0
7.1	**Standard Drug**	1	0
7.2	**Standard Drug**	1	0
8	**Standard Drug**	1	0
10.6	**New Drug**	0	1
6.6	**New Drug**	0	1
8	**New Drug**	0	1
8	**New Drug**	0	1
6.8	**New Drug**	0	1
6.6	**New Drug**	0	1

Reference group model

SUMMARY OUTPUT

Regression Statistics	
Multiple R	0.8169
R Square	0.6673
Adjusted R Square	0.6229
Standard Error	1.0500
Observations	18

Regression ANOVA outputs

Units of measurement
= hours sleep.

ANOVA

	df	*SS*	*MS*	*F*	*Sign F*
Regression	2	33.1633	16.582	15.041	0.0003
Residual	15	16.5367	1.102		
Total	17	49.7000			

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	4.9833	0.429	11.626	0.0000	4.070	5.897	4.070	5.897
Dummy 1	2.9667	0.606	4.894	0.0002	1.675	4.259	1.675	4.259
Dummy 2	2.7833	0.606	4.591	0.0004	1.491	4.075	1.491	4.075

$$\begin{aligned} y_{tj} &= \mu_t + \varepsilon_{tj} && i = t \\ y_{ij} &= \mu_t + \beta_i + \varepsilon_{ij} && i = 1,2,\cdots,t-1 \end{aligned}$$

Mean difference between standard drug and placebo.

Mean difference between new drug and placebo.

Placebo mean.

What about difference between new drug and standard drug?

Equivalence between regression and ANOVA

Regression in dummy coding gives the same results as ANOVA

Why ANOVA?

- **Computational advantage**
- **Intuitive underlying logic for complex designs**
- **Historical traditions**

SAS example

Investigate the effect of 3 different strains of Rhizobium on the nitrogen content in clover

Which is the best strain?

	Strain 1	Strain 2 $\bar{y}_3$	Strain 3		
Nitrogen content	y_{11} y_{12} $\vdots$ y_{1n_1}	y_{21} y_{22} $\vdots$ y_{2n_2}	y_{31} y_{32} $\vdots$ y_{3n_3}		
Means per strain $\bar{y}_i$	$\bar{y}_1$	$\bar{y}_2$	$\bar{y}_3$	$\bar{y}$	**Overal mean** $\bar{y}_{..}$

Dummy variable approach: one factor with 2 levels

- **Comparison of a one-way ANOVA with a simple regression model in dummy coding**

● **SAS program**

● **SAS output**

Dummy variable approach: one factor with 3 levels

- **Comparison of a one-way ANOVA with a simple regression model in dummy coding**

● **SAS program**

● **SAS output**

Multiple Comparisons

If we reject H_0 of no differences in treatment mean in favor of H_a, we conclude that at least one of the t population means differ from the other t-1.

Which means differ from each other?
Which treatment level is the best?

Multiple comparison procedures have been developed to help determine which means are significantly different from each other.

Many different approaches - not all produce the same result.

Duncan, LSD, Bonferroni, Scheffe, Tukey, …

Problems with the confidence assumed for the comparisons.

Problems with the confidence assumed for the multiple comparisons

Suppose we make c mutually orthogonal comparisons, each with Type I (comparisonwise) error rate of α. The experimentwise error rate can be approximated by:

$$e = 1 - (1 - \alpha)^c$$

Error Rates

Number of comparsons	Type I Error Rate	Experimentwise Error Rate
1	0.05	0.050
2	0.05	0.098
3	0.05	0.143
4	0.05	0.185
5	0.05	0.226
6	0.05	0.265
7	0.05	0.302
8	0.05	0.337
9	0.05	0.370
10	0.05	0.401
11	0.05	0.431
12	0.05	0.460
13	0.05	0.487
14	0.05	0.512
15	0.05	0.537
16	0.05	0.560
17	0.05	0.582
18	0.05	0.603
19	0.05	0.623
20	0.05	0.642

Multiple Comparison Procedures

- The major differences among all of the different MCPs is in the calculation of the "**yardstick**" used to determine if two means are significantly different. The yardstick can generically be referred to as the least significant difference. Any two means greater than this difference are declared significantly different.

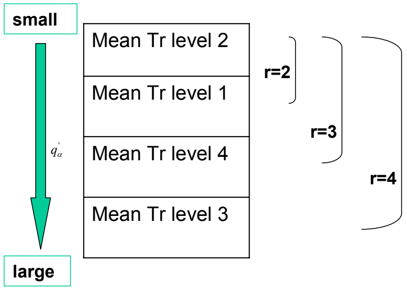
$$\left|\bar{y}_i - \bar{y}_j\right| > \text{"yardstick"} = \text{"TabledValue"} \times \text{"SEofdifference"}$$

- Yardsticks are composed of a standard error term and a critical value from some tabulated statistic.
- Some procedures have "fixed" yardsticks, some have "variable" yardsticks. The variable yardsticks will depend on how far apart two observed means are in a rank ordered list of the mean values.

Duncan's Multiple Range Test

Based on a ranking of the observed means.

Number of steps that means are apart r
2
3
4
5
6
7



$$\left|\bar{y}_i - \bar{y}_j\right| \geq W_r$$

$$W_r = q'_\alpha(r, n_T - t)\sqrt{\frac{MSE}{n}}$$

{ q'_α Tabled values}

Duncan's multiple range test tabled values

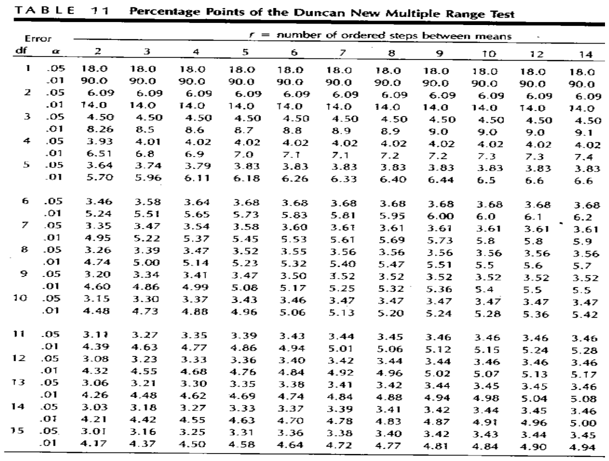


TABLE 11 Percentage Points of the Duncan New Multiple Range Test

Error df	α	2	3	4	5	6	7	8	9	10	12	14
		r = number of ordered steps between means										
1	.05	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
	.01	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
2	.05	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09
	.01	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
3	.05	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
	.01	8.26	8.5	8.6	8.7	8.8	8.9	8.9	9.0	9.0	9.0	9.1
4	.05	3.93	4.01	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02
	.01	6.51	6.8	6.9	7.0	7.1	7.1	7.2	7.2	7.3	7.3	7.4
5	.05	3.64	3.74	3.79	3.83	3.83	3.83	3.83	3.83	3.83	3.83	3.83
	.01	5.70	5.96	6.11	6.18	6.26	6.33	6.40	6.44	6.5	6.6	6.6
6	.05	3.46	3.58	3.64	3.68	3.68	3.68	3.68	3.68	3.68	3.68	3.68
	.01	5.24	5.51	5.65	5.73	5.83	5.81	5.95	6.00	6.0	6.1	6.2
7	.05	3.35	3.47	3.54	3.58	3.60	3.61	3.61	3.61	3.61	3.61	3.61
	.01	4.95	5.22	5.37	5.45	5.53	5.61	5.69	5.73	5.8	5.8	5.9
8	.05	3.26	3.39	3.47	3.52	3.55	3.56	3.56	3.56	3.56	3.56	3.56
	.01	4.74	5.00	5.14	5.23	5.32	5.40	5.47	5.51	5.5	5.6	5.7
9	.05	3.20	3.34	3.41	3.47	3.50	3.52	3.52	3.52	3.52	3.52	3.52
	.01	4.60	4.86	4.99	5.08	5.17	5.25	5.32	5.36	5.4	5.5	5.5
10	.05	3.15	3.30	3.37	3.43	3.46	3.47	3.47	3.47	3.47	3.47	3.47
	.01	4.48	4.73	4.88	4.96	5.06	5.13	5.20	5.24	5.28	5.36	5.42
11	.05	3.11	3.27	3.35	3.39	3.43	3.44	3.45	3.46	3.46	3.46	3.46
	.01	4.39	4.63	4.77	4.86	4.94	5.01	5.06	5.12	5.15	5.24	5.28
12	.05	3.08	3.23	3.33	3.36	3.40	3.42	3.44	3.44	3.46	3.46	3.46
	.01	4.32	4.55	4.68	4.76	4.84	4.92	4.96	5.02	5.07	5.13	5.17
13	.05	3.06	3.21	3.30	3.35	3.38	3.41	3.42	3.44	3.45	3.45	3.46
	.01	4.26	4.48	4.62	4.69	4.74	4.84	4.88	4.94	4.98	5.04	5.08
14	.05	3.03	3.18	3.27	3.33	3.37	3.39	3.41	3.42	3.44	3.45	3.46
	.01	4.21	4.42	4.55	4.63	4.70	4.78	4.83	4.87	4.91	4.96	5.00
15	.05	3.01	3.16	3.25	3.31	3.36	3.38	3.40	3.42	3.43	3.44	3.45
	.01	4.17	4.37	4.50	4.58	4.64	4.72	4.77	4.81	4.84	4.90	4.94

Example of multiple comparison

Study Objective:	Test six varieties of wheat for resistance to a particular race of stem rust.
Experimental factor	Wheat Variety
Levels:	A(i=1), B (i=2), C (i=3), D (i=4), E (i=5)
Experimental Unit:	Pot of well mixed potting soil.
Replication:	Four pots per treatment, four plants per pot.
Randomization:	Varieties randomized to 24 pots (CRD)
Response:	Yield (Y_{ij}) (in grams) of wheat variety(i) at maturity in pot (j).
Implementation Notes:	Six seeds of a variety are planted in a pot. Once plants emerge, the four most vigorous are retained and inoculated with stem rust.

Statistics and AOV Table

Rank	Variety	Mean Yield
5	A	50.3
4	B	69.0
6	C	24.0
2	D	94.0
3	E	75.0
1	F	95.3

$n_1=n_2=n_3=n_4=n_5=n=4$

ANOVA Table

Source	df	MeanSquare	F
Variety	5	2976.44	24.80**
Error	18	120.00	

Duncan's Multiple Comparison Test

$$W_r = q'_\alpha(r, n_T - t)\sqrt{MSE \bullet \left(\tfrac{1}{n}\right)} = q'_{0.05}(r, 18)\sqrt{30}$$

Table
row Error df=18
α = 0.05
col = r

Neighbors

One between

Two between

r	2	3	4	5	6
$q'_\alpha(r,n_T-t)$	2.97	3.12	3.21	3.27	3.32
W_r	16.27	17.09	17.58	17.91	18.18

Duncan'sTest

r	2	3	4	5	6
$q'_\alpha(r,n_T-t)$	2.97	3.12	3.21	3.27	3.32
W_r	16.27	17.09	17.58	17.91	18.18

		C 24.0	A 50.3	B 69.0	E 75.0	D 94.0	F 95.3
C	24.0	--	26.3 ‡	45.0 ‡	51.0 ‡	70.0 ‡	71.3 ‡
A	50.3		--	18.7 ‡	24.7 ‡	43.7 ‡	45.0 ‡
B	69.0			--	6.0	25.0 ‡	26.3 ‡
E	75.0				--	19.0 ‡	20.3 ‡
D	94.0					--	1.3
F	95.3						--

‡ Implies that the two treatment level means are statistically different at the α = 0.05 level.

C^a	A^b	B^c	E^c	D^d	F^d
24.0	50.3	69.0	75.0	94.0	95.3

Duncan grouping: **Means with the same letter are not significantly different**

What's expected to be understood

- **Design and analysis in Multiple regression in Response Surface Methodology. Dependent and independent variables are numeric. The predicted response is studied. Including mixtures.**
- **One way ANOVA including multiple comparison tests. Dependent variable is numeric, independent variables are categorical. Differences between treatment level means (model parameters) are investigated for one experimental factor. Equivalence between regression and ANOVA.**
- **In all approaches Completely Randomised Designs are assumed.**

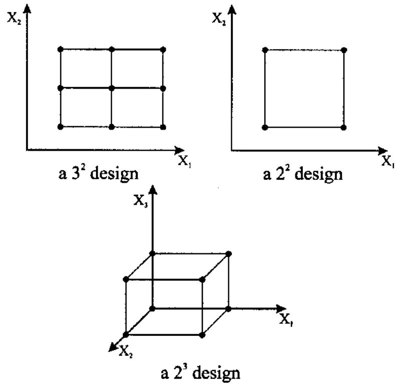
What's next?

Generalisation to more experimental factors and more complicated experimental designs.

Factorial Experiment

Factorial Experiment - an experiment in which the response *y* is observed at all factor level combinations.

Factorial Experiment



Number of treatments

a*b*c*d …

with

a number of levels of factor A
b number of levels of factor B
c number of levels of factor C
d number of levels of factor D

Size of the experiment

R*(a*b*c*d)

with

R number of replications

General Data Layout
Two Factor (a x b) Factorial Design

Column Factor (B)

Row Factor (A)	1	2	3	…	b	Totals
1	T_{11}	T_{12}	T_{13}	…	T_{1b}	$A_{1.}$
2	T_{21}	T_{22}	T_{23}	…	T_{2b}	$A_{2.}$
3	T_{31}	T_{32}	T_{33}	…	T_{3b}	$A_{3.}$
…	…	…	…	…	…	…
a	T_{a1}	T_{a2}	T_{a3}	…	T_{ab}	$A_{a.}$
Totals	B_1	B_2	B_3	…	B_b	G

Possible sums

$$T_{ij} = \sum_{k=1}^{n} y_{ijk} = y_{ij\bullet}$$

$$A_i = \sum_{j=1}^{b} T_{ij} = y_{i\bullet\bullet}$$

$$B_j = \sum_{i=1}^{a} T_{ij} = y_{\bullet j\bullet}$$

$$G = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk} = y_{\bullet\bullet\bullet}$$

y_{ijk}= observed value for the kth replicate for the treatment T_{ij} defined by the combination of the ith level of the row factor and the jth level of the column factor.

n = number of replications.

Model

$$y_{ijk} = \mu_{ij} + \varepsilon_{ijk}$$

μ_{ij} = mean of the ij th table cell, expected value of the response for the combination for the i th row factor level and the j th column factor level.

Overall Test of no treatment differences

$H_0: \quad \mu_{ij} = \mu_{i'j'}$ for all $i, j \neq i', j'$

$H_a: \mu_{ij} \neq \mu_{i'j'}$ for at least one $i, j \neq i', j'$

Test just as for a completely randomized design with a x b treatments.

Sums of Squares (CRD with a x b treatments)

$$TSS = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{\bullet\bullet\bullet})^2 = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n} y_{ijk}^2 - \frac{G^2}{abn}$$

$$SSCells = n\sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij\bullet} - \bar{y}_{\bullet\bullet\bullet})^2 = \frac{1}{n}\sum_{i=1}^{a}\sum_{j=1}^{b} T_{ij}^2 - \frac{G^2}{abn}$$

$$SSW = SSE = \sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n}(y_{ijk} - \bar{y}_{ij\bullet})^2 = TSS - SSCells$$

$$df_{total} = abn - 1$$

$$df_{cells} = ab - 1$$

$$df_{within} = ab(n-1)$$

$$\boxed{\begin{gathered} \text{MSE} = \frac{\text{SSE}}{\text{df}_{\text{within}}} = \hat{\sigma}_{\varepsilon}^2 \\ \text{F} = \frac{\left(\text{SSCells}/\text{df}_{\text{cells}}\right)}{\text{MSE}} \sim \text{F}_{\text{df}_{\text{cells}},\text{df}_{\text{within}}} \end{gathered}}$$

After the Overall F test

As with any experiment, if the hypothesis of equal cell means is rejected, the next step is to determine where the differences are.

In a factorial experiment, there are a number of effects that are always of interest.

- **Main Effect of Treatment Factor A** - Are there differences in the means of the factor A levels (averaged over the levels of factor B).
- **Main Effect of Treatment Factor B** - Are there differences in the means of the factor B levels (averaged over the levels of factor A).
- **Interaction Effects of Factor A with Factor B** - Are the differences between the levels of factor A the same for all levels of factor B? (or equivalently, are the differences among the levels of factor B the same for all levels of factor A?

Main Effect

	Column Factor (B)					
Row Factor(A)	1	2	3	…	b	
1	μ_{11}	μ_{12}	μ_{13}	…	μ_{1b}	$\mu_{1\bullet}$
2	μ_{21}	μ_{22}	μ_{13}	…	μ_{1b}	$\mu_{2\bullet}$
3	μ_{31}	μ_{32}	μ_{13}	…	μ_{1b}	$\mu_{3\bullet}$
…	…	…	…	…	…	…
a	μ_{a1}	μ_{a2}	μ_{a3}	…	μ_{ab}	$\mu_{a\bullet}$
Totals	$\mu_{\bullet 1}$	$\mu_{\bullet 2}$	$\mu_{\bullet 3}$	…	$\mu_{\bullet b}$	$\mu_{\bullet\bullet}$

$$\mu_{1\bullet} = (\tfrac{1}{b})\mu_{11} + (\tfrac{1}{b})\mu_{12} + \cdots + (\tfrac{1}{b})\mu_{1b} = \frac{\sum_{j=1}^{b}\mu_{1j}}{b}$$

$$H_0: \mu_{1\bullet} = \mu_{2\bullet} = \cdots = \mu_{a\bullet}$$

Testing via a set of linear comparison.

Partition of Sums of Squares
Main effect SS for factor A

There are **a** levels of Treatment Factor A. The Sums of Squares for the main effect for treatment differences among levels of Factor A is computed as follows:

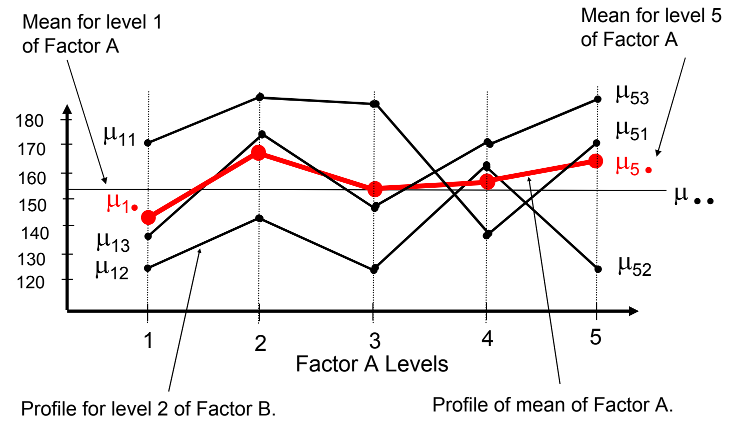
$$SSA = bn\sum_{i=1}^{a}(\bar{y}_{i\bullet\bullet} - \bar{y}_{\bullet\bullet\bullet})^2 \qquad df_A = a - 1$$

$$MSA = \frac{SSA}{df_A}$$

$$F = \frac{MSA}{MSE} \sim F_{df_A, df_{Error}} = F_{(a-1),ab(n-1)}$$

$\boxed{\text{Reject } H_0 \text{ if } F > F_{(a-1),ab(n-1),\alpha}}$

Profile Analysis for Factor A



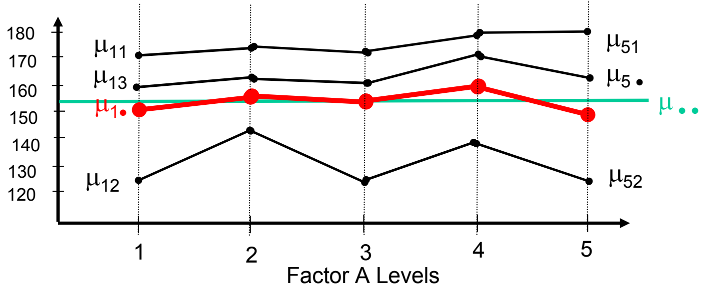
Mean for level 1 of Factor A

Mean for level 5 of Factor A

Profile for level 2 of Factor B.

Profile of mean of Factor A.

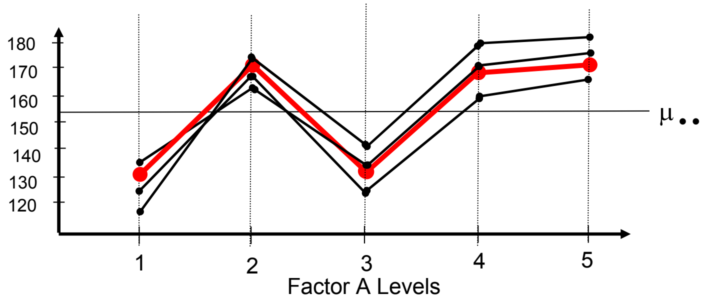
Main Effect for Factor A (2)



Is there strong evidence for a Main Effect for Factor A?

[Significant differences in means at Factor A levels? Compared to the residual variability.]

Main Effect for Factor A (3)



Is there strong evidence for a Main Effect for Factor A?

[Significant differences in means at Factor A levels? Compared to the residual variability.]

Main Effect Linear Comparisons-Factor A

	Column Factor (B)			
Row Factor(A)	1	2	b=3	
1	μ_{11}	μ_{12}	μ_{13}	$\mu_{1\bullet}$
2	μ_{21}	μ_{22}	μ_{13}	$\mu_{2\bullet}$
3	μ_{31}	μ_{32}	μ_{13}	$\mu_{3\bullet}$
4	μ_{41}	μ_{42}	μ_{43}	$\mu_{4\bullet}$
a=5	μ_{51}	μ_{52}	μ_{53}	$\mu_{5\bullet}$
Totals	$\mu_{\bullet 1}$	$\mu_{\bullet 2}$	$\mu_{\bullet 3}$	$\mu_{\bullet\bullet}$

$$\mu_{1\bullet} = (\tfrac{1}{3})\mu_{11} + (\tfrac{1}{3})\mu_{12} + (\tfrac{1}{3})\mu_{13}$$

Model: $E(y_{ijk}) = \mu_{ij} = \mu + \alpha_i + \beta_j$

$$H_0 : \mu_{1\bullet} = \mu_{2\bullet} = \cdots = \mu_{a\bullet}$$

Testing via a set of linear comparison.

$$\text{L1}: \quad \mu_{1\bullet} - \mu_{5\bullet} = 0 \equiv \alpha_1 - \alpha_5 = 0$$
$$\text{L2}: \quad \mu_{2\bullet} - \mu_{5\bullet} = 0 \equiv \alpha_2 - \alpha_5 = 0$$
$$\text{L3}: \quad \mu_{3\bullet} - \mu_{5\bullet} = 0 \equiv \alpha_3 - \alpha_5 = 0$$
$$\text{L4}: \quad \mu_{4\bullet} - \mu_{5\bullet} = 0 \equiv \alpha_4 - \alpha_5 = 0$$

Not mutually orthogonal, but together they represent a-1=4 dimensions of comparison.

Partition of Sums of Squares
Main effect SS for factor B

There are b levels of Treatment Factor B. The Sums of Squares for the main effect for treatment differences among levels of Factor B is computed as follows:

$$SSB = an\sum_{j=1}^{b}(\bar{y}_{\bullet j\bullet} - \bar{y}_{\bullet\bullet\bullet})^2 \qquad df_B = b - 1$$

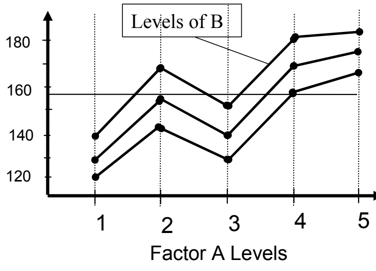
$$MSB = \frac{SSB}{df_B}$$

$$F = \frac{MSB}{MSE} \sim F_{df_B, df_{Error}} = F_{(b-1),ab(n-1)}$$

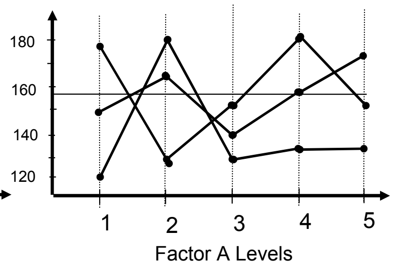
Reject H_0 if $F > F_{(b-1),ab(n-1),\alpha}$

Interaction

Two Factors, A and B, are said to **interact** if the difference in mean response for two levels of one factor is not consistent across levels of the second factor.



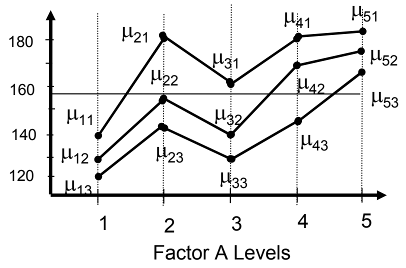
Differences between levels of Factor B do **not** depend on the level of Factor A.



Differences between levels of Factor B **do** depend on the level of Factor A.

Interaction Linear Comparisons

Interaction is inconsistency in differences between two levels of Factor B across levels of Factor A.



$$(\mu_{11} - \mu_{12}) - (\mu_{51} - \mu_{52}) = 0$$
$$(\mu_{21} - \mu_{22}) - (\mu_{51} - \mu_{52}) = 0$$
$$(\mu_{31} - \mu_{32}) - (\mu_{51} - \mu_{52}) = 0$$
$$(\mu_{41} - \mu_{42}) - (\mu_{51} - \mu_{52}) = 0$$

These four linear comparisons tested simultaneously is equivalent to testing that the profile line for level 1 of B is parallel to profile line for level 2 of B.

Four more similar contrasts would be needed to test the profile line for level 1 of B to that of level 3 of B.

Overall Test for Interaction

$$\mathbf{SSAB} = \mathbf{SSCells} - \mathbf{SSA} - \mathbf{SSB}$$
$$= n\sum_{i=1}^{a}\sum_{i=1}^{b}(\bar{y}_{ij\bullet} - \bar{y}_{i\bullet\bullet} - \bar{y}_{\bullet j\bullet} + \bar{y}_{\bullet\bullet\bullet})^2$$
$$= \tfrac{1}{n}\sum_{i=1}^{a}\sum_{j=1}^{b}T_{ij}^2 - SSA - SSB - \frac{G^2}{abn}$$

H_0: No interaction.
H_A: Interaction exists.

$$F = \frac{\frac{\mathrm{SSAB}}{(a-1)(b-1)}}{\mathrm{MSE}} = \frac{\mathrm{MSAB}}{\mathrm{MSE}}$$

$$F > F_{(a-1)(b-1),ab(n-1),\alpha}$$

Critical note: Replications per cell are necessary, otherwise SSAB not estimable in two factor ANOVA

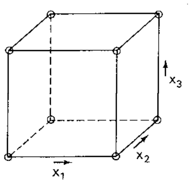
Partitioning of Total Sums of Squares ANOVA table

TSS = SSCells + SSE
= SSA + SSB + SSAB + SSE

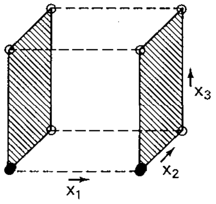
ANOVA Table

Source	df	SS	MS	F
Between Cells	ab-1	SSCells	MSCells	MSCELLS/MSE
Factor A	a-1	SSA	MSA	MSA/MSE
Factor B	b-1	SSB	MSB	MSB/MSE
Interaction	(a-1)(b-1)	SSAB	MSAB	MSAB/MSE
Error(Within Cells)	ab(n-1)	SSE	MSE	
Total (corrected)	abn-1	TSS		

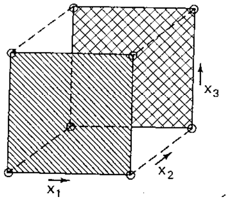
Effects are linear comparisons



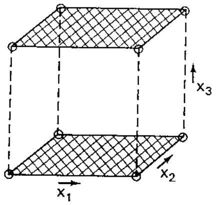
X$_1$ effect



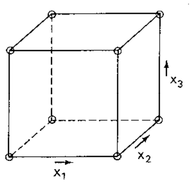
X$_2$ effect



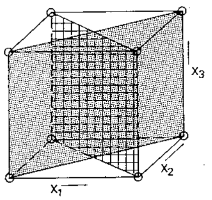
X$_3$ effect



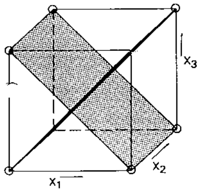
Effects are linear comparisons



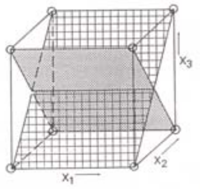
X$_1$X$_2$ effect



X$_1$X$_3$ effect



X$_2$X$_3$ effect



An example of a two way ANOVA

Objectives of the experiment. Investigate the

effect of different insertions of gene A on yield

effect of different insertions of gene B on yield

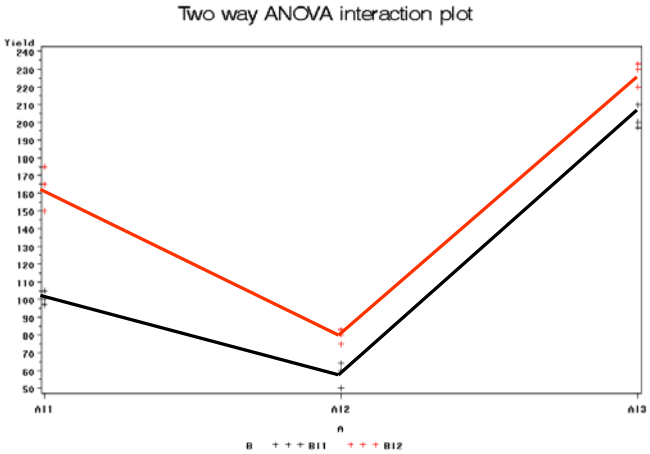
possible interaction between gene A and gene B

- Experimental factors: gene A and gene B
- Factor levels: 3 different insertions for gene A
  2 different insertions for gene B
- Treatments: all possible combinations of insertions of gene A with insertions of gene B
- Replications: 3 plants were harvested per treatment
- Dependent variable: yield
- Size of the experiment: 3*2*3=18 experimental units

An example of a two way ANOVA

- SAS Program
- SAS Output

An example of a two way ANOVA



N-way ANOVA

- Factorial experiment with N experimental factors: A, B, C, D, …
- Completely randomised design
- High order interactions
  - AB, AC, AD, … BC, BD, …
  - ABC, ABD, …
  - ABCD, …
  - …
- Generalisation is straightforward

What’s expected to be understood

- Multiple regression in Response Surface Methodology. Dependent and independent variables are numeric. The predicted response is studied.
- N- way ANOVA including multiple comparison tests. Dependent variable is numeric, independent variables are categorical or fixed numeric. Differences between treatment level means are investigated for N experimental factors, including possible interaction effects between factors. Model parameters are investigated.
- In all approaches Completely Randomised Designs are assumed.

What’s next?

Generalisation to more complicated experimental designs: Randomised Block and Latin Squares Designs