An **ANOVA** is an analysis of the variation present in an experiment. It is a test of the hypothesis that the variation in an experiment is no greater than that due to normal variation of individuals' characteristics and error in their measurement.

Statistics: based on philosophy and the testing of hypothesis.

NO WRONG ANALYSIS

you just might not be testing the hypothesis you think you are testing

Ultimate goal is to say:
"significant differences were found in ... with a probability <0.05."

Hypothesis Testing (Excel exercise):

Donovan TM. & Welden CW. 2002. Spreadsheet Exercises in Ecology and Evolution, Sinauer (now online: http://www.uvm.edu/envnr/vtcfwru/spreadsheets/

	Reject H_0	Fail to reject H_0
H_0 is true:	Type I error (α)	Correct decision. Other ideas?
H_0 is false:	Correct decision. Nobel Prize!	Type II error (β)

Your on-line reference:

A Field Guide to Experimental Designs

http://www.tfrec.wsu.edu/ANOVA/index.html

The Completely Randomized Design (CRD)

The CRD is the simplest of all designs. It is equivalent to a t-test when only two treatments are examined.

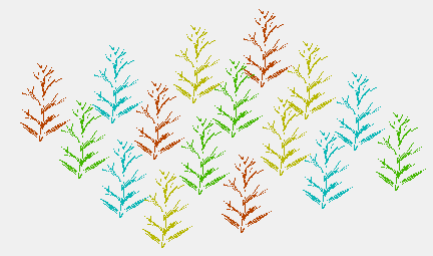
Field marks:

•Replications of treatments are assigned completely at random to independent experimental subjects.
•Adjacent subjects could potentially have the same treatment.

Sample layout:

Different colors represent different treatments. There are 4 (A-D) treatments with 4 replications (1- 4) each.

A1 B1 C1 A2
D1 A3 D2 C2
B2 D3 C3 B3
C4 A4 B4 D4



ANOVA table format:

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
Treatments (*Tr*)	t-1	SSQ_{Tr}	$SSQ_{Tr}/(t-1)$	MS_{Tr}/MS_E
Error (*E*)	t*(r-1)	SSQ_E	$SSQ_E/(t*(r-1))$	
Total (*Tot*)	t*r-1	SSQ_{Tot}		
[a]where t=number of treatments and r=number of replications per treatment.				

Example for Completely Randomized Design (CRD)

A1 B1 C1 A2
D1 A3 D2 C2
B2 D3 C3 B3
C4 A4 B4 D4

Set 1

Treat	Repl	Measure
1	1	10
2	1	5
3	1	12
1	2	11
3	1	1
1	3	7
4	2	1
3	2	14
2	2	3
4	3	2
3	3	15
2	3	3
3	4	17
1	4	9
2	4	4
4	4	1

Example for Completely Randomized Design (CRD)

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	273.471[a]	3	91.157	6.398	.008
Intercept	660.920	1	660.920	46.389	.000
Treat	273.471	3	91.157	6.398	.008
Error	170.967	12	14.247		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .615 (Adjusted R Squared = .519)

aF test with 3,12 degrees of freedom at *P*=0.05 is 3.49

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
Treatments (*Tr*)	t-1	SSQ_{Tr}	$SSQ_{Tr}/(t-1)$	MS_{Tr}/MS_E
Error (*E*)	t*(r-1)	SSQ_E	$SSQ_E/(t*(r-1))$	
Total (*Tot*)	t*r-1	SSQ_{Tot}		

[a]where t=number of treatments and r=number of replications per treatment.

Tests of Between-Subjects Effects

Dependent Variable: Measure

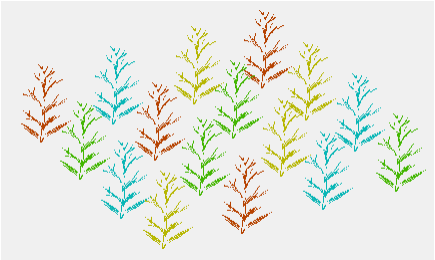
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	273.471[a]	3	91.157	6.398	.008
Intercept	660.920	1	660.920	46.389	.000
Treat	273.471	3	91.157	6.398	.008
Error	170.967	12	14.247		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .615 (Adjusted R Squared = .519)

aF test with 3,12 degrees of freedom at *P*=0.05 is 3.49

The Completely Randomized Design (CRD)

A1 B1 C1 A2
D1 A3 D2 C2
B2 D3 C3 B3
C4 A4 B4 D4



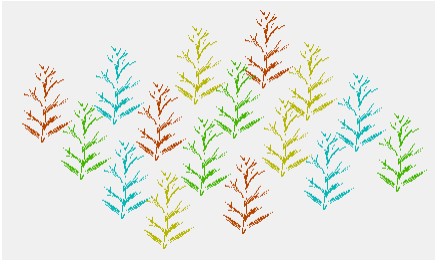
Signal and Noise

Noise Reduction: What if the area is not homogenous?

Signal Promotion: How to get a stronger signal?

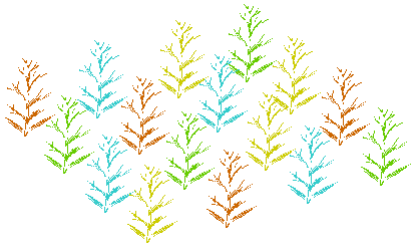
The Completely Randomized Design (CRD)

A1 B1 C1 A2
D1 A3 D2 C2
B2 D3 C3 B3
C4 A4 B4 D4



Gradient?

What's different?



Block I A B C D
Block II D A B C
Block III B D C A
Block IV C A B D

The Randomized Complete Block design (RCB)

Standard design for agricultural experiments. The field or orchard is divided into units to account for any variation in the field. Treatments are then assigned at random to the subjects in the blocks-once in each block.

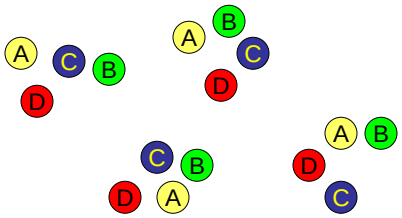
Field marks:

- Treatments are assigned at random within blocks of adjacent subjects, each treatment once per block (non-replicated, replication are possible though).
- The number of blocks is the number of replications.
- Any treatment can be adjacent to any other treatment, but not to the same treatment within the block.
- Used to control variation in an experiment by accounting for spatial effects.

Sample layout:

Different colors represent different treatments; each near horizontal row represents a block. There are 4 blocks (I-IV) and 4 treatments (A-D) in this example.

Block I A B C D
Block II D A B C
Block III B D C A
Block IV C A B D



The Completely Randomized Design (CRD)

ANOVA table format:

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
Blocks (*B*)	b-1	SSQ_B	$SSQ_B/(b-1)$	MS_B/MS_E
Treatments (*Tr*)	t-1	SSQ_{Tr}	$SSQ_{Tr}/(t-1)$	MS_{Tr}/MS_E
Error (*E*)	(t-1)*(b-1)	SSQ_E	$SSQ_E/((t-1)*(b-1))$	
Total (*Tot*)	t*b-1	SSQ_{Tot}		

[a]where t=number of treatments and b=number of blocks or replications.

Set 2

Treat	Repl Now Block	Measure
1	1	10.00
2	1	5.00
3	1	12.00
1	2	11.00
3	1	1.00
1	3	7.00
4	2	1.00
3	2	14.00
2	2	3.00
4	3	2.00
3	3	15.00
2	3	3.00
3	4	17.00
1	4	9.00
2	4	4.00
4	4	1.00

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	302.863[a]	6	50.477	3.209	.057
Intercept	642.604	1	642.604	40.851	.000
Block	29.393	3	9.798	.623	.618
Treat	300.676	3	100.225	6.371	.013
Error	141.574	9	15.730		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .681 (Adjusted R Squared = .469)

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	302.863[a]	6	50.477	3.209	.057
Intercept	642.604	1	642.604	40.851	.000
Block	29.393	3	9.798	.623	.618
Treat	300.676	3	100.225	6.371	.013
Error	141.574	9	15.730		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .681 (Adjusted R Squared = .469)

ANOVA table format:

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
Blocks (*B*)	b-1	SSQ_B	$SSQ_B/(b-1)$	MS_B/MS_E
Treatments (*Tr*)	t-1	SSQ_{Tr}	$SSQ_{Tr}/(t-1)$	MS_{Tr}/MS_E
Error (*E*)	(t-1)*(b-1)	SSQ_E	$SSQ_E/((t-1)*(b-1))$	
Total (*Tot*)	t*b-1	SSQ_{Tot}		

[a]where t=number of treatments and b=number of blocks or replications.

Randomized Complete Block

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	302.863[a]	6	50.477	3.209	.057
Intercept	642.604	1	642.604	40.851	.000
Block	29.393	3	9.798	.623	.618
Treat	300.676	3	100.225	6.371	.013
Error	141.574	9	15.730		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .681 (Adjusted R Squared = .469)

Completely Randomized Design

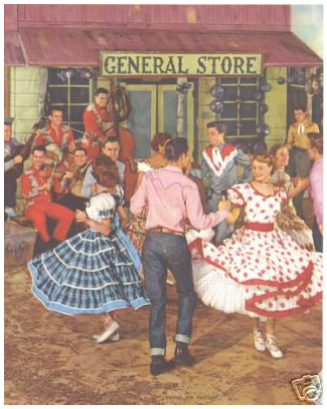
Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	273.471[a]	3	91.157	6.398	.008
Intercept	660.920	1	660.920	46.389	.000
Treat	273.471	3	91.157	6.398	.008
Error	170.967	12	14.247		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .615 (Adjusted R Squared = .519)

The Latin Square design



Stained glass window in the dining hall of Caius College, in Cambridge, commemorating Ronald Fisher and representing a Latin Square

The Latin Square design

The Latin square design is used where the researcher desires to control the variation in an experiment that is related to rows and columns in the field.

Field marks:

•Treatments are assigned at random within rows and columns, with each treatment once per row and once per column.

•There are equal numbers of rows, columns, and treatments.

•Useful where the experimenter desires to control variation in two different directions

Sample layout:

Different colors represent different treatments. There are 4 treatments (A-D) assigned to 4 rows (I-IV) and 4 columns (1-4).

Row I	A	B	C	D
Row II	C	D	A	B
Row III	D	C	B	A
Row IV	B	A	D	C
Column	1	2	3	4

The Latin Square design

ANOVA table format:

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
Rows (*R*)	r-1	SSQ_R	$SSQ_R/(r-1)$	MS_R/MS_E
Columns (*C*)	r-1	SSQ_C	$SSQ_C/(r-1)$	MS_C/MS_E
Treatments (*Tr*)	r-1	SSQ_{Tr}	$SSQ_{Tr}/(r-1)$	MS_{Tr}/MS_E
Error (*E*)	(r-1)(r-2)	SSQ_E	$SSQ_E/((r-1)(r-2))$	
Total (*Tot*)	r^2-1	SSQ_{Tot}		
[a]where r=number of treatments, rows, and columns.				

Set 3

Treat	Measure	Row	Column
1	10	1	1
2	5	1	2
3	12	1	2
4	1	1	3
1	11	2	3
2	3	2	1
3	14	2	4
4	1	2	4
1	7	3	4
2	3	3	4
3	15	3	1
4	2	3	2
1	9	4	2
2	4	4	3
3	17	4	3
4	1	4	1

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	428.509[a]	9	47.612	17.935	.001
Intercept	826.563	1	826.563	311.351	.000
Row	1.134	3	.378	.142	.931
Colum	7.134	3	2.378	.896	.496
Treat	419.188	3	139.729	52.633	.000
Error	15.929	6	2.655		
Total	1271.000	16			
Corrected Total	444.438	15			

a. R Squared = .964 (Adjusted R Squared = .910)

More design: see website and books

Nested design, Split-Plot … etc. all meant to reduse noise (or to account for other possible factors that increase the error)

But we have not used another r rather “simple design” yet ….

Factorial design

Two way Factorial arrangement (on a CRD)

Field marks:

•Treatments are combinations of two factors, such as chemical compositions and methods of applying chemicals. This is an arrangement of treatments within a CRD design.

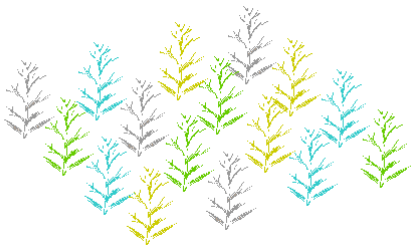
•Replications of treatments are assigned completely at random to independent experimental subjects.

•Adjacent subjects could potentially have the same combination of treatments.

Sample layout:

Different colors represent different combinations of treatments; each combination is randomly assigned to a subject. There are 4 replications (1-4) and 4 treatment combinations (Aa, Ba, Ab and Bb) in this example.

Aa1 Ba1 Ab1 Aa2
Bb1 Aa3 Bb2 Ab2
Ba2 Bb3 Ab3 Ba3
Ab4 Aa4 Ba4 Bb4



ANOVA table format:

Source of variation	Degrees of freedom[a]	Sums of squares (SSQ)	Mean square (MS)	F
First factor (*F1*)	f-1	SSQ_{F1}	$SSQ_{F1}/(f-1)$	MS_{F1}/MS_E
Second factor (*F2*)	s-1	SSQ_{F2}	$SSQ_{F2}/(s-1)$	MS_{F2}/MS_E
First X Second (*FxS*)	(f-1)*(s-1)	SSQ_{FxS}	$SSQ_{FxS}/((f-1)*(s-1))$	MS_{FxS}/MS_E
Error (*E*)	f*s*(r-1)	SSQ_E	$SSQ_E/(f*s*(r-1))$	
Total (*Tot*)	f*s*r-1	SSQ_{Tot}		

[a]where f=number of treatments in the first factor, s=number of treatments in the second factor and r=number of replications.

Nutrient	Water	Repl	Mass	Set 4
0	0	1	3	
0	0	2	2	
0	0	3	4	
0	0	4	2	
0	0	5	3	
1	0	1	7	
1	0	2	6	
1	0	3	5	
1	0	4	7	
1	0	5	6	
0	1	1	2	
0	1	2	2	
0	1	3	3	
0	1	4	1	
0	1	5	2	
1	1	1	12	
1	1	2	14	
1	1	3	17	
1	1	4	14	
1	1	5	16	

Tests of Between-Subjects Effects

Dependent Variable: Measure

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	498.000[a]	3	166.000	116.491	.000
Intercept	819.200	1	819.200	574.877	.000
NutrienT	320.000	1	320.000	224.561	.000
Water	72.200	1	72.200	50.667	.000
NutrienT * Water	105.800	1	105.800	74.246	.000
Error	22.800	16	1.425		
Total	1340.000	20			
Corrected Total	520.800	19			

a. R Squared = .956 (Adjusted R Squared = .948)

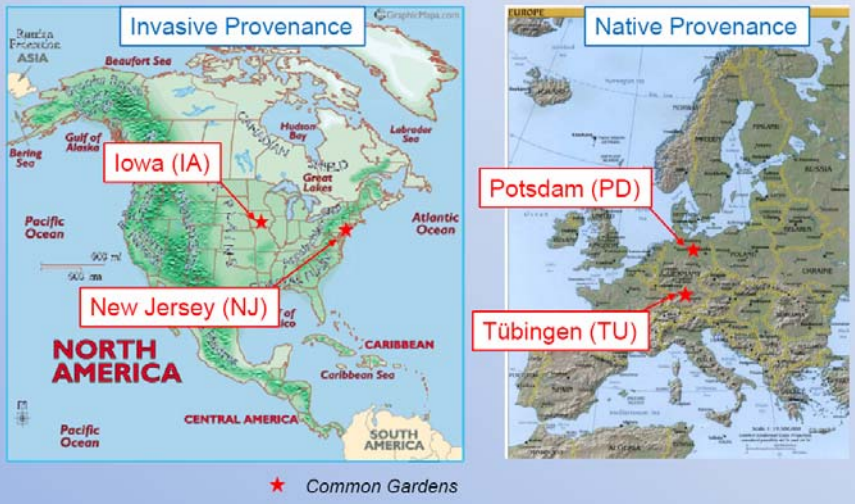
Graphs!!! What does the significant ***Interaction Term*** mean?

And now for something completely different ….

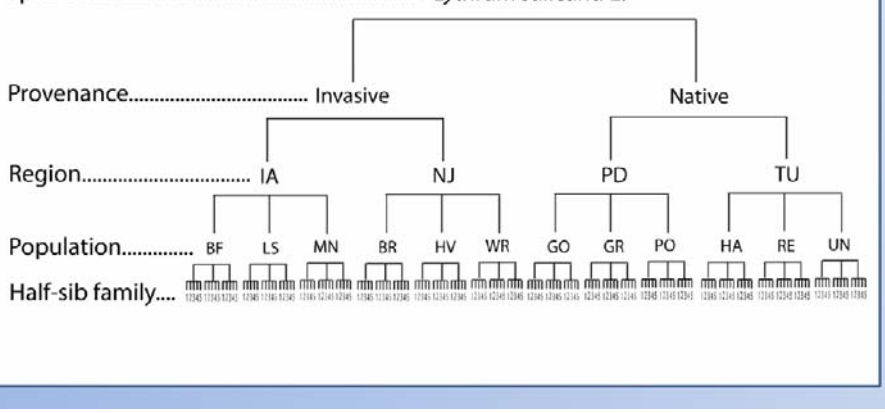
….. Loosestrife

Region

(within Provenance)



Complete Seed Source Design



The loosestrife roof garden data set

Lythrum data 2007 class.xls

To do:

Explore and analyse the data set

1. *Import from Excel into SPSS (I help).*
2. *Calculate "relative reproductive mass" Rel_prod (reproductive mass / total mass) (I will help)*
3. *Plot (bar graph) these two parameters (means totmass)in dependence of Continent , Nut, Wat, etc, …*
4. *Run factorial design ANOVA with block factor (2 block here only)*

…. Think of your own analysis

ENJOY

Set 5

LEGEND (the (al)most important part!)

Lythrum data 2007 class.xls

Blck	Block
TRT	Treatment
Nut	Nutrient level: h and l
Wat	Water level: h and l
Continent	n - North America, e - Europa
Orig	Origin: NJ, IA, PD, TU
Pop	name of population
Fam	number of halfsib -family
SIB -ID	comprehensive name: Orig + Pop + Fam
NewPool	pool number
d_to_flw	days till flowering
date H	date of harvest
d_to_hrv	days till harvest
Height	height in cm
flow_no	number of flowering twigs/shoots
vegmass	vegetative mass
flowmass	generative (flowering) mass

What is the dirtiest word in Statistical Ecology?

Pseudoreplication