

# ANOVA

Three way ANOVA

Latin square design

Hierarchical (nested) ANOVA

Split-plot ANOVA

Repeated measures ANOVA

Mixed-effects models

## The table for one way ANOVA

Source	SS	d.f.	MS	F	p-value
Treat	$SS_T$	$t-1$	$MS_T$	$MS_T/MS_E$	
Error	$SS_E$	$(n-t)$	$MS_E$		

## The table for randomized block experiment

Source	SS	d.f.	MS	F	p-value
Treat	$SS_T$	$t-1$	$MS_T$	$MS_T/MS_E$	
Block	$SS_B$	$b-1$	$MS_B$	$MS_B/MS_E$	
Error	$SS_E$	$(t-1)(b-1)$	$MS_E$		

# Quiz

```
data(mtcars)
```

```
nrow(mtcars) # 32
```

```
mtcars$cyl = as.factor(mtcars$cyl)
```

```
levels(mtcars$cyl) # "4" "6" "8"
```

```
model = aov(mpg~cyl, data = mtcars)
```

```
summary(model)
```

	mpg	cyl	displacement	horsepower	weight	quarter mile time	vs	automatic	gear	carburetors
Mazda RX4	21	6	160	110	2.62	16.46	0	1	4	4
Mazda RX4 Wag	21	6	160	110	2.875	17.02	0	1	4	4
Datsun 710	22.8	4	108	93	3.85	2.32	1	1	4	1
Hornet 4 Drive	21.4	6	258	110	3.08	3.215	1	0	3	1
Hornet Sportabout	18.7	8	360	175	3.15	3.44	0	0	3	2
Valiant	18.1	6	225	105	2.76	3.46	1	0	3	1
Duster 360	14.3	8	360	245	3.21	3.57	0	0	3	4
Merc 240D	24.4	4	146.7	62	3.69	3.19	1	0	4	2
Merc 230	22.8	4	140.8	95	3.92	3.15	1	0	4	2
Merc 280	19.2	6	167.6	123	3.92	3.44	1	0	4	4
Merc 280C	17.8	6	167.6	123	3.92	3.44	1	0	4	4
Merc 450SE	16.4	8	275.8	180	3.07	4.07	0	0	3	3
Merc 450SL	17.3	8	275.8	180	3.07	3.73	0	0	3	3
Merc 450SLC	15.2	8	275.8	180	3.07	3.78	0	0	3	3
Cadillac Fleetwood	10.4	8	472	205	2.93	5.25	0	0	3	4
Lincoln Continental	10.4	8	460	215	3	5.424	0	0	3	4
Chrysler Imperial	14.7	8	440	230	3.23	5.345	0	0	3	4
Fiat 128	32.4	4	78.7	66	4.08	2.2	1	1	4	1
Honda Civic	30.4	4	75.7	52	4.93	1.615	1	1	4	2
Toyota Corolla	33.9	4	71.1	65	4.22	1.835	1	1	4	1
Toyota Corona	21.5	4	120.1	97	3.7	2.465	1	0	3	1
Dodge Challenger	15.5	8	318	150	2.76	3.52	0	0	3	2
AMC Javelin	15.2	8	304	150	3.15	3.435	0	0	3	2
Camaro Z28	13.3	8	350	245	3.73	3.84	0	0	3	4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
cyl					4.98 E-09
Residuals		301.3			
Total		1126.1			

# Quiz

```
data(mtcars)
```

```
nrow(mtcars) # 32
```

```
mtcars$cyl = as.factor(mtcars$cyl)
```

```
levels(mtcars$cyl) # "4" "6" "8"
```

```
model = aov(mpg~cyl, data = mtcars)
```

```
summary(model)
```

	mpg	cyl	disp	hp	drat	wt	qsec	vs	am	gear	carb
Mazda RX4	21	6	160	110	3.9	2.62	16.46	0	1	4	4
Mazda RX4 Wag	21	6	160	110	3.9	2.875	17.02	0	1	4	4
Datsun 710	22.8	4	108	93	3.85	2.32	18.61	1	1	4	1
Hornet 4 Drive	21.4	6	258	110	3.08	3.215	19.44	1	0	3	1
Hornet Sportabout	18.7	8	360	175	3.15	3.44	17.02	0	0	3	2
Valiant	18.1	6	225	105	2.76	3.46	20.22	1	0	3	1
Duster 360	14.3	8	360	245	3.21	3.57	15.84	0	0	3	4
Merc 240D	24.4	4	146.7	62	3.69	3.19	20	1	0	4	2
Merc 230	22.8	4	140.8	95	3.92	3.15	22.9	1	0	4	2
Merc 280	19.2	6	167.6	123	3.92	3.44	18.3	1	0	4	4
Merc 280C	17.8	6	167.6	123	3.92	3.44	18.9	1	0	4	4
Merc 450SE	16.4	8	275.8	180	3.07	4.07	17.4	0	0	3	3
Merc 450SL	17.3	8	275.8	180	3.07	3.73	17.6	0	0	3	3
Merc 450SLC	15.2	8	275.8	180	3.07	3.78	18	0	0	3	3
Cadillac Fleetwood	10.4	8	472	205	2.93	5.25	17.98	0	0	3	4
Lincoln Continental	10.4	8	460	215	3	5.424	17.82	0	0	3	4
Chrysler Imperial	14.7	8	440	230	3.23	5.345	17.42	0	0	3	4
Fiat 128	32.4	4	78.7	66	4.08	2.2	19.47	1	1	4	1
Honda Civic	30.4	4	75.7	52	4.93	1.615	18.52	1	1	4	2
Toyota Corolla	33.9	4	71.1	65	4.22	1.835	19.9	1	1	4	1
Toyota Corona	21.5	4	120.1	97	3.7	2.465	20.01	1	0	3	1
Dodge Challenger	15.5	8	318	150	2.76	3.52	16.87	0	0	3	2
AMC Javelin	15.2	8	304	150	3.15	3.435	17.3	0	0	3	2
Camaro Z28	13.3	8	350	245	3.73	3.84	15.41	0	0	3	4

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
cyl	2	824.8	412.4	39.7	4.98E-09
Residuals	29	301.3	10.4		
Total	31	1126.1			

# Three way ANOVA

# Three way ANOVA

Species	Temp	Sex	Rate
1	M	F	2.6
1	H	M	2.9
1	H	M	2.8
1	H	M	3.4
.	.	.	.
1	H	F	3
1	H	F	2.7
2	L	M	2.1
2	L	M	2.2
2	L	F	2.3
2	L	F	2

species temp sex rate

1 L M 1.9  
 1 L M 1.8  
 1 L M 1.6  
 1 L M 1.4  
 1 L F 1.8  
 1 L F 1.7  
 1 L F 1.4  
 1 L F 1.5  
 1 M M 2.3  
 1 M M 2.1  
 1 M M 2.0  
 1 M M 2.6  
 1 M F 2.4  
 1 M F 2.7  
 1 M F 2.4  
 1 M F 2.6  
 1 H M 2.9  
 1 H M 2.8  
 1 H M 3.4  
 1 H M 3.2  
 1 H F 3.0  
 1 H F 3.1  
 1 H F 3.0  
 1 H F 2.7  
 2 L M 2.1  
 2 L M 2.0  
 2 L M 1.8  
 2 L M 2.2  
 2 L F 2.3  
 2 L F 2.0  
 2 L F 1.9  
 2 L F 1.7  
 2 M M 2.4  
 2 M M 2.6  
 2 M M 2.7  
 2 M M 2.3  
 2 M F 2.0  
 2 M F 2.3  
 2 M F 2.1  
 2 M F 2.4  
 2 H M 3.6  
 2 H M 3.1  
 2 H M 3.4  
 2 H M 3.2  
 2 H F 3.1  
 2 H F 3.0  
 2 H F 2.8  
 2 H F 3.2  
 3 L M 1.1  
 3 L M 1.2  
 3 L M 1.0  
 3 L M 1.4  
 3 L F 1.4  
 3 L F 1.0  
 3 L F 1.3  
 3 L F 1.2  
 3 M M 2.0  
 3 M M 2.1  
 3 M M 1.9  
 3 M M 2.2  
 3 M F 2.4  
 3 M F 2.6  
 3 M F 2.3  
 3 M F 2.2  
 3 H M 2.9  
 3 H M 2.8  
 3 H M 3.0  
 3 H M 3.1  
 3 H F 3.2  
 3 H F 2.9  
 3 H F 2.8  
 3 H F 2.9

# Model

$$\begin{aligned}\text{Rate} = & \beta_0 + \beta_{\text{species}} \times \text{species} \\ & + \beta_{\text{temp}} \times \text{temp} + \beta_{\text{sex}} \times \text{sex} \\ & + \beta_{\text{temp} \times \text{species}} \times \text{temp} \times \text{species} \\ & + \beta_{\text{sex} \times \text{species}} \times \text{sex} \times \text{species} \\ & + \beta_{\text{temp} \times \text{sex}} \times \text{temp} \times \text{sex} \\ & + \beta_{\text{temp} \times \text{species} \times \text{sex}} \times \text{temp} \times \text{species} \times \text{sex} \\ & + \text{error}\end{aligned}$$

# R script

```
# Three way ANOVA
```

```
Dat = read.table('d:/ioz/statistics/2015/3way.ANOVA.txt',  
  sep=' ', header=T)
```

```
Dat$species <- as.factor(Dat$species)
```

```
model <- aov(rate ~ species * temp* sex, data=Dat)
```

```
summary(model)
```

```
summary.lm(model)
```

Species	Temp	Sex	Rate
1	M	F	2.6
1	H	M	2.9
1	H	M	2.8
1	H	M	3.4
.	.	.	.
1	H	F	3
1	H	F	2.7
2	L	M	2.1
2	L	M	2.2
2	L	F	2.3
2	L	F	2

```
species temp sex rate  
1 L M 1.9  
1 L M 1.8  
1 L M 1.6  
1 L M 1.4  
1 L F 1.8  
1 L F 1.7  
1 L F 1.4  
1 L F 1.5  
1 M M 2.3  
1 M M 2.1  
1 M M 2.0  
1 M M 2.6  
1 M F 2.4  
1 M F 2.7  
1 M F 2.4  
1 M F 2.6  
1 H M 2.9  
1 H M 2.8  
1 H M 3.4  
1 H M 3.2  
1 H F 3.0  
1 H F 3.1  
1 H F 3.0  
1 H F 2.7  
2 L M 2.1  
2 L M 2.0  
2 L M 1.8  
2 L M 2.2  
2 L F 2.3  
2 L F 2.0  
2 L F 1.9  
2 L F 1.7  
2 M M 2.4  
2 M M 2.6  
2 M M 2.7  
2 M M 2.3  
2 M F 2.0  
2 M F 2.3  
2 M F 2.1  
2 M F 2.4  
2 H M 3.6  
2 H M 3.1  
2 H M 3.4  
2 H M 3.2  
2 H F 3.1  
2 H F 3.0  
2 H F 2.8  
2 H F 3.2  
3 L M 1.1  
3 L M 1.2  
3 L M 1.0  
3 L M 1.4  
3 L F 1.4  
3 L F 1.0  
3 L F 1.3  
3 L F 1.2  
3 M M 2.0  
3 M M 2.1  
3 M M 1.9  
3 M M 2.2  
3 M F 2.4  
3 M F 2.6  
3 M F 2.3  
3 M F 2.2  
3 H M 2.9  
3 H M 2.8  
3 H M 3.0  
3 H M 3.1  
3 H F 3.2  
3 H F 2.9  
3 H F 2.8  
3 H F 2.9
```



# Results

`summary(model)`

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
species	2	1.8175	0.9088	24.4751	2.72E-08***
temp	2	24.6558	12.3279	332.0237	2.20E-16***
sex	1	0.0089	0.0089	0.2394	0.6266
species:temp	4	1.1017	0.2754	7.4177	7.75E-05***
species:sex	2	0.3703	0.1851	4.9863	0.0103*
temp:sex	2	0.1753	0.0876	2.3603	0.1041
species:temp:sex	4	0.2206	0.0551	1.485	0.2196
Residuals	54	2.005	0.0371		

```
model <- aov(rate ~ species * temp* sex  
             - species : temp: sex,  
             data=Dat)
```

# Results

`summary.lm(model)`

	<b>Estimate</b>	<b>Std. Error</b>	<b>t value</b>	<b>Pr(&gt; t )</b>
(Intercept)	2.95E+00	9.64E-02	30.619	2.00E-16 ***
species2	7.50E-02	1.36E-01	0.55	0.58428
species3	-6.99E-17	1.36E-01	0	1
tempL	-1.35E+00	1.36E-01	-9.908	9.47E-14 ***
tempM	-4.25E-01	1.36E-01	-3.119	0.00291 **
sexM	1.25E-01	1.36E-01	0.917	0.36301
species2:tempL	3.00E-01	1.93E-01	1.557	0.12534
species3:tempL	-3.75E-01	1.93E-01	-1.946	0.05685 .
species2:tempM	-4.00E-01	1.93E-01	-2.076	0.04268 *
species3:tempM	-1.50E-01	1.93E-01	-0.778	0.4397
species2:sexM	1.75E-01	1.93E-01	0.908	0.36781
species3:sexM	-1.25E-01	1.93E-01	-0.649	0.51928
tempL:sexM	-5.00E-02	1.93E-01	-0.259	0.79625
tempM:sexM	-4.00E-01	1.93E-01	-2.076	0.04268 *
species2:tempL:sexM	-2.00E-01	2.73E-01	-0.734	0.46617
species3:tempL:sexM	-7.07E-17	2.73E-01	0	1
species2:tempM:sexM	4.00E-01	2.73E-01	1.468	0.14794
species3:tempM:sexM	7.50E-02	2.73E-01	0.275	0.78419

# Model selection

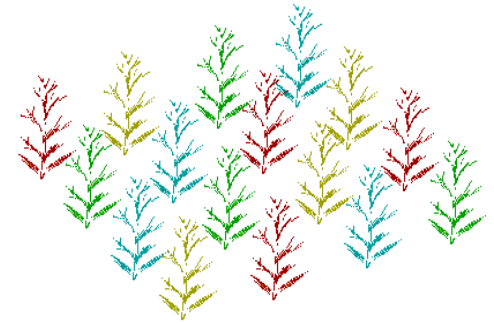
```
model <- aov(rate ~ species * temp* sex  
- species : temp: sex - temp:sex - sex,  
data=Dat)
```

	<b>Df</b>	<b>Sum Sq</b>	<b>Mean Sq</b>	<b>F value</b>	<b>Pr(&gt;F)</b>
species	2	1.817	0.909	22.711	4.54e-08 ***
temp	2	24.656	12.328	308.091	< 2e-16 ***
species:temp	4	1.102	0.275	6.883	0.000125 ***
species:sex	3	0.379	0.126	3.159	0.031070 *
Residuals	60	2.401	0.040		

# Latin square design

An orthogonal experimental design

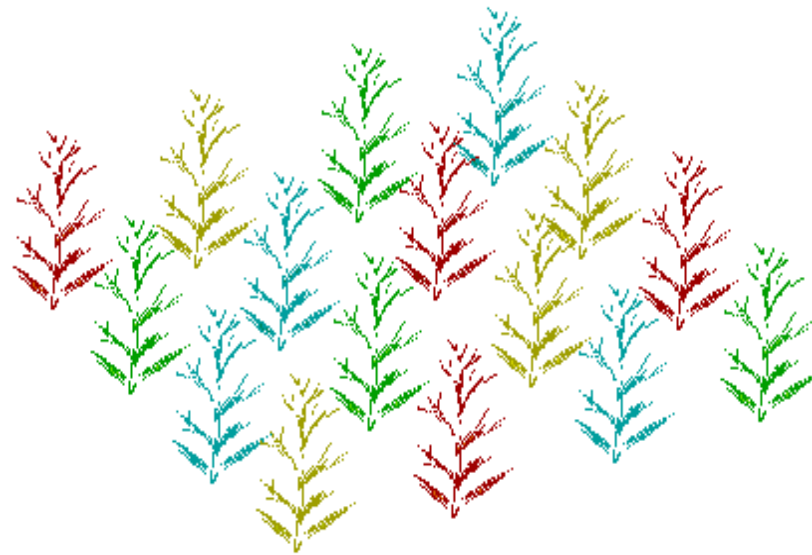
# Latin square design



- Treatments are assigned within rows and columns, with each treatment once per row and once per column.
- There are equal numbers of rows, columns, and treatments (orthogonally designed).
- Useful where the experimenter desires to control variation in two different directions.

# 4×4 Latin square design

Different colors represent different treatments. There are 4 treatments (A-D) assigned to 4 rows (I-IV, e.g. independent days) and 4 columns (1-4, e.g. different species).



# Latin square design solutions

(digits indicate treatments)

$$[1] \quad \begin{bmatrix} 1 & 2 \\ 2 & 1 \end{bmatrix} \quad \begin{bmatrix} 1 & 2 & 3 \\ 2 & 3 & 1 \\ 3 & 1 & 2 \end{bmatrix}$$

$$\begin{bmatrix} 1 & 2 & 3 & 4 \\ 2 & 1 & 4 & 3 \\ 3 & 4 & 1 & 2 \\ 4 & 3 & 2 & 1 \end{bmatrix} \quad \begin{bmatrix} 1 & 2 & 3 & 4 \\ 2 & 4 & 1 & 3 \\ 3 & 1 & 4 & 2 \\ 4 & 3 & 2 & 1 \end{bmatrix}$$

$$\begin{bmatrix} 1 & 2 & 3 & 4 & 5 \\ 2 & 3 & 5 & 1 & 4 \\ 3 & 5 & 4 & 2 & 1 \\ 4 & 1 & 2 & 5 & 3 \\ 5 & 4 & 1 & 3 & 2 \end{bmatrix} \quad \begin{bmatrix} 1 & 2 & 3 & 4 & 5 \\ 2 & 4 & 1 & 5 & 3 \\ 3 & 5 & 4 & 2 & 1 \\ 4 & 1 & 5 & 3 & 2 \\ 5 & 3 & 2 & 1 & 4 \end{bmatrix}$$

# Variance partation

Source of variation	Degrees of freedom <sup>a</sup>	Sums of squares (SS)	Mean square (MS)	F
Rows ( $R$ )	$r-1$	$SS_R$	$SS_R/(r-1)$	$MS_R/MS_E$
Columns ( $C$ )	$r-1$	$SS_C$	$SS_C/(r-1)$	$MS_C/MS_E$
Treatments ( $Tr$ )	$r-1$	$SS_{Tr}$	$SS_{Tr}/(r-1)$	$MS_{Tr}/MS_E$
Error ( $E$ )	$(r-1)(r-2)$	$SS_E$	$SS_E/((r-1)(r-2))$	
Total ( $Tot$ )	$r^2-1$	$SS_{Tot}$		

where  $r$ =number of treatments, rows, and columns.



Example

Change in Blood Sugar Levels in Mice:  
Four individuals of mice, four days, and four treatments are arranged in a Latin square design. The response is the mean change in blood sugar for 4 animals. The treatments are levels of insulin coded as follows:

Level	A	for 150 micro units
	B	300
	C	600
	D	1200

1	I	B	-4.5
1	II	D	92.33
1	III	C	59.83
1	IV	A	-45.
2	I	C	91.83
2	II	A	-48.33
2	III	D	168.99
2	IV	B	89.
3	I	D	86.16
3	II	B	-78.16
3	III	A	-24.17
3	IV	C	101.0
4	I	A	-.17
4	II	C	68.83
4	III	B	25.17
4	IV	D	177.17

Mouse	Day							
	1		2		3		4	
I	B	-4.5	C	91.83	D	86.16	A	-0.17
II	D	92.33	A	-48.33	B	-78.16	C	68.83
III	C	59.83	D	168.99	A	-24.17	B	25.17
IV	A	-45	B	89	C	101	D	177.17

## Model

$$\begin{aligned}\text{Sugar} = & \beta_0 + \beta_{\text{insulin}} \times \text{insulin} \\ & + \beta_{\text{day}} \times \text{day} \\ & + \beta_{\text{group}} \times \text{group} \\ & + \text{error}\end{aligned}$$

Treatment factor: insulin

Intrinsic factor: day

Nonspecific factor: group

*Cox, D. Interaction. International Statistical Review 52(1): 1-24*

# Book “Linear Models with R” by Faraway

library(faraway)

data(abrasion)

lines <-

"id run position material wear

1 1 1 C 235

2 1 2 D 236

3 1 3 B 218

4 1 4 A 268

5 2 1 A 251

6 2 2 B 241

7 2 3 D 227

8 2 4 C 229

9 3 1 D 234

10 3 2 C 273

11 3 3 A 274

12 3 4 B 226

13 4 1 B 195

14 4 2 A 270

15 4 3 C 230

16 4 4 D 225"

abrasion.data <- read.table(con <-

textConnection(lines), header=TRUE)

close(con)

## R script for another example

matrix(abrasion.data\$material, 4, 4)

abrasion.data\$run = as.factor(abrasion.data\$run)

abrasion.data\$position = as.factor(abrasion.data\$position)

fit1 = aov(wear ~ run + position + material, abrasion.data)

fit2 = lm (wear ~ run + position + material, abrasion.data)

summary(fit1)

summary(fit2)

# R results

```
matrix(abrasion.data$material, 4, 4)
```

```
      [,1] [,2] [,3] [,4]
[1,] "C"  "A"  "D"  "B"
[2,] "D"  "B"  "C"  "A"
[3,] "B"  "D"  "A"  "C"
[4,] "A"  "C"  "B"  "D"
```

```
summary(fit2)
```

Coefficients:

	Estimate	Std.Error	t value	Pr(> t )	
(Intercept)	254.750	6.187	41.174	1.37e-08	***
run2	-2.250	5.534	-0.407	0.698423	
run3	12.500	5.534	2.259	0.064657	.
run4	-9.250	5.534	-1.671	0.145658	
position2	26.250	5.534	4.743	0.003180	**
position3	8.500	5.534	1.536	0.175454	
position4	8.250	5.534	1.491	0.186608	
materialB	-45.750	5.534	-8.267	0.000169	***
materialC	-24.000	5.534	-4.337	0.004892	**
materialD	-35.250	5.534	-6.370	0.000703	***

id	run	position	material	wear
1	1	1	C	235
2	1	2	D	236
3	1	3	B	218
4	1	4	A	268
5	2	1	A	251
6	2	2	B	241
7	2	3	D	227
8	2	4	C	229
9	3	1	D	234
10	3	2	C	273
11	3	3	A	274
12	3	4	B	226
13	4	1	B	195
14	4	2	A	270
15	4	3	C	230
16	4	4	D	225

# **Hierarchical (nested) ANOVA**

# Hierarchical (nested) ANOVA

- In some two-factor experiments the level of one factor, say B, is not “cross” or “cross classified” with the other factor, say A, but is “NESTED” with it.
- The levels of B are different for different levels of A.
  - For example: 2 Areas (Study vs Control)
    - 4 sites per area, each with 5 replicates.
    - There is no link from any sites on one area to any sites on another area.

## Example

- There are 8 sites, not 2, not 4 either.



X = replications

Number of sites (S)/replications need not be equal with each sites.

Analysis is carried out using a nested ANOVA not a two-way ANOVA.

# Nested ANOVA vs. two-way ANOVA

- A Nested design is not the same as a two-way ANOVA which is represented by:

	A1	A2	A3
B1	X X X X X	X X X X X	X X X X X
B2	X X X X X	X X X X X	X X X X X
B3	X X X X X	X X X X X	X X X X X

Nested, or hierarchical designs are very common in environmental effects monitoring studies. There are several “Study” and several “Control” Areas.



# Objectives

- The nested design tests two things: (1) difference between “Study” and “Control” areas, and (2) the variability of the sites within areas.
- If we fail to find a significant variability among the sites within areas, then a significant difference between areas would suggest that there is an treatment effect.
- In other words, the variability is due to differences between areas (treatment) and not to variability among the sites.

Treatment	
Area 1	Area 2
Site1	Site3
Site2	Site4

# Notes

- In this kind of situation, however, it is highly likely that we will find variability among the sites.
- Even if it should be significant, however, we can still test to see whether the difference between the areas is significantly larger than the variability among the sites with areas.

# Statistical Model

$$Y_{ijk} = \mu + A_i + B_{(i)j} + \varepsilon_{(ij)k}$$

$i$  indexes “A” (often called the “major factor”)

$(i)j$  indexes “B” within “A” (B is often called the “minor factor”)

$(ij)k$  indexes replication

$$i = 1, 2, \dots, M$$

$$j = 1, 2, \dots, m$$

$$k = 1, 2, \dots, n$$

## Model (continued)

$$Y_{ijk} = \bar{Y}_{...} + (\bar{Y}_{i..} - \bar{Y}_{...}) + (\bar{Y}_{ij.} - \bar{Y}_{i..}) + (Y_{ijk} - \bar{Y}_{ij.})$$

and

$$\begin{aligned} \sum_i \sum_j \sum_k (Y_{ijk} - \bar{Y}_{...})^2 &= \sum_i \sum_j \sum_k (\bar{Y}_{i..} - \bar{Y}_{...})^2 + \sum_i \sum_j \sum_k (\bar{Y}_{ij.} - \bar{Y}_{i..})^2 \\ &\quad + \sum_i \sum_j \sum_k (Y_{ijk} - \bar{Y}_{ij.})^2 \end{aligned}$$

# Model (continued)

Or,

$$TSS = SS_A + SS_{(A)B} + SS_{W_{error}}$$

M <sub>1</sub>				M <sub>2</sub>				M <sub>3</sub>				Areas	j
<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	Sites	j
10	12	8	13	11	13	9	10	13	14	7	10		
14	8	10	12	14	11	10	9	10	13	9	7	Repl.	k
9	10	12	11	8	9	8	8	16	12	5	4		
11	10	10	12	11	11	9	9	13	13	7	7		
10.75				10.0				10.0					
				10.25									

$$= m.n \sum_{i=1}^M (\bar{Y}_{i..} - \bar{Y}_{...})^2 + n \sum_{i=1}^M \sum_{j=1}^m (\bar{Y}_{ij.} - \bar{Y}_{i..})^2 + \sum_{i=1}^M \sum_{j=1}^m \sum_{k=1}^n (Y_{ijk} - \bar{Y}_{ij.})^2$$

Degrees of freedom:

$$M.m.n - 1 = (M-1) + M(m-1) + Mm(n-1)$$

In lecture 5, Two-way ANOVA:

$$SS_A = \sum n_B n_{AB} (\bar{X}_A - \bar{\bar{X}})^2$$

$$SS_B = \sum n_A n_{AB} (\bar{X}_B - \bar{\bar{X}})^2$$

# Example

$M=3, m=4, n=3$ ; 3 Areas, 4 sites within each area, 3 replications per site, total of ( $M.m.n = 36$ ) data points

M <sub>1</sub>				M <sub>2</sub>				M <sub>3</sub>				Areas
<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	Sites
10	12	8	13	11	13	9	10	13	14	7	10	
14	8	10	12	14	11	10	9	10	13	9	7	Repl.
9	10	12	11	8	9	8	8	16	12	5	4	
11	10	10	12	11	11	9	9	13	13	7	7	$\bar{Y}_{ij.}$
10.75				10.0				10.0				$\bar{Y}_{i..}$
				10.25								$\bar{Y}_{...}$

# Example (continue)

$$SS_A = 4 \times 3 [(10.75-10.25)^2 + (10.0-10.25)^2 + (10.0-10.25)^2]$$

$$= 12 (0.25 + 0.0625 + 0.625) = 4.5$$

$$SS_{(A)B} = 3 [(11-10.75)^2 + (10-10.75)^2 + (10-10.75)^2 + (12-10.75)^2 +$$

$$(11-10)^2 + (11-10)^2 + (9-10)^2 + (9-10)^2 +$$

$$(13-10)^2 + (13-10)^2 + (7-10)^2 + (7-10)^2]$$

$$= 3 (42.75) = 128.25$$

$$TSS = 240.75$$

$$SSW_{\text{error}} = 108.0$$

M <sub>1</sub>				M <sub>2</sub>				M <sub>3</sub>				Areas
<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	Sites
10	12	8	13	11	13	9	10	13	14	7	10	
14	8	10	12	14	11	10	9	10	13	9	7	Repl.
9	10	12	11	8	9	8	8	16	12	5	4	
11	10	10	12	11	11	9	9	13	13	7	7	$\bar{Y}_{ij}$ .
10.75				10.0				10.0				$\bar{Y}_{i..}$
10.25								10.25				$\bar{Y}_{...}$

## ANOVA Table for Example

### Nested ANOVA: Observations versus Area, Sites

Source	DF	SS	MS	F	P
Area	2	4.50	2.25	0.158	0.856
Sites (A)B	9	128.25	14.25	3.167	0.012**
Error	24	108.00	4.50		
Total	35	240.75			

$= MS_A / MS_{(A)B}$

$= MS_{(A)B} / MSW_{\text{error}}$



# Summary

- Nested designs are very common in environmental monitoring
- It is a refinement of the one-way ANOVA
- All assumptions of ANOVA hold: normality of residuals, constant variance, etc.
- Can be easily computed using R, SAS, MINITAB, etc.
- Need to be careful about the proper ratio of the Mean squares.
- Always use graphical methods e.g. boxplots and normal plots as visual aids to aid analysis.

**Example:**  
**Hierarchical (nested)**  
**ANOVA**

Length	Mosquito	Cage
58.5	1	1
59.5	1	1
77.8	2	1
80.9	2	1
84.0	3	1
83.6	3	1
70.1	4	1
68.3	4	1
69.8	1	2
69.8	1	2
56.0	2	2
54.5	2	2
50.7	3	2
49.3	3	2
63.8	4	2
65.8	4	2
56.6	1	3
57.5	1	3
77.8	2	3
79.2	2	3
69.9	3	3
69.2	3	3
62.1	4	3
64.5	4	3

## Model

$$\begin{aligned}\text{Length} = & \beta_0 + \beta_{\text{cage}} \times \text{cage} \\ & + \beta_{\text{mosquito}(\text{cage})} \times \text{mosquito}(\text{cage}) \\ & + \text{error}\end{aligned}$$

df?

```
lines <-
"Length  Mosquito  Cage
58.5      1         1
59.5      1         1
77.8      2         1
80.9      2         1
84.0      3         1
83.6      3         1
70.1      4         1
68.3      4         1
69.8      1         2
69.8      1         2
56.0      2         2
54.5      2         2
50.7      3         2
49.3      3         2
63.8      4         2
65.8      4         2
56.6      1         3
57.5      1         3
77.8      2         3
79.2      2         3
69.9      3         3
69.2      3         3
62.1      4         3
64.5      4         3 "
```

```
dat <- read.table(con <-
  textConnection(lines),
  header=TRUE); close(con)
```

# R code

```
dat$Mosquito = as.factor(dat$Mosquito)
dat$Cage = as.factor(dat$Cage)
```

```
# two way ANOVA
```

```
summary(aov(Length ~ Cage * Mosquito, dat))
```

```
# nested ANOVA
```

```
summary(aov(Length ~ Cage / Mosquito, dat))
```

```
> summary(aov(Length ~ Cage * Mosquito, dat))
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Cage	2	665.7	332.8	255.70	1.45e-10 ***
Mosquito	3	260.2	86.7	66.63	9.44e-08 ***
Cage:Mosquito	6	1460.5	243.4	187.00	3.86e-11 ***
Residuals	12	15.6	1.3		

```
> summary(aov(Length ~ Cage / Mosquito, dat))
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Cage	2	665.7	332.8	255.7	1.45e-10 ***
Cage:Mosquito	9	1720.7	191.2	146.9	6.98e-11 ***
Residuals	12	15.6	1.3		

## R results

R does not compute the correct F-statistics, because it uses the residual MS for the denominator in all calculations, which is not applicable for nested ANOVA.

The interaction sum of squares in the nested case is the sum of the subject (nested in) effect and interaction in the crossed model case, as are the degrees of freedom.

**# Manually computation of the F-statistic and p value is needed.**

```
fit = summary(aov(Length ~ Cage / Mosquito, dat))
```

```
F.value = fit[[1]] [1, 3] / fit[[1]] [2, 3]
```

```
p = pf(F.value, fit[[1]] [1, 1], fit[[1]] [2, 1], lower=FALSE); p # 0.23
```

Or, lme() or lmer() are preferred.

**# Another version: nested ANOVA**

```
summary(aov(Length ~ Cage +  
            Error(Cage / Mosquito),  
            dat))
```

Error: Cage

	Df	Sum Sq	Mean Sq
Cage	2	665.7	332.8

Error: Cage:Mosquito

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	9	1721	191.2		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	12	15.62	1.302		

# Split-plot ANOVA

# Split-plot ANOVA

The split-plot design involves two experimental factors, A and B. Levels of A are randomly assigned to whole plots (main plots), and levels of B are randomly assigned to split plots (subplots) within each whole plot.

The design provides more precise information about B than about A.

The Experiment on the Tensile Strength of Paper

A	Pulp Preparation Method	Replicate (or Block) 1			Replicate (or Block) 2			Replicate (or Block) 3		
		1	2	3	1	2	3	1	2	3
B	Temperature (°F)									
	200	30	34	29	28	31	31	31	35	32
	225	35	41	26	32	36	30	37	40	34
	250	37	38	33	40	42	32	41	39	39
	275	36	42	36	41	40	40	40	44	45

# The Split-plot design

- Each replicate (sometimes called **blocks**) has been divided into three parts, called the **whole plots**
- Pulp preparation methods is the **whole plot treatment**, it is a **hard-to-change** factor
- Each whole plot has been divided into four **subplots** or **split-plots**
- Temperature is the **subplot treatment**
- Generally, the hard-to-change factor is assigned to the whole plots

The Experiment on the Tensile Strength of Paper

A	Pulp Preparation Method	Replicate (or Block) 1			Replicate (or Block) 2			Replicate (or Block) 3		
		1	2	3	1	2	3	1	2	3
B	Temperature (°F)									
	200	30	34	29	28	31	31	31	35	32
	225	35	41	26	32	36	30	37	40	34
	250	37	38	33	40	42	32	41	39	39
	275	36	42	36	41	40	40	40	44	45



# Split-plot ANOVA

Recall that nested ANOVA has specific error variance.

M <sub>1</sub>				M <sub>2</sub>				M <sub>3</sub>				Areas
1	2	3	4	5	6	7	8	9	10	11	12	Sites
10	12	8	13	11	13	9	10	13	14	7	10	Repl.
14	8	10	12	14	11	10	9	10	13	9	7	
9	10	12	11	8	9	8	8	16	12	5	4	
11	10	10	12	11	11	9	9	13	13	7	7	
10.75				10.0				10.0				
10.25												

As to split-plot ANOVA, each different plot size is associated with its own error variance, so instead of having one error variance (as in all the ANOVA tables up to this point), we have as **many error terms** as there are different plot sizes.

The analysis is presented as a series of component ANOVA tables, one for each plot size, in a hierarchy from the largest plot size with the lowest replication at the top, down to the smallest plot size with the greatest replication at the bottom.

# Example

- Example – tensile strength in paper manufacturing
  - Three pulp preparation methods
  - Four different temperatures
  - Each replicate requires 12 runs
  - The experimenters want to use three replicates

The Experiment on the Tensile Strength of Paper

A	Pulp Preparation Method	Replicate (or Block) 1			Replicate (or Block) 2			Replicate (or Block) 3		
		1	2	3	1	2	3	1	2	3
		Temperature (°F)								
B	200	30	34	29	28	31	31	31	35	32
	225	35	41	26	32	36	30	37	40	34
	250	37	38	33	40	42	32	41	39	39
	275	36	42	36	41	40	40	40	44	45

# R script

```
# Split-plot
# Tensile strength in paper manufacturing
Y <- c(30,35,37,36,34,41,38,42,29,26,33,36,
      28,32,40,41,31,36,42,40,31,30,32,40,
      31,37,41,40,35,40,39,44,32,34,39,45)

block <- gl(3,12,36) # Three blocks
A      <- gl(3,4,36) # Three pulp preparation methods
B      <- gl(4,1,36) # Four different temperatures
Dat    <- cbind(Y, block, A, B)
fit     <- aov(Y ~ A*B + Error(block/A))
summary(fit)

# Compare regular ANOVA
summary(aov(Y ~ A*B + block))
```

	Y	block	A	B
[1,]	30	1	1	1
[2,]	35	1	1	2
[3,]	37	1	1	3
[4,]	36	1	1	4
[5,]	34	1	2	1
[6,]	41	1	2	2
[7,]	38	1	2	3
[8,]	42	1	2	4
[9,]	29	1	3	1
[10,]	26	1	3	2
[11,]	33	1	3	3
[12,]	36	1	3	4
[13,]	28	2	1	1
[14,]	32	2	1	2
[15,]	40	2	1	3
[16,]	41	2	1	4
[17,]	31	2	2	1
[18,]	36	2	2	2
[19,]	42	2	2	3
[20,]	40	2	2	4
[21,]	31	2	3	1
[22,]	30	2	3	2
[23,]	32	2	3	3
[24,]	40	2	3	4
[25,]	31	3	1	1
[26,]	37	3	1	2
[27,]	41	3	1	3
[28,]	40	3	1	4
[29,]	35	3	2	1
[30,]	40	3	2	2
[31,]	39	3	2	3
[32,]	44	3	2	4
[33,]	32	3	3	1
[34,]	34	3	3	2
[35,]	39	3	3	3
[36,]	45	3	3	4

# R results

```
summary(aov(Y ~ A*B + Error(block/A))) # spilt plot
```

Error: block

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	2	77.56	38.78		

Error: block:A

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
A	2	128.39	64.19	7.078	0.0485 *
Residuals	4	36.28	9.07		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
B	3	434.1	144.69	36.427	7.45e-08 ***
A:B	6	75.2	12.53	3.154	0.0271 *
Residuals	18	71.5	3.97		

```
summary(aov(Y ~ (A + B + block)^2)) # regular ANOVA
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
A	2	128.4	64.19	15.154	0.000521	***
B	3	434.1	144.69	34.157	3.71E-06	***
block	2	77.6	38.78	9.154	0.003852	**
A:B	6	75.2	12.53	2.957	0.051971	.
A:block	4	36.3	9.07	2.141	0.138153	
B:block	6	20.7	3.44	0.813	0.579669	
Residuals	12	50.8	4.24			

The Experiment on the Tensile Strength of Paper									
Pulp Preparation Method	Replicate (or Block) 1			Replicate (or Block) 2			Replicate (or Block) 3		
	1	2	3	1	2	3	1	2	3
Temperature (°F)									
200	30	34	29	28	31	31	31	35	32
225	35	41	26	32	36	30	37	40	34
250	37	38	33	40	42	32	41	39	39
275	36	42	36	41	40	40	40	44	45

# F Ratios

- F ratios are computed somewhat differently because there are two errors
- $F_R = MS_{Block} / MSE_{A:block}$  tests the effectiveness of blocking
- $F_A = MS_A / MSE_{A:block}$  tests the sig. of the A main effect
- $F_B = MS_B / MSE_{A:B:block}$  tests the sig. of the B main effect
- $F_{AB} = MS_{AB} / MSE_{A:B:block}$  tests the sig. of the AB interaction

The Experiment on the Tensile Strength of Paper									
Pulp Preparation Method	Replicate (or Block) 1			Replicate (or Block) 2			Replicate (or Block) 3		
	1	2	3	1	2	3	1	2	3
Temperature (°F)									
200	30	34	29	28	31	31	31	35	32
225	35	41	26	32	36	30	37	40	34
250	37	38	33	40	42	32	41	39	39
275	36	42	36	41	40	40	40	44	45

	yield	block	irrigation	density	fertilizer
1	90	A	control	low	N
2	95	A	control	low	P
3	107	A	control	low	NP
4	92	A	control	medium	N
5	89	A	control	medium	P
6	92	A	control	medium	NP
7	81	A	control	high	N
8	92	A	control	high	P
9	93	A	control	high	NP
10	80	A	irrigated	low	N
11	87	A	irrigated	low	P
12	100	A	irrigated	low	NP
13	121	A	irrigated	medium	N
14	110	A	irrigated	medium	P
15	119	A	irrigated	medium	NP
16	78	A	irrigated	high	N
17	98	A	irrigated	high	P
18	122	A	irrigated	high	NP
19	83	B	control	low	N
20	80	B	control	low	P
21	95	B	control	low	NP
22	98	B	control	medium	N
23	98	B	control	medium	P
24	106	B	control	medium	NP
25	74	B	control	high	N
26	81	B	control	high	P
27	74	B	control	high	NP
28	102	B	irrigated	low	N
29	109	B	irrigated	low	P
30	105	B	irrigated	low	NP
31	99	B	irrigated	medium	N
32	94	B	irrigated	medium	P
33	123	B	irrigated	medium	NP
34	136	B	irrigated	high	N
35	133	B	irrigated	high	P
36	132	B	irrigated	high	NP
37	85	C	control	low	N
38	88	C	control	low	P
39	88	C	control	low	NP
40	112	C	control	medium	N
41	104	C	control	medium	P
42	91	C	control	medium	NP
43	82	C	control	high	N
44	78	C	control	high	P
45	94	C	control	high	NP
46	60	C	irrigated	low	N
47	104	C	irrigated	low	P
48	114	C	irrigated	low	NP
49	90	C	irrigated	medium	N
50	118	C	irrigated	medium	P
51	113	C	irrigated	medium	NP
52	119	C	irrigated	high	N
53	122	C	irrigated	high	P
54	136	C	irrigated	high	NP
55	86	D	control	low	N
56	78	D	control	low	P
57	89	D	control	low	NP
58	79	D	control	medium	N
59	86	D	control	medium	P
60	87	D	control	medium	NP
61	85	D	control	high	N
62	89	D	control	high	P
63	83	D	control	high	NP
64	73	D	irrigated	low	N
65	114	D	irrigated	low	P
66	114	D	irrigated	low	NP
67	109	D	irrigated	medium	N
68	131	D	irrigated	medium	P
69	126	D	irrigated	medium	NP
70	116	D	irrigated	high	N
71	136	D	irrigated	high	P
72	133	D	irrigated	high	NP

# Split-plot ANOVA example

The R Book (Second edition) Page 520 (Crawley 2013)

	yield	block	irrigation	density	fertilizer
1	90	A	control	low	N
2	95	A	control	low	P
3	107	A	control	low	NP
4	92	A	control	medium	N
5	89	A	control	medium	P
6	92	A	control	medium	NP
7	81	A	control	high	N
8	92	A	control	high	P
9	93	A	control	high	NP
10	80	A	irrigated	low	N
11	87	A	irrigated	low	P
12	100	A	irrigated	low	NP
13	121	A	irrigated	medium	N
14	110	A	irrigated	medium	P
15	119	A	irrigated	medium	NP
16	78	A	irrigated	high	N
17	98	A	irrigated	high	P
18	122	A	irrigated	high	NP
19	83	B	control	low	N

# Split-plot ANOVA example

The R Book (Second edition) Page 520 (Crawley 2013)

```
model <- aov(yield ~ irrigation * density * fertilizer + Error(block / irrigation / density))
summary(model)
```

Error: block

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	3	194.4	64.81		

Error: block:irrigation

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
irrigation	1	8278	8278	17.59	0.0247 *
Residuals	3	1412	471		

Error: block:irrigation:density

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
density	2	1758	879.2	3.784	0.0532 .
irrigation:density	2	2747	1373.5	5.912	0.0163 *
Residuals	12	2788	232.3		

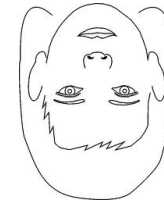
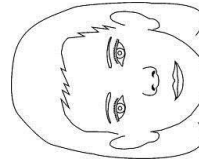
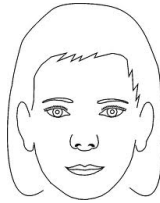
Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
fertilizer	2	1977.4	988.7	11.449	0.000142 ***
irrigation:fertilizer	2	953.4	476.7	5.520	0.008108 **
density:fertilizer	4	304.9	76.2	0.883	0.484053
irrigation:density:fertilizer	4	234.7	58.7	0.680	0.610667
Residuals	36	3108.8	86.4		

# Repeated measures ANOVA



# Face recognition experiment



Subject	regular	90deg	inverted
1	360	320	440
2	120	220	240
3	280	340	400
4	100	80	260
5	240	260	300
6	300	320	380
7	120	140	240
8	400	400	460

m1 = 240 ms

m2 = 260 ms

m3 = 340 ms

# Repeated Measures

- ‘Default’ design: 10 subjects (students) give an observation for condition A, 10 **other** subjects give an observation for condition B, and **another** 10 subjects give an observation for condition C.
- Repeated measures design: 10 subjects give an observation for condition A and the **same** 10 give an observation for condition B and C.
- Condition A, B, and C are usually associated.

# Why should we change the “regular” ANOVA to RM designs?

Repeated measures ANOVA is used when all members of a random sample are measured under a number of different conditions. As the sample is exposed to each condition in turn, the measurement of the dependent variable is repeated.

Using a standard ANOVA in this case is not appropriate because it fails to model the correlation between the repeated measures: the data violate the ANOVA assumption of independence.

Keep in mind that some ANOVA designs combine repeated measures factors and nonrepeated factors. If *any* repeated factor is present, then repeated measures ANOVA should be used.

## “Regular” ANOVA

- Face recognition experiment:
  - Subject1 does condition A, subject2 does condition B
  - Mean rate for A = 500 ms, for B = 600 ms
- Interpretation 1: condition A is easier
- Interpretation 2: subject1 is faster

# Increase power

- In an ANOVA we're always looking at the size of the ratio between the variance between conditions and the 'rest of' the variance—the **unexplained** part (estimated by the average variability within a given condition).
- The more of the unexplained part you can explain and get out of the denominator, the better the chance for the ratio to come out big enough to pass the F test.
- Explaining the variance due to individual differences between subjects is one way of doing this.

# Applications

First, some research hypotheses require repeated measures.

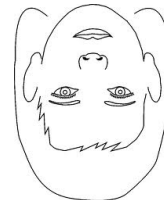
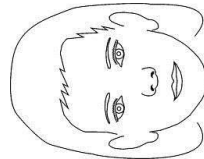
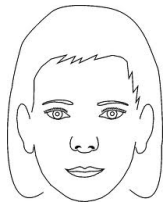
Longitudinal research, for example, measures each sample member at each of several ages. In this case, age would be a repeated factor.

Second, in cases where there is a great deal of variation between sample members, error variance estimates from standard ANOVAs are large. Repeated measures of each sample member provides a way of accounting for this variance, thus reducing error variance.

Third, when sample members are difficult to recruit, repeated measures designs are economical because each member is measured under all conditions.

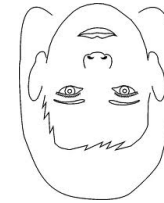
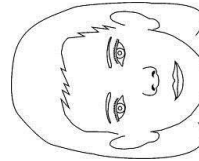
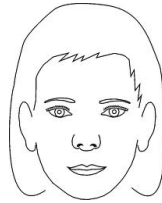
# Example: face recognition experiment

- 8 participants
- 3 conditions: normal, 90-degree turn, 180-degree turn (inverted)



- Measure: rate to recognize

# Data of face recognition experiment



Subject	regular	90deg	inverted
1	360	320	440
2	120	220	240
3	280	340	400
4	100	80	260
5	240	260	300
6	300	320	380
7	120	140	240
8	400	400	460

**m1 = 240 ms**

**m2 = 260 ms**

**m3 = 340 ms**

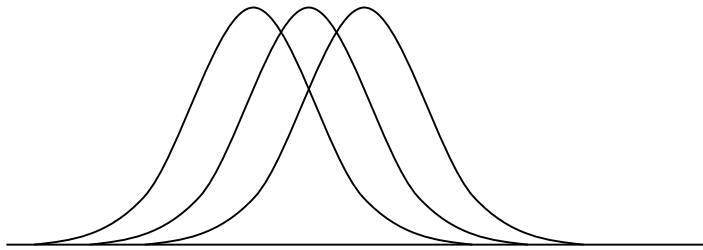


# Regular 1-way ANOVA

- Global Mean = 280 ms
- Total variance ( $SS_{tot}$ ) = 277600
  - Variance for all 24 data points from each other
- Between-conditions variance ( $SS_{bet}$ ) = 44800
  - Variance of the three condition mean rates (means for regular, 90deg, & inverted)

# Regular 1-way ANOVA

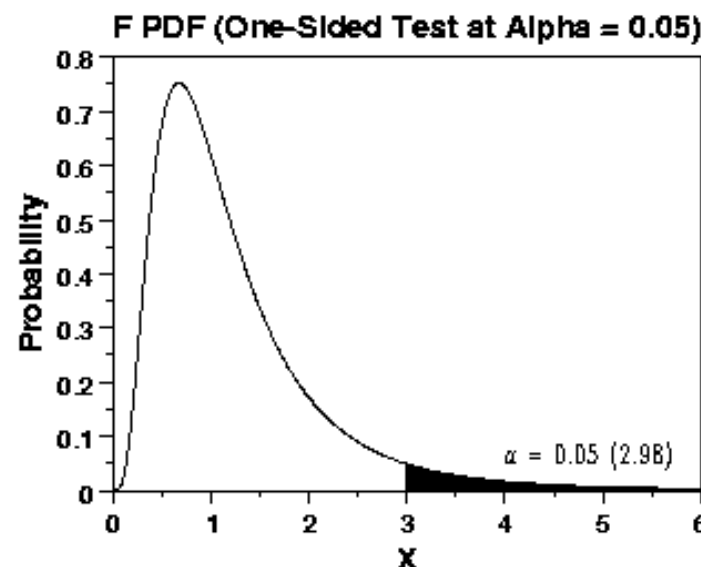
- Within condition variance ( $SS_w$ ) = 232800
  - ‘left-over’ or ‘error’ or ‘unexplained’ variance
  - Conceptually, sum of the variances for each of the three conditions considered separately



- Calculated by just subtracting the  $SS_{bet}$  from the  $SS_{tot}$  (that's why it's ‘left over’)

# Regular 1-way ANOVA

- $MS_{bet} = SS_{bet} / df_{bet}$   
 $= 44800 / (\# \text{ of conditions} - 1)$   
 $= 44800 / 2 = 22400$
- $MS_w = SS_w / df_w$   
 $= 232800 / (\# \text{ of datapoints} - \# \text{ conditions})$   
 $= 232800 / 21 = 11180$
- $F \text{ ratio} = MS_{bet} / MS_w$   
 $= 2.02$
- $F = 2.02$  not significant  
at  $\alpha = .05$ !




# What's the problem?

	regular	90deg	inverted
<b>1</b>	360	320	440
<b>2</b>	120	220	240
<b>3</b>	280	340	400
<b>4</b>	100	80	260
<b>5</b>	240	260	300
<b>6</b>	300	320	380
<b>7</b>	120	140	240
<b>8</b>	400	400	460

Each subject shows the same pattern (regular fastest, inverted slowest), but there's a lot of variability in the absolute times because the subjects differ in their average speed.

# Repeated measures ANOVA

- Within condition variance ( $SS_w$ ) = 232800
- But now we know how to explain some of the ‘unexplained’ variance
  - If we take the subject variance out of  $SS_w$ , there’ll be less variance ‘left over’
- Break up the  $SS_w$  into  $SS_{subj}$  and  $SS_{inter}$



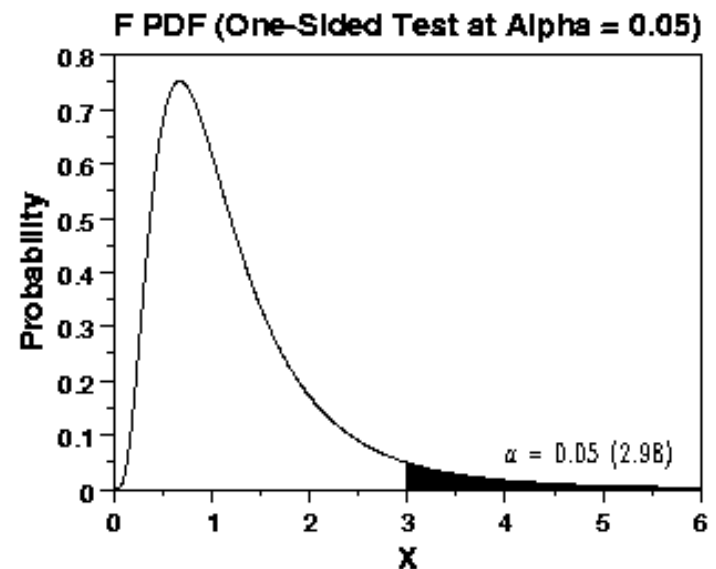
Often known as  
 $SS_{err}$

# Repeated measures ANOVA

- Remember that  $SS_w = 232800$
- Subject variance ( $SS_{subj}$ ) = 219200
  - Variance of the mean rates (averaged over all conditions) for each of the 8 subjects
  - = 94% of the original  $SS_w$  variance!
- Interaction (error) variance ( $SS_{inter}$ ) = 13600
  - Leftovers after subject variance subtracted out ( $SS_w - SS_{subj}$ )

# Repeated measures ANOVA

- $MS_{bet} = SS_{bet} / df_{bet}$   
 $= 44800 / (\# \text{ of conditions} - 1)$   
 $= 22400$
- $MS_{inter} = SS_{inter} / df_{inter}$   
 $= 13600 / (df_{subj}=7 \times df_{bet}=2)$   
 $= 971$
- $F \text{ ratio} = MS_{bet} / MS_{inter}$   
 $= 23.0$
- $F = 23.0$  way above significance cutoff!



# Repeated measures ANOVA

- In a repeated measures design it is possible to partition subject variability from the **treatment** and **error** terms.
- In such a case, variability can be broken down into **between-treatments variability** (or within-subjects effects, excluding individual differences) and **within-treatments variability**.
- The within-treatments variability can be further partitioned into **between-subjects variability** (individual differences) and **error** (excluding the individual differences)



# Assumptions of RMANOVA

- Independent random sampling
- Normal distribution (doesn't really matter if sample is not tiny)
- Homogeneity of variance (need same number of observations for each condition)
- Homogeneity of covariance (sphericity)
  - This actually matters and can be a pain

# Sphericity

- If homogeneity of covariance holds, your population/sample is said to have sphericity.
  - Basic idea of sphericity: there is no tendency for subjects to pattern together more on some conditions than on others
  - Unfortunately, it is pretty common in the real world that you do NOT have sphericity

	regular	90deg	inverted
1	360	320	440
2	120	220	240
3	280	340	400
4	100	80	260
5	240	260	300
6	300	320	380
7	120	140	240
8	400	400	460

# Sphericity

- Sphericity is the property that the degree of interaction (covariance) between any two different levels of the independent variable is the same.
- Sphericity is critical for RM ANOVA because the error term is the average of the pairwise interactions.
- Violations generally lead to inflated F statistics (and hence inflated Type I error).

# Sphericity

- How to find out whether you have it or not: Mauchly test. `mauchly.test()`
- How to correct: use a more conservative F value, e.g. Greenhouse-Geisser or Huynh-Feldt
- Multivariate ANOVA (MANOVA) is an alternative to RMANOVA which is not so sensitive to violations of sphericity

## mauchly.test()

# data

```
Group <- c("A","A","A","A","A","A","A","A","B","B","B","B","B","B","B","B",  
          "C","C","C","C","C","C","C","C")
```

```
Value <- c(1,2,4,1,1,2,2,3,3,4,4,2,3,4,4,3,4,5,3,5,5,3,4,6)
```

```
Participant <- c("1","2","3","4","5","6","7","8","1","2","3","4","5","6","7","8",  
               "1","2","3","4","5","6","7","8")
```

```
data <- data.frame(Participant, Group, Value)
```

# make a matrix such that the rows are the within-subject factor (Participant)

# and the columns are the groups to compare (Group)

```
matrix <- with(data, cbind(Value[Group == "A"], Value[Group == "B"], Value[Group == "C"]))
```

# build a multivariate linear model with the matrix you've just created

```
model <- lm(matrix ~ 1)
```

# define the design of the study, make a list of the independent variable

```
design <- factor(c("A", "B", "C"))
```

# load car package, which has Anova() function including Mauchly's test

```
library(car)
```

```
options(contrasts=c("contr.sum", "contr.poly"))
```

```
aov <- Anova(model, idata = data.frame(design), idesign = ~design, type = "III")
```

```
summary(aov, multivariate = F)
```

## mauchly.test()

Participant	Group	Value
1	A	1
2	A	2
3	A	4
4	A	1
5	A	1
6	A	2
7	A	2
8	A	3
1	B	3
2	B	4
3	B	4
4	B	2
5	B	3
6	B	4
7	B	4
8	B	3
1	C	4
2	C	5
3	C	3
4	C	5
5	C	5
6	C	3
7	C	4
8	C	6

### Univariate Type III Repeated-Measures ANOVA Assuming Sphericity

	SS	num Df	Error SS	den Df	F	Pr(>F)
(Intercept)	253.50	1	5.1667	7	343.45	3.304e-07 ***
design	22.75	2	14.5833	14	10.92	0.001388 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

### Mauchly Tests for Sphericity

	Test statistic	p-value
design	0.63791	0.25958

### Greenhouse-Geisser and Huynh-Feldt Corrections for Departure from Sphericity

	GG eps	Pr(>F[GG])
design	0.73417	0.00452 **

---

Signif. codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05 '.' 0.1 ' ' 1

	HF eps	Pr(>F[HF])
design	0.8809871	0.002348975

# R – repeated ANOVA

## # Repeated measures ANOVA

```
face = read.table("d:/ioz/statistics/repeated_ANOVA/face.csv", header = T, sep = ",")
```

```
face$aspect <- as.factor(face$aspect)
```

```
face$id <- as.factor(face$id)
```

## # id / aspect (aspect within id)

```
face.aov = aov(time ~ aspect + Error(id / aspect), data = face)
```

```
face.aov = aov(time ~ aspect + Error(id), data = face) # same
```

```
summary(face.aov)
```

## # pairwise comparison

```
with(face, pairwise.t.test(time, aspect, p.adjust.method="holm", paired=T))
```

id	aspect	time
1	0	360
2	0	120
3	0	280
4	0	100
5	0	240
6	0	300
7	0	120
8	0	400
1	90	320
2	90	220
3	90	340
4	90	80
5	90	260
6	90	320
7	90	140
8	90	400
1	180	440
2	180	240
3	180	400
4	180	260
5	180	300
6	180	380
7	180	240
8	180	460

## Error: factor(id) (Between Subjects Effects)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Residuals	7	219200	31314		

## Error: Within (Within Subjects Effects)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
factor(aspect)	2	44800	22400	23.1	3.7e-05 ***
Residuals	14	13600	971		

## Summary of GLM

### Commonly used tests, based on the General Linear Model.

Analysis	Response Variable	Explanatory Variable	Interaction	Comments
t-test	1 ratio	1 nominal	Absent	compares two means
1-way ANOVA	1 ratio	1 nominal	Absent	compares 3 or more means in 1 category
2-way ANOVA	1 ratio	2 nominal	Present	tests for interactive effects compares means in 2 categories, if no interaction
Paired Comparison	1 ratio	2 nominal	Assumed Absent	compare 2 means in 1 category, controlled for 2nd category(blocks or units)
Randomized Blocks	1 ratio	2 nominal	Assumed Absent	compare 3 or more means in 1 category, controlled for 2nd category(blocks or sampling units)
Hierarchical ANOVA	1 ratio	$\geq 2$ nominal	Absent	nested comparisons of means
ANCOVA	1 ratio	$\geq 1$ ratio $\geq 1$ nominal	present	compares two or more slopes
			Absent	compares means, controlled for slopes
Regression	1 ratio	1 ratio	Absent	tests linear relation of response to explanatory
Multiple Regression	1 ratio	$\geq 2$ ratio	Assumed Absent	tests linear relation to 2 explanatory variables relation expressed as a plane



# Mixed effect models

# Mixed-effects models

Fisher's ANOVA treated all categorical explanatory variables as if they were the same.

It was Eisenhart (1947) who realized that there were actually two fundamentally different sorts of categorical explanatory variables: he called these fixed effects and random effects.

- Fixed model: generalize the results to experimental values used in the study.
  - drug, placebo; male, female;
- Random model: make inferences beyond the particular values of the study
  - sites, individuals

# Fixed and random effects

- **Mixed model: fixed + random**
  - fixed effects influence only the mean of  $y$ ;
  - random effects influence only the variance of  $y$ .

The random effects come from a large population, there is no point in concentrating on estimating means of our small subset of factor levels, and no point in comparing individual pairs of means for different factor levels.

Much better to recognize them for what they are, random samples from a much larger population, and to concentrate on their variance.

## Random effects model: a one-way ANOVA

$$Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \quad i = 1, \dots, a \text{ levels}; j = 1, \dots, n \text{ obs.}$$

where the  $\alpha$  and  $\varepsilon$  have mean zero, but variances  $\sigma^2_{\alpha}$  and  $\sigma^2_{\varepsilon}$  respectively.

$$\sigma^2_{\varepsilon} = \text{SSE}/(a(n-1)) = \text{MSE}$$

$$\sigma^2_{\alpha} = (\text{MSA} - \text{MSE}) / n$$

# Common cases

- Mixed-effects models are particularly useful in cases where there is
  - temporal pseudoreplication (repeated measurements)
  - spatial pseudoreplication (e.g. nested designs or split-plot experiments).

# R script - randomized block design

```
# Randomized Block Design
# Carbon dioxide density, 8 incubators and 4 treatments
CO2 <- data.frame(ID=1:32, group=NA, treat=NA, density=NA)
n <- 0
for(i in 1:8){
  for(j in c('A','B','C','D')){
    n <- n+1
    CO2$group[n] = i
    CO2$treat[n] = j
  }
}
CO2$group <- factor(CO2$group)
CO2$treat <- factor(CO2$treat)
CO2$density <- c(5.27,5.27,5.94,5.53,5.27,5.22,4.88,4.96,5.88,5.83,
  5.38,5.53,5.44, 5.38,5.27,5.32,5.66, 5.44,5.38,4.88,6.22,
  6.22,5.61,5.92,5.83,5.72,5.38,4.88,5.27,5.11,5.12,4.44)

fit1 <- aov(density ~ treat, data = CO2) # one way ANOVA
fit2 <- aov(density ~ group + treat, data = CO2) # Randomized Block Design
library(lme4) # package
fit3 <- lmer(density ~ treat + (1|group), CO2) # mixed effect model
summary(fit3)
```

Linear mixed model fit by REML ['lmerMod']  
 Formula: density ~ treat + (1 | group)  
 Data: CO2  
 REML criterion at convergence: 23.114  
 Random effects:  

Groups	Name	Variance	Std.Dev.
group	(Intercept)	0.08514	0.2918
Residual		<b>0.06228</b>	0.2496

 Number of obs: 32, groups: group, 8

Fixed effects:

	Estimate	Std. Error	t value
(Intercept)	5.60500	0.13574	41.29
treatB	-0.08125	0.12478	-0.65
treatC	-0.23500	0.12478	-1.88
treatD	-0.42250	0.12478	-3.39

ID	group	treat	density
1	1	A	5.27
2	1	B	5.27
3	1	C	5.94
4	1	D	5.53
5	2	A	5.27
6	2	B	5.22
7	2	C	4.88
8	2	D	4.96
9	3	A	5.88
10	3	B	5.83
11	3	C	5.38
12	3	D	5.53
13	4	A	5.44
14	4	B	5.38
15	4	C	5.27
16	4	D	5.32
17	5	A	5.66
18	5	B	5.44
19	5	C	5.38
20	5	D	4.88
21	6	A	6.22
22	6	B	6.22
23	6	C	5.61
24	6	D	5.92
25	7	A	5.83
26	7	B	5.72
27	7	C	5.38
28	7	D	4.88
29	8	A	5.27
30	8	B	5.11
31	8	C	5.12
32	8	D	4.44

## Randomized block design: compare with results from mixed-effect model

```
summary(fit2) # fit2 <- aov (density ~ group + treat, data = CO2)
```

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
group	7	2.8197	0.4028	6.468	0.000386 ***
treat	3	0.8312	<b>0.2771</b>	4.449	0.014343 *
Residuals	21	1.3078	<b>0.0623</b>		

```
summary(fit3) # lmer(density ~ treat + (1|group), CO2)
```

Random effects:

Groups	Name	Variance	Std.Dev.
group	(Intercept)	0.08514	0.2918
Residual		<b>0.06228</b>	0.2496

```
anova(fit3) # lmer(density ~ treat + (1|group), CO2)
```

Analysis of Variance Table

	Df	Sum Sq	Mean Sq	F value
treat	3	0.83116	<b>0.27705</b>	4.4487

## R script – split plot

### # Crop products

```
Y <- c(30,35,37,36,34,41,38,42,29,26,33,36,
      28,32,40,41,31,36,42,40,31,30,32,40,
      31,37,41,40,35,40,39,44,32,34,39,45)
```

```
block <- gl(3,12,36) # Three blocks
```

```
A <- gl(3,4,36) # Three different fertilizers
```

```
B <- gl(4,1,36) # Four different pesticides
```

```
Dat <- data.frame(Y, block, A, B); head(Dat)
```

```
model <- aov(Y ~ A*B + Error(block/A)) # split plot
```

### # Mixed-effect models

```
library(nlme)
```

```
model1 <- lme(Y ~ A*B, random=~1|block/A, data=Dat)
```

```
summary(model1)
```

```
library(lme4)
```

```
model2 <- lmer(Y ~ A*B+(1|block/A), data=Dat)
```

```
summary(model2)
```

```
anova(model2)
```

Y	block	A	B
30	1	1	1
35	1	1	2
37	1	1	3
36	1	1	4
34	1	2	1
41	1	2	2
38	1	2	3
42	1	2	4
29	1	3	1
26	1	3	2
33	1	3	3
36	1	3	4
28	2	1	1
32	2	1	2
40	2	1	3
41	2	1	4
31	2	2	1
36	2	2	2
42	2	2	3
40	2	2	4
31	2	3	1
30	2	3	2
32	2	3	3
40	2	3	4
31	3	1	1
37	3	1	2
41	3	1	3
40	3	1	4
35	3	2	1
40	3	2	2
39	3	2	3
44	3	2	4
32	3	3	1
34	3	3	2
39	3	3	3
45	3	3	4



# Results of the split plot design

## summary(model2)

Linear mixed model fit by REML

Formula: Y ~ A \* B + (1 | block/A)

Random effects:

Groups	Name	Variance	Std.Dev.
A:block	(Intercept)	1.2743	1.1289
block	(Intercept)	2.4757	1.5734
Residual		3.9722	1.9930

Number of obs: 36, groups: A:block, 9; block, 3

Fixed effects: Y ~ A \* B

## anova(model1)

	numDF	denDF	F-value	p-value
(Intercept)	1	18	1205.021	<.0001
A	2	4	7.0781	0.0485
B	3	18	36.4266	<.0001
A:B	6	18	3.1538	0.0271

	Value	Std.Error	DF	t-value	p-value
(Intercept)	29.66667	1.604392	18	18.49091	0
A2	3.666667	1.87021	4	1.960564	0.1215
A3	1	1.87021	4	0.534699	0.6212
B2	5	1.627313	18	3.072549	0.0066
B3	9.666667	1.627313	18	5.940262	0
B4	9.333333	1.627313	18	5.735425	0
A2:B2	0.666667	2.301369	18	0.289683	0.7754
A3:B2	-5.66667	2.301369	18	-2.4623	0.0241
A2:B3	-3.33333	2.301369	18	-1.44841	0.1647
A3:B3	-5.66667	2.301369	18	-2.4623	0.0241
A2:B4	-0.66667	2.301369	18	-0.28968	0.7754
A3:B4	0.333333	2.301369	18	0.144841	0.8864

# Repeated measure design for time series data

# Crop products

```
Y <- c(30,35,37,36,34,41,38,42,29,26,33,36,
      28,32,40,41,31,36,42,40,31,30,32,40,
      31,37,41,40,35,40,39,44,32,34,39,45)
```

```
block <- gl(3,12,36) # Three blocks
```

```
time <- gl(8,2,36); time <- as.numeric(time) # 8 time periods
```

```
B <- gl(4,1,36) # Four different pesticides
```

```
Dat <- data.frame(Y, block, time, B); head(Dat)
```

# Mixed-effect models

```
library(nlme)
```

```
model <- lme(Y ~ B, random = ~ time | block, data = Dat)
```

```
summary(model)
```

Y	block	time	B
30	1	1	1
35	1	1	2
37	1	2	3
36	1	2	4
34	1	3	1
41	1	3	2
38	1	4	3
42	1	4	4
29	1	5	1
26	1	5	2
33	1	6	3
36	1	6	4
28	2	7	1
32	2	7	2
40	2	8	3
41	2	8	4
31	2	1	1
36	2	1	2
42	2	2	3
40	2	2	4
31	2	3	1
30	2	3	2
32	2	4	3
40	2	4	4
31	3	5	1
37	3	5	2
41	3	6	3
40	3	6	4
35	3	7	1
40	3	7	2
39	3	8	3
44	3	8	4
32	3	1	1
34	3	1	2
39	3	2	3
45	3	2	4

# Repeated measure design for time series data

```
summary(model)
```

Random effects:

Formula: ~time | block

Structure: General positive-definite, Log-Cholesky parametrization

	StdDev	Corr
(Intercept)	2.821343e-05	(Intr)
time	3.563997e-01	-0.034
Residual	3.094827e+00	

Fixed effects: Y ~ B

	Value	Std.Error	DF	t-value	p-value
(Intercept)	31.352421	1.173379	30	26.719775	0.0000
B2	3.333333	1.458915	30	2.284803	0.0296
B3	6.742636	1.467119	30	4.595834	0.0001
B4	9.298191	1.467119	30	6.337720	0.0000

# Mixed effect models for spatially autocorrelated data

*# plot 5 autocorrelation types in package nlme*

```
library(nlme)
```

```
par(mfrow=c(2,3))
```

```
D <- seq(from = 0, to = 1, by = 0.1); Mydata <- data.frame(D = D)
```

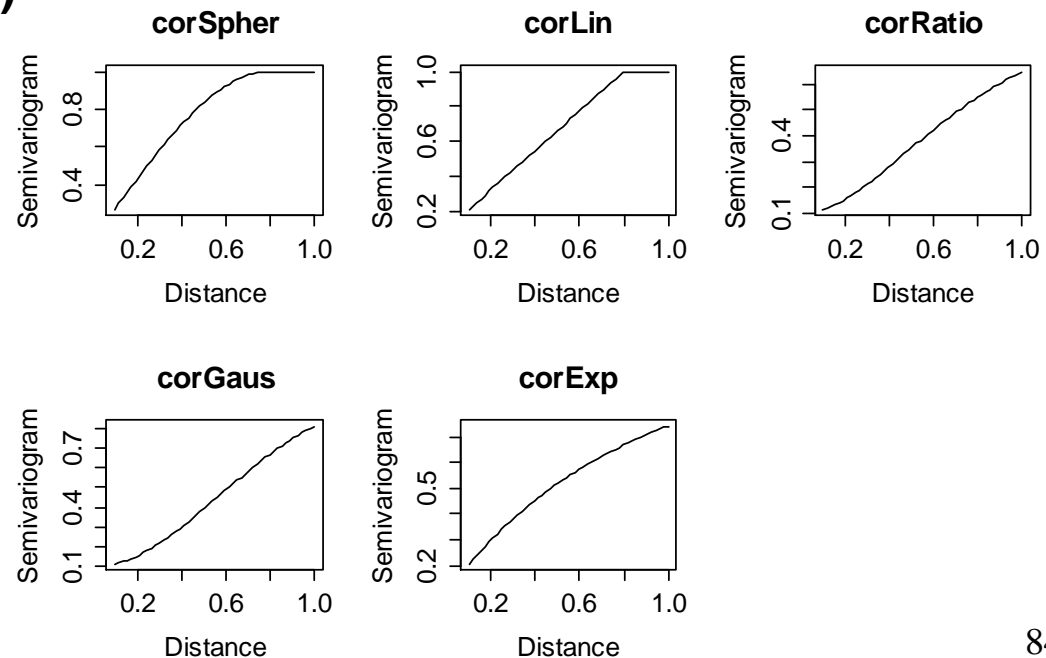
```
autocor <- corSpher(c(0.8, 0.1), form = ~ D, nugget = TRUE)
```

```
autocor <- Initialize(autocor, data = Mydata)
```

```
semivar <- Variogram(autocor)
```

```
plot(semivar[,2], semivar[,1], type = "l", col = 1, xlab = 'Distance', ylab =  
'Semivariogram', main = 'corSpher')
```

- Exponential correlation using the function `corExp`.
- Gaussian correlation using the function `corGaus`.
- Linear correlation using the function `corLin`.
- Rational quadratic correlation using the function `corRatio`.
- Spherical correlation using the function `corSpher`.



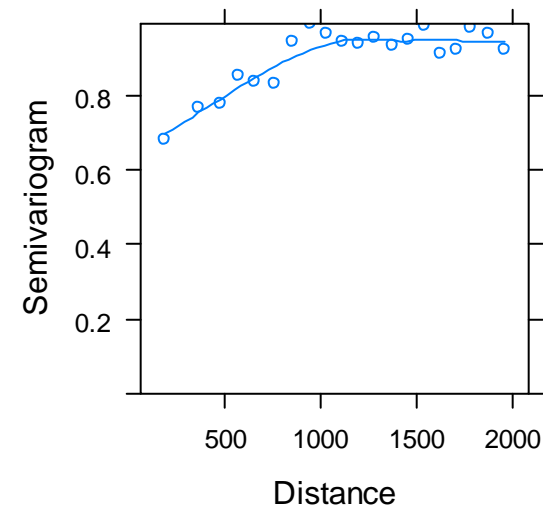
# Mixed effect models for spatially autocorrelated data

*# Mixed effect model (Zuur)*

```
Boreality <- read.table('D:/softwares/R/library/AED/data/Boreality.txt', header=T)
head(Boreality)
```

*# Mixed effect models (Zuur, Page 168)*

```
library(nlme)
f1 <- formula(nBor ~ Wet)
B1.gls <- gls(f1, data = Boreality)
Vario.gls <- Variogram(B1.gls, form =~ x + y,
                      robust = TRUE, maxDist = 2000,
                      resType = "pearson")
plot(Vario.gls, smooth = TRUE)
```



```
B1A <- gls(f1, correlation = corSpher(form =~ x + y, nugget = TRUE), data = Boreality)
B1B <- gls(f1, correlation = corLin (form =~ x + y, nugget = TRUE), data = Boreality)
B1C <- gls(f1, correlation = corRatio (form =~ x + y, nugget = TRUE), data = Boreality)
B1D <- gls(f1, correlation = corGaus (form =~ x + y, nugget = TRUE), data = Boreality)
B1E <- gls(f1, correlation = corExp (form =~ x + y, nugget = TRUE), data = Boreality)
AIC(B1A, B1B, B1C, B1D, B1E)
```

## R script – nested ANOVA

Distinct names for the variables in factorB, like this; then just having factorB as a random effect, is sufficient.

factorA	factorB	response
bob	bob1	6
bob	bob2	8
bob	bob3	12
jane	jane1	9
jane	jane2	7
jane	jane3	10

```
lme(response ~ factorA, random=~1|factorB)
#library(nlme)
```

Same coding for the variables in factorB for each level of factorA, then just having factorB as a random effect is not correct; you instead need the random effect to be the interaction between them.

factorA	factorB
bob	rep1
bob	rep2
bob	rep3
jane	rep1
jane	rep2
jane	rep3

```
lme(response ~ factorA,
      random=~1|factorA:factorB)
```

# R code

## # Split-plot design

```
Model = aov(Y ~ A*B + Error(block/A ), data = D)
library(nlme)
model1 = lme(Y ~ A*B, random=~1|block/A, data=D)
library(lme4)
model2 = lmer(Y ~ A*B+(1|block/A), data=D)
```

## # Hierarchical ANOVA

```
Model = aov(Y ~ A / B, data = D)
```

## # Repeated measures ANOVA

```
Model = aov(Y ~ Time + Error(Subject / Time), data = D)
Model = aov(Y ~ Time + Error(Subject), data = D) # same
model = lme(Y ~ Treat*Time, random = ~1| Subject, data = D)
```

## # Randomized Block Design

```
library(lme4)
Model <- lmer(Y ~ treat + (1|group), data = D) # mixed effect model
```

## ## SS - residual sum of squares for mixed effect models

```
sum(resid(Model)^2)
```

# Notes for experiment design

## Control

We recommend planned comparisons (contrasts) rather than unplanned multiple comparisons. In most cases, you are not interested in comparing all possible groups, but can identify particular questions that are of greater interest.

## Balance

Aim for equal sample sizes. The linear model calculations can easily handle unequal samples, but the analysis is more sensitive to the underlying assumptions and parameter estimates and hypothesis tests will be more reliable if sample sizes are equal.

## Normality

For moderate violations of normality and homogeneity of variances, we recommend proceeding with the analysis, but being cautious about results that are marginally significant or non-significant. Otherwise we recommend using generalized linear models when the underlying distribution of the response variable can be determined, or one of the robust tests.

## Homogeneity of variances

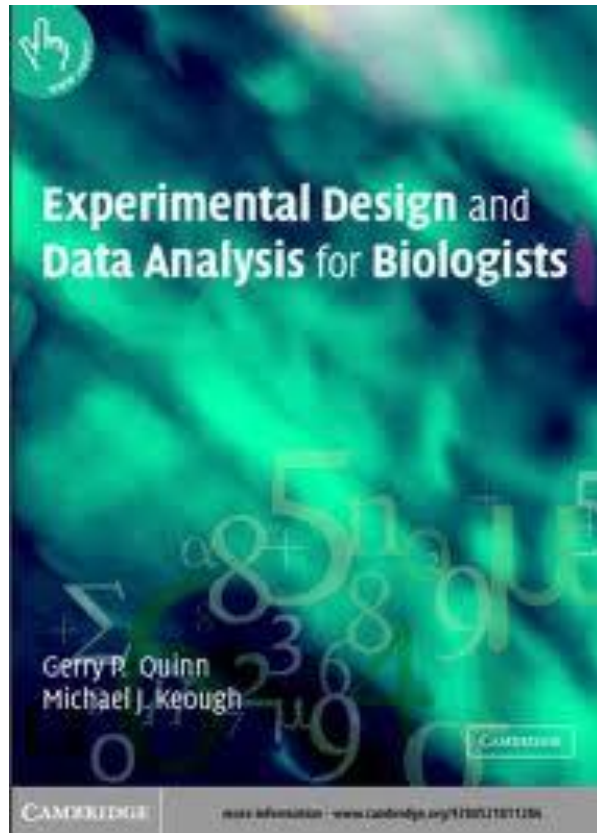
Homogeneity of variances is an important assumption. ANOVA is robust to small and moderate violations (especially with equal sample sizes), but big differences (e.g. manyfold differences between largest and smallest variances) will alter the Type I error rate of the  $F$  test.

## Checking assumptions

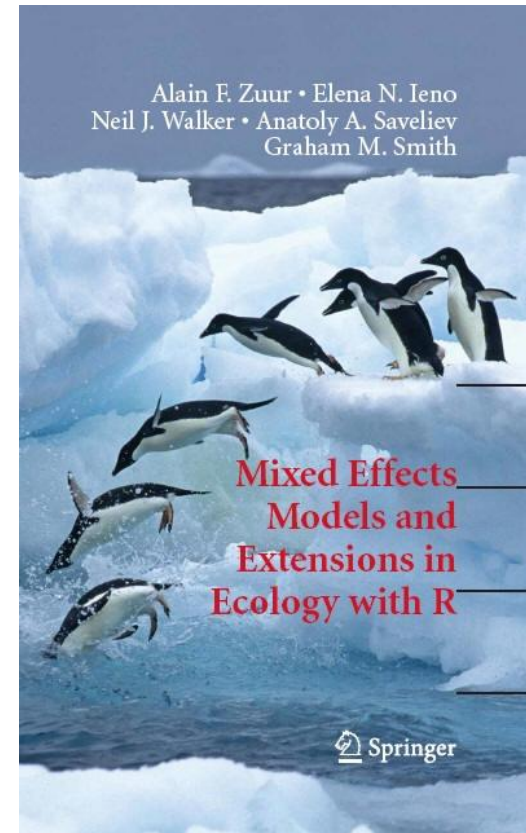
Examine homogeneity of variances with exploratory graphical methods, e.g. look at the spread of boxplots, plot group variances or standard deviations against group means, or plot residuals against group means and look for patterns.



## Suggested books for ANOVA



Quinn, G. P. and Keough, M. J. 2002. Experimental design and data analysis for biologists. Cambridge University Press.



Zuur, A., et al. 2009. Mixed effects models and extensions in ecology with R. Springer.

# Assignment

## General objectives: learn two-way ANOVA.

- Generate your own data (In order to better understand an interaction in a 2-way ANOVA, you create data that has an interaction with a  $P < .05$ . Please describe the experiment and data for interpretation purposes. )
- Provide a brief introduction to the data set,
- Formally state the hypotheses that you are going to test ( $H_0$ 's and  $H_a$ 's),
- Satisfy assumptions of normality of residuals, homogeneity of variances, and independency of residuals, homogeneous of residuals
- Provide a print out's of the data set, programs and their output.
- Indicate in your results and discussion section what you found, i.e. did you reject your null, and the conclusions that you have drawn from the analysis. Since there will be an interaction ( $P < 0.05$ ) then you must break apart your factors and do 1-way ANOVA.

# R script

```
# Bartlett Test of Homogeneity of Variances (parametric)
bartlett.test(split(Dat$y, list(Dat$x1, Dat$x2)))
bartlett.test(Dat$y ~ Dat$x1 * Dat$x2)

# Two Way ANOVA
weight.gain=data.frame(ID=1:60, amount=NA, food=NA, gain=NA)
n=0
for(i in c('high','low')){
  for(j in c('beef','cereal','port')){
    for(k in 1:10){
      n=n+1
      weight.gain$amount[n]=i
      weight.gain$food[n]=j
    }
  }
}
weight.gain$gain=c(73,102,118,104,81,107,100,87,117,111,
98,74,56,111,95,88,82,77,86,92, 94,79,96,98,102,102,108,91,120,105,
90,76,90,64,86,51,72,90,95,78, 107,95,97,80,98,74,74,67,89,58,
49,82,73,86,81,97,106,70,61,82)

fit = aov(gain ~ amount + food + amount:food, data=weight.gain)
fit <- aov(gain ~ amount * food, data=weight.gain) # same thing

summary(fit)

par(mfrow=c(2,2)); plot(fit)
```

## Checking assumptions

```
mtcars$cyl = factor(mtcars$cyl)
mtcars$am = factor(mtcars$am)
fit = lm(mpg~ cyl*am, data=mtcars)
shapiro.test(fit$residuals)
```

Shapiro-Wilk normality test

data: fit\$residuals  
W = 0.96277, p-value = 0.3263

```
library(car)
durbinWatsonTest(fit) # Autocorrelation
```

lag	Autocorrelation	D-W Statistic	p-value
1	0.07110978	1.670616	0.174

Alternative hypothesis: rho != 0

```
leveneTest(fit) # Homogeneity of Variance
```

Levene's Test for Homogeneity of Variance (center = median)

	Df	F value	Pr(>F)
group	5	2.736	0.04086 *
	26		