## Design & Analysis of Experiments Experimental Designs & AN(C)OVA & MAN(C)OVA

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#### **Outline**

- Designs
  - One-way Between-Groups ANOVA (Analysis of Variance)
  - One-way Within-Groups ANOVA
  - Two-way Factorial ANOVA
  - ANCOVA: Adjusting for Covariates
  - MANOVA: Multivariate Analysis of Variable (>1 Response Vars)
  - MANCOVA: Multivariate Analysis of Covariance
- Fitting ANOVA and ANCOVA Models in R
  - Formulas for different types of designs
  - Order matters: Type I, II, and III ordering of formula terms
  - Examples: ANOVA and ANCOVA
  - Assessing ANOVA and ANCOVA Assumptions
- Fitting MANOVA Models in R
  - Examples: One-way MANOVA
  - Assessing MANOVA Assumptions

# Experimental Designs AN(C)OVA & MAN(C)OVA

<u>Focus</u>: Design of experiments = ANalysis Of VAriance

#### Design #1: One-way Between-Groups ANOVA

- Examplar Study: Goal: To study two treatments of anxiety
  - **Treatment (Independent Variable)**: Two treatments
    - CBT: Cognitive Behavior Therapy
    - EMDR: Eye Movement Desensitization & Reprocessing Therapy
  - **Response (Dependent Variable)**: (collected after 5 weeks of treatment)
    - STAI: State-Trait Anxiety Inventory; a self-report measure of anxiety
  - Subjects:
    - Randomly divided between two independent groups: CBT & EMDR

		Subjects
Treatment	CBT	$s_1$
		$s_2$
		•••
		$s_{n/2}$
	EMDR	$s_{n/2+1}$
		•••
		•••
		$s_n$

#### **One-way Between-Groups Balanced ANOVA**

- Treatment is a **between-groups** factor with two levels
- Balanced design: equal number of subjects in each treatment condition; otherwise, unbalanced
- One-way: because a single classification variable

#### F-tests to assess the effects in ANOVA designs

- If the F-test for Treatment is significant then reject the null hypothesis:  $H_0$ : the mean STAI scores are the same
- Conclude: the mean STAI scores changed over time for two therapies differed after five weeks of

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#### Design #2: One-way Within-Groups ANOVA

- Examplar Study: Goal: To study longitudinally one treatment of anxiety
  - Treatment (Independent Variable): One treatment
    - CBT: Cognitive Behavior Therapy
  - Response (Dependent Variable): (collected after 5 weeks of treatment)
    - STAI: State-Trait Anxiety Inventory; a self-report measure of anxiety
  - Subjects:
    - The same subjects over different time points (dependent groups)
  - Time: Two different time points: 5 weeks & 6 months

#### **One-way Within-Groups Balanced ANOVA**

 $\begin{array}{c|c} & \text{Time} \\ \hline \text{Time} \\ \hline \text{Sueeks} & 6 \text{ months} \\ \hline s_1 & s_1 \\ \hline s_2 & s_2 \\ \hline \dots & \dots \\ \hline \end{array}$ 

 $S_n$ 

- Time is a within-groups factor with two levels: each subject is measured under both levels
  - Repeated measures ANOVA
- One-way: because a single classification variable

#### Paired F-tests to assess effects in ANOVA designs

- If the F-test for Treatment is significant then reject the null hypothesis:  $H_0$ : the mean STAI scores are the same
- Conclude: the mean STAI scores change over time: between 5 weeks and 6 months

 $S_n$ 

### Design #3: Factorial (Mixed-Model) ANOVA

- Examplar Study: Goal: To study two treatments of anxiety longitudinally
  - Treatment (Independent Variable): Two treatments
    - CBT: Cognitive Behavior Therapy
    - EMDR: Eye Movement Desensitization & Reprocessing Therapy
  - Response (Dependent Variable): (collected after 5 weeks of treatment)
    - STAI: State-Trait Anxiety Inventory; a self-report measure of anxiety
  - **Subjects**: Randomly assigned to two **independent groups**: CBT & EMDR
  - Time: Two dependent groups over time: 5 weeks & 6 months

		5 wks	6 mo.
ment	CBT	$s_1$	$s_1$
		$s_2$	$s_2$
	S	•••	•••
		$s_{n/2}$	$s_{n/2}$
Treatment		$s_{n/2+1}$	$s_{n/2+1}$
	EMDR	•••	•••
	EM	•••	•••
		$s_n$	$s_n$

#### **Two-way Factorial ANOVA Design**

- Main effects: Impact of Therapy (averaged across Time) and Time (averaged across Therapy type)
- Interaction effect: Interaction of Therapy & Time
- Factorial ANOVA: cross 2 factors (two-way) or more

#### Three F-tests to assess ANOVA design effects

- F-test for Therapy: Significant→ CBI & EMDR differ
- F-test for Time: Significant -> change over time
- F-test for Therapy x Time interaction: Significant → two treatments had a differential impact over time: different change from 5 wks to 6 mo. for 2 therapies<sub>6</sub>

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## Design #4: ANCOVA

- Goal: To study the treatments of anxiety with confounding factors
- Confounding Factor (Nuisance Variable): Other factors (than Treatment) that could explain the post-therapy differences on the dependent variable
  - Depression level:
    - A self-reported measure such as Beck Depression Inventory (BDI)
    - Although subjects were assigned randomly to treatment conditions, it is possible that two therapy groups differed in patient depression levels at the start of the study
    - Any post-therapy differences might be due to the preexisting depression differences and not to experimental manipulations

#### **ANCOVA** Design

- Because depression could also explain the group difference on the dependent variable, it is a confounding factor
- Because the study is not interested in depression, this confounding factor is called a nuisance variable

## Design #5-6: MANOVA & MANCOVA

- Goal: To study the treatments of anxiety with multiple dependent variables and/or confounding factors
- Multiple Dependent Variables: To increase the validity of the study
  - STAI: One dependent variable
  - Family ratings: Another dependent variable
  - Therapy ratings: Yet another dependent variable
  - A measure assessing the impact of anxiety on the daily functioning
- MANOVA: Multivariate Analysis of Variance
  - There is more than one dependent variable
- MANCOVA: Multivariate Analysis of Covariance
  - Besides multiple dependent variables, there are covariates present

# **Experimental Designs FITTING ANOVA & ANCOVA MODELS**

#### **ANOVA and ANCOVA in R: Formula**

aov (formula, data = dataframe

Design	Formula
One-way ANOVA	Response.STAI ~ Treatments.CBT.EMDR
One-way ANCOVA with one covariate	Response.STAI ~ Covariate.Depression + Treatments.CBT.EMDR
Two-way Factorial ANOVA	Response.STAI ~ Treatments.CBT.EMDR * Times.5wks.6mo
Two-way Factorial ANCOVA with two covariates	Response.STAI ~ Covariate.Depression + Covariate.Gender + Treatments.CBT.EMDR * Times.5wks.6mo
One-way within groups ANOVA	Response.STAI ~ Times.5wks.6mo + Error (Subject/Times.5wks.6mo)
Repeated measures ANOVA with one within groups factor (W) and one between-groups factor (B)	Response ~ B * W + Error (Subject/W)

(C)

#### Formula Terms: Order Counts!

- The order in which the effects appear in a formula matters when
  - More than one factor
  - Design is unbalanced: the greater the imbalance in sample sizes, the greater the impact the order of the terms will have on the results
  - Covariates are present
  - $y \sim A * B$  and  $y \sim B * A$  will produce different results
- Type I (sequential) Ordering:  $y \sim A + B + A * B$ 
  - The impact of A on y
  - The impact of B on y, controlling for A
  - The interaction of A and B, controlling for the A and B main effects
  - Summary: the effects are adjusted for those factors that appear earlier in the formula. *A* is unadjusted. *B* is adjusted for the *A*. The *A*: *B* interaction is adjusted for *A* and *B*.

#### Best Practices:

- Strive for balanced designs
- Place more fundamental effects earlier in the formula
- List covariates first in the formula
- Followed by main factors, followed by two-way interactions, followed three-way interactions, and so on

## Other Formula Term Ordering Schemes

- Type I (sequential) Ordering:  $y \sim A + B + A * B$ 
  - Effects are adjusted for those factors that appear earlier in the formula. *A* is unadjusted. *B* is adjusted for *A*. The *A*: *B* interaction is adjusted for *A* and *B*.
  - R's aov() function deploys Type I approach by default

#### Type II (hierarchical) Ordering:::

- Effects are adjusted for other effects at the same or lower level. A is adjusted for B and B is adjusted for A. The A: B interaction is adjusted for both A and B.
- help(Anova, package="car"): allows to choose Type I, II, or III

#### Type III (hierarchical) Ordering: :

- Each effect is adjusted for every other effect in the model. A is adjusted for B and A: B. B is adjusted for A and A: B. The A: B interaction is adjusted for both A and B.
- SAS and SPSS employ Type III approach by default

# Fitting ANOVA Models ONE-WAY BETWEEN-GROUPS

## Ex: One-way Between-Groups ANOVA

```
10 # install.packages("multcomp")
                                   Treatments (trt: Dependent Var):
11 require (multcomp)
                                     * Same drug but administered differently
12 attach (cholesterol)
                                         - 20 mg once per day (1time)
13 head (cholesterol)
                                         - 10 mg twice per day (2times)
                                         - 5 mg four times per day (4times)
                                      drugD
                                     * drugE
                                   Response Var: Cholesterol Reduction
> table(trt)
trt
 1time 2times 4times drugD drugE
                         10
    10
           10
                  10
                                 10
> aggregate (response, by=list(trt), FUN=mean)
  Group.1
                 Х
    1time 5.78197
 2times 9.22497
 4times 12.37478
  drugD 15.36117
    drugE 20.94752
  aggregate (response, by=list(trt), FUN=sd)
  Group.1
                 Х
    1time 2.878113
  2times 3.483054
   4times 2.923119
    drugD 3.454636
                          File: Design_Experiments.ANOVA.MANOVA.R
                                                                         14
    drugE 3.345003
```

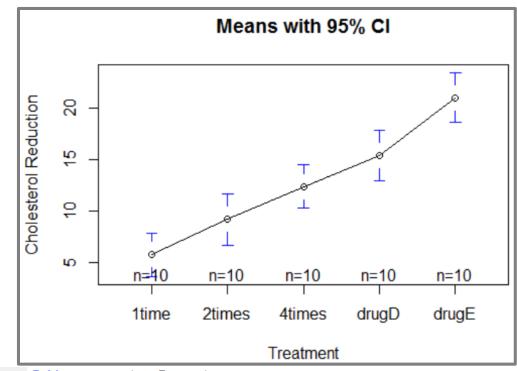
#### Ex: One-way Between-Groups ANOVA (cont.)

36

37

38

- F-test is significant:
  - five treatments are not equally effective
  - it does not tell which treatments differ from one another
- drugE appears to produce the greatest cholesterol reduction based on the plotmeans()



## Multiple Comparisons: TukeyHSD(): pairwise

- F-test is significant:
  - Conclusion: five treatments are not equally effective
  - But: it does not tell which treatments differ from one another

TukeyHSD() provides a test of all pairwise differences between group means

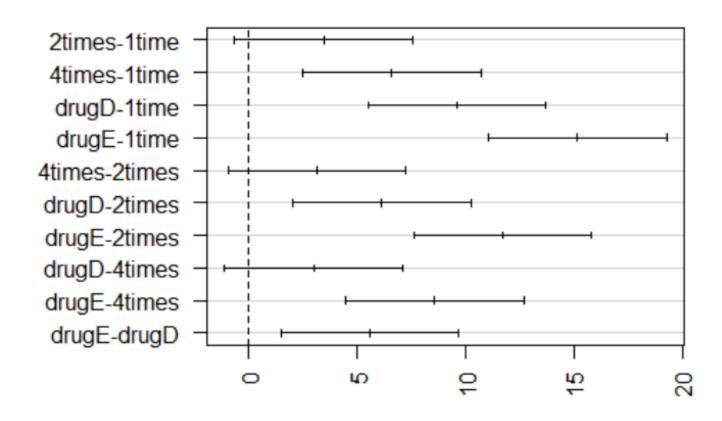
```
> TukeyHSD(fit)
         Tukey multiple comparisons of means
           95% family-wise confidence level
       Fit: aov(formula = response ~ trt)
       $trt
                          diff
                                      lwr
                                                upr
       2times-1time
                       3.44300 -0.6582817
                                           7.544282 0.1380949
                                          10.694092 0.0003542
       4times-1time
                       6.59281
                                2.4915283
                                5.4779183 13.680482 0.0000003
       drugD-1time
                      9.57920
       drugE-1time
                     15.16555 11.0642683
                                          19.266832 0.0000000
       4times-2times
                      3.14981 -0.9514717
                                           7.251092 0.2050382
       drugD-2times
                      6.13620
                                2.0349183 10.237482 0.0009611
                                7.6212683 15.823832 0.0000000
       drugE-2times
                     11.72255
       drugD-4times
                      2.98639 -1.1148917
                                          7.087672 0.2512446
       drugE-4times
                      8.57274
                                4.4714583 12.674022 0.0000037
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                                1.4850683
                       5.58635
                                           9.687632 0.0030633
```

16

## Plot: TukeyHSD(): pairwise comparisons

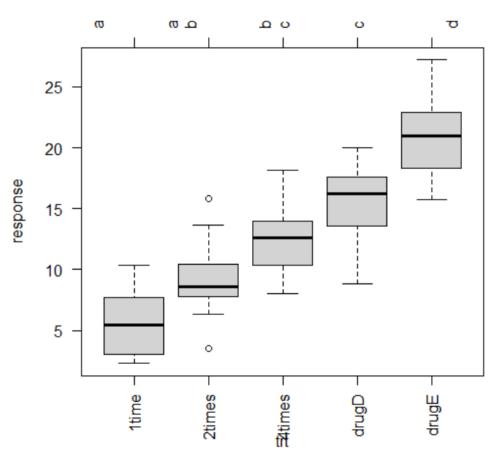
```
50 par(las=2)
51 par (mar=c(5,8,4,2))
52 plot (TukeyHSD(fit))
```

#### 95% family-wise confidence level



## Multiple Comparisons: glht()

**ghlt()** provides a test of **comparing multiple** group means



- 1time & 2times are not significantly different (share letter a)
- 2times & 4times are not significantly different (share letter b)
- 1time & 4times are different (they do not share a letter)
- drugE is superior and is different from all the competitors

```
55 library(multicomp)
56 par (mar = c(5,4,6,2))
57 comp <- glht(fit, linfct=mcp(trt="Tukey"))
58 plot (cld(comp, level=0.05), col="lightgrey")</pre>
```

### **Assumptions: One-way ANOVA**

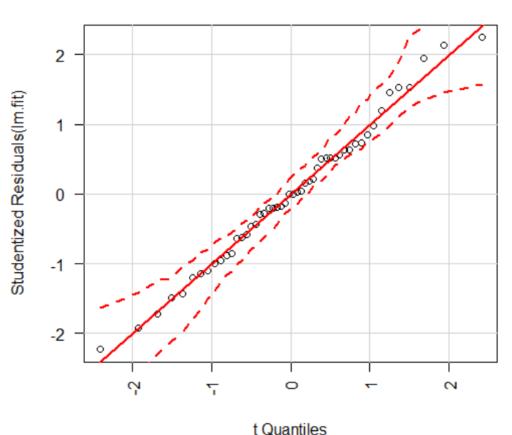
- Assumptions underlying the statistical tests:
  - Dependent variable is normally distributed
  - Dependent variable has equal variance in each trt group
  - ANOVA results could be sensitive to outliers

## **Normality Assumption: One-way ANOVA**

Dependent variable must be normally distributed

Data falls within 95% CI > normality assumption is met

Q-Q Plot



#### **Equal Variance Assumption: One-way ANOVA**

Dependent variable must have equal variance for each group

```
> bartlett.test (response ~ trt, data=cholesterol)
Bartlett test of homogeneity of variances
data: response by trt
Bartlett's K-squared = 0.5797, df = 4, p-value = 0.9653
```

- Fail to reject the null hypothesis (p—value=0.96)
- H<sub>0</sub>: Equality / homogeneity of variances

#### Lack of Outliers Assumption: One-way ANOVA

ANOVA can be sensitive to the presence of outliers

- Fail to reject the null hypothesis (NA: p-value>1)
- H<sub>0</sub>: There is no outliers in the data

# Fitting ANCOVA Models ONE-WAY BETWEEN-GROUPS

### Ex: One-way Between-Groups ANCOVA

- Treatments: four doses of drugs administered to pregnant mice
- **Response**: Post-birth weight
- Covariate: Gestation time

```
87 data (litter, package="multcomp")
88 attach(litter)
89 head(litter)
90 table(dose)
91 fit <- aov(weight ~ gesttime + dose)
92 summary(fit)</pre>
```

```
Df Sum Sq Mean Sq F value Pr(>F)
gesttime 1 134.3 134.30 8.049 0.00597 **
dose 3 137.1 45.71 2.739 0.04988 *
Residuals 69 1151.3 16.69
---
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'
0.1 ' ' 1
```

- Gestation time was related to birth weight
- Drug dosage was related to birth weight after controlling for gestation time
- Mean birth weight isn't the same for each of the drug dosages, after controlling for gestation time:
  - F-test does not say which treatments do not have the same mean birth weight, i.e. which means differ from one another
  - → use glht() for multiple comparisons

## **ANCOVA: Adjusting Group Means**

Original grop means before adjusting for the covariate

Adjusted group means after partialing out the effects of the covariate

```
99 install.packages("effects")
100 library (effects)
101 effect ("dose", fit)
```

```
dose effect
dose
0 5 50 500
32.35367 28.87672 30.56614 29.33460
```

### **ANCOVA:** Multiple Comparisons

- Mean birth weight isn't the same for each of the drug dosages, after controlling for gestation time:
  - F-test does not say which treatments do not have the same mean birth weight, i.e. which means differ from one another
  - → use glht() for multiple comparisons

```
# c(3, -1, -1, -1): comparison of the no-drug group
# with the average of the other three drug groups
library(multicomp)
contrast <- rbind("no drug vs drug" = c(3, -1, -1, -1))
summary(glht(fit, linfct=mcp(dose=contrast)))</pre>
```

```
Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: User-defined Contrasts

Fit: aov(formula = weight ~ gesttime + dose)

Linear Hypotheses:

Estimate Std. Error t value Pr(>|t|)

no drug vs drug == 0 8.284 3.209 2.581 0.012 *

---

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

(Adjusted p values reported -- single-step method)
```

- Significant t-statistic
   (p-value<0.05) →</li>
- Fail to reject H<sub>0</sub>
- No-drug group has higher birth weight than drug conditions

## Assumptions: One-way ANCOVA

- Assumptions underlying the statistical tests:
  - Dependent/response variable is normally distributed
  - Response variable has equal variance in each group
  - ANOVA results could be sensitive to outliers
  - Homogeneity / equality of regression slopes between response variable and covariate for each group

## Homogeneity of Regression Slopes: ANCOVA

- Fail to reject H<sub>0</sub>
- The gestation\*dose interaction is nonsignificant supporting the assumption of equality of slopes

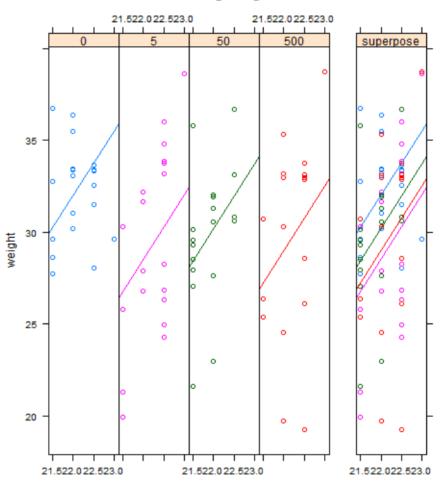
If  $H_0$  were rejected then other tests could be tried out:

nonparametric ANCOVA: help(sm.ancova, package="sm")

#### **ANCOVA: Visualization**

```
131 library (HH)
132 ancova (weight ~ gesttime + dose, data=litter)
```

#### weight ~ gesttime + dose



Relationship between the dependent variable (birth weight), the covariate (gestation time), and the factor (dose)



 Regression lines for predicting birth weight from gestation time are parallel in each group but have different intercepts

# Fitting ANOVA Models TWO-WAY FACTORIAL

#### Ex: Two-Way Between-Groups Factorial ANOVA

#### Subjects are assigned to groups from the cross-classification of factors

- Cross-classification of factors (Independent variable):
  - dose: Three levels of ascorbic acid (0.5, 1, and 2)
  - supp: Two delivery methods (Orange Juice (OJ) and Vitamic C (VC))
- Dependent Variable: Response
  - Tooth length (len) for pigs

```
139 attach (ToothGrowth)
140 table (supp, dose)

dose
supp 0.5 1 2 balanced
0J 10 10 10
VC 10 10 10 design
```

```
aggregate (len,
           by=list(supp,dose),
           FUN=mean)
Group.1 Group.2
            0.5 13.23
     OJ
            0.5 7.98
     VC
           1.0 22.70
     OJ
           1.0 16.77
     VC
            2.0 26.06
     OJ
            2.0 26.14
     VC
```

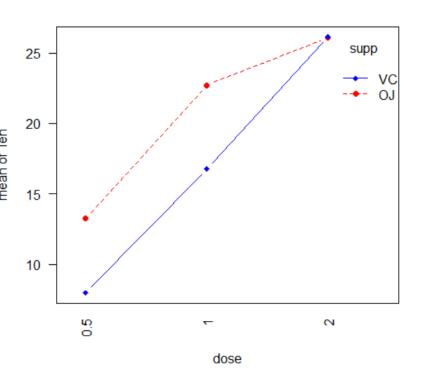
```
> fit <- aov(len ~ supp*dose)</pre>
> summary(fit)
           Df Sum Sq Mean Sq F value
                                       Pr(>F)
            1 205.4
                       205.4 12.317 0.000894 ***
supp
          1 2224.3 2224.3 133.415
dose
supp:dose 1 88.9
                        88.9
                               5.333 0.024631 *
Residuals 56 933.6
                        16.7
               0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Signif. codes:
```

- Main effects: sup and dose
  - significant (p-value < 0.05)</li>
- Interaction between factors: sup\*dose
  - significant (p-value < 0.05)</li>
- Reject the null hypothesis of equal means

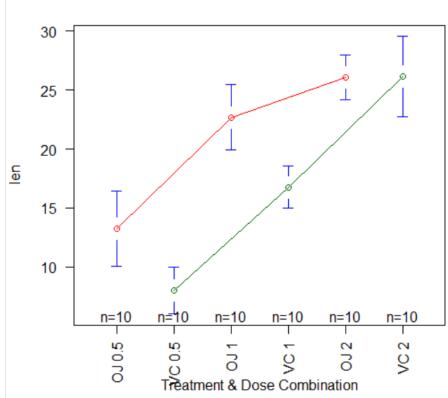
### Visualization: Two-Way Factorial ANOVA

```
interaction.plot (dose, supp, len, type="b",
col=c("red","blue"), pch=c(16,18),
main="Interaction: Dose & Supplement Type")
```

#### Interaction: Dose & Supplement Type

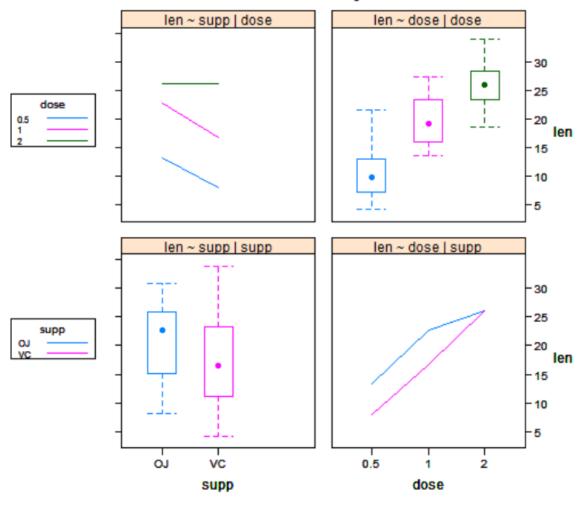


#### Interaction with 95% CI



#### Visualization: Two-Way Factorial ANOVA





167 library (HH) 168 interaction2wt (len~supp\*dose)

## Fitting ANOVA Models

### REPEATED MEASURES

The same subjects are measured more than once

#### Ex: Repeated Measures ANOVA

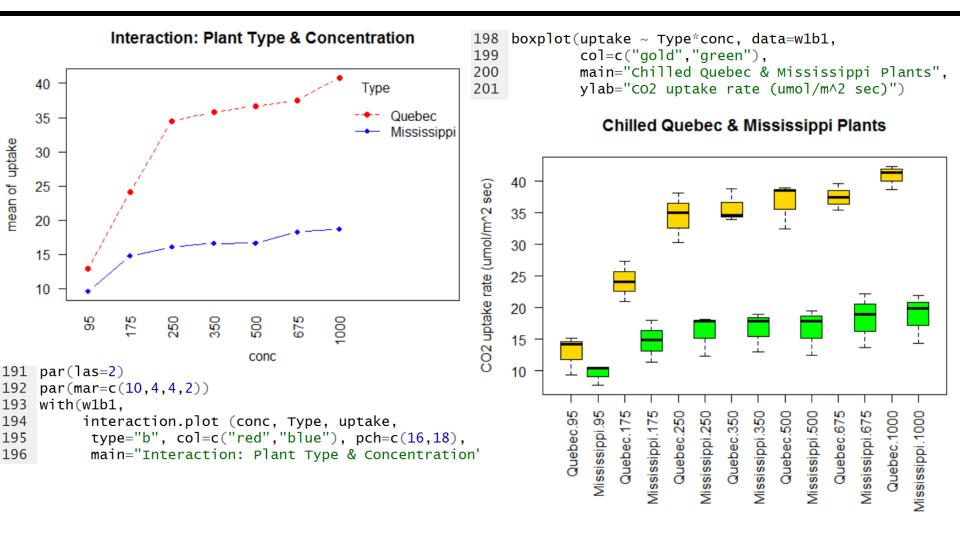
#### Subjects are measured more than once

- Factors (Independent variables):
  - Type: Quebec vs Mississippi (Between-Group)
  - Conc:  $CO_2$  concentration (Within-Group)
- Dependent Variable / Response:
  - Uptake: CO<sub>2</sub> uptake by plants

- The Type and concentration main effects are significant
- Type x conc interaction is significant

```
Frror: Plant
         Df Sum Sq Mean Sq F value Pr(>F)
          1 2667.2
                    2667.2
                             60.41 0.00148 **
Type
Residuals 4 176.6
                      44.1
               0 '***' 0.001 '**' 0.01 '*' 0.05
Signif. codes:
Error: Plant:conc
         Df Sum Sq Mean Sq F value Pr(>F)
                     888.6 215.46 0.000125
          1 888.6
conc
conc:Type 1 239.2 239.2 58.01 0.001595 **
Residuals 4 16.5
                       4.1
               0 '***' 0.001 '**' 0.01 '*' 0.05
Signif. codes:
Error: Within
         Df Sum Sq Mean Sq F value Pr(>F)
Residuals 30
               869
                     28.97
```

#### Visualization: Repeated Measures ANOVA



# Experimental Designs FITTING MANOVA & MANCOVA

There is more than one dependent (outcome) variable

#### Ex: MANOVA: Multivariate Analysis of Variance

#### There is more than one dependent (outcome) variable

- Factors (Independent variables):
  - shelf: Store shelf (1 is bottom; 2 is middle, 3 is top) (Between-Group)
- Dependent Variables / Outcome:
  - calories
  - fat
  - sugars

```
208 library(MASS)
209 attach(Uscereal)
210 y <- cbind(calories, fat, sugars)
211 aggregate (y, by=list(shelf), FUN=mean)

Group.1 calories fat sugars
1 119.4774 0.6621338 6.295493
2 2129.8162 1.3413488 12.507670
3 3 180.1466 1.9449071 10.856821</pre>
```

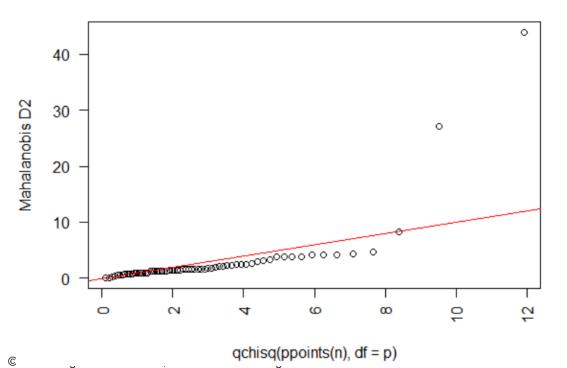
- Significant F-value indicates that the three groups differ on the set of nutritional measures
  - Use summary.aov(fit) for pairwise comparison
  - Use TukeyHSD() to determine which shelves differ from each other

## **Assumptions: One-way MANOVA**

- Assumptions underlying the statistical tests:
  - Multivariate normality of response
  - Homogeneity / equality of variance-covariance matrices:
    - Use Box M test: sensitive to normality assumption
  - Multivariate outliers
- If any of the assumptions are not met:
  - Consider robust nonparametric MANOVA provided by Wilks.test() in the "rrcov" package
  - Also, Adonis() function in the "vegan" package

#### Multivariate Normality Assumption: MANOVA

#### Q-Q Plot: Multivariate Normality



- The data appears to violate multivariate normality (due to a couple of outliers):
  - Wheaties Honey Gold
  - Wheaties
- Delete them and re-run

## **Outliers: One-way MANOVA**

```
library(mvoutlier)
  237
          outliers <- aq.plot(y)</pre>
  238
   239
          outliers
                                     8
                                                                     32
                                     2
                                     9
                                                                                 0.0
                                                        100
                                                                 200
                                                                          300
                                                                                                      60
                                                                                                                 100
                                                                                                                      120
                                                                                           Ordered squared robust distance
                                           Outliers based on 97.5% quantile
                                                                                      Outliers based on adjusted quantile
                                     30
                                                                                 8
                                                                     32
                                                                                                                 32
                                     2
                                                                                 2
                                     9
                                                                                 9
                                                                                 9
                                                                 200
                                                        100
                                                                          300
                                                                                                     100
                                                                                                             200
                                                                                                                      300
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```

#### **Robust MANOVA**

```
248 library (rrcov)
   249 Wilks.test(y, shelf, method="mcd")
Robust One-way MANOVA (Bartlett Chi2)
data:
     X
Wilks' Lambda = 0.5107, Chi2-Value = 23.311, DF =
4.845, p-value = 0.0002549
sample estimates:
 calories fat sugars
1 119.8210 0.7010828 5.663143
2 128.0407 1.1849576 12.537533
3 160.8604 1.6524559 10.352646
```

## Summary

- Designs
  - One-way Between-Groups ANOVA (Analysis of Variance)
  - One-way Within-Groups ANOVA
  - Two-way Factorial ANOVA
  - ANCOVA: Adjusting for Covariates
  - MANOVA: Multivariate Analysis of Variable (>1 Response Vars)
  - MANCOVA: Multivariate Analysis of Covariance
- Fitting ANOVA and ANCOVA Models in R
  - Formulas for different types of designs
  - Order matters: Type I, II, and III ordering of formula terms
  - Examples: ANOVA and ANCOVA
  - Assessing ANOVA and ANCOVA Assumptions
- Fitting MANOVA Models in R
  - Examples: One-way MANOVA
  - Assessing MANOVA Assumptions