STAT 516 Lec 07

Unbalanced two-way factorial design

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2025-03-27

Mice example

Change in mRNA expression of esr1 in wild-type vs gen-modified mice on low- and high-fat diets.

Data from Trey Hope in Dr. Enos' lab in the USC School of Medicine.

Wild, low fat

GMO mouse low

Wild High

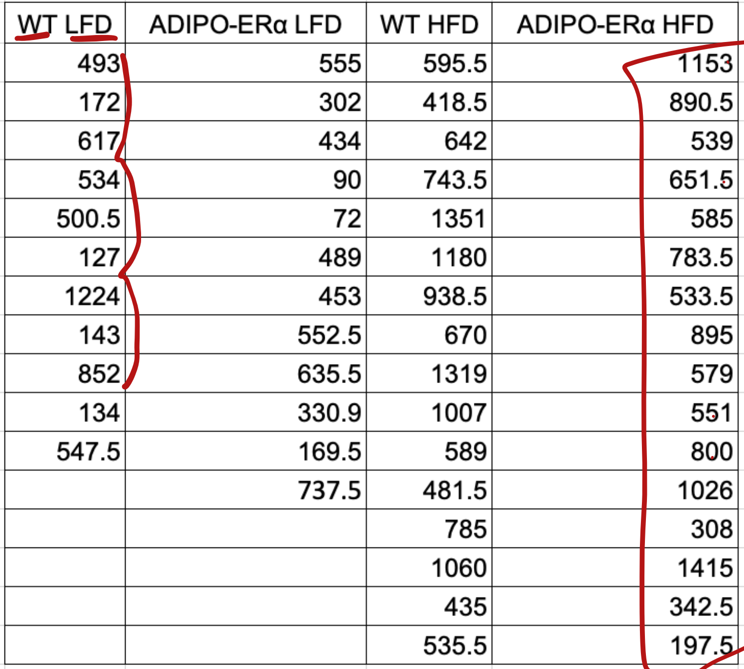
ADIPO × HFD

(A) Diet : LFD , HFD

(B) Gene : WT , ADIPO

a=2

b=2



WT LFD	ADIPO-ERα LFD	WT HFD	ADIPO-ERα HFD
493	555	595.5	1153
172	302	418.5	890.5
617	434	642	539
534	90	743.5	651.5
500.5	72	1351	585
127	489	1180	783.5
1224	453	938.5	533.5
143	552.5	670	895
852	635.5	1319	579
134	330.9	1007	551
547.5	169.5	589	800
	737.5	481.5	1026
		785	308
		1060	1415
		435	342.5
		535.5	197.5

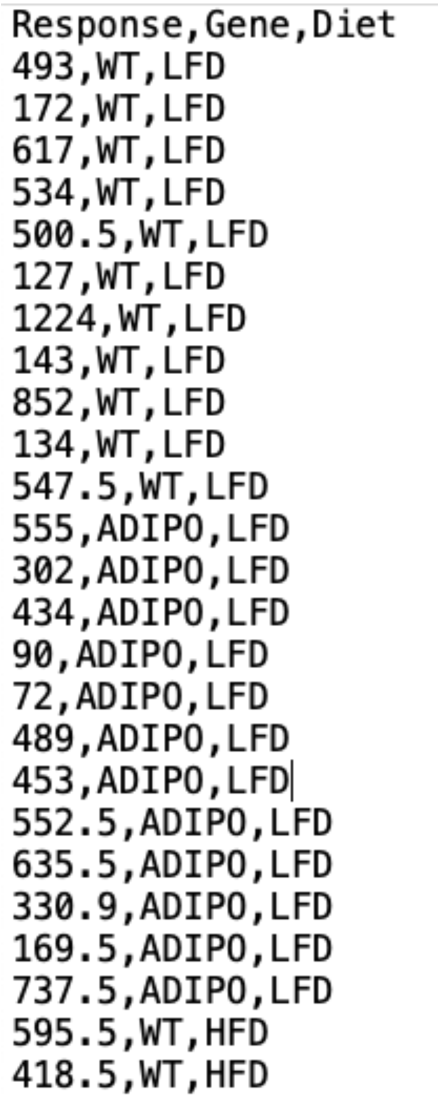
$n_{11}=11$ $n=12$ $n_{21}=16$ $n_{22}=16$

Don't have

$n_{ij} = n$.

Does the diet affect the response? The genetic modification? Is the effect of the diet the same in the wild-type and the gen-modified mice?

Now in a `.csv` file with one column for each factor.

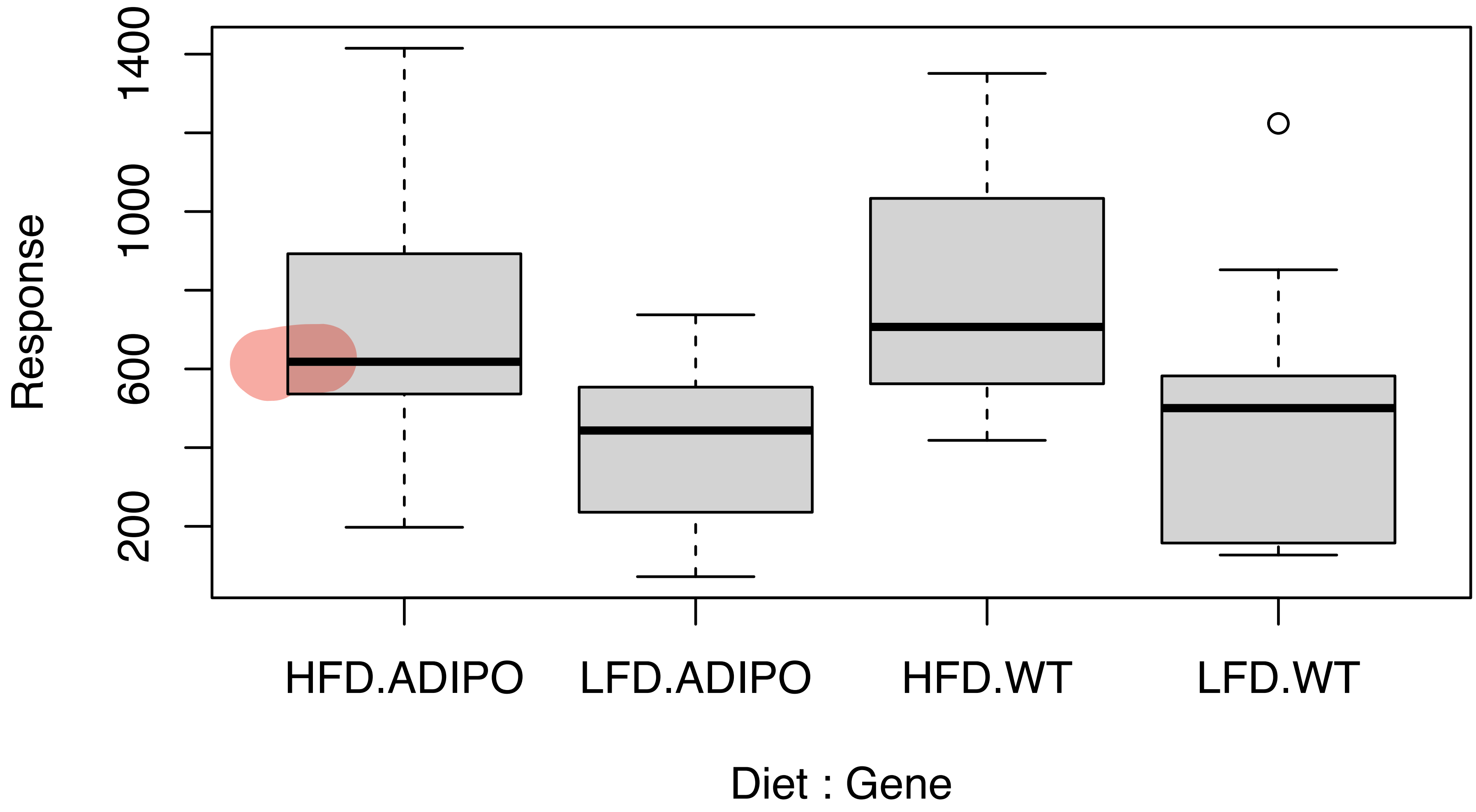


```
Response,Gene,Diet
493,WT,LFD
172,WT,LFD
617,WT,LFD
534,WT,LFD
500.5,WT,LFD
127,WT,LFD
1224,WT,LFD
143,WT,LFD
852,WT,LFD
134,WT,LFD
547.5,WT,LFD
555,ADIPO,LFD
302,ADIPO,LFD
434,ADIPO,LFD
90,ADIPO,LFD
72,ADIPO,LFD
489,ADIPO,LFD
453,ADIPO,LFD
552.5,ADIPO,LFD
635.5,ADIPO,LFD
330.9,ADIPO,LFD
169.5,ADIPO,LFD
737.5,ADIPO,LFD
595.5,WT,HFD
418.5,WT,HFD
```

```r
link <- url("https://people.stat.sc.edu/gregorkb/data/ADIPOER.csv")
adipoer <- read.csv(link)
head(adipoer,n = 14)
```

```
   Response  Gene Diet
1     493.0    WT  LFD
2     172.0    WT  LFD
3     617.0    WT  LFD
4     534.0    WT  LFD
5     500.5    WT  LFD
6     127.0    WT  LFD
7    1224.0    WT  LFD
8     143.0    WT  LFD
9     852.0    WT  LFD
10    134.0    WT  LFD
11    547.5    WT  LFD
12    555.0 ADIPO  LFD
13    302.0 ADIPO  LFD
14    434.0 ADIPO  LFD
```

```r
boxplot(Response ~ Diet + Gene, data = adipoer)
```



```r
grp_means <- aggregate(Response ~ Diet + Gene, data = adipoer, mean)
grp_means
```

```
  Diet  Gene Response
1  HFD ADIPO 703.1250
2  LFD ADIPO 401.7417
3  HFD    WT 796.9375
4  LFD    WT 485.8182
```

```r
grp_counts <- aggregate(Response ~ Diet + Gene, data = adipoer, length)
grp_counts
```

```
  Diet  Gene Response
1  HFD ADIPO       16
2  LFD ADIPO       12
3  HFD    WT       16
4  LFD    WT       11
```

n_{ij}

$N = 55$

Two-way treatment effects model

The two-way treatment effects model gives

$$Y_{ijk} = \underline{\mu} + \tau_i + \gamma_j + (\tau\gamma)_{ij} + \varepsilon_{ijk}$$

for $i = 1, \dots, a$, $j = 1, \dots, b$, and $k = 1, \dots, n_{ij}$, where

- Y_{ijk} is the response for EU k under level i of A and level j of B.
- μ represents a baseline or overall mean.
- The τ_i are the main effects for Factor A.
- The γ_j are the main effects for Factor B.
- The $(\tau\gamma)_{ij}$ are the interaction effects between A and B.
- The ε_{ijk} are Normal$(0, \sigma^2)$ error terms.

Now suppose n_{ij} are *not* all the same, so the design is unbalanced.

Parameter constraints in the unbalanced case

We have $1 + a + b + ab$ parameters for ab treatment means...

Treatment effects model has $a + b + ab + 1$ parameters for ab means...

To identify the parameters uniquely, we usually set

$$\tau_1 = 0, \quad \gamma_1 = 0, \qquad \text{and} \quad (\tau\gamma)_{\underline{1j}} = (\tau\gamma)_{\underline{i1}} = 0 \text{ for all } i, j.$$

Then μ is the mean of the baseline treatment at level 1 of each factor.

Mice example (cont)

$$\mu_{ij} = \mu + \tau_i + \gamma_j + (\tau\gamma)_{ij}$$

for $i = 1, 2$,
$j = 1, 2$.

For the mice example, we have $\underline{a = b = 2}$, so the group means

		Gene	
		1	2
Diet	1	μ_{11}	μ_{12}
	2	μ_{21}	μ_{22}

becomes baseline

A total of 4 means: $\mu_{11}, \mu_{12}, \mu_{21}, \mu_{22}$

$\underline{R}$: set $\tau_1 = 0$
$\gamma_1 = 0$
set $(\tau\gamma)_{11} = 0$
$(\tau\gamma)_{12} = 0$
$(\tau\gamma)_{21} = 0$

are parameterized under the μ-as-baseline constraint as:

	1	2
1	μ	$\mu + \gamma_2$
2	$\mu + \tau_2$	$\mu + \tau_2 + \gamma_2 + (\tau\gamma)_{22}$

interaction effect

A total of 4 parameters, μ, τ_2, γ_2, $(\tau\gamma)_{22}$

Estimating the error term variance

An unbiased estimator of the error term variance σ^2 is given by

$$\hat{\sigma}^2 = \frac{1}{\boxed{N - ab}} \sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{k=1}^{n_{ij}} \underbrace{(Y_{ijk} - \bar{Y}_{ij.})^2}_{\hat{\varepsilon}_{ijk}^2},$$

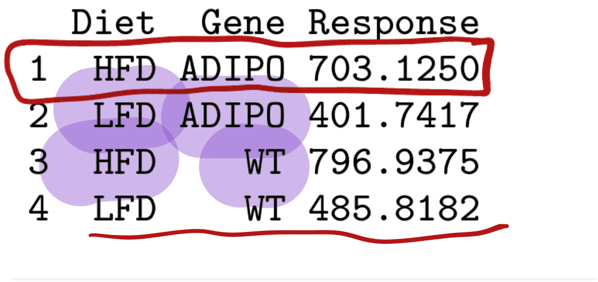
where $N = \sum_{i=1}^{a} \sum_{j=1}^{b} n_{ij}$.

In balanced case, $n_{ij} = n \; \forall i,j$.

we had $N = abn$,

so $N - ab = abn - ab$

$= ab(n-1)$.



```
  Diet   Gene Response
1  HFD  ADIPO 703.1250
2  LFD  ADIPO 401.7417
3  HFD     WT 796.9375
4  LFD     WT 485.8182
```

Mice example (cont)

R uses the μ-as-baseline constraint. A

	ADIPO	WT
HFD	703.125	796.94
LFD	401.7417	495.82

$703.125 + (-301.383)$

```r
lm_out <- lm(Response ~ Diet + Gene + Diet:Gene, data = adipoer)
summary(lm_out)
```

A B A:B

μ_{11}	μ_{12}
μ_{21}	μ_{22}

```
Call:
lm(formula = Response ~ Diet + Gene + Diet:Gene, data = adipoer)

Residuals:
    Min      1Q  Median      3Q     Max
-505.62 -220.09  -11.94  170.32  738.18

Coefficients:
               Estimate Std. Error t value Pr(>|t|)
(Intercept)     703.125     75.516   9.311 1.39e-12 ***
DietLFD        -301.383    115.352  -2.613   0.0118 *
GeneWT           93.813    106.795   0.878   0.3838
DietLFD:GeneWT   -9.736    165.238  -0.059   0.9532
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 302.1 on 51 degrees of freedom
Multiple R-squared:  0.2289,    Adjusted R-squared:  0.1835
F-statistic: 5.045 on 3 and 51 DF,  p-value: 0.003882
```

μ	$\mu + \beta_{WT}$
$\mu + \tau_{LFD}$	$\mu + \tau_{LFD} + \beta_{WT} + (\tau\beta)_{LFD \times WT}$

$\hat{\mu}$ = mean of

$\hat{\tau}_{LFD}$

$\hat{\beta}_{WT}$

$\hat{\sigma} = 302.1$

$\widehat{(\tau\beta)}_{LFD \times WT}$

Sums of squares in the unbalanced design

Balanced $n_{ij} = n \quad \forall \quad i,j.$

"for all"

Sum of squares	Symbol	Formula
Total	$\mathrm{SS}_{\mathrm{Tot}}$	$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n_{ij}}(Y_{ijk}-\bar{Y}_{...})^2$
Treatment	$\mathrm{SS}_{\mathrm{Trt}}$	$\sum_{i=1}^{a}\sum_{j=1}^{b}n_{ij}(\bar{Y}_{ij.}-\bar{Y}_{...})^2$
Error	$\mathrm{SS}_{\mathrm{Error}}$	$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{n_{ij}}(Y_{ijk}-\bar{Y}_{ij.})^2$

We have the decomposition $\mathrm{SS}_{\mathrm{Tot}} = \mathrm{SS}_{\mathrm{Trt}} + \mathrm{SS}_{\mathrm{Error}}$.

The Analysis of Variance (ANOVA) table

Obtain the MS values by dividing the SS values by the Df values.

Source	Df	SS	MS	F value
Treatments	$ab - 1$	$\mathrm{SS}_{\mathrm{Trt}}$	$\mathrm{MS}_{\mathrm{Trt}}$	$F_{\mathrm{stat}} = \mathrm{MS}_{\mathrm{Trt}} \,/\, \mathrm{MS}_{\mathrm{Error}}$
Error	$N - ab$	$\mathrm{SS}_{\mathrm{Error}}$	$\mathrm{MS}_{\mathrm{Error}}$	
Total	$N - 1$	$\mathrm{SS}_{\mathrm{Tot}}$		

In balanced case, N = abn

Reject H_0: μ_{ij} all the same if $F_{\mathrm{stat}} > F_{ab-1,\underline{N-ab},\alpha}$.

In the unbalanced case we have $N = \sum_{i=1}^{a} \sum_{j=1}^{b} n_{ij}$ in place of abn.

Main effect sums of squares under unbalancedness

- In the balanced case, we decomposed $\mathrm{SS}_{\mathrm{Trt}}$ as $\mathrm{SS}_{\mathrm{A}} + \mathrm{SS}_{\mathrm{B}} + \mathrm{SS}_{\mathrm{AB}}$.
- No such “clean” decomposition of $\mathrm{SS}_{\mathrm{Trt}}$ under unbalancedness.
- Specifically, it is not obvious how to define SS_{A} and SS_{B}.
- Three versions of SS_{A} and SS_{B} measure main effect importance:
  1. Sequentially
  2. After other main effect in the *absence* of interaction
  3. After other main effect in the *presence* of interaction

  These are called Type I, Type II, and Type III sums of squares.

Defining Type I/II/III sums of squares for main effects

- Each is a full/reduced model difference in SS.
- To define them, we introduce the notation:

$\mathrm{SS}_{\mathrm{Error}}(A)$ is $\mathrm{SS}_{\mathrm{Error}}$ from model with only A main effects
$\mathrm{SS}_{\mathrm{Error}}(B)$ is $\mathrm{SS}_{\mathrm{Error}}$ from model with only B main effects
$\mathrm{SS}_{\mathrm{Error}}(A, B)$ is $\mathrm{SS}_{\mathrm{Error}}$ from model with A,B main effects
$\mathrm{SS}_{\mathrm{Error}}(A, B, AB)$ is $\mathrm{SS}_{\mathrm{Error}}$ from full model

Type I SS (Sequential)

↑ Do not use if design is unbalanced (not all n_{ij} are the same).

Default output of anova( ) function.

- Meaningless for unbalanced designs!
- The Type I sums of squares $\boxed{\mathrm{SS}^{\mathrm{I}}_{\mathrm{A}}}$ and $\boxed{\mathrm{SS}^{\mathrm{I}}_{\mathrm{B}}}$ are defined as follows:
  1. If A enters first and then B, then

Mice: 1270105 (Diet) → $\mathrm{SS}^{\mathrm{I}}_{\mathrm{A}} = \boxed{\mathrm{SS}_{\mathrm{Tot}}} - \boxed{\mathrm{SS}_{\mathrm{Error}}(A)}$ (change in SS_{Error} by adding A to the model)

110658.5 (Gene) → $\mathrm{SS}^{\mathrm{I}}_{\mathrm{B}} = \mathrm{SS}_{\mathrm{Error}}(A) - \mathrm{SS}_{\mathrm{Error}}(A, B)$

  2. If B enters first and then A, then

(change in SS_{Error} by adding B after A is already in the model).

$$\mathrm{SS}^{\mathrm{I}}_{\mathrm{B}} = \mathrm{SS}_{\mathrm{Tot}} - \mathrm{SS}_{\mathrm{Error}}(B)$$

$$\mathrm{SS}^{\mathrm{I}}_{\mathrm{A}} = \mathrm{SS}_{\mathrm{Error}}(B) - \mathrm{SS}_{\mathrm{Error}}(A, B)$$

- When the design is balanced the order of entrance does not matter.

Mice example (cont)

Obtain the Type I SS when Gene is added and then Diet

```
# fit model with no effects
lm_none <- lm(Response ~ 1, data = adipoer)
SST <- sum(lm_none$residuals^2)

# fit model with just Gene
lm_Gene <- lm(Response ~ Gene, data = adipoer)
SSE_Gene <- sum(lm_Gene$residuals^2)
SS_Gene <- SST - SSE_Gene # take difference in SSE
SS_Gene
```

```
[1] 127271.2
```

```
# now add Diet
lm_Gene_Diet <- lm(Response ~ Gene + Diet, data = adipoer)
SSE_Gene_Diet <- sum(lm_Gene_Diet$residuals^2)
SS_Diet <- SSE_Gene - SSE_Gene_Diet # take difference in SSE
SS_Diet
```

```
[1] 1253492
```

Obtain the Type I SS when Diet is added and then Gene

```
# fit model with just Diet
lm_Diet <- lm(Response ~ Diet, data = adipoer)
SSE_Diet <- sum(lm_Diet$residuals^2)
SS_Diet <- SST - SSE_Diet # take difference in SSE
SS_Diet
```

```
[1] 1270105
```

```
# now add Gene
lm_Gene_Diet <- lm(Response ~ Gene + Diet, data = adipoer)
SSE_Gene_Diet <- sum(lm_Gene_Diet$residuals^2)
SS_Gene <- SSE_Diet - SSE_Gene_Diet # take difference in SSE
SS_Gene
```

```
[1] 110658.6
```

Mice example (cont)

Obtain the Type I SS on the mice data using `anova()` function.

```
anova(lm(Response ~ Gene + Diet + Gene:Diet, data = adipoer))
```

```
Analysis of Variance Table

Response: Response
          Df  Sum Sq Mean Sq F value    Pr(>F)    
Gene       1  127271  127271  1.3949 0.2430621    
Diet       1 1253492 1253492 13.7381 0.0005184 ***
Gene:Diet  1     317     317  0.0035 0.9532452    
Residuals 51 4653342   91242                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
anova(lm(Response ~ Diet + Gene + Diet:Gene, data = adipoer))
```

```
Analysis of Variance Table

Response: Response
          Df  Sum Sq Mean Sq F value    Pr(>F)    
Diet       1 1270105 1270105 13.9202 0.0004804 ***
Gene       1  110659  110659  1.2128 0.2759481    
Diet:Gene  1     317     317  0.0035 0.9532452    
Residuals 51 4653342   91242                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type II SS

optional

- Measures contribution of each main effect in *absence* of interaction.
- The Type II sums of squares $\mathrm{SS}_{\mathrm{A}}^{\mathrm{II}}$ and $\mathrm{SS}_{\mathrm{B}}^{\mathrm{II}}$ are given by

$$
\begin{aligned}
\mathrm{SS}_{\mathrm{A}}^{\mathrm{II}} &= \mathrm{SS}_{\mathrm{Error}}(\overset{B}{\cancel{A}}) - \boxed{\mathrm{SS}_{\mathrm{Error}}(A, B)} \\
\mathrm{SS}_{\mathrm{B}}^{\mathrm{II}} &= \mathrm{SS}_{\mathrm{Error}}(\underset{A}{\cancel{B}}) - \boxed{\mathrm{SS}_{\mathrm{Error}}(A, B)}
\end{aligned}
$$

Error SS from model with both main effects but without interaction.

Mice example (cont)

optional

Obtain the Type II SS on the mice data

```
# fit model with both main effects
lm_Diet_Gene <- lm(Response ~ Diet + Gene, data = adipoer)
SSE_Diet_Gene <- sum(lm_Diet_Gene$residuals^2)

# fit model with just Gene
lm_Gene <- lm(Response ~ Gene, data = adipoer)
SSE_Gene <- sum(lm_Gene$residuals^2)
SS_Diet <- SSE_Gene - SSE_Diet_Gene # take difference in SSE
SS_Diet
```

```
[1] 1253492
```

```
# fit model with just Diet
lm_Diet <- lm(Response ~ Diet, data = adipoer)
SSE_Diet <- sum(lm_Diet$residuals^2)
SS_Gene <- SSE_Diet - SSE_Diet_Gene # take difference in SSE
SS_Gene
```

```
[1] 110658.6
```

optional

Obtain Type II SS with `Anova()` function from R package `car`.

```
library(car)
Anova(lm(Response ~ Diet + Gene + Diet:Gene, data = adipoer),type = "II")
```

```
Anova Table (Type II tests)

Response: Response
           Sum Sq Df F value    Pr(>F)
Diet      1253492  1 13.7381 0.0005184 ***
Gene       110659  1  1.2128 0.2759481
Diet:Gene     317  1  0.0035 0.9532452
Residuals 4653342 51
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Type III SS

- In the unbalanced case we should use the so-called Type III SS.
- Measures contribution of each main effect in *presence* of interaction.
- The Type III sums of squares $\mathrm{SS}_{\mathrm{A}}^{\mathrm{III}}$ and $\mathrm{SS}_{\mathrm{B}}^{\mathrm{III}}$ are given by

$$\begin{aligned} \mathrm{SS}_{\mathrm{A}}^{\mathrm{III}} &= \boxed{\mathrm{SS}_{\mathrm{Error}}(\overset{B}{\cancel{A}}, AB)} - \mathrm{SS}_{\mathrm{Error}}(A, B, AB) \\ \mathrm{SS}_{\mathrm{B}}^{\mathrm{III}} &= \mathrm{SS}_{\mathrm{Error}}(\underset{A}{\cancel{B}}, AB) - \mathrm{SS}_{\mathrm{Error}}(A, B, AB) \end{aligned}$$

SSE from full model with A, B main effects and interaction.

Tedious to compute

Type III SS (cont)

- Type III SS are tedious to obtain in the unbalanced case; they are like full-reduced model differences in the error sum of squares, where the reduced model is the model without the main effect in question.
- The `anova()` or `aov()` functions in R give sequential SS, which are not meaningful in the unbalanced case; however, the Type III SS are equal to the sequential (i.e. Type I) SS when $\boxed{n_{ij} = n \ \forall ij.}$
- Under unbalancedness $\boxed{\mathrm{SS}_{\mathrm{Trt}} \neq \mathrm{SS}_{\mathrm{A}}^{\mathrm{III}} + \mathrm{SS}_{\mathrm{B}}^{\mathrm{III}} + \mathrm{SS}_{\mathrm{AB}}.}$

Type III sums of squares for unbalanced designs

ANOVA table with Type III SS and MS for A and B:

Treatments

Source	Df	SS	MS	F value
A	$a-1$	$\text{SS}_\text{A}^\text{III}$	$\text{MS}_\text{A}^\text{III}$	$F_\text{A} = \text{MS}_\text{A}^\text{III} / \text{MS}_\text{Error}$
B	$b-1$	$\text{SS}_\text{B}^\text{III}$	$\text{MS}_\text{B}^\text{III}$	$F_\text{B} = \text{MS}_\text{B}^\text{III} / \text{MS}_\text{Error}$
AB	$(a-1)(b-1)$	SS_AB	MS_AB	$F_\text{AB} = \text{MS}_\text{AB} / \text{MS}_\text{Error}$
Error	$N-ab$	SS_Error	MS_Error	
Total	$N-1$	SS_Tot		

in balanced case we have $N = abn$

1. Reject H_0: $\bar{\mu}_{1.} = \cdots = \bar{\mu}_{a.}$ if $F_\text{A} > F_{\underline{a-1},\underline{N-ab},\alpha}$. H_0: No factor A main effect
2. Reject H_0: $\bar{\mu}_{.1} = \cdots = \bar{\mu}_{.b}$ if $F_\text{B} > F_{\underline{b-1},\underline{N-ab},\alpha}$. H_0: No factor B main effect.
3. Reject H_0: $\mu_{ij} = \bar{\mu}_{i.} + \bar{\mu}_{.j} - \bar{\mu}_{..} \quad \forall ij$ if $F_\text{AB} > F_{\underline{(a-1)(b-1)},\underline{N-ab},\alpha}$.

H_0: No interaction

Mice example (cont)

Obtain the Type III SS on the mice data.

```
# fit model with both main effects---must specify constraint type!
lm_full <- lm(Response ~ Diet + Gene + Diet:Gene,
              contrasts = list(Gene = contr.sum,Diet = contr.sum),
              data = adipoer)
SSE_full <- sum(lm_full$residuals^2)

# drop main effect of Gene from the model
drop1(lm_full,~Gene,test="F",data = adipoer)
```

```
Single term deletions

Model:
Response ~ Diet + Gene + Diet:Gene
       Df Sum of Sq     RSS    AIC F value Pr(>F)
<none>               4653342 632.02
Gene    1    105749 4759091 631.25   1.159 0.2867
```

```r
# drop main effect of Diet from the model
drop1(lm_full,~Diet,test="F",data = adipoer)
```

```
Single term deletions

Model:
Response ~ Diet + Gene + Diet:Gene
       Df Sum of Sq     RSS    AIC F value    Pr(>F)
<none>               4653342 632.02
Diet    1   1253698 5907040 643.14   13.74 0.0005179 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Obtain Type III SS with `Anova()` from the R package `car`.

Important to specify all the options exactly!

```r
library(car)
Anova(lm(Response ~ Gene + Diet + Gene:Diet,
         contrasts = list(Gene = contr.sum, Diet = contr.sum),# specify constraint
         data = adipoer),
      type = "III")
```

```
Anova Table (Type III tests)

Response: Response
              Sum Sq Df  F value    Pr(>F)
(Intercept) 19050576  1 208.7917 < 2.2e-16 ***
Gene          105749  1   1.1590 0.2867411
Diet         1253698  1  13.7404 0.0005179 ***
Gene:Diet        317  1   0.0035 0.9532452
Residuals    4653342 51
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

H_0: no effect of Gene

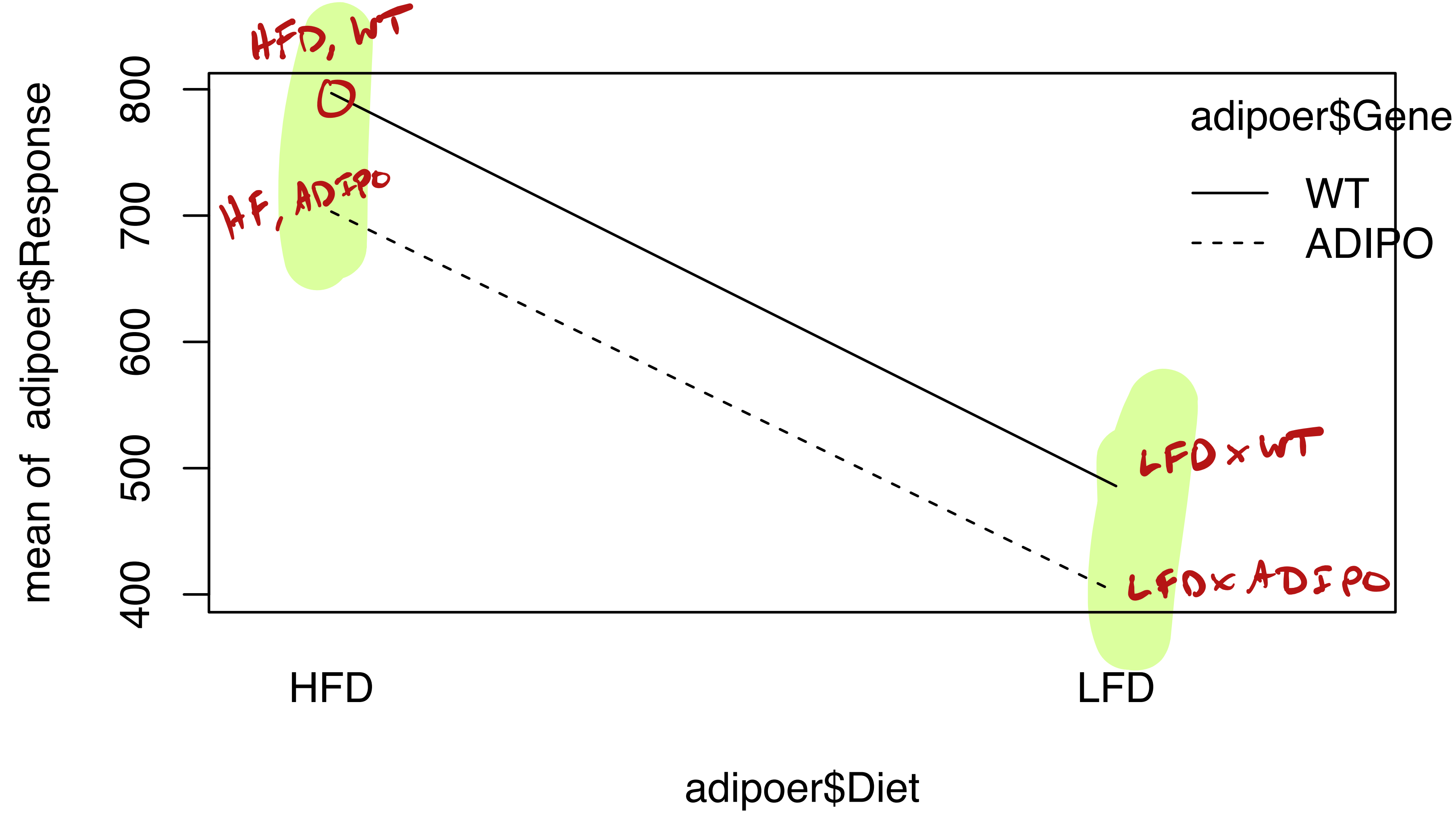
H_0: no effect of Diet

H_0: no interaction

Since interaction is not significant, we can focus on main effects...

Mice example (cont)

```
interaction.plot(adipoer$Diet, adipoer$Gene, adipoer$Response)
```



Estimates of cell and marginal means in unbalanced case

optional

The estimators of the cell and marginal means are given by

- $\hat{\mu}_{ij} = \bar{Y}_{ij.}$, $i = 1, \ldots, a$, $j = 1, \ldots, b$.
- $\hat{\hat{\mu}}_{i.} = \frac{1}{b} \sum_{j=1}^{b} \hat{\mu}_{ij}$, $i = 1, \ldots, a$.
- $\hat{\hat{\mu}}_{.j} = \frac{1}{a} \sum_{i=1}^{a} \hat{\mu}_{ij}$, $j = 1, \ldots, b$.

We estimate $\hat{\hat{\mu}}_{i.}$ with $\bar{Y}_{i..}$ (and $\hat{\hat{\mu}}_{.j}$ with $\bar{Y}_{.j.}$) only when $n_{ij} = n \ \forall ij$.

Exercise: Write $\hat{\hat{\mu}}_{i.}$ in terms of Y_{ijk} and find $\operatorname{Var} \hat{\hat{\mu}}_{i.}$.

Some CI formulas (without familywise adjustment)

optional

Under unbalancedness our CI formulas become more complicated.

Target	$(1-\alpha)100\%$ confidence interval
μ_{ij}	$\hat{\mu}_{ij} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{n_{ij}}}$
$\mu_{ij} - \mu_{i'j'}$	$\hat{\mu}_{ij} - \hat{\mu}_{i'j'} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{n_{ij}} + \frac{1}{n_{i'j'}}}$
$\bar{\mu}_{i.}$	$\hat{\bar{\mu}}_{i.} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{b^2}\sum_{j=1}^{b}\frac{1}{n_{ij}}}$
$\bar{\mu}_{.j}$	$\hat{\bar{\mu}}_{.j} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{a^2}\sum_{i=1}^{a}\frac{1}{n_{ij}}}$
$\bar{\mu}_{i.} - \bar{\mu}_{i'.}$	$\hat{\bar{\mu}}_{i.} - \hat{\bar{\mu}}_{i'.} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{b^2}\sum_{j=1}^{b}(\frac{1}{n_{ij}} + \frac{1}{n_{i'j}})}$
$\bar{\mu}_{.j} - \bar{\mu}_{.j'}$	$\hat{\bar{\mu}}_{.j} - \hat{\bar{\mu}}_{.j'} \pm t_{N-ab,\alpha/2}\hat{\sigma}\sqrt{\frac{1}{a^2}\sum_{i=1}^{a}(\frac{1}{n_{ij}} + \frac{1}{n_{i'j}})}$

In the above $\hat{\sigma} = \sqrt{\text{MS}_{\text{Error}}}$.

Mice example (cont)

The interaction appears to be negligible, so we can focus on main effects.

Build 95% CIs for the differences in marginal means for

- Diet: $\hat{\bar{\mu}}_{1.} - \hat{\bar{\mu}}_{2.} \pm t_{55-4,0.05/2}\hat{\sigma}\sqrt{\frac{1}{2^2}\sum_{j=1}^{2}(\frac{1}{n_{1j}} + \frac{1}{n_{2j}})}$
- Gene: $\hat{\bar{\mu}}_{.1} - \hat{\bar{\mu}}_{.2} \pm t_{55-4,0.05/2}\hat{\sigma}\sqrt{\frac{1}{2^2}\sum_{i=1}^{2}(\frac{1}{n_{i1}} + \frac{1}{n_{i2}})}$,

where $\hat{\sigma} = \sqrt{\mathrm{MS}_{\mathrm{Error}}}$.

```
mu_hat <- grp_means$Response
nn <- grp_counts$Response
mu11_hat <- mu_hat[1] ; n11 <- nn[1] # HFD x ADIPO
mu21_hat <- mu_hat[2] ; n21 <- nn[2] # LFD x ADIPO
mu12_hat <- mu_hat[3] ; n12 <- nn[3] # HFD x WT
mu22_hat <- mu_hat[4] ; n22 <- nn[4] # LFD x WT

mu1.hat <- (mu11_hat + mu12_hat)/2 # HFD mean
mu2.hat <- (mu21_hat + mu22_hat)/2 # LFD mean
mu.1hat <- (mu11_hat + mu21_hat)/2 # ADIPO mean
mu.2hat <- (mu12_hat + mu22_hat)/2 # WT mean

N <- sum(nn)
a <- 2
b <- 2
MSE <- sum(lm_out$residuals^2)/(N - a*b)
alpha <- 0.05

me <- qt(1-alpha/2,N-a*b)*sqrt(MSE)*sqrt(1/2^2*(1/n11 + 1/n12 + 1/n11 + 1/n22))

ci_diet <- c(mu1.hat - mu2.hat - me, mu1.hat - mu2.hat + me)
ci_gene <- c(mu.1hat - mu.2hat - me, mu.1hat - mu.2hat + me)
```

We obtain the intervals:

- Diet: $(146.27, 466.24)$
- Gene: $(-248.93, 71.04)$

Unbalanced tensile strength data from Kuehl (2000)

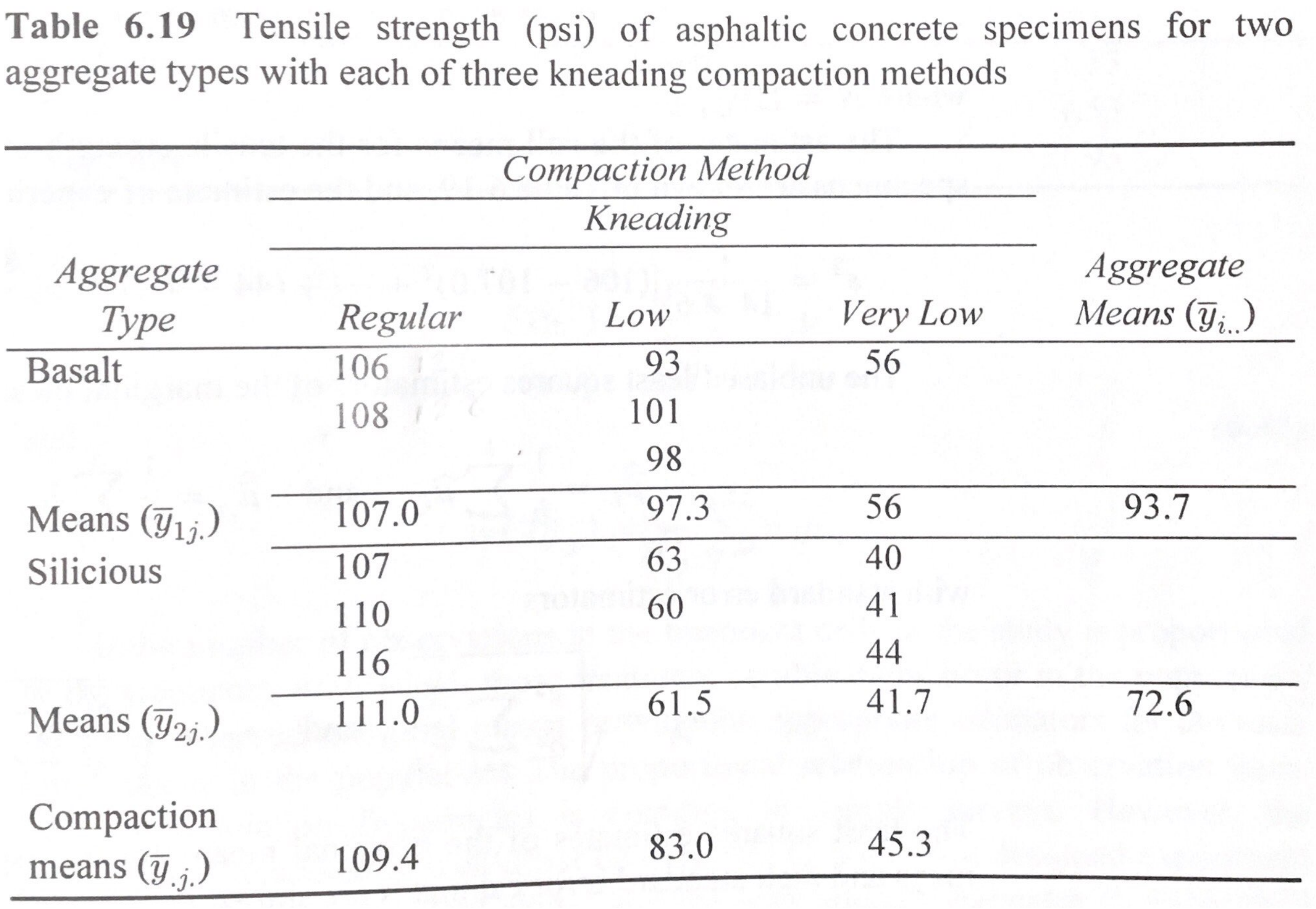


Table 6.19 Tensile strength (psi) of asphaltic concrete specimens for two aggregate types with each of three kneading compaction methods

	Compaction Method			
	Kneading			
Aggregate Type	*Regular*	*Low*	*Very Low*	*Aggregate Means* ($\overline{y}_{i..}$)
Basalt	106	93	56	
	108	101		
		98		
Means ($\overline{y}_{1j.}$)	107.0	97.3	56	93.7
Silicious	107	63	40	
	110	60	41	
	116		44	
Means ($\overline{y}_{2j.}$)	111.0	61.5	41.7	72.6
Compaction means ($\overline{y}_{.j.}$)	109.4	83.0	45.3	

```
y <- c(106,108,93,101,98,56,107,110,116,63,60,40,41,44)
agg <- as.factor(c("b","b","b","b","b","b","s","s","s","s","s","s","s","s"))
comp <- as.factor(c("r","r","l","l","l","vl","r","r","r","l","l","vl","vl","vl"))
tensile <- data.frame(y = y, agg = agg, comp = comp)
```

Compute group means:

```
aggregate(y ~ agg + comp,data = tensile, mean)
```

```
  agg comp         y
1   b    l  97.33333
2   s    l  61.50000
3   b    r 107.00000
4   s    r 111.00000
5   b   vl  56.00000
6   s   vl  41.66667
```

Unbalanced tensile strength data (cont)

Again the meaninglessness of sequential SS under unbalancedness:

```r
anova(lm(y ~ agg + comp + agg:comp, data = tensile))
```

```
Analysis of Variance Table

Response: y
          Df Sum Sq Mean Sq F value    Pr(>F)    
agg        1 1518.0  1518.0 135.184 2.726e-06 ***
comp       2 8401.9  4201.0 374.112 1.252e-08 ***
agg:comp   2  953.4   476.7  42.454 5.497e-05 ***
Residuals  8   89.8    11.2                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```r
anova(lm(y ~ comp + agg + agg:comp, data = tensile))
```

```
Analysis of Variance Table

Response: y
          Df Sum Sq Mean Sq F value    Pr(>F)    
comp       2 9159.3  4579.6 407.834 8.899e-09 ***
agg        1  760.7   760.7  67.740 3.557e-05 ***
comp:agg   2  953.4   476.7  42.454 5.497e-05 ***
Residuals  8   89.8    11.2                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Unbalanced tensile strength data (cont)

Obtain Type III SS with `Anova()` from the R package car.

```
library(car)
Anova(lm(y ~ agg + comp + agg:comp, data = tensile,
         contrasts = list(agg = contr.sum, comp = contr.sum)),
      type = "III")
```

```
Anova Table (Type III tests)

Response: y
            Sum Sq Df  F value    Pr(>F)    
(Intercept)  75050  1 6683.495 5.589e-13 ***
agg            710  1   63.269 4.551e-05 ***
comp          6806  2  303.070 2.879e-08 ***
agg:comp       953  2   42.454 5.497e-05 ***
Residuals       90  8                       
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Now we could make comparisons of means, noting the interaction.

References

Kuehl, R. O. 2000. *Design of Experiments: Statistical Principles of Research Design and Analysis*. Duxbury/Thomson Learning.