STAT 525

Chapter 23
Two-Factor Studies with Unequal Sample Sizes

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Data for Two-Factor ANOVA

- Y is the response variable

- Factor A has levels $i = 1, 2, ..., a$

- Factor B has levels $j = 1, 2, ..., b$

- Now $k = 1, 2, ..., n_{ij}$

Example (Page 954)

- Synthetic growth horomone administered to growth horomone deficient pre-pubescent children

- Interested in two factors

  - Gender ($a = 2$)

  - Bone development level ($b = 3$)

- Y is the difference between growth rate during treatment and prior to treatment

- Set up as balanced design ($n = 3$) but four children were unable to complete the study

  - $i = 1, 2$ and $j = 1, 2, 3$

  - $n_{ij} = 3, 2, 2, 1, 3, 3$

Recall: General Plan of Two-Factor ANOVA

- Construct scatterplot / interaction plot

- Run <u>full</u> model

- Check assumptions

  - Residual plots

  - Histogram / QQplot

  - Ordered residuals plot

- Check significance of interaction

```
options nocenter;
data a1; infile 'u:\.www\datasets525\CH23TA01.txt';
    input growth gender bone;

proc print data=a1; run; quit;
```

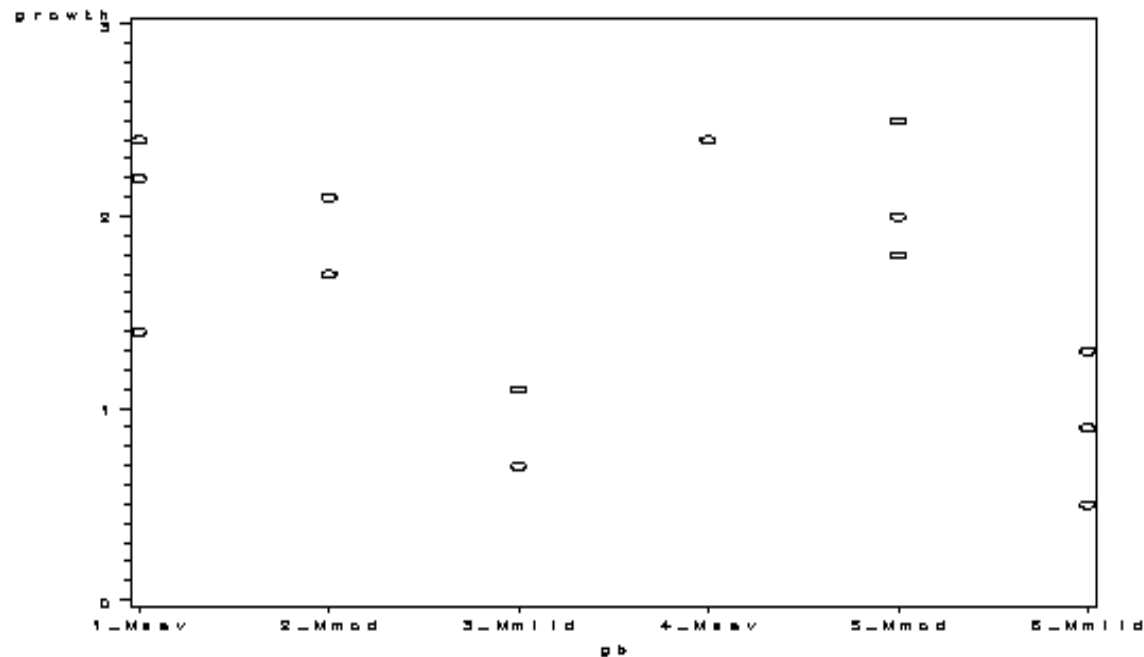| Obs	growth	gender	bone
1	1.4	1	1
2	2.4	1	1
3	2.2	1	1
4	2.1	1	2
5	1.7	1	2
6	0.7	1	3
7	1.1	1	3
8	2.4	2	1
9	2.5	2	2
10	1.8	2	2
11	2.0	2	2
12	0.5	2	3
13	0.9	2	3
14	1.3	2	3

```
data a1; set a1;
   if (gender eq 1)*(bone eq 1) then gb='1_Msev ';
   if (gender eq 1)*(bone eq 2) then gb='2_Mmod ';
   if (gender eq 1)*(bone eq 3) then gb='3_Mmild';
   if (gender eq 2)*(bone eq 1) then gb='4_Msev ';
   if (gender eq 2)*(bone eq 2) then gb='5_Mmod ';
   if (gender eq 2)*(bone eq 3) then gb='6_Mmild';

/*----------- Scatterplot -----------*/
symbol1 v=circle i=none c=black;
proc gplot data=a1;
   plot growth*gb/frame;
run; quit;
```
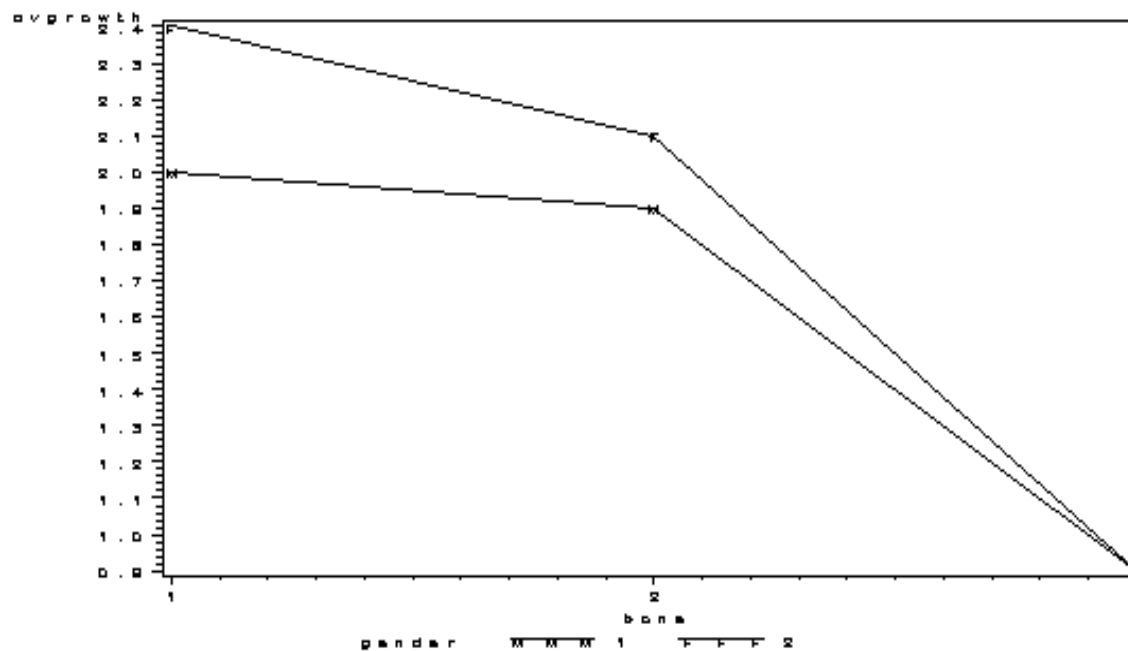
```
proc means data=a1;
    output out=a2 mean=avgrowth;
    by gender bone;

/*----------- Interaction Plot -----------*/
symbol1 v='M' i=join c=black;
symbol2 v='F' i=join c=black;
proc gplot data=a2;
    plot avgrowth*bone=gender/frame;
run; quit;
```

The Cell Means Model

- Expressed numerically

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

  where μ_{ij} is the theoretical mean or expected value of all observations in cell (i, j)

- The ε_{ijk} are iid $N(0, \sigma^2)$ which implies the Y_{ijk} are independent $N(\mu_{ij}, \sigma^2)$

- Parameters

  $- \{\mu_{ij}\}$, $i = 1, 2, .., a$, $j = 1, 2, ..., b$

  $- \sigma^2$

Estimates

- Estimate μ_{ij} by the sample mean of the observations in cell (i, j)

$$\widehat{\mu}_{ij} = \overline{Y}_{ij.}$$

- For each cell (i, j), also estimate of the variance

$$s_{ij}^2 = \sum (Y_{ijk} - \overline{Y}_{ij.})^2 / (n_{ij} - 1)$$

- These s_{ij}^2 are pooled to estimate σ^2

Example (Page 954)

```
proc glm data=a1;
    class gender bone;
    model growth=gender|bone/solution;
run; quit;
```

- The `solution` option gives parameter estimates for the factor effects model under the GLM constraints (aka SAS approach)

  - $\alpha_2 = 0$

  - $\beta_3 = 0$

  - $(\alpha\beta)_{13} = (\alpha\beta)_{23} = (\alpha\beta)_{21} = (\alpha\beta)_{22} = 0$

- Produces the cell means in the usual way

```
                    Sum of
Source            DF    Squares   Mean Square  F Value  Pr > F
Model              5  4.47428571   0.89485714     5.51  0.0172
Error              8  1.30000000   0.16250000
Corrected Total   13  5.77428571


Source            DF   Type I SS   Mean Square  F Value  Pr > F
gender             1  0.00285714   0.00285714     0.02   0.8978
bone               2  4.39600000   2.19800000    13.53   0.0027
gender*bone        2  0.07542857   0.03771429     0.23   0.7980


Source            DF Type III SS  Mean Square  F Value  Pr > F
gender             1  0.12000000   0.12000000     0.74   0.4152
bone               2  4.18971429   2.09485714    12.89   0.0031
gender*bone        2  0.07542857   0.03771429     0.23   0.7980


Parameter            Estimate    Std Error    t Value    Pr > |t|
Intercept            0.9000 B   0.23273733      3.87      0.0048
gender      1       -0.0000 B   0.36799004     -0.00      1.0000
gender      2        0.0000 B       .             .         .
bone        1        1.5000 B   0.46547467      3.22      0.0122
bone        2        1.2000 B   0.32914029      3.65      0.0065
bone        3        0.0000 B       .             .         .
gender*bone 1 1     -0.4000 B   0.59336610     -0.67      0.5192
gender*bone 1 2     -0.2000 B   0.52041650     -0.38      0.7108
gender*bone 1 3      0.0000 B       .             .         .
gender*bone 2 1      0.0000 B       .             .         .
gender*bone 2 2      0.0000 B       .             .         .
gender*bone 2 3      0.0000 B       .             .         .
```

Comments

- For studies with equal sample sizes, Type I and Type III SS were identical, due to the fact that the sample sizes were all the same made the variables completely orthogonal.

- When sample sizes are unequal, the SS do not break down in the usual way. The various SS that we can calculate will not necessarily add up to the SSM.

  - Type I: $0.003 + 4.396 + 0.075 = 4.474 =$ Model SS

  - Type III: $0.120 + 4.190 + 0.075 = 4.385 \neq$ Model SS

- SAS actually calculates four types of SS (I, II, III, IV). It does `ss1` and `ss3` by default but you can also ask for `ss2` and `ss4`.

- We will focus on Type I and Type III in ANOVA.

  - Type I and Type III hypotheses are different

  - Most prefer Type III analysis

  - Can be misleading if n_{ij} widely different

  - Use contrasts to understand the difference

Type I

- Type I SS refer to the difference in SS when variables are added sequentially in the model, i.e. $SS(A)$, $SS(B|A)$, $SS(A \times B|A, B)$.

- Similar to Type I SS for `reg` procedure.

- Type I test for main effect A: $F = MS(A)/MSE$ corresponds to

$$H_0 : \sum_{j=1}^{b} n_{ij}\mu_{ij} / \sum_{j=1}^{b} n_{ij} = \text{constant for all } i.$$

- Type I test weights each observation equally, with the result that the treatments are weighted in proportion to their n_{ij}.

- Type I test for main effect B: $F = MS(B|A)/MSE$ corresponds to

$$H_0 : \sum_{i=1}^{a} (n_{ij} - n_{ij}^2/n_{i.})\mu_{ij} = \sum_{h \neq j} \sum_{i=1}^{a} n_{ij} n_{ih} \mu_{ih}/n_{i.}, \quad j = 1, \ldots, b.$$

Type III

- Type III SS referred to the difference in SSM when a variable is included last in the model or not (i.e $SS(A|B, A \times B)$, $SS(B|A, A \times B)$, $SS(A \times B|A, B)$)

- Type III test for main effect A: $F = MS(A|B, A \times B)/MSE$ corresponds to

$$H_0 : \sum_{j=1}^{b} \mu_{ij}/b = \text{constant for all } i.$$

- Type III test adjusts for the cells having different n_{ij}, by weighting each treatment equally, so that the observations are weighted differently.

- When the sample sizes are unequal, Type III SS are more informative about the treatments than Type I.

- In Sections 23.2-3, KNNL are discussing Type III SS (they don't call them that; the type numbers are a SAS convention).

Additional Comments

- SAS has a rigorous mathematical way to define parameter constraints, type I/III contrast, which is more complicated than our lecture

  For example, our discussion on parameter constraint or type I SS doesn't apply to `model y = A*B A B;` or `model y =A A*B B;`

Type II

- Type II SS referred to the reduction in the SSE due to adding the effect to a model that contains all other effects except higher order interaction effects that contain the effect being tested, (i.e $SS(A|B)$, $SS(B|A)$, $SS(A \times B|A, B)$.

Type IV

- Type IV SS are like Type III, except that Type IV additionally take into account possibly empty cells ($n_{i,j} = 0$).

- If there are empty cells, then Type IV SS are preferred. See KNNL Section 23.4 about empty cells.

Contrast for $A * B$

- Same for Type I and Type III, corresponding to tests

$$H_0 : \mu_{ij} - \mu_{kj} = \mu_{ih} - \mu_{kh}, \text{for all } i, j, k, h.$$

- Null Hypothesis is that the mean profiles are parallel (recall interaction plot)

- For the growth example,

$$\mu_{12} - \mu_{11} \quad = \quad \mu_{22} - \mu_{21}$$
$$\text{and}$$
$$\mu_{13} - \mu_{12} \quad = \quad \mu_{23} - \mu_{22}$$

```
contrast 'gender*bone Type I&III'
   gender*bone 1 -1 0 -1 1 0,  gender*bone 0 1 -1 0 -1 1;
```

Type III Contrast for A

- Null Hypothesis is that the marginal gender means are the same

- Null hypothesis can be expressed

$$
\begin{aligned}
1 \times \mu_{11} &= 1(\mu + \alpha_1 + \beta_1 + (\alpha\beta)_{11}) \\
1 \times \mu_{12} &= 1(\mu + \alpha_1 + \beta_2 + (\alpha\beta)_{12}) \\
1 \times \mu_{13} &= 1(\mu + \alpha_1 + \beta_3 + (\alpha\beta)_{13}) \\
-1 \times \mu_{21} &= -1(\mu + \alpha_2 + \beta_1 + (\alpha\beta)_{21}) \\
-1 \times \mu_{22} &= -1(\mu + \alpha_2 + \beta_2 + (\alpha\beta)_{22}) \\
-1 \times \mu_{23} &= -1(\mu + \alpha_2 + \beta_3 + (\alpha\beta)_{23}) \\
\hline
&= 3\alpha_1 - 3\alpha_2 + (\alpha\beta)_{1.} - (\alpha\beta)_{2.}
\end{aligned}
$$

```
contrast 'gender Type III'
  gender 3 -3  gender*bone 1 1 1 -1 -1 -1;
estimate 'gender Type III'
  gender 3 -3  gender*bone 1 1 1 -1 -1 -1;
```

Type I Contrast for A

- Null Hypothesis is that the "weighted" marginal gender means are the same

- Null hypothesis can be expressed

$$
\begin{aligned}
3 \times \mu_{11} &= 3(\mu + \alpha_1 + \beta_1 + (\alpha\beta)_{11}) \\
2 \times \mu_{12} &= 2(\mu + \alpha_1 + \beta_2 + (\alpha\beta)_{12}) \\
2 \times \mu_{13} &= 2(\mu + \alpha_1 + \beta_3 + (\alpha\beta)_{13}) \\
-1 \times \mu_{21} &= -1(\mu + \alpha_2 + \beta_1 + (\alpha\beta)_{21}) \\
-3 \times \mu_{22} &= -3(\mu + \alpha_2 + \beta_2 + (\alpha\beta)_{22}) \\
-3 \times \mu_{23} &= -3(\mu + \alpha_2 + \beta_3 + (\alpha\beta)_{23}) \\
\hline
&= 7\alpha_1 - 7\alpha_2 + 2\beta_1 - \beta_2 - \beta_3 + \\
&\quad 3(\alpha\beta)_{11} + 2(\alpha\beta)_{12} + 2(\alpha\beta)_{13} - \\
&\quad (\alpha\beta)_{21} - 3(\alpha\beta)_{22} - 3(\alpha\beta)_{23}
\end{aligned}
$$

```
contrast 'gender Type I'
   gender 7 -7  bone 2 -1 -1  gender*bone 3 2 2 -1 -3 -3;
estimate 'gender Type I'
   gender 7 -7  bone 2 -1 -1  gender*bone 3 2 2 -1 -3 -3;
```

```
proc glm data=a1;
    class gender bone;
    model growth=gender|bone;
    contrast 'gender*bone Type I and III'
      gender*bone 1 -1 0 -1 1 0,  gender*bone 0 1 -1 0 -1 1;
    contrast 'gender Type III'
      gender 3 -3  gender*bone 1 1 1 -1 -1 -1;
    estimate 'gender Type III'
      gender 3 -3  gender*bone 1 1 1 -1 -1 -1;
    contrast 'gender Type I'
      gender 7 -7  bone 2 -1 -1  gender*bone 3 2 2 -1 -3 -3;
    estimate 'gender Type I'
      gender 7 -7  bone 2 -1 -1  gender*bone 3 2 2 -1 -3 -3;
    contrast 'bone Type III'
      bone 2 -2 0  gender*bone 1 -1 0 1 -1 0,
      bone 0 2 -2  gender*bone 0 1 -1 0 1 -1;
run; quit;
```

	DF	Contrast SS	Mean Square	F Value	Pr > F
gender*bone Type I&III	2	0.07542857	0.03771429	0.23	0.7980
gender Type III	1	0.12000000	0.12000000	0.74	0.4152
gender Type I	1	0.00285714	0.00285714	0.02	0.8978
bone Type III	2	4.18971429	2.09485714	12.89	0.0031

Parameter	Estimate	Standard Error	t Value	Pr > |t|
gender Type III	-0.60000000	0.69821200	-0.86	0.4152
gender Type I	0.20000000	1.50831031	0.13	0.8978

Recall: If Interaction Not Significant

- Determine whether pooling is beneficial

  – If yes, rerun analysis without interaction

- Check significance of main effects

  – If factor insignificant, determine whether pooling is bene-ficial

    ∗ If yes, rerun analysis as one-way ANOVA

  – If statistically significant factor has more than two levels, use multiple comparison procedure to assess differences

    ∗ Contrasts and linear combinations can also be used

```
proc glm data=a1;
    class gender bone;
    model growth=gender bone/solution;
run; quit;
```

| | | Sum of | | | |
Source	DF	Squares	Mean Square	F Value	Pr > F
Model	3	4.39885714	1.46628571	10.66	0.0019
Error	10	1.37542857	0.13754286		
Corrected Total	13	5.77428571			

R-Square	Coeff Var	Root MSE	growth Mean
0.761801	22.57456	0.370868	1.642857

Source	DF	Type I SS	Mean Square	F Value	Pr > F
gender	1	0.00285714	0.00285714	0.02	0.8883
bone	2	4.39600000	2.19800000	15.98	0.0008

Source	DF	Type III SS	Mean Square	F Value	Pr > F
gender	1	0.09257143	0.09257143	0.67	0.4311
bone	2	4.39600000	2.19800000	15.98	0.0008

| | | | | Standard | | |
Parameter		Estimate		Error	t Value	Pr > |t|
Intercept		0.968571429	B	0.18572796	5.22	0.0004
gender	1	-0.171428571	B	0.20896028	-0.82	0.4311
gender	2	0.000000000	B	.	.	.
bone	1	1.260000000	B	0.25931289	4.86	0.0007
bone	2	1.120000000	B	0.23455733	4.77	0.0008
bone	3	0.000000000	B	.	.	.

```
proc glm data=a1;
    class gender bone;
    model growth=gender bone;
    means gender bone/ tukey lines;
run; quit;
```

Tukey's Studentized Range (HSD) Test for growth

```
Alpha                                      0.05
Error Degrees of Freedom                     10
Error Mean Square                      0.137543
Critical Value of Studentized Range   3.15106
Minimum Significant Difference          0.4417
```

	Mean	N	gender
A	1.6571	7	1
A			
A	1.6286	7	2

```
Alpha                                      0.05
Error Degrees of Freedom                     10
Error Mean Square                      0.137543
Critical Value of Studentized Range   3.87676
Minimum Significant Difference          0.6692
Harmonic Mean of Cell Sizes           4.615385
```

	Mean	N	bone
A	2.1000	4	1
A			
A	2.0200	5	2
B	0.9000	5	3

```
proc glm data=a1;
    class gender bone;
    model growth=gender bone;
    lsmeans gender bone/ adjust=tukey pdiff;
run; quit;
```

Adjustment for Multiple Comparisons: Tukey-Kramer

gender	growth LSMEAN	HO:LSMean1= LSMean2 Pr > |t|
1	1.59047619	0.4311
2	1.76190476	

bone	growth LSMEAN	LSMEAN Number
1	2.14285714	1
2	2.00285714	2
3	0.88285714	3

Least Squares Means for effect bone
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: growth

i/j	1	2	3
1		0.8538	0.0017
2	0.8538		0.0020
3	0.0017	0.0020	

With ODS GRAPHICS ON, the `lsmeans` gives the following pairwise comparison representation.

growth Comparisons for bone
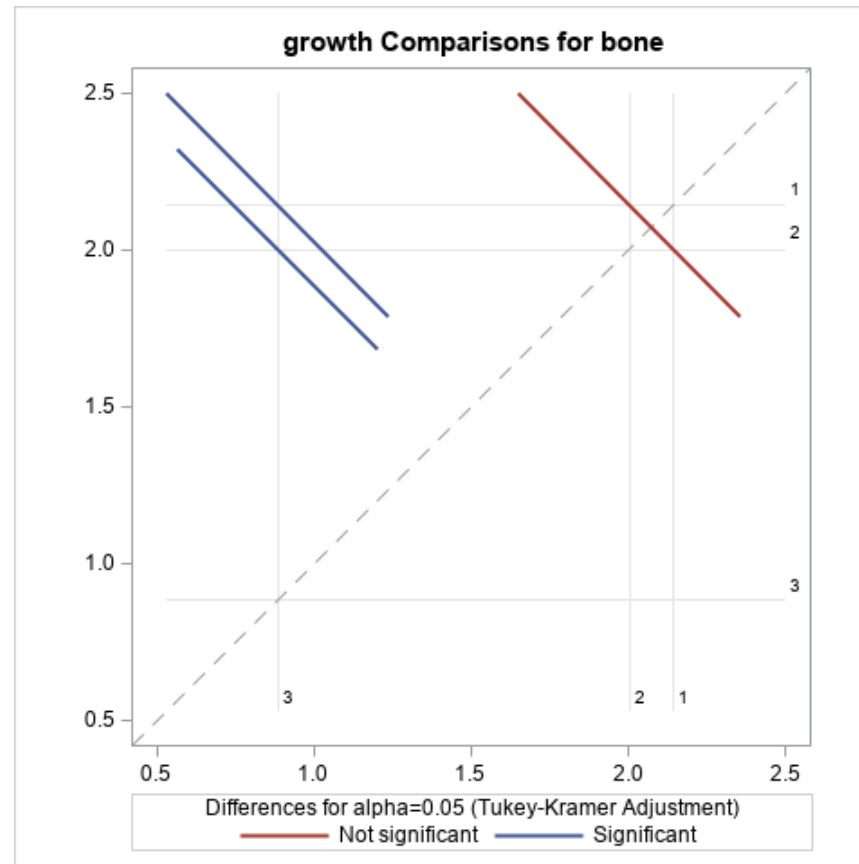
Comments

- The `means` and `lsmeans` are not the same

  - `means` : raw sample mean - similar to a weighted average of cell means (Type I)

  - `lsmeans`: uses parameter estimates - similar to Type III approach

- `lsmeans` most commonly used

Chapter Review

- Two-factor ANOVA with unequal sample sizes

  – Data

  – Model

  – Parameter Estimates

  – Inference