

# STAT 401A - Statistical Methods for Research Workers

## Two-way ANOVA

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# Data

An experiment was run on tomato plants to determine the effect of

- 3 different varieties (A,B,C) and
- 4 different planting densities (10,20,30,40)

on yield.

There is an expectation that planting density will have a different effect depending on the variety. Therefore a **balanced, complete, randomized** design was used.

- complete: each treatment (variety  $\times$  density) is represented in the experiment
- balanced: each treatment in the experiment has the same number of replications
- randomized: treatment was randomly assigned to the plot

This is also referred to as a **full factorial** or **fully crossed** design.

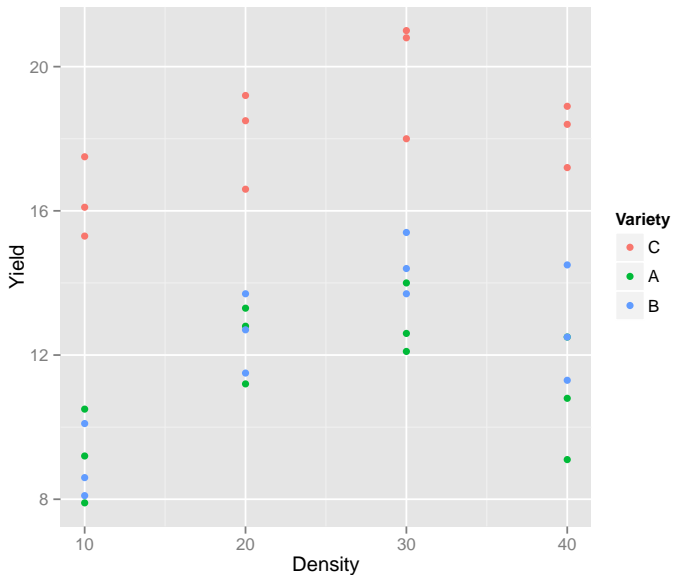
# Hypotheses

- Does variety affect mean yield?
  - Is the mean yield for variety A different from B **on average**?
  - Is the mean yield for variety A different from B **at a particular value for density**?
- Does density affect mean yield?
  - Is the mean yield for density 10 different from density 20 **on average**?
  - Is the mean yield for density 10 different from density 20 **at a particular value for variety**?
- Does density affect yield differently for each variety?

For all of these questions, we want to know

- is there any effect and
- if yes, what is the nature of the effect.

Confidence intervals can answer these questions.



# Summary statistics

## Number of replicates

	Variety	10	20	30	40
1	C	3	3	3	3
2	A	3	3	3	3
3	B	3	3	3	3

## Mean Yield

	Variety	10	20	30	40
1	C	16.300000	18.10000	19.93333	18.16667
2	A	9.200000	12.43333	12.90000	10.80000
3	B	8.933333	12.63333	14.50000	12.76667

## Standard deviation of yield

	Variety	10	20	30	40
1	C	1.113553	1.345362	1.6772994	0.8736895
2	A	1.300000	1.096966	0.9848858	1.7000000
3	B	1.040833	1.101514	0.8544004	1.6165808

# Two-way ANOVA

- Setup: Two categorical explanatory variables with I and J levels
- Model:

$$Y_{ijk} \stackrel{ind}{\sim} N(\mu_{ij}, \sigma^2)$$

where  $Y_{ijk}$  is the

- $k$ th observation at the
- $i$ th level of variable 1 (variety) with  $i = 1, \dots, I$  and the
- $j$ th level of variable 2 (density) with  $j = 1, \dots, J$ .

Consider the models:

- Additive:  $\mu_{ij} = \mu + \nu_i + \delta_j$
- Cell-means:  $\mu_{ij} = \mu + \nu_i + \delta_j + \gamma_{ij}$

	10	20	30	40
A	$\mu_{11}$	$\mu_{12}$	$\mu_{13}$	$\mu_{14}$
B	$\mu_{21}$	$\mu_{22}$	$\mu_{23}$	$\mu_{24}$
C	$\mu_{31}$	$\mu_{32}$	$\mu_{33}$	$\mu_{34}$

# As a regression model

- 1 Assign a reference level for both variety (C) and density (40).
- 2 Let  $V_i$  and  $D_i$  be the variety and density for observation  $i$ .
- 3 Build indicator variables, e.g.  $I(V_i = A)$  and  $I(D_i = 10)$ .
- 4 The additive model:

$$\mu_i = \beta_0 + \beta_1 I(V_i = A) + \beta_2 I(V_i = B) \\ + \beta_3 I(D_i = 10) + \beta_4 I(D_i = 20) + \beta_5 I(D_i = 30).$$

$\beta_1$  is the expected difference in yield between varieties A and C at any fixed density

- 5 The cell-means model:

$$\mu_i = \beta_0 + \beta_1 I(V_i = A) + \beta_2 I(V_i = B) \\ + \beta_3 I(D_i = 10) + \beta_4 I(D_i = 20) + \beta_5 I(D_i = 30) \\ + \beta_6 I(V_i = A)I(D_i = 10) + \beta_7 I(V_i = A)I(D_i = 20) + \beta_8 I(V_i = A)I(D_i = 30) \\ + \beta_9 I(V_i = B)I(D_i = 10) + \beta_{10} I(V_i = B)I(D_i = 20) + \beta_{11} I(V_i = B)I(D_i = 30)$$

$\beta_1$  is the expected difference in yield between varieties A and C at a density of 40

# ANOVA Table

## ANOVA Table - Additive model

Source	SS	df	MS	F
Factor A	SSA	(I-1)	$SSA/(I-1)$	$MSA/MSE$
Factor B	SSB	(J-1)	$SSB/(J-1)$	$MSB/MSE$
Error	SSE	n-I-J-1	$SSE/(n-I-J-1)$	
Total	SST	n-1		

## ANOVA Table - Cell-means model

Source	SS	df	MS	
Factor A	SSA	I-1	$SSA/(I-1)$	$MSA/MSE$
Factor B	SSB	J-1	$SSB/(J-1)$	$MSB/MSE$
Interaction AB	SSAB	(I-1)(J-1)	$SSAB/(I-1)(J-1)$	$MSAB/MSE$
Error	SSE	n-IJ	$SSE/(n-IJ)$	
Total	SST	n-1		



## Additive vs cell-means

Opinions differ on whether to use an additive vs a cell-means model when the interaction is not significant. Remember that an insignificant test does not prove that there is no interaction.

	Additive	Cell-means
Interpretation	Direct	Complicated
Estimate of $\sigma^2$	Biased	Unbiased

We will continue using the cell-means model to answer the scientific questions of interest.

## Two-way ANOVA using PROC GLM

```
DATA tomato;  
  INFILE 'Ch13-tomato.csv' DSD FIRSTOBS=2;  
  INPUT variety $ density yield;  
  
PROC GLM DATA=tomato PLOTS=all;  
  CLASS variety density;  
  MODEL yield = variety|density / SOLUTION;  
  LSMEANS variety / cl adjust=tukey;  
  LSMEANS density / cl adjust=tukey;  
  LSMEANS variety*density / cl adjust=tukey;  
RUN;
```

# Two-way ANOVA using PROC GLM

## The GLM Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	422.3155556	38.3923232	24.22	<.0001
Error	24	38.0400000	1.5850000		
Corrected Total	35	460.3555556			

R-Square	Coeff Var	Root MSE	yield Mean
0.917368	9.064568	1.258968	13.88889

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety	2	327.5972222	163.7986111	103.34	<.0001
density	3	86.6866667	28.8955556	18.23	<.0001
variety*density	6	8.0316667	1.3386111	0.84	0.5484

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	2	327.5972222	163.7986111	103.34	<.0001
density	3	86.6866667	28.8955556	18.23	<.0001
variety*density	6	8.0316667	1.3386111	0.84	0.5484

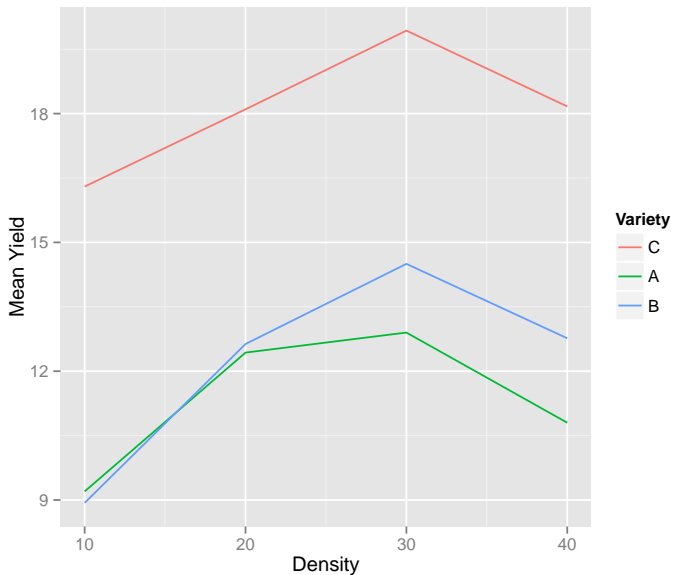
The Type I and Type III SS are equal because the design is balanced.

# Two-way ANOVA using PROC GLM

```
MODEL yield = variety|density / SOLUTION;
```

The GLM Procedure

Parameter		Estimate	Standard Error	t Value	Pr >  t
Intercept		18.16666667 B	0.72686542	24.99	<.0001
variety	A	-7.36666667 B	1.02794293	-7.17	<.0001
variety	B	-5.40000000 B	1.02794293	-5.25	<.0001
variety	C	0.00000000 B	.	.	.
density	10	-1.86666667 B	1.02794293	-1.82	0.0819
density	20	-0.06666667 B	1.02794293	-0.06	0.9488
density	30	1.76666667 B	1.02794293	1.72	0.0986
density	40	0.00000000 B	.	.	.
variety*density	A 10	0.26666667 B	1.45373083	0.18	0.8560
variety*density	A 20	1.70000000 B	1.45373083	1.17	0.2537
variety*density	A 30	0.33333333 B	1.45373083	0.23	0.8206
variety*density	A 40	0.00000000 B	.	.	.
variety*density	B 10	-1.96666667 B	1.45373083	-1.35	0.1887
variety*density	B 20	-0.06666667 B	1.45373083	-0.05	0.9638
variety*density	B 30	-0.03333333 B	1.45373083	-0.02	0.9819
variety*density	B 40	0.00000000 B	.	.	.
variety*density	C 10	0.00000000 B	.	.	.



# Is the mean yield for variety A different from B on average?

```
LSMEANS variety / cl adjust=tukey;
```

Least Squares Means

Adjustment for Multiple Comparisons: Tukey

...

Least Squares Means for effect variety

Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: yield

i/j	1	2	3
1		0.2249	<.0001
2	0.2249		<.0001
3	<.0001	<.0001	

variety	yield LSMEAN	95% Confidence Limits	
A	11.333333	10.583245	12.083422
B	12.208333	11.458245	12.958422
C	18.125000	17.374912	18.875088

Least Squares Means for Effect variety

Difference

Simultaneous 95%

Between

Confidence Limits for

Means

LSMean(i)-LSMean(j)

i	j			
1	2	-0.875000	-2.158534	0.408534
1	3	-6.791667	-8.075201	-5.508132
2	3	-5.916667	-7.200201	-4.633132

# Is the mean yield at density 10 different from density 20 on average?

```
LSMEANS density / cl adjust=tukey;
```

Least Squares Means  
Adjustment for Multiple Comparisons: Tukey  
...

density	yield LSMEAN	95% Confidence Limits	
10	11.477778	10.611650	12.343905
20	14.388889	13.522762	15.255016
30	15.777778	14.911650	16.643905
40	13.911111	13.044984	14.777238

Least Squares Means for Effect density				
		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
i	j	Means	LSMean(i)-LSMean(j)	
1	2	-2.911111	-4.548299	-1.273923
1	3	-4.300000	-5.937188	-2.662812
1	4	-2.433333	-4.070521	-0.796145
2	3	-1.388889	-3.026077	0.248299
2	4	0.477778	-1.159410	2.114966
3	4	1.866667	0.229479	3.503855

# Is mean yield different for particular combinations?

```
LSMEANS variety*density / cl adjust=tukey;
```

variety	density	yield LSMEAN	95% Confidence Limits	
A	10	9.200000	7.699824	10.700176
A	20	12.433333	10.933157	13.933510
A	30	12.900000	11.399824	14.400176
A	40	10.800000	9.299824	12.300176
B	10	8.933333	7.433157	10.433510
B	20	12.633333	11.133157	14.133510
B	30	14.500000	12.999824	16.000176
B	40	12.766667	11.266490	14.266843
C	10	16.300000	14.799824	17.800176
C	20	18.100000	16.599824	19.600176
C	30	19.933333	18.433157	21.433510
C	40	18.166667	16.666490	19.666843



# Is mean yield different for particular combinations?

```
LSMEANS variety*density / cl adjust=tukey;
```

Least Squares Means for Effect variety\*density

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
		Means	LSMean(i)-LSMean(j)	
i	j			
1	2	-3.233333	-6.939704	0.473037
1	3	-3.700000	-7.406371	0.006371
1	4	-1.600000	-5.306371	2.106371
1	5	0.266667	-3.439704	3.973037
1	6	-3.433333	-7.139704	0.273037
1	7	-5.300000	-9.006371	-1.593629
1	8	-3.566667	-7.273037	0.139704
1	9	-7.100000	-10.806371	-3.393629
1	10	-8.900000	-12.606371	-5.193629
1	11	-10.733333	-14.439704	-7.026963
1	12	-8.966667	-12.673037	-5.260296
2	3	-0.466667	-4.173037	3.239704
2	4	1.633333	-2.073037	5.339704
2	5	3.500000	-0.206371	7.206371
2	6	-0.200000	-3.906371	3.506371
2	7	-2.066667	-5.773037	1.639704
2	8	-0.333333	-4.039704	3.373037
2	9	-3.866667	-7.573037	-0.160296
2	10	-5.666667	-9.373037	-1.960296
2	11	-7.500000	-11.206371	-3.793629
2	12	-5.733333	-9.439704	-2.026963
3	4	2.100000	-1.606371	5.806371
3	5	3.966667	0.260296	7.673037
3	6	0.266667	-3.439704	3.973037

```
tomato$Density = factor(tomato$Density)
m = lm(Yield~Variety*Density, tomato)
anova(m)
```

#### Analysis of Variance Table

Response: Yield

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	327.60	163.799	103.3430	1.608e-12 ***
Density	3	86.69	28.896	18.2306	2.212e-06 ***
Variety:Density	6	8.03	1.339	0.8445	0.5484
Residuals	24	38.04	1.585		

---

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

```
library(lsmmeans)
lsmeans(m, pairwise~Variety)
```

```
$lsmeans
  Variety    lsmean      SE df lower.CL upper.CL
C         18.12500 0.3634327 24  17.37491  18.87509
A         11.33333 0.3634327 24  10.58325  12.08342
B         12.20833 0.3634327 24  11.45825  12.95842
```

Results are averaged over the levels of: Density  
Confidence level used: 0.95

```
$contrasts
  contrast      estimate      SE df t.ratio p.value
C - A      6.791667 0.5139715 24   13.214 <.0001
C - B      5.916667 0.5139715 24   11.512 <.0001
A - B     -0.875000 0.5139715 24   -1.702  0.2249
```

Results are averaged over the levels of: Density  
P value adjustment: tukey method for a family of 3 means

```
lsmeans(m, pairwise~Density)
```

```
$lsmeans
```

Density	lsmean	SE	df	lower.CL	upper.CL
10	11.47778	0.4196559	24	10.61165	12.34391
20	14.38889	0.4196559	24	13.52276	15.25502
30	15.77778	0.4196559	24	14.91165	16.64391
40	13.91111	0.4196559	24	13.04498	14.77724

Results are averaged over the levels of: Variety

Confidence level used: 0.95

```
$contrasts
```

contrast	estimate	SE	df	t.ratio	p.value
10 - 20	-2.9111111	0.5934831	24	-4.905	0.0003
10 - 30	-4.3000000	0.5934831	24	-7.245	<.0001
10 - 40	-2.4333333	0.5934831	24	-4.100	0.0022
20 - 30	-1.3888889	0.5934831	24	-2.340	0.1169
20 - 40	0.4777778	0.5934831	24	0.805	0.8514
30 - 40	1.8666667	0.5934831	24	3.145	0.0213

Results are averaged over the levels of: Variety

P value adjustment: tukey method for a family of 4 means

```
lsmeans(m, pairwise~Variety*Density)
```

```
$lsmeans
```

Variety	Density	lsmean	SE	df	lower.CL	upper.CL
C	10	16.300000	0.7268654	24	14.799824	17.80018
A	10	9.200000	0.7268654	24	7.699824	10.70018
B	10	8.933333	0.7268654	24	7.433157	10.43351
C	20	18.100000	0.7268654	24	16.599824	19.60018
A	20	12.433333	0.7268654	24	10.933157	13.93351
B	20	12.633333	0.7268654	24	11.133157	14.13351
C	30	19.933333	0.7268654	24	18.433157	21.43351
A	30	12.900000	0.7268654	24	11.399824	14.40018
B	30	14.500000	0.7268654	24	12.999824	16.00018
C	40	18.166667	0.7268654	24	16.666490	19.66684
A	40	10.800000	0.7268654	24	9.299824	12.30018
B	40	12.766667	0.7268654	24	11.266490	14.26684

Confidence level used: 0.95

```
$contrasts
```

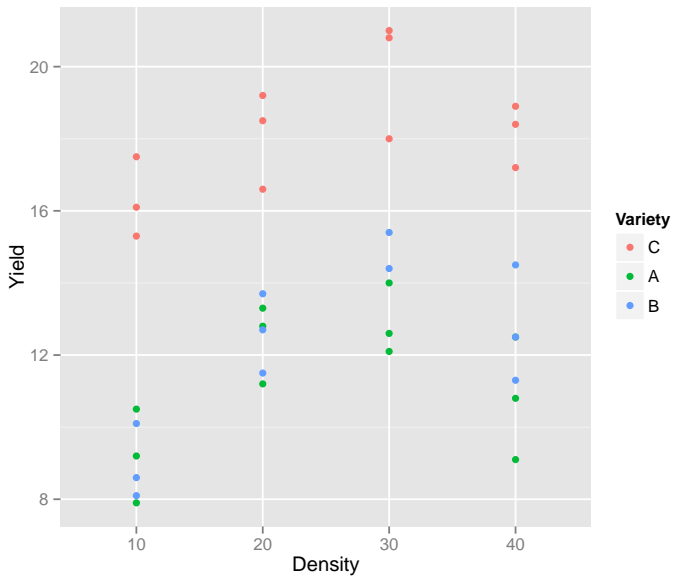
contrast	estimate	SE	df	t.ratio	p.value
C,10 - A,10	7.10000000	1.027943	24	6.907	<.0001
C,10 - B,10	7.36666667	1.027943	24	7.166	<.0001
C,10 - C,20	-1.80000000	1.027943	24	-1.751	0.8276
C,10 - A,20	3.86666667	1.027943	24	3.762	0.0356
C,10 - B,20	3.66666667	1.027943	24	3.567	0.0543
C,10 - C,30	-3.63333333	1.027943	24	-3.535	0.0582
C,10 - A,30	3.40000000	1.027943	24	3.308	0.0932
C,10 - B,30	1.80000000	1.027943	24	1.751	0.8276
C,10 - C,40	-1.86666667	1.027943	24	-1.816	0.7947
C,10 - A,40	5.50000000	1.027943	24	5.350	0.0008
C,10 - B,40	3.53333333	1.027943	24	3.437	0.0714
A,10 - B,10	0.26666667	1.027943	24	0.259	1.0000
A,10 - C,20	-8.90000000	1.027943	24	-8.658	<.0001
A,10 - A,20	-3.23333333	1.027943	24	-3.145	0.1284

# Summary

- Use LSMEANS to answer questions of scientific interest.
- Check model assumptions
- Consider alternative models, e.g. treating density as continuous

# Unbalanced design

Suppose for some reason that a variety B, density 30 sample was contaminated. Although you started with a balanced design, the data is now unbalanced. Fortunately, we can still use the tools we have used previously.





# Summary statistics

## Number of replicates

	Variety	10	20	30	40
1	C	3	3	3	3
2	A	3	3	3	3
3	B	3	3	2	3

## Mean Yield

	Variety	10	20	30	40
1	C	16.300000	18.10000	19.93333	18.16667
2	A	9.200000	12.43333	12.90000	10.80000
3	B	8.933333	12.63333	14.90000	12.76667

## Standard deviation of yield

	Variety	10	20	30	40
1	C	1.113553	1.345362	1.6772994	0.8736895
2	A	1.300000	1.096966	0.9848858	1.7000000
3	B	1.040833	1.101514	0.7071068	1.6165808

# Two-way ANOVA using PROC GLM

```
DATA tomato;
  INFILE 'Ch13-tomato.csv' DSD FIRSTOBS=2;
  INPUT variety $ density yield;
  i = _n_;

PROC GLM DATA=tomato PLOTS=all;
  WHERE i ^= 19; /* not equal to 19 */
  CLASS variety density;
  MODEL yield = variety|density / SOLUTION;
  LSMEANS variety / cl adjust=tukey;
  LSMEANS density / cl adjust=tukey;
  LSMEANS variety*density / cl adjust=tukey;
RUN;
```

# Two-way ANOVA using PROC GLM

## The GLM Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	423.2388571	38.4762597	23.87	<.0001
Error	23	37.0800000	1.6121739		
Corrected Total	34	460.3188571			

R-Square	Coeff Var	Root MSE	yield Mean
0.919447	9.138391	1.269714	13.89429

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety	2	329.9878723	164.9939361	102.34	<.0001
density	3	84.4486608	28.1495536	17.46	<.0001
variety*density	6	8.8023241	1.4670540	0.91	0.5052

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	2	320.0374679	160.0187340	99.26	<.0001
density	3	86.0657613	28.6885871	17.79	<.0001
variety*density	6	8.8023241	1.4670540	0.91	0.5052

# Two-way ANOVA using PROC GLM

Parameter		Estimate	Standard Error	t Value	Pr >  t
Intercept		18.16666667 B	0.73306978	24.78	<.0001
variety	A	-7.36666667 B	1.03671723	-7.11	<.0001
variety	B	-5.40000000 B	1.03671723	-5.21	<.0001
variety	C	0.00000000 B	.	.	.
density	10	-1.86666667 B	1.03671723	-1.80	0.0849
density	20	-0.06666667 B	1.03671723	-0.06	0.9493
density	30	1.76666667 B	1.03671723	1.70	0.1018
density	40	0.00000000 B	.	.	.
variety*density	A 10	0.26666667 B	1.46613956	0.18	0.8573
variety*density	A 20	1.70000000 B	1.46613956	1.16	0.2581
variety*density	A 30	0.33333333 B	1.46613956	0.23	0.8222
variety*density	A 40	0.00000000 B	.	.	.
variety*density	B 10	-1.96666667 B	1.46613956	-1.34	0.1929
variety*density	B 20	-0.06666667 B	1.46613956	-0.05	0.9641
variety*density	B 30	0.36666667 B	1.55507584	0.24	0.8157
variety*density	B 40	0.00000000 B	.	.	.
variety*density	C 10	0.00000000 B	.	.	.
variety*density	C 20	0.00000000 B	.	.	.
variety*density	C 30	0.00000000 B	.	.	.
variety*density	C 40	0.00000000 B	.	.	.

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

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

Dependent Variable: yield

i/j	1	2	3
1		0.1839	<.0001
2	0.1839		<.0001
3	<.0001	<.0001	

variety	yield LSMEAN	95% Confidence Limits	
A	11.333333	10.575098	12.091569
B	12.308333	11.504103	13.112563
C	18.125000	17.366765	18.883235

Least Squares Means for Effect variety

		Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
i	j			
1	2	-0.975000	-2.313097	0.363097
1	3	-6.791667	-8.089811	-5.493522
2	3	-5.816667	-7.154763	-4.478570

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

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

Dependent Variable: yield

i/j	1	2	3	4
1		0.0004	<.0001	0.0025
2	0.0004		0.0967	0.8545
3	<.0001	0.0967		0.0189
4	0.0025	0.8545	0.0189	

density	yield LSMEAN	95% Confidence Limits	
10	11.477778	10.602243	12.353312
20	14.388889	13.513354	15.264423
30	15.911111	14.965426	16.856797
40	13.911111	13.035577	14.786646

Least Squares Means for Effect density

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
i	j	Means	LSMean(i)-LSMean(j)	
1	2	-2.911111	-4.567433	-1.254789
1	3	-4.433333	-6.157288	-2.709379
1	4	-2.433333	-4.089656	-0.777011
2	3	-1.522222	-3.246177	0.201733

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

variety	density	yield LSMEAN	LSMEAN Number
A	10	9.2000000	1
A	20	12.4333333	2
A	30	12.9000000	3
A	40	10.8000000	4
B	10	8.9333333	5
B	20	12.6333333	6
B	30	14.9000000	7
B	40	12.7666667	8
C	10	16.3000000	9
C	20	18.1000000	10
C	30	19.9333333	11
C	40	18.1666667	12

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

Least Squares Means for Effect variety\*density

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
		Means	LSMean(i)-LSMean(j)	
i	j			
1	11	-10.733333	-14.487164	-6.979502
1	12	-8.966667	-12.720498	-5.212836
2	3	-0.466667	-4.220498	3.287164
2	4	1.633333	-2.120498	5.387164
2	5	3.500000	-0.253831	7.253831
2	6	-0.200000	-3.953831	3.553831
2	7	-2.466667	-6.663577	1.730244
2	8	-0.333333	-4.087164	3.420498
2	9	-3.866667	-7.620498	-0.112836
2	10	-5.666667	-9.420498	-1.912836
2	11	-7.500000	-11.253831	-3.746169
2	12	-5.733333	-9.487164	-1.979502
3	4	2.100000	-1.653831	5.853831
3	5	3.966667	0.212836	7.720498
3	6	0.266667	-3.487164	4.020498
3	7	-2.000000	-6.196911	2.196911
3	8	0.133333	-3.620498	3.887164
3	9	-3.400000	-7.153831	0.353831
3	10	-5.200000	-8.953831	-1.446169
3	11	-7.033333	-10.787164	-3.279502
3	12	-5.266667	-9.020498	-1.512836
4	5	1.866667	-1.887164	5.620498



```
m = lm(Yield~Variety*Density, tomato)
anova(m)
```

# Analysis of Variance Table

Response: Yield

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	327.60	163.799	103.3430	1.608e-12 ***
Density	3	86.69	28.896	18.2306	2.212e-06 ***
Variety:Density	6	8.03	1.339	0.8445	0.5484
Residuals	24	38.04	1.585		

---

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

```
lsmeans(m, pairwise~Variety)
```

```
$lsmeans
```

Variety	lsmean	SE	df	lower.CL	upper.CL
C	18.12500	0.3634327	24	17.37491	18.87509
A	11.33333	0.3634327	24	10.58325	12.08342
B	12.20833	0.3634327	24	11.45825	12.95842

Results are averaged over the levels of: Density  
Confidence level used: 0.95

```
$contrasts
```

contrast	estimate	SE	df	t.ratio	p.value
C - A	6.791667	0.5139715	24	13.214	<.0001
C - B	5.916667	0.5139715	24	11.512	<.0001
A - B	-0.875000	0.5139715	24	-1.702	0.2249

Results are averaged over the levels of: Density  
P value adjustment: tukey method for a family of 3 means

```
lsmeans(m, pairwise~Density)
```

```
$lsmeans
```

Density	lsmean	SE	df	lower.CL	upper.CL
10	11.47778	0.4196559	24	10.61165	12.34391
20	14.38889	0.4196559	24	13.52276	15.25502
30	15.77778	0.4196559	24	14.91165	16.64391
40	13.91111	0.4196559	24	13.04498	14.77724

Results are averaged over the levels of: Variety

Confidence level used: 0.95

```
$contrasts
```

contrast	estimate	SE	df	t.ratio	p.value
10 - 20	-2.9111111	0.5934831	24	-4.905	0.0003
10 - 30	-4.3000000	0.5934831	24	-7.245	<.0001
10 - 40	-2.4333333	0.5934831	24	-4.100	0.0022
20 - 30	-1.3888889	0.5934831	24	-2.340	0.1169
20 - 40	0.4777778	0.5934831	24	0.805	0.8514
30 - 40	1.8666667	0.5934831	24	3.145	0.0213

Results are averaged over the levels of: Variety

P value adjustment: tukey method for a family of 4 means

```
lsmeans(m, pairwise~Variety*Density)
```

```
$lsmeans
```

Variety	Density	lsmean	SE	df	lower.CL	upper.CL
C	10	16.300000	0.7268654	24	14.799824	17.80018
A	10	9.200000	0.7268654	24	7.699824	10.70018
B	10	8.933333	0.7268654	24	7.433157	10.43351
C	20	18.100000	0.7268654	24	16.599824	19.60018
A	20	12.433333	0.7268654	24	10.933157	13.93351
B	20	12.633333	0.7268654	24	11.133157	14.13351
C	30	19.933333	0.7268654	24	18.433157	21.43351
A	30	12.900000	0.7268654	24	11.399824	14.40018
B	30	14.500000	0.7268654	24	12.999824	16.00018
C	40	18.166667	0.7268654	24	16.666490	19.66684
A	40	10.800000	0.7268654	24	9.299824	12.30018
B	40	12.766667	0.7268654	24	11.266490	14.26684

Confidence level used: 0.95

```
$contrasts
```

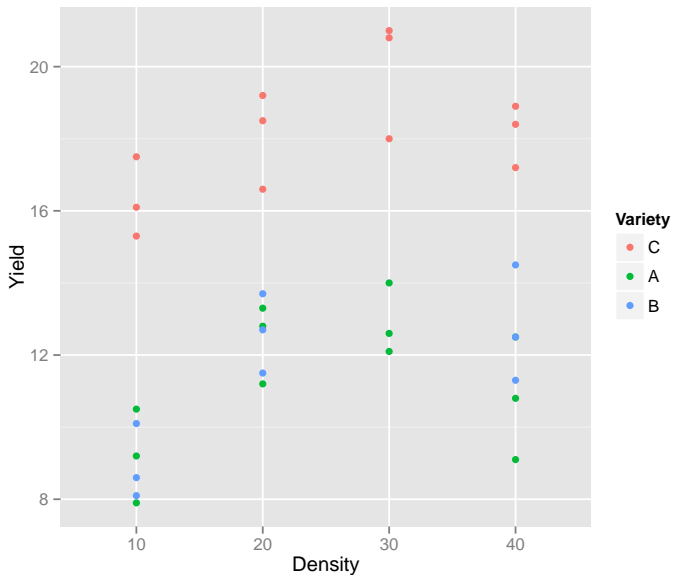
contrast	estimate	SE	df	t.ratio	p.value
C,10 - A,10	7.10000000	1.027943	24	6.907	<.0001
C,10 - B,10	7.36666667	1.027943	24	7.166	<.0001
C,10 - C,20	-1.80000000	1.027943	24	-1.751	0.8276
C,10 - A,20	3.86666667	1.027943	24	3.762	0.0356
C,10 - B,20	3.66666667	1.027943	24	3.567	0.0543
C,10 - C,30	-3.63333333	1.027943	24	-3.535	0.0582
C,10 - A,30	3.40000000	1.027943	24	3.308	0.0932
C,10 - B,30	1.80000000	1.027943	24	1.751	0.8276
C,10 - C,40	-1.86666667	1.027943	24	-1.816	0.7947
C,10 - A,40	5.50000000	1.027943	24	5.350	0.0008
C,10 - B,40	3.53333333	1.027943	24	3.437	0.0714
A,10 - B,10	0.26666667	1.027943	24	0.259	1.0000
A,10 - C,20	-8.90000000	1.027943	24	-8.658	<.0001
A,10 - A,20	-3.23333333	1.027943	24	-3.145	0.1284

# Summary

The analysis can be completed just like the balanced design using LSMEANS to answer scientific questions of interest.

# Incomplete design

Suppose none of the samples from Variety B, density 30 were obtained. Now the analysis becomes more complicated.



# Summary statistics

## Number of replicates

	Variety	10	20	30	40
1	C	3	3	3	3
2	A	3	3	3	3
3	B	3	3	0	3

## Mean Yield

	Variety	10	20	30	40
1	C	16.300000	18.10000	19.93333	18.16667
2	A	9.200000	12.43333	12.90000	10.80000
3	B	8.933333	12.63333	NaN	12.76667

## Standard deviation of yield

	Variety	10	20	30	40
1	C	1.113553	1.345362	1.6772994	0.8736895
2	A	1.300000	1.096966	0.9848858	1.7000000
3	B	1.040833	1.101514	NA	1.6165808



# Two-way ANOVA using PROC GLM

```
DATA tomato;
  INFILE 'Ch13-tomato.csv' DSD FIRSTOBS=2;
  INPUT variety $ density yield;

PROC GLM DATA=tomato PLOTS=all;
  WHERE ~(variety='B' & density=30);
  CLASS variety density;
  MODEL yield = variety|density / SOLUTION;
  LSMEANS variety / cl adjust=tukey;
  LSMEANS density / cl adjust=tukey;
  LSMEANS variety*density / cl adjust=tukey;
RUN;
```

# Two-way ANOVA using PROC GLM

## The GLM Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	421.0933333	42.1093333	25.33	<.0001
Error	22	36.5800000	1.6627273		
Corrected Total	32	457.6733333			

R-Square	Coeff Var	Root MSE	yield Mean
0.920074	9.321454	1.289468	13.83333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety	2	347.3819444	173.6909722	104.46	<.0001
density	3	66.6531019	22.2177006	13.36	<.0001
variety*density	5	7.0582870	1.4116574	0.85	0.5300

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety	2	321.2233796	160.6116898	96.60	<.0001
density	3	66.6531019	22.2177006	13.36	<.0001
variety*density	5	7.0582870	1.4116574	0.85	0.5300

# Two-way ANOVA using PROC GLM

Parameter		Estimate		Standard Error	t Value	Pr >  t
Intercept		18.16666667	B	0.74447460	24.40	<.0001
variety	A	-7.36666667	B	1.05284607	-7.00	<.0001
variety	B	-5.40000000	B	1.05284607	-5.13	<.0001
variety	C	0.00000000	B	.	.	.
density	10	-1.86666667	B	1.05284607	-1.77	0.0901
density	20	-0.06666667	B	1.05284607	-0.06	0.9501
density	30	1.76666667	B	1.05284607	1.68	0.1075
density	40	0.00000000	B	.	.	.
variety*density	A 10	0.26666667	B	1.48894919	0.18	0.8595
variety*density	A 20	1.70000000	B	1.48894919	1.14	0.2658
variety*density	A 30	0.33333333	B	1.48894919	0.22	0.8249
variety*density	A 40	0.00000000	B	.	.	.
variety*density	B 10	-1.96666667	B	1.48894919	-1.32	0.2001
variety*density	B 20	-0.06666667	B	1.48894919	-0.04	0.9647
variety*density	B 40	0.00000000	B	.	.	.
variety*density	C 10	0.00000000	B	.	.	.
variety*density	C 20	0.00000000	B	.	.	.
variety*density	C 30	0.00000000	B	.	.	.
variety*density	C 40	0.00000000	B	.	.	.

Notice the missing variety\*density B 30 line.

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

variety	yield LSMEAN	LSMEAN Number
A	11.3333333	1
B	Non-est	2
C	18.1250000	3

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

variety	yield LSMEAN	95% Confidence Limits	
A	11.333333	10.561360	12.105306
B	.	.	.
C	18.125000	17.353027	18.896973

Least Squares Means for Effect variety

		Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)	
i	j			
1	2	.	.	.
1	3	-6.791667	-7.883358	-5.699975
2	3	.	.	.

# Two-way ANOVA using PROC GLM

The GLM Procedure  
 Least Squares Means  
 Adjustment for Multiple Comparisons: Tukey-Kramer

		LSMEAN	
	density	yield LSMEAN	Number
	10	11.4777778	1
	20	14.3888889	2
	30	Non-est	3
	40	13.9111111	4

	density	yield LSMEAN	95% Confidence Limits	
	10	11.477778	10.586380	12.369175
	20	14.388889	13.497491	15.280286
	30	.	.	.
	40	13.911111	13.019714	14.802509

Least Squares Means for Effect density

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
i	j	Means	LSMean(i)-LSMean(j)	
1	2	-2.911111	-4.438096	-1.384126
1	3	.	.	.
1	4	-2.433333	-3.960319	-0.906348
2	3	.	.	.
2	4	0.477778	-1.049207	2.004763
3	4	.	.	.

# Two-way ANOVA using PROC GLM

The GLM Procedure  
Least Squares Means  
Adjustment for Multiple Comparisons: Tukey

variety	density	yield LSMEAN	LSMEAN Number
A	10	9.2000000	1
A	20	12.4333333	2
A	30	12.9000000	3
A	40	10.8000000	4
B	10	8.9333333	5
B	20	12.6333333	6
B	40	12.7666667	7
C	10	16.3000000	8
C	20	18.1000000	9
C	30	19.9333333	10

# Two-way ANOVA using PROC GLM

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
i	j	Means	LSMean(i)-LSMean(j)	
1	2	-3.233333	-6.997053	0.530387
1	3	-3.700000	-7.463720	0.063720
1	4	-1.600000	-5.363720	2.163720
1	5	0.266667	-3.497053	4.030387
1	6	-3.433333	-7.197053	0.330387
1	7	-3.566667	-7.330387	0.197053
1	8	-7.100000	-10.863720	-3.336280
1	9	-8.900000	-12.663720	-5.136280
1	10	-10.733333	-14.497053	-6.969613
1	11	-8.966667	-12.730387	-5.202947
2	3	-0.466667	-4.230387	3.297053
2	4	1.633333	-2.130387	5.397053
2	5	3.500000	-0.263720	7.263720
2	6	-0.200000	-3.963720	3.563720
2	7	-0.333333	-4.097053	3.430387
2	8	-3.866667	-7.630387	-0.102947
2	9	-5.666667	-9.430387	-1.902947
2	10	-7.500000	-11.263720	-3.736280
2	11	-5.733333	-9.497053	-1.969613
3	4	2.100000	-1.663720	5.863720
3	5	3.966667	0.202947	7.730387
3	6	0.266667	-3.497053	4.030387
3	7	0.133333	-3.630387	3.897053
3	8	-3.400000	-7.163720	0.363720
3	9	-5.200000	-8.963720	-1.436280
3	10	-7.033333	-10.797053	-3.269613
3	11	-5.266667	-9.030387	-1.502947
4	5	1.866667	-1.897053	5.630387

## Treat as a One-way ANOVA

When the data are incomplete, use a one-way ANOVA combined with contrasts to answer questions of interest. For example, to compare the average difference between B and C, we want to only compare at densities 10, 20, and 40.

	10	20	30	40
A	$\mu_{11}$	$\mu_{12}$	$\mu_{13}$	$\mu_{14}$
B	$\mu_{21}$	$\mu_{22}$	$\mu_{23}$	$\mu_{24}$
C	$\mu_{31}$	$\mu_{32}$	$\mu_{33}$	$\mu_{34}$

Thus, the contrast is

$$\begin{aligned}\gamma &= \frac{1}{3}(\mu_{31} + \mu_{32} + \mu_{34}) - \frac{1}{3}(\mu_{21} + \mu_{22} + \mu_{24}) \\ &= \frac{1}{3}(\mu_{31} + \mu_{32} + \mu_{34} - \mu_{21} - \mu_{22} - \mu_{24})\end{aligned}$$



# Two-way ANOVA using PROC GLM

```
DATA tomato;
  INFILE 'Ch13-tomato.csv' DSD FIRSTOBS=2;
  INPUT variety $ density yield;

PROC GLM DATA=tomato PLOTS=all;
  WHERE ~(variety='B' & density=30);
  CLASS variety density;
  MODEL yield = variety*density / SOLUTION CLPARM;
  LSMEANS variety*density / cl adjust=tukey;
  /*
      A10 A20 A30 A40 B10 B20 B40 C10 C20 C30 C40 */
  ESTIMATE 'C-B' variety*density  0  0  0  0 -1 -1 -1  1  1  0  1 / DIVISOR=3;
  ESTIMATE 'C-A' variety*density -1 -1 -1 -1  0  0  0  1  1  1  1 / DIVISOR=4;
  ESTIMATE 'B-A' variety*density -1 -1  0 -1  1  1  1  0  0  0  0 / DIVISOR=3;
  /* we could do the densities similarly */
RUN;
```

# Two-way ANOVA using PROC GLM

Dependent Variable: yield

The GLM Procedure

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	421.0933333	42.1093333	25.33	<.0001
Error	22	36.5800000	1.6627273		
Corrected Total	32	457.6733333			

	R-Square	Coeff Var	Root MSE	yield Mean
	0.920074	9.321454	1.289468	13.83333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
variety*density	10	421.0933333	42.1093333	25.33	<.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
variety*density	10	421.0933333	42.1093333	25.33	<.0001

# Two-way ANOVA using PROC GLM

Parameter	Estimate	Standard Error	t Value	Pr >  t	95% Confidence Limits	
Intercept	18.16666667 B	0.74447460	24.40	<.0001	16.62272085	19.71061248
variety*density A 10	-8.96666667 B	1.05284607	-8.52	<.0001	-11.15013578	-6.78319756
variety*density A 20	-5.73333333 B	1.05284607	-5.45	<.0001	-7.91680244	-3.54986422
variety*density A 30	-5.26666667 B	1.05284607	-5.00	<.0001	-7.45013578	-3.08319756
variety*density A 40	-7.36666667 B	1.05284607	-7.00	<.0001	-9.55013578	-5.18319756
variety*density B 10	-9.23333333 B	1.05284607	-8.77	<.0001	-11.41680244	-7.04986422
variety*density B 20	-5.53333333 B	1.05284607	-5.26	<.0001	-7.71680244	-3.34986422
variety*density B 40	-5.40000000 B	1.05284607	-5.13	<.0001	-7.58346911	-3.21653089
variety*density C 10	-1.86666667 B	1.05284607	-1.77	0.0901	-4.05013578	0.31680244
variety*density C 20	-0.06666667 B	1.05284607	-0.06	0.9501	-2.25013578	2.11680244
variety*density C 30	1.76666667 B	1.05284607	1.68	0.1075	-0.41680244	3.95013578
variety*density C 40	0.00000000 B	.	.	.	.	.

# The Regression model

The regression model here considers variety-density combination as a single explanatory variable with 11 levels: A10, A20, A30, A40, B10, B20, B40, C10, C20, C30, and C40. By default, SAS chose C40 as our reference level. For observation  $i$ , let

- $Y_i$  be the yield
- $V_i$  be the variety
- $D_i$  be the density

The model is then  $Y_i \stackrel{ind}{\sim} N(\mu_i, \sigma^2)$  and

$$\begin{aligned} \mu_i = \beta_0 & + \beta_1 I(V_i = A, D_i = 10) & + \beta_2 I(V_i = A, D_i = 20) & + \beta_3 I(V_i = A, D_i = 30) & + \beta_4 I(V_i = A, D_i = 40) \\ & + \beta_5 I(V_i = B, D_i = 10) & + \beta_6 I(V_i = B, D_i = 20) & & + \beta_7 I(V_i = B, D_i = 40) \\ & + \beta_8 I(V_i = C, D_i = 10) & + \beta_9 I(V_i = C, D_i = 20) & + \beta_{10} I(V_i = C, D_i = 30) \end{aligned}$$

# Two-way ANOVA using PROC GLM

## The GLM Procedure

Dependent Variable: yield

Parameter	Estimate	Standard Error	t Value	Pr >  t	95% Confidence Limits	
C-B	6.07777778	0.60786096	10.00	<.0001	4.81715130	7.33840426
C-A	6.79166667	0.52642304	12.90	<.0001	5.69993211	7.88340122
B-A	0.63333333	0.60786096	1.04	0.3088	-0.62729315	1.89395981

# Two-way ANOVA using PROC GLM

The GLM Procedure  
Least Squares Means  
Adjustment for Multiple Comparisons: Tukey

variety	density	yield LSMEAN	LSMEAN Number
A	10	9.2000000	1
A	20	12.4333333	2
A	30	12.9000000	3
A	40	10.8000000	4
B	10	8.9333333	5
B	20	12.6333333	6
B	40	12.7666667	7
C	10	16.3000000	8
C	20	18.1000000	9
C	30	19.9333333	10

# Two-way ANOVA using PROC GLM

		Difference	Simultaneous 95%	
		Between	Confidence Limits for	
i	j	Means	LSMean(i)-LSMean(j)	
1	2	-3.233333	-6.997053	0.530387
1	3	-3.700000	-7.463720	0.063720
1	4	-1.600000	-5.363720	2.163720
1	5	0.266667	-3.497053	4.030387
1	6	-3.433333	-7.197053	0.330387
1	7	-3.566667	-7.330387	0.197053
1	8	-7.100000	-10.863720	-3.336280
1	9	-8.900000	-12.663720	-5.136280
1	10	-10.733333	-14.497053	-6.969613
1	11	-8.966667	-12.730387	-5.202947
2	3	-0.466667	-4.230387	3.297053
2	4	1.633333	-2.130387	5.397053
2	5	3.500000	-0.263720	7.263720
2	6	-0.200000	-3.963720	3.563720
2	7	-0.333333	-4.097053	3.430387
2	8	-3.866667	-7.630387	-0.102947
2	9	-5.666667	-9.430387	-1.902947
2	10	-7.500000	-11.263720	-3.736280
2	11	-5.733333	-9.497053	-1.969613
3	4	2.100000	-1.663720	5.863720
3	5	3.966667	0.202947	7.730387
3	6	0.266667	-3.497053	4.030387
3	7	0.133333	-3.630387	3.897053
3	8	-3.400000	-7.163720	0.363720
3	9	-5.200000	-8.963720	-1.436280
3	10	-7.033333	-10.797053	-3.269613
3	11	-5.266667	-9.030387	-1.502947
4	5	1.866667	-1.897053	5.630387

```
m = lm(Yield~Variety:Density, tomato, subset!=(Variety=='B' & Density==30))
anova(m)
```

Analysis of Variance Table

Response: Yield

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Variety:Density	10	421.09	42.109	25.326	8.563e-10 ***
Residuals	22	36.58	1.663		

---

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



```
tomato$VarietyDensity = factor(paste(tomato$Variety, tomato$Density, sep=""))
# Note the -1 in order to construct the contrast
m = lm(Yield~VarietyDensity-1, tomato, subset=!(Variety=='B' & Density==30))
#           A10 A20 A30 A40 B10 B20 B40 C10 C20 C30 C40
K = rbind('C-B' = c( 0, 0, 0, 0, -1, -1, -1, 1, 1, 0, 1)/3,
          'C-A' = c(-1, -1, -1, -1, 0, 0, 0, 1, 1, 1, 1)/4,
          'B-A' = c(-1, -1, 0, -1, 1, 1, 1, 0, 0, 0, 0)/3)

library(multcomp)
t = glht(m, linfct=K)
#summary(t)
confint(t, calpha=univariate_calpha())
```

### Simultaneous Confidence Intervals

```
Fit: lm(formula = Yield ~ VarietyDensity - 1, data = tomato, subset = !(Variety ==
"B" & Density == 30))
```

Quantile = 2.0739

95% confidence level

### Linear Hypotheses:

	Estimate	lwr	upr
C-B == 0	6.0778	4.8172	7.3384
C-A == 0	6.7917	5.6999	7.8834
B-A == 0	0.6333	-0.6273	1.8940

```
m = lm(Yield~Variety:Density, tomato, subset=!(Variety=='B' & Density==30))
lsmeans(m, pairwise~Variety:Density)
```

```
$lsmeans
```

Variety	Density	lsmean	SE	df	lower.CL	upper.CL
C	10	16.300000	0.7444746	22	14.756054	17.84395
A	10	9.200000	0.7444746	22	7.656054	10.74395
B	10	8.933333	0.7444746	22	7.389388	10.47728
C	20	18.100000	0.7444746	22	16.556054	19.64395
A	20	12.433333	0.7444746	22	10.889388	13.97728
B	20	12.633333	0.7444746	22	11.089388	14.17728
C	30	19.933333	0.7444746	22	18.389388	21.47728
A	30	12.900000	0.7444746	22	11.356054	14.44395
B	30	NA	NA	NA	NA	NA
C	40	18.166667	0.7444746	22	16.622721	19.71061
A	40	10.800000	0.7444746	22	9.256054	12.34395
B	40	12.766667	0.7444746	22	11.222721	14.31061

Confidence level used: 0.95

```
$contrasts
```

contrast	estimate	SE	df	t.ratio	p.value
C,10 - A,10	7.10000000	1.052846	22	6.744	<.0001
C,10 - B,10	7.36666667	1.052846	22	6.997	<.0001
C,10 - C,20	-1.80000000	1.052846	22	-1.710	0.8458
C,10 - A,20	3.86666667	1.052846	22	3.673	0.0465
C,10 - B,20	3.66666667	1.052846	22	3.483	0.0688
C,10 - C,30	-3.63333333	1.052846	22	-3.451	0.0734
C,10 - A,30	3.40000000	1.052846	22	3.229	0.1136
C,10 - B,30	NA	NA	NA	NA	NA
C,10 - C,40	-1.86666667	1.052846	22	-1.773	0.8156
C,10 - A,40	5.50000000	1.052846	22	5.224	0.0014
C,10 - B,40	3.53333333	1.052846	22	3.356	0.0887
A,10 - B,10	0.26666667	1.052846	22	0.253	1.0000
A,10 - C,20	-8.90000000	1.052846	22	-8.453	<.0001

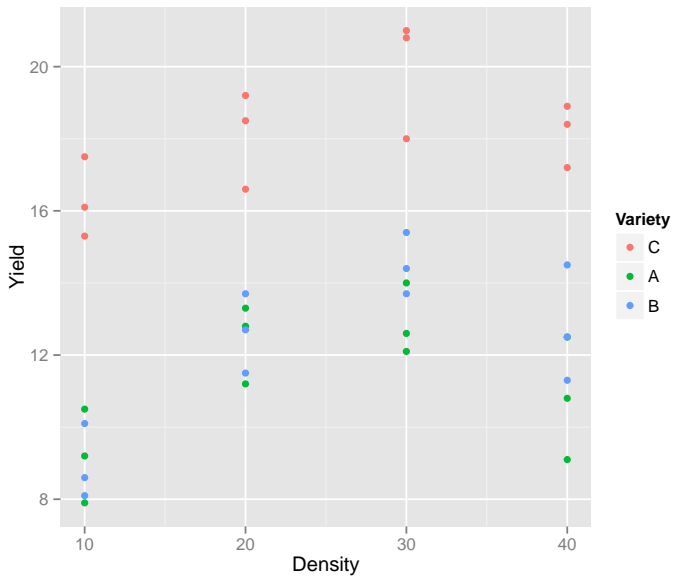
# Summary

When dealing with an incomplete design, it is often easier to treat the analysis as a one-way ANOVA and use contrasts to answer scientific questions of interest.

# Optimal yield

Now suppose you have the same data set, but your scientific question is different. Specifically, you are interested in choosing a variety and density that provide the optimal yield.

You can use the ANOVA analysis to choose from amongst the 3 varieties and one of the 4 densities, but there is no reason to believe that the optimal density will be one of those 4.



# Modeling

Considering a single variety, if we assume a linear relationship between Yield ( $Y_i$ ) and Density ( $D_i$ ) then the maximum Yield will occur at either  $-\infty$  or  $+\infty$  which is unreasonable. The easiest way to have a maximum (or minimum) is to assume a quadratic relationship, e.g.

$$E[Y_i] = \mu_i = \beta_0 + \beta_1 D_i + \beta_2 D_i^2$$

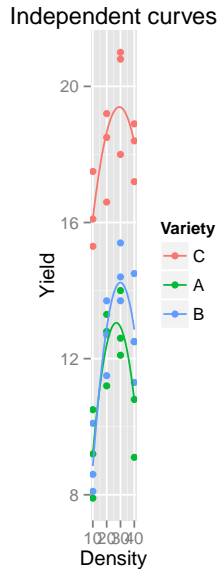
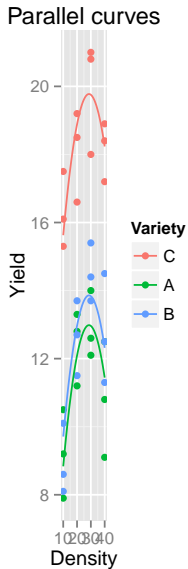
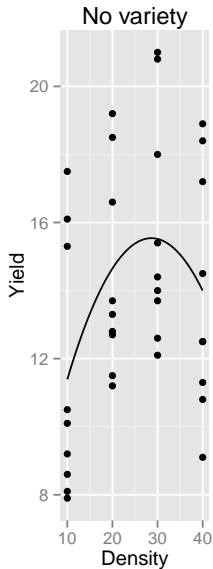
Now we can incorporate Variety ( $V_i$ ) in many ways. Two options are parallel curves or completely independent curves.

Parallel curves:

$$\begin{aligned} \mu_i = & \beta_0 + \beta_1 D_i + \beta_2 D_i^2 \\ & + \beta_3 I(V_i = A) + \beta_4 I(V_i = B) \end{aligned}$$

Independent lines:

$$\mu_i = \beta_0 + \beta_1 D_i + \beta_2 D_i^2$$



# Finding the maximum

For a particular variety, there will be an equation like

$$E[Y_i] = \mu_i = \beta_0 + \beta_1 D_i + \beta_2 D_i^2$$

where these  $\beta_1$  and  $\beta_2$  need not correspond to any particular  $\beta_1$  and  $\beta_2$  we have discussed thus far.

If  $\beta_2 < 0$ , then the quadratic curve has a maximum and it occurs at  $-\beta_1/2\beta_2$ .



# Analysis in SAS

```
DATA tomato;
  INFILE 'Ch13-tomato.csv' DSD FIRSTOBS=2;
  INPUT variety $ density yield;

/* No variety */
PROC GLM DATA=tomato PLOTS=all;
  CLASS variety; /* density is no longer here */
  MODEL yield = density|density / SOLUTION;
RUN;

/* Parallel curves */
PROC GLM DATA=tomato PLOTS=all;
  CLASS variety; /* density is no longer here */
  MODEL yield = density|density variety/ SOLUTION;
RUN;

/* Independent curves */
PROC GLM DATA=tomato PLOTS=all;
  CLASS variety; /* density is no longer here */
  MODEL yield = density|density|variety/ SOLUTION;
RUN;
```

# No variety

## The GLM Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	85.3346667	42.6673333	3.75	0.0340
Error	33	375.0208889	11.3642694		
Corrected Total	35	460.3555556			

...

Source	DF	Type III SS	Mean Square	F Value	Pr > F
density	1	65.30344358	65.30344358	5.75	0.0223
density*density	1	51.36111111	51.36111111	4.52	0.0411

Parameter	Estimate	Standard Error	t Value	Pr >  t
Intercept	5.744444444	3.12824210	1.84	0.0753
density	0.684111111	0.28538383	2.40	0.0223
density*density	-0.011944444	0.00561849	-2.13	0.0411

# Parallel curves

## The GLM Procedure

Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	412.9318889	103.2329722	67.48	<.0001
Error	31	47.4236667	1.5297957		
Corrected Total	35	460.3555556			

...

Source	DF	Type III SS	Mean Square	F Value	Pr > F
density	1	65.3034436	65.3034436	42.69	<.0001
density*density	1	51.3611111	51.3611111	33.57	<.0001
variety	2	327.5972222	163.7986111	107.07	<.0001

Parameter		Estimate	Standard Error	t Value	Pr >  t
Intercept		9.980555556	1.18419286	8.43	<.0001
density		0.684111111	0.10470690	6.53	<.0001
density*density		-0.011944444	0.00206142	-5.79	<.0001
variety	A	-6.791666667	0.50494153	-13.45	<.0001
variety	B	-5.916666667	0.50494153	-11.72	<.0001
variety	C	0.000000000	.	.	.

# Independent curves

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	419.8612222	52.4826528	34.99	<.0001
Error	27	40.4943333	1.4997901		
Corrected Total	35	460.3555556			

...

Source	DF	Type III SS	Mean Square	F Value	Pr > F
density	1	65.30344358	65.30344358	43.54	<.0001
density*density	1	51.36111111	51.36111111	34.25	<.0001
variety	2	21.66539427	10.83269713	7.22	0.0031
density*variety	2	2.07850215	1.03925108	0.69	0.5088
densit*densit*variet	2	1.65388889	0.82694444	0.55	0.5825

Parameter		Estimate	Standard Error	t Value	Pr >  t
Intercept		11.80833333 B	1.96836425	6.00	<.0001
density		0.52016667 B	0.17957029	2.90	0.0074
density*density		-0.00891667 B	0.00353529	-2.52	0.0179
variety	A	-8.45833333 B	2.78368742	-3.04	0.0052
variety	B	-9.73333333 B	2.78368742	-3.50	0.0016
variety	C	0.00000000 B	.	.	.
density*variety	A	0.19916667 B	0.25395073	0.78	0.4397
density*variety	B	0.29266667 B	0.25395073	1.15	0.2592
density*variety	C	0.00000000 B	.	.	.
densit*densit*variet	A	-0.00441667 B	0.00499965	-0.88	0.3848
densit*densit*variet	B	-0.00466667 B	0.00499965	-0.93	0.3589
densit*densit*variet	C	0.00000000 B	.	.	.

# No variety

```
Call:
lm(formula = Yield ~ Density + I(Density^2), data = tomato)

Residuals:
    Min       1Q   Median       3Q      Max
-4.898 -2.721 -1.320  3.364  6.109

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)   5.744444    3.128242   1.836  0.0753 .
Density        0.684111    0.285384   2.397  0.0223 *
I(Density^2)  -0.011944    0.005618  -2.126  0.0411 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3.371 on 33 degrees of freedom
Multiple R-squared:  0.1854, Adjusted R-squared:  0.136
F-statistic: 3.755 on 2 and 33 DF,  p-value: 0.03395
```

# Parallel curves

```
Call:
lm(formula = Yield ~ Density + I(Density^2) + Variety, data = tomato)
```

Residuals:

	Min	1Q	Median	3Q	Max
	-2.3422	-0.9039	0.1744	0.8082	2.1828

Coefficients:

	Estimate	Std. Error	t value	Pr(> t )
(Intercept)	9.980556	1.184193	8.428	1.61e-09 ***
Density	0.684111	0.104707	6.534	2.71e-07 ***
I(Density^2)	-0.011944	0.002061	-5.794	2.21e-06 ***
VarietyA	-6.791667	0.504942	-13.450	1.76e-14 ***
VarietyB	-5.916667	0.504942	-11.718	6.39e-13 ***

---

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

Residual standard error: 1.237 on 31 degrees of freedom  
 Multiple R-squared: 0.897, Adjusted R-squared: 0.8837  
 F-statistic: 67.48 on 4 and 31 DF, p-value: 7.469e-15

# Independent curves

```
Call:
lm(formula = Yield ~ Density * Variety + I(Density^2) * Variety,
    data = tomato)
```

```
Residuals:
    Min       1Q   Median       3Q      Max
-2.04500 -0.82125 -0.01417  0.94000  1.71000
```

```
Coefficients:
```

	Estimate	Std. Error	t value	Pr(> t )	
(Intercept)	11.808333	1.968364	5.999	2.12e-06	***
Density	0.520167	0.179570	2.897	0.00739	**
VarietyA	-8.458333	2.783687	-3.039	0.00523	**
VarietyB	-9.733333	2.783687	-3.497	0.00165	**
I(Density^2)	-0.008917	0.003535	-2.522	0.01787	*
Density:VarietyA	0.199167	0.253951	0.784	0.43971	
Density:VarietyB	0.292667	0.253951	1.152	0.25924	
VarietyA:I(Density^2)	-0.004417	0.005000	-0.883	0.38482	
VarietyB:I(Density^2)	-0.004667	0.005000	-0.933	0.35889	

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

```
Residual standard error: 1.225 on 27 degrees of freedom
Multiple R-squared:  0.912, Adjusted R-squared:  0.886
F-statistic: 34.99 on 8 and 27 DF,  p-value: 2.678e-12
```

## Completely randomized design (CRD)

This semester, we have assumed a completely randomized design. As an example, consider 36 plots and we are randomly assigning our variety-density combinations to the plots such that we have 3 reps of each combination. The result may look something like this

A20	A30	A40	C20	A40	B40
C20	C40	C40	B30	A10	A40
B40	C30	B40	C10	A20	C10
C10	B20	B20	A30	B10	A20
A10	C40	A10	B10	A30	B10
C20	B30	B20	B30	C30	C30



# Complete randomized block design (RBD)

A randomized block design is appropriate when there is a nuisance factor that you want to control for. In our example, imagine you had 12 plots at 3 different locations and you expect these locations would have impact on yield. A randomized block design might look like this.

A30	B40
C10	B10
C30	C20
B30	B20
A10	A20
C40	A40

Block 1

A20	B40
C10	B20
C30	C40
A10	A30
B30	A40
C20	B10

Block 2

A10	B40
C20	B30
C10	A40
A20	C40
A30	B10
B20	C30

Block 3

## RBD Analysis

Generally, you will want to model a randomized block design using an additive model for the treatment and blocking factor. If you have the replication, you should test for an interaction. Let's compute the degrees of freedom for the ANOVA tables for this current design considering the variety-density combination as the treatment.

V+D+B		T+B		Cell-means	
Factor	df	Factor	df	Factor	df
Variety	2				
Density	3	Treatment	11	Treatment	11
Block	2	Block	2	Block	2
				Treatment x Block	22
Error	28	Error	22	Error	0
Total	35	Total	35	Total	35

The cell-means model does not have enough degrees of freedom to estimate the interaction because there is no replication of the treatment within a block.

# Why blocking helps

Estimation of treatment effects is performed within block because all treatments are available within a block.

*Block what you can; randomize what you cannot.*