

STAT 401A - Statistical Methods for Research Workers

Two-way ANOVA

Jarad Niemi (Dr. J)

Iowa State University

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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.

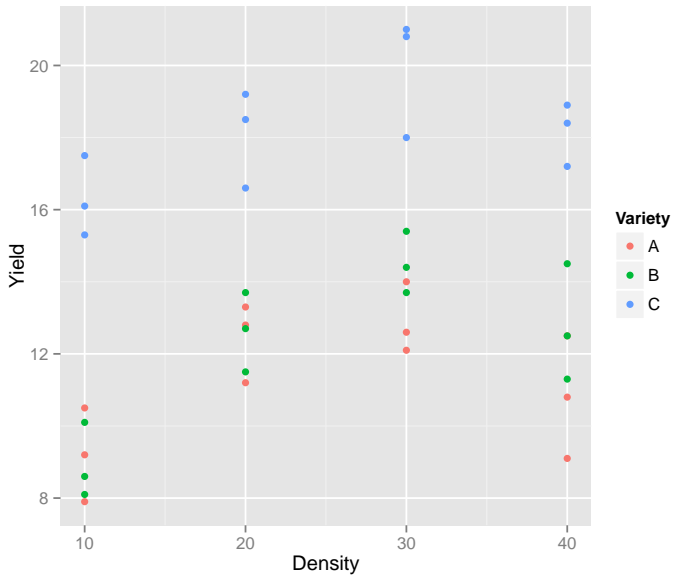
Hypotheses

- 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	A	3	3	3	3
2	B	3	3	3	3
3	C	3	3	3	3

Mean Yield

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

Standard deviation of yield

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

Two-way ANOVA

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

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

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	μ_{11}	μ_{12}	μ_{13}	μ_{14}
B	μ_{21}	μ_{22}	μ_{23}	μ_{24}
C	μ_{31}	μ_{32}	μ_{33}	μ_{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:

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

β_1 is the expected difference in yield between varieties A and C at any fixed density

- ⑤ The cell-means model:

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

β_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 σ^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	422.315556	38.3923232	24.22	<.0001
Error	24	38.040000	1.5850000		
Corrected Total	35	460.355556			

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

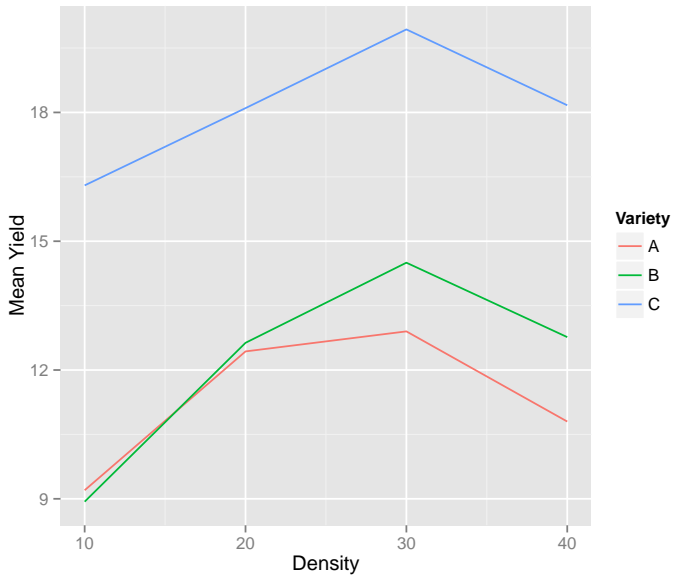
The Type I and Type III SS are equal because the design is balanced.

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	
i	j	Means	LSMean(i)-LSMean(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	
i	j	Means	LSMean(i)-LSMean(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
A         11.33333 0.3634327 24 10.58325 12.08342
B         12.20833 0.3634327 24 11.45825 12.95842
C         18.12500 0.3634327 24 17.37491 18.87509
```

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

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

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

Confidence level used: 0.95

```
$contrasts
```

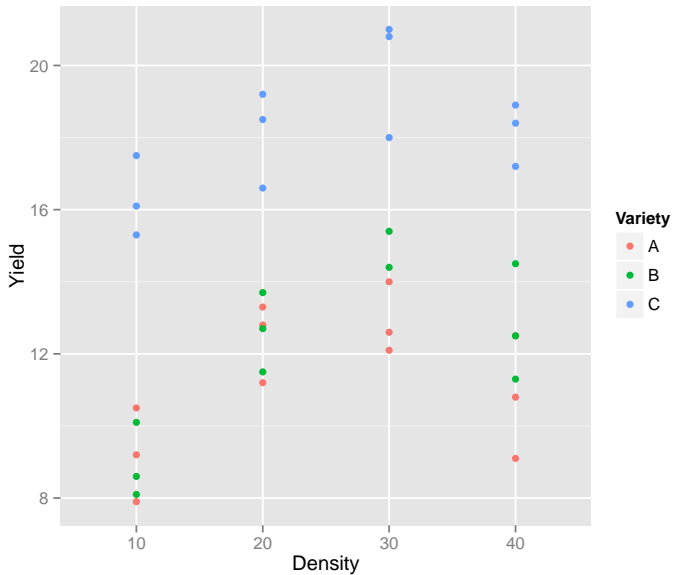
contrast	estimate	SE	df	t.ratio	p.value
A,10 - B,10	0.2666667	1.027943	24	0.259	1.0000
A,10 - C,10	-7.1000000	1.027943	24	-6.907	<.0001
A,10 - A,20	-3.2333333	1.027943	24	-3.145	0.1284
A,10 - B,20	-3.4333333	1.027943	24	-3.340	0.0873
A,10 - C,20	-8.9000000	1.027943	24	-8.658	<.0001
A,10 - A,30	-3.7000000	1.027943	24	-3.599	0.0507
A,10 - B,30	-5.3000000	1.027943	24	-5.156	0.0013
A,10 - C,30	-10.7333333	1.027943	24	-10.442	<.0001
A,10 - A,40	-1.6000000	1.027943	24	-1.557	0.9085
A,10 - B,40	-3.5666667	1.027943	24	-3.470	0.0668
A,10 - C,40	-8.9666667	1.027943	24	-8.723	<.0001
B,10 - C,10	-7.3666667	1.027943	24	-7.166	<.0001
B,10 - A,20	-3.5000000	1.027943	24	-3.405	0.0764
B,10 - B,20	-2.7000000	1.027943	24	-2.599	0.0507
B,10 - C,20	-7.1666667	1.027943	24	-6.969	<.0001
B,10 - A,30	-3.5000000	1.027943	24	-3.405	0.0764
B,10 - B,30	-1.8000000	1.027943	24	-1.750	0.9100
B,10 - C,30	-7.0333333	1.027943	24	-6.853	<.0001
B,10 - A,40	-1.9000000	1.027943	24	-1.847	0.8300
B,10 - B,40	-1.9333333	1.027943	24	-1.879	0.8000
B,10 - C,40	-6.2000000	1.027943	24	-6.032	<.0001
C,10 - C,20	2.0000000	1.027943	24	1.944	0.9600
C,10 - C,30	2.6000000	1.027943	24	2.528	0.1200
C,10 - C,40	1.9000000	1.027943	24	1.847	0.8300
C,20 - C,30	1.3000000	1.027943	24	1.264	0.9200
C,20 - C,40	0.6000000	1.027943	24	0.584	0.9800
C,30 - C,40	0.3000000	1.027943	24	0.292	0.9900

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 one of the variety B, density 30 samples 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	A	3	3	3	3
2	B	3	3	2	3
3	C	3	3	3	3

Mean Yield

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

Standard deviation of yield

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

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

variety	yield LSMEAN	LSMEAN Number
A	11.3333333	1
B	12.3083333	2
C	18.1250000	3

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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for LSMean(i)-LSMean(j)
---	---	--------------------------------	--

Two-way ANOVA using PROC GLM

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

	density	yield LSMEAN	LSMEAN Number
10		11.4777778	1
20		14.3888889	2
30		15.9111111	3
40		13.9111111	4

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

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
A	11.33333	0.3634327	24	10.58325	12.08342
B	12.20833	0.3634327	24	11.45825	12.95842
C	18.12500	0.3634327	24	17.37491	18.87509

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

```
$contrasts
```

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

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

Confidence level used: 0.95

```
$contrasts
```

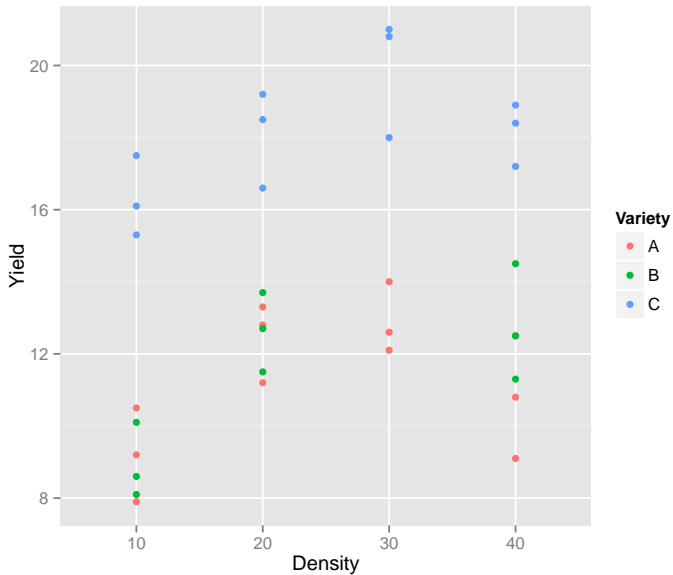
contrast	estimate	SE	df	t.ratio	p.value
A,10 - B,10	0.2666667	1.027943	24	0.259	1.0000
A,10 - C,10	-7.1000000	1.027943	24	-6.907	<.0001
A,10 - A,20	-3.2333333	1.027943	24	-3.145	0.1284
A,10 - B,20	-3.4333333	1.027943	24	-3.340	0.0873
A,10 - C,20	-8.9000000	1.027943	24	-8.658	<.0001
A,10 - A,30	-3.7000000	1.027943	24	-3.599	0.0507
A,10 - B,30	-5.3000000	1.027943	24	-5.156	0.0013
A,10 - C,30	-10.7333333	1.027943	24	-10.442	<.0001
A,10 - A,40	-1.6000000	1.027943	24	-1.557	0.9085
A,10 - B,40	-3.5666667	1.027943	24	-3.470	0.0668
A,10 - C,40	-8.9666667	1.027943	24	-8.723	<.0001
B,10 - C,10	-7.3666667	1.027943	24	-7.166	<.0001
B,10 - A,20	-3.5000000	1.027943	24	-3.405	0.0764
B,10 - B,20	-2.7000000	1.027943	24	-2.599	0.0507
B,10 - C,20	-6.1666667	1.027943	24	-5.985	<.0001
B,10 - A,30	-2.5000000	1.027943	24	-2.433	0.0224
B,10 - B,30	-1.8000000	1.027943	24	-1.746	0.0913
B,10 - C,30	-7.2333333	1.027943	24	-7.023	<.0001
B,10 - A,40	-0.9000000	1.027943	24	-0.877	0.3867
B,10 - B,40	0.0000000	1.027943	24	0.000	1.0000
B,10 - C,40	-7.3666667	1.027943	24	-7.166	<.0001
C,10 - A,20	-3.2000000	1.027943	24	-3.119	0.0032
C,10 - B,20	-4.7000000	1.027943	24	-4.570	<.0001
C,10 - C,20	1.8000000	1.027943	24	1.746	0.0913
C,10 - A,30	-1.7000000	1.027943	24	-1.653	0.1087
C,10 - B,30	-3.2000000	1.027943	24	-3.119	0.0032
C,10 - C,30	8.4000000	1.027943	24	8.173	<.0001
C,10 - A,40	-2.0000000	1.027943	24	-1.943	0.0613
C,10 - B,40	4.1666667	1.027943	24	4.053	<.0001
C,10 - C,40	10.1666667	1.027943	24	9.885	<.0001

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	A	3	3	3	3
2	B	3	3	0	3
3	C	3	3	3	3

Mean Yield

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

Standard deviation of yield

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

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	11.333333	1
B	Non-est	2
C	18.125000	3

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

Dependent Variable: yield

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

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

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

Two-way ANOVA using PROC GLM

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

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

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

Dependent Variable: yield

i/j	1	2	3	4
1		0.0002	.	0.0017
2	0.0002		.	0.7153
3	.	.		.
4	0.0017	0.7153	.	

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%

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	
		Means	LSMean(i)-LSMean(j)	
i	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	μ_{11}	μ_{12}	μ_{13}	μ_{14}
B	μ_{21}	μ_{22}	μ_{23}	μ_{24}
C	μ_{31}	μ_{32}	μ_{33}	μ_{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

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*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

$$\begin{array}{llllll} \mu_i & = \beta_0 & +\beta_1\mathbf{I}(V_i = A, D_i = 10) & +\beta_1\mathbf{I}(V_i = A, D_i = 20) & +\beta_1\mathbf{I}(V_i = A, D_i = 30) & +\beta_1\mathbf{I}(V_i = A, D_i = 40) \\ & & +\beta_1\mathbf{I}(V_i = B, D_i = 10) & +\beta_1\mathbf{I}(V_i = B, D_i = 20) & & +\beta_1\mathbf{I}(V_i = B, D_i = 40) \\ & & +\beta_1\mathbf{I}(V_i = C, D_i = 10) & +\beta_1\mathbf{I}(V_i = C, D_i = 20) & +\beta_1\mathbf{I}(V_i = C, D_i = 30) & +\beta_1\mathbf{I}(V_i = C, D_i = 40) \end{array}$$

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	
		Means	LSMean(i)-LSMean(j)	
i	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

$K = 1$

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

```
$lsmeans
```

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

Confidence level used: 0.95

```
$contrasts
```

contrast	estimate	SE	df	t.ratio	p.value
A,10 - B,10	0.2666667	1.052846	22	0.253	1.0000
A,10 - C,10	-7.1000000	1.052846	22	-6.744	<.0001
A,10 - A,20	-3.2333333	1.052846	22	-3.071	0.1529
A,10 - B,20	-3.4333333	1.052846	22	-3.261	0.1069
A,10 - C,20	-8.9000000	1.052846	22	-8.453	<.0001
A,10 - A,30	-3.7000000	1.052846	22	-3.514	0.0645
A,10 - B,30	NA	NA	NA	NA	NA
A,10 - C,30	-10.7333333	1.052846	22	-10.195	<.0001
A,10 - A,40	-1.6000000	1.052846	22	-1.520	0.9193
A,10 - B,40	-3.5666667	1.052846	22	-3.388	0.0833
A,10 - C,40	-8.9666667	1.052846	22	-8.517	<.0001
B,10 - C,10	-7.3666667	1.052846	22	-6.997	<.0001
B,10 - A,20	-3.5000000	1.052846	22	-3.324	0.0945
B,10 - B,20	2.7000000	1.052846	22	2.514	0.0645
B,10 - C,20	5.6666667	1.052846	22	5.333	<.0001
B,10 - A,30	-0.8000000	1.052846	22	-0.759	0.9500
B,10 - B,30	3.7000000	1.052846	22	3.514	0.0645
B,10 - C,30	8.2333333	1.052846	22	7.767	<.0001
B,10 - A,40	-0.2000000	1.052846	22	-0.189	0.9899
B,10 - B,40	1.1666667	1.052846	22	1.107	0.9193
B,10 - C,40	6.2000000	1.052846	22	5.867	<.0001
C,10 - C,20	1.8000000	1.052846	22	1.708	0.9899
C,10 - C,30	3.6333333	1.052846	22	3.444	0.0645
C,10 - C,40	1.8666667	1.052846	22	1.769	0.9899
C,20 - C,30	7.5000000	1.052846	22	7.099	<.0001
C,20 - C,40	5.6666667	1.052846	22	5.333	<.0001
C,30 - C,40	7.0000000	1.052846	22	6.625	<.0001

Summary

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