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.

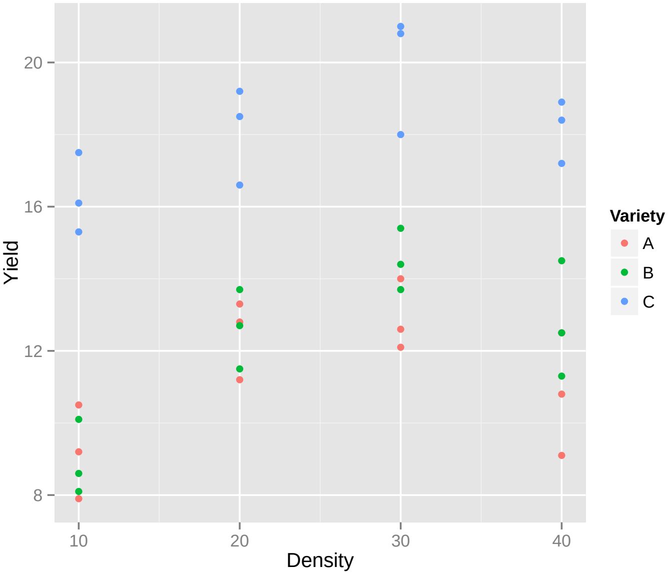
Hypotheses

- 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.


Yield
20
16
12
8
10
20
30
40
Density
Variety
A
B
C

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:

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

where Y_{ijk} is the

- kth observation at the
- ith level of variable 1 (variety) with $i = 1, \ldots, \mathrm{I}$ and the
- jth level of variable 2 (density) with $j = 1, \ldots, \mathrm{J}$.

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. $\mathrm{I}(V_i = A)$ and $\mathrm{I}(D_i = 10)$.
4. The additive model:

$$\begin{aligned}\mu_i = \quad & \beta_0 + \beta_1 \mathrm{I}(V_i = A) + \beta_2 \mathrm{I}(V_i = B) \\ & + \beta_3 \mathrm{I}(D_i = 10) + \beta_4 \mathrm{I}(D_i = 20) + \beta_5 \mathrm{I}(D_i = 30).\end{aligned}$$

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

5. The cell-means model:

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

   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

                                          Sum of
        Source                      DF         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
```

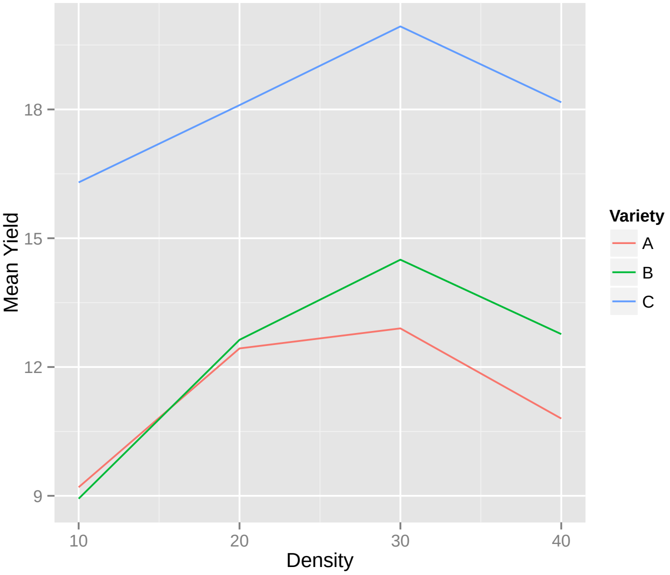
The Type I and Type III SS are equal because the design is balanced.

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	.	.	.


Mean Yield
18
15
12
9
10
20
30
40
Density
Variety
A
B
C

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(lsmeans)
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
```

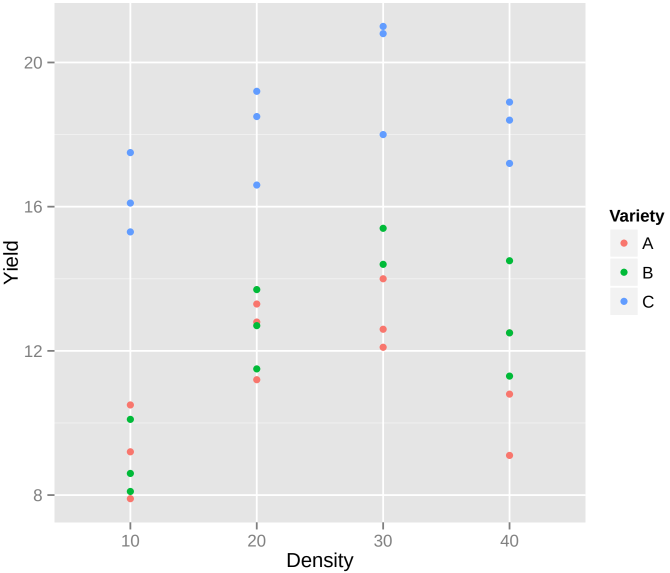
```
lsmeans(m, pairwise~Variety*Density)

$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   0.26666667 1.027943 24   0.259  1.0000
 A,10 - C,10  -7.10000000 1.027943 24  -6.907  <.0001
 A,10 - A,20  -3.23333333 1.027943 24  -3.145  0.1284
 A,10 - B,20  -3.43333333 1.027943 24  -3.340  0.0873
 A,10 - C,20  -8.90000000 1.027943 24  -8.658  <.0001
 A,10 - A,30  -3.70000000 1.027943 24  -3.599  0.0507
 A,10 - B,30  -5.30000000 1.027943 24  -5.156  0.0013
 A,10 - C,30 -10.73333333 1.027943 24 -10.442  <.0001
 A,10 - A,40  -1.60000000 1.027943 24  -1.557  0.9085
 A,10 - B,40  -3.56666667 1.027943 24  -3.470  0.0668
 A,10 - C,40  -8.96666667 1.027943 24  -8.723  <.0001
 B,10 - C,10  -7.36666667 1.027943 24  -7.166  <.0001
 B,10 - A,20  -3.50000000 1.027943 24  -3.405  0.0764
 B,10 - B,20  -3.70000000 1.027943 24  -3.599  0.0507
```

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.



20
16
12
8
Yield
10
20
30
40
Density
Variety
A
B
C

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

                                        Sum of
       Source                 DF       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         Simultaneous 95%
                   Between      Confidence Limits for
 i    j              Means       LSMean(i)-LSMean(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

                                                  LSMEAN
variety     density      yield 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
i     j              Means       LSMean(i)-LSMean(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
```

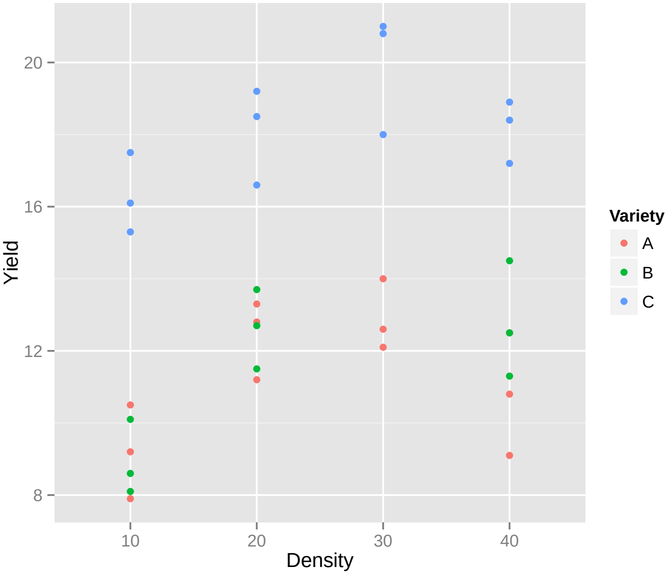
```
lsmeans(m, pairwise~Variety*Density)


$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   0.26666667 1.027943 24   0.259  1.0000
 A,10 - C,10  -7.10000000 1.027943 24  -6.907  <.0001
 A,10 - A,20  -3.23333333 1.027943 24  -3.145  0.1284
 A,10 - B,20  -3.43333333 1.027943 24  -3.340  0.0873
 A,10 - C,20  -8.90000000 1.027943 24  -8.658  <.0001
 A,10 - A,30  -3.70000000 1.027943 24  -3.599  0.0507
 A,10 - B,30  -5.30000000 1.027943 24  -5.156  0.0013
 A,10 - C,30 -10.73333333 1.027943 24 -10.442  <.0001
 A,10 - A,40  -1.60000000 1.027943 24  -1.557  0.9085
 A,10 - B,40  -3.56666667 1.027943 24  -3.470  0.0668
 A,10 - C,40  -8.96666667 1.027943 24  -8.723  <.0001
 B,10 - C,10  -7.36666667 1.027943 24  -7.166  <.0001
 B,10 - A,20  -3.50000000 1.027943 24  -3.405  0.0764
 B,10 - B,20  -3.70000000 1.027943 24  -3.599  0.0507
```

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.


Yield
20
16
12
8
10
20
30
40
Density
Variety
A
B
C

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
                                         Sum of
     Source                     DF      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

                                               LSMEAN
             variety     yield 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         Simultaneous 95%
                     Between      Confidence Limits for
   i    j              Means       LSMean(i)-LSMean(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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for 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	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
                                             Sum of
      Source                      DF        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

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

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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for 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
 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   0.26666667 1.052846 22   0.253  1.0000
 A,10 - C,10  -7.10000000 1.052846 22  -6.744  <.0001
 A,10 - A,20  -3.23333333 1.052846 22  -3.071  0.1529
 A,10 - B,20  -3.43333333 1.052846 22  -3.261  0.1069
 A,10 - C,20  -8.90000000 1.052846 22  -8.453  <.0001
 A,10 - A,30  -3.70000000 1.052846 22  -3.514  0.0645
 A,10 - B,30           NA       NA NA      NA      NA
 A,10 - C,30 -10.73333333 1.052846 22 -10.195  <.0001
 A,10 - A,40  -1.60000000 1.052846 22  -1.520  0.9193
 A,10 - B,40  -3.56666667 1.052846 22  -3.388  0.0833
 A,10 - C,40  -8.96666667 1.052846 22  -8.517  <.0001
 B,10 - C,10  -7.36666667 1.052846 22  -6.997  <.0001
 B,10 - A,20  -3.50000000 1.052846 22  -3.324  0.0945
```

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.