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.

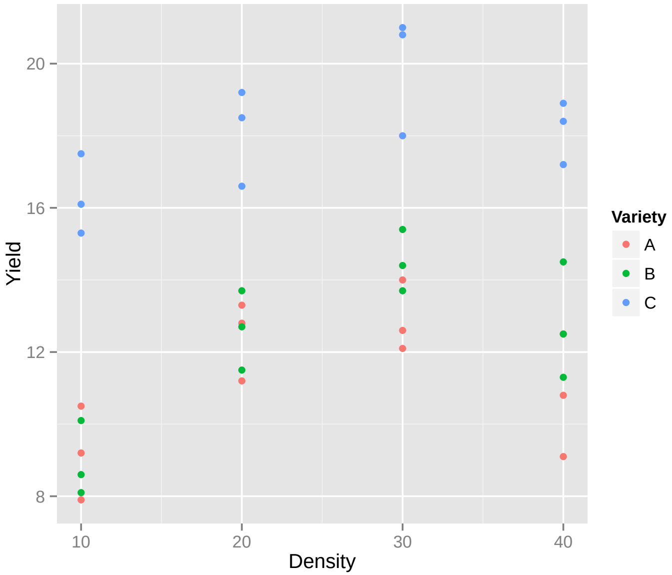
Hypotheses

- 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.


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  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: $\mu_{ij} = \mu + \alpha_i + \eta_j$
  - Cell-means: $\mu_{ij} = \mu + \alpha_i + \eta_j + \gamma_{ij}$

	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:

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

   β_1 is the expected difference in yield between varieties A and C at any fixed density
5. The cell-means model:

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

   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
```

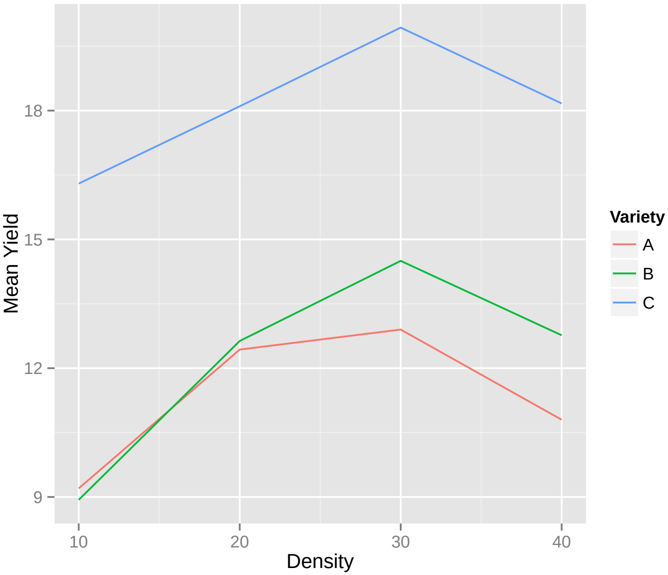
The Type I and Type III SS are equal because the design is balanced.

Two-way ANOVA using PROC GLM

```
MODEL yield = variety|density / SOLUTION;
                                        The GLM Procedure

                                                             Standard
         Parameter                     Estimate                 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

i	j	Difference Between Means	Simultaneous 95% Confidence Limits for 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

Summary

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