STAT 222 Lecture 26

Block Designs w/ Factorial Treatments

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Problem 13.7 on p. 348-349, Oehlert's Text

- Plant shoots can be encouraged in tissue culture by exposing the cotyledons of plant embryos to cytokinin, a plant growth hormone. However, some shoots become watery, soft, and unviable; this is **vitrification**
- **Goal**: to how the `orientation` of the embryo during exposure to cytokinin and the type of growth medium after exposure to cytokinin affect the rate of vitrification.
- **6 treatments** = 2×3 combinations of
  - `orientation` (standard and experimental), and
  - `medium` (1, 2, 3)
- On a given day, the experimenters extract embryos from white pine seeds and randomize them to the 6 treatments. The embryos are exposed using the selected orientation for 1 week, and then go onto the selected medium. The experiment was repeated 22 times on different starting `days`.

Data

- **Block** = `day`: starting day of exposure
- **Response** = `fracnormal` = fraction of shoots that are normal

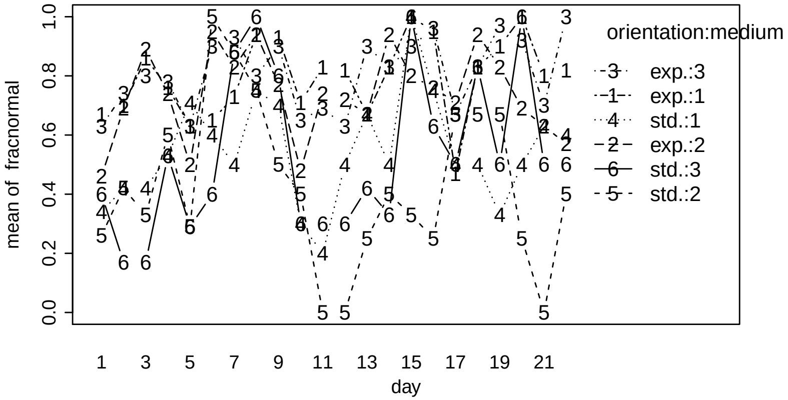
	Medium 1		Medium 2		Medium 3	
Day	Exp.	Std.	Exp.	Std.	Exp.	Std.
1	.67	.34	.46	.26	.63	.40
2	.70	.42	.69	.42	.74	.17
3	.86	.42	.89	.33	.80	.17
⋮	⋮	⋮	⋮	⋮	⋮	⋮
22	.82	.60	.57	.40	1.00	.50

Loading data to R:

```
shoot = read.table(
  "http://www.stat.uchicago.edu/~yibi/s222/pr13_7.txt", h=T)
shoot$day = as.factor(shoot$day)
shoot$medium = as.factor(shoot$medium)
shoot$orientation = as.factor(shoot$orientation)
```

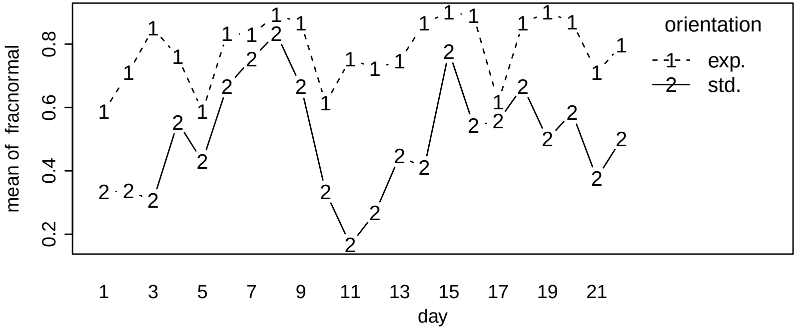
Interaction Plot — Treatment v.s. Day (Block)

```r
par(mai=c(.6,.6,.05,.5),mgp=c(2,.5,0))
with(shoot,
     interaction.plot(day, orientation:medium, fracnormal, type="b"))
```



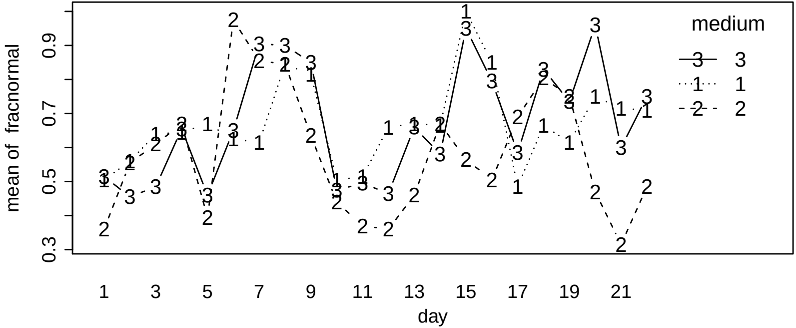
Interaction Plots — Orientation v.s. Day (Block)

```
par(mai=c(.6,.6,.05,.1),mgp=c(2,.5,0))
with(shoot,interaction.plot(day, orientation, fracnormal, type="b"))
```



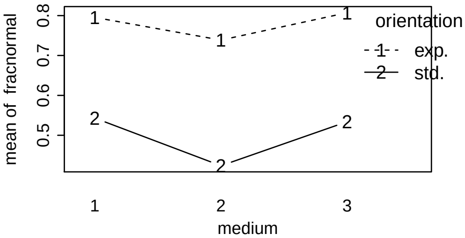
Interaction Plot — Medium v.s. Day (Block)

```r
par(mai=c(.6,.6,.05,.1),mgp=c(2,.5,0))
with(shoot,interaction.plot(day, medium, fracnormal, type="b"))
```



Interaction Plot — Medium v.s. Orientation

```
par(mai=c(.6,.6,.05,.4),mgp=c(2,.5,0))
with(shoot,interaction.plot(medium, orientation, fracnormal, type="b"))
```



Model

RCBD blocking on the starting `day` and the 6 treatments are 2×3 combination of `orientation` and `medium`

$$y_{ijk} = \mu + \underbrace{\alpha_i}_{\text{(orientation)}} + \underbrace{\beta_j}_{\text{(medium)}} + \underbrace{\alpha\beta_{ij}}_{\text{(interaction)}} + \underbrace{\gamma_k}_{\text{(day)}} + \varepsilon_{ijk}$$

- no treatment-block interaction (`orientation:day`, `medium:day`, `orientation:medium:day`)
- interaction between treatment factors (`orientation:medium`) is allowed

Estimation of Parameters (1)

$$y_{ijk} = \mu + \underset{\text{(orientation)}}{\alpha_i} + \underset{\text{(medium)}}{\beta_j} + \underset{\text{(interaction)}}{\alpha\beta_{ij}} + \underset{\text{(day)}}{\gamma_k} + \varepsilon_{ijk}$$

Under the zero-sum constraint, estimates for μ, α_i, β_j, and $\alpha\beta_{ij}$ can be calculated as in a 2×3 factorial model with 22 replicates, ignoring the blocking variable `day`.

$$\widehat{\mu} = \overline{y}_{\bullet\bullet\bullet}$$

$$\widehat{\alpha}_i = \overline{y}_{i\bullet\bullet} - \overline{y}_{\bullet\bullet\bullet}$$

$$\widehat{\beta}_j = \overline{y}_{\bullet j\bullet} - \overline{y}_{\bullet\bullet\bullet}$$

$$\widehat{\alpha\beta}_{ij} = \overline{y}_{ij\bullet} - \overline{y}_{i\bullet\bullet} - \overline{y}_{\bullet j\bullet} + \overline{y}_{\bullet\bullet\bullet}$$

Estimates for the blocking effects γ_k:

$$\widehat{\gamma}_k = \overline{y}_{\bullet\bullet k} - \overline{y}_{\bullet\bullet\bullet}$$

Estimation of Parameters (2)

Estimate for μ:

```
library(mosaic)
muhat = mean(fracnormal ~ 1, data=shoot); muhat
      1
0.64038
```

Estimates for α_i:

```
mean(fracnormal ~ orientation, data=shoot) - muhat
    exp.     std.
 0.14008 -0.14008
```

Estimates for β_i:

```
mean(fracnormal ~ medium, data=shoot) - muhat
        1         2         3
 0.028939 -0.059242  0.030303
```

Estimates for γ_k (22 estimates, results not shown to save space):

```
mean(fracnormal ~ day, data=shoot) - muhat
```

Estimation of Parameters (3)

	Medium			
Orientation	1	2	3	Mean
experimental	$\bar{y}_{e1\bullet} \approx 0.79545$	$\bar{y}_{e2\bullet} \approx 0.73864$	$\bar{y}_{e3\bullet} \approx 0.80727$	$\bar{y}_{e\bullet\bullet} \approx 0.78045$
standard	$\bar{y}_{s1\bullet} \approx 0.54318$	$\bar{y}_{s2\bullet} \approx 0.42364$	$\bar{y}_{s3\bullet} \approx 0.53409$	$\bar{y}_{s\bullet\bullet} \approx 0.50030$
Mean	$\bar{y}_{\bullet 1\bullet} \approx 0.66932$	$\bar{y}_{\bullet 2\bullet} \approx 0.58114$	$\bar{y}_{\bullet 3\bullet} \approx 0.67068$	$\bar{y}_{\bullet\bullet\bullet} \approx 0.64038$

$$\widehat{\alpha\beta}_{e1} = \bar{y}_{e1\bullet} - \bar{y}_{e\bullet\bullet} - \bar{y}_{\bullet 1\bullet} + \bar{y}_{\bullet\bullet\bullet} = 0.79545 - 0.78045 - 0.66932 + 0.64038 = -0.01394$$

$$\widehat{\alpha\beta}_{e2} = \bar{y}_{e2\bullet} - \bar{y}_{e\bullet\bullet} - \bar{y}_{\bullet 2\bullet} + \bar{y}_{\bullet\bullet\bullet} = 0.73864 - 0.78045 - 0.58114 + 0.64038 = 0.01743$$

$$\begin{aligned}\widehat{\alpha\beta}_{e3} &= -(\widehat{\alpha\beta}_{e1} + \widehat{\alpha\beta}_{e2}) \\ &= -(-0.01394 + 0.01743) = -0.00349 \quad \text{(since } \alpha\beta_{e1} + \alpha\beta_{e2} + \alpha\beta_{e3} = 0)\end{aligned}$$

$$\widehat{\alpha\beta}_{s1} = -\widehat{\alpha\beta}_{e1} = 0.01394 \qquad \text{(since } \alpha\beta_{e1} + \alpha\beta_{s1} = 0)$$

$$\widehat{\alpha\beta}_{s2} = -\widehat{\alpha\beta}_{e2} = -0.01743 \quad \text{(since } \alpha\beta_{e2} + \alpha\beta_{s2} = 0)$$

$$\widehat{\alpha\beta}_{s3} = -\widehat{\alpha\beta}_{e3} = 0.00349 \qquad \text{(since } \alpha\beta_{e3} + \alpha\beta_{s3} = 0)$$

Sum of Squares

The sum of squares for `orientation` and `medium` and their interactions can be computed as in a 2×3 factorial model with 22 replicates, ignoring the blocking variable `day`.

$$\text{SS}_{orientation} = \sum_{i=1}^{2}\sum_{j=1}^{3}\sum_{k=1}^{22}(\widehat{\alpha}_i)^2 \approx (3)(22)[0.14008^2 + (-0.14008)^2] \approx 2.5902.$$

$$\text{SS}_{medium} = \sum_{i=1}^{2}\sum_{j=1}^{3}\sum_{k=1}^{22}(\widehat{\beta}_j)^2 \approx (2)(22)[0.02894^2 + (-0.05924)^2 + 0.0303^2] \approx 0.2317$$

$$\begin{aligned}\text{SS}_{interaction} &= \sum_{i=1}^{2}\sum_{j=1}^{3}\sum_{k=1}^{22}(\widehat{\alpha\beta}_{ij})^2 = 22\sum_{i=1}^{2}\sum_{j=1}^{3}(\widehat{\alpha\beta}_{ij})^2 \\ &\approx 22[(-0.01394)^2 + 0.01743^2 + (-0.00349)^2 + 0.01394^2 \\ &\qquad + (-0.01743)^2 + 0.00349^2] \\ &\approx 0.02245\end{aligned}$$

Sum of Squares

The SS for the blocking factor day is

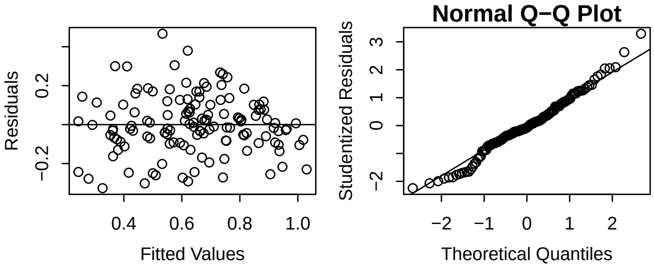
$$\text{SS}_{day} = \sum_{i=e,s} \sum_{j=1}^{3} \sum_{k=1}^{22} (\widehat{\gamma}_k)^2 = (2)(3) \sum_{k=1}^{22} (\widehat{\gamma}_k)^2 \approx 2.017$$

```
2*3*sum((mean(fracnormal ~ day, data=shoot) - muhat)^2)
[1] 2.017
```

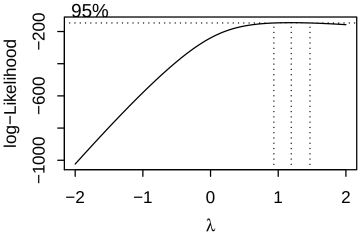
```
anova(lm(fracnormal ~ orientation*medium+day, data=shoot))
Analysis of Variance Table

Response: fracnormal
                    Df  Sum Sq Mean Sq F value    Pr(>F)    
orientation          1 2.59000 2.59000 93.6792 3.225e-16 ***
medium               2 0.23168 0.11584  4.1899   0.01776 *  
day                 21 2.01696 0.09605  3.4739 1.233e-05 ***
orientation:medium   2 0.02244 0.01122  0.4059   0.66744    
Residuals          105 2.90299 0.02765                      
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Checking Model Assumptions




```r
library(MASS);par(mai=c(.6,.6,.15,.01),mgp=c(2,.7,0))
boxcox(lm(I(fracnormal+0.01) ~ orientation*medium+day, data=shoot))
```



95% CI for the Orientation Effect $\alpha_e - \alpha_s$

If embryos are exposed to cytokinin in the experimental rather than the standard orientation, the fraction of normal shoots increases by $\alpha_e - \alpha_s$, estimated to be

$$\widehat{\alpha}_e - \widehat{\alpha}_s = (\bar{y}_{e\bullet\bullet} - \bar{y}_{\bullet\bullet\bullet}) - (\bar{y}_{s\bullet\bullet} - \bar{y}_{\bullet\bullet\bullet}) = \bar{y}_{e\bullet\bullet} - \bar{y}_{s\bullet\bullet} = 0.78045 - 0.5003 = 0.28015$$

w/ the standard error

$$\text{SE} = \sqrt{\text{MSE}\left(\frac{1}{r} + \frac{1}{r}\right)} = \sqrt{0.02765\left(\frac{1}{3 \times 22} + \frac{1}{3 \times 22}\right)} \approx 0.02895$$

where $r = 3 \times 22$ is the # of obs. used to calculate the 2 means.

The 95% confidence interval for $\alpha_e - \alpha_s$ is

$$\begin{aligned} \bar{y}_{e\bullet\bullet} - \bar{y}_{s\bullet\bullet} \pm t_{0.05/2,\text{df of MSE}}\text{SE.} &= 0.78045 - 0.50030 \pm 1.9828 \times 0.02895 \\ &\approx 0.28015 \pm 0.05740 \approx (0.223, 0.338) \end{aligned}$$

```
qt(0.025, df = 105, lower.tail = F)
[1] 1.983
```

95% CI for the Orientation Effect $\alpha_e - \alpha_s$

Interpretation of the 95% CI $(0.223, 0.338)$ for $\alpha_e - \alpha_s$:

With 95% confidence, the rate of vitrification is reduced by 22.3% to 33.8% on average if the shoots are exposed to cytokinin in the experimental orientation rather than in the standard orientation, for all 3 types of medium

LSD for Comparing the 3 medium

The 95% LSD for comparing the medium effects $\beta_{j_1} - \beta_{j_2}$ is

$$\begin{aligned}\text{LSD} &= t_{0.05/2,\text{df of MSE}} \sqrt{\text{MSE}\left(\frac{1}{r} + \frac{1}{r}\right)} \\ &= t_{0.05/2,105} \sqrt{0.02765\left(\frac{1}{2 \times 22} + \frac{1}{2 \times 22}\right)} \\ &\approx 1.9828 \times 0.03545 \approx 0.07029\end{aligned}$$

Here $r = 2 \times 22$ is the # of obs. used to calculate the 3 means below.

```
sort(mean(fracnormal ~ medium, data = shoot))
     2      1      3
0.5811 0.6693 0.6707
```

Conclusion: Medium 2 is significantly lower than Medium 1 and 3 (by more than 1 LSD) but Medium 1 and 3 are not significantly different.

Tukey's HSD for Comparing the 3 Medium (1)

If one wants to account for multiple comparison (Chapter 5), Tukey's 95% HSD for comparing the medium effects $\beta_{j_1} - \beta_{j_2}$ is

$$\begin{aligned}\text{HSD} &= \frac{q(0.05, g, \text{df of MSE})}{\sqrt{2}} \sqrt{\text{MSE}\left(\frac{1}{r} + \frac{1}{r}\right)} \\ &= \frac{q(0.05, 3, 105)}{\sqrt{2}} \sqrt{0.02765\left(\frac{1}{2 \times 22} + \frac{1}{2 \times 22}\right)} \\ &\approx 2.3774 \times 0.03545 \approx 0.08428\end{aligned}$$

```
qtukey(0.05, 3, 105, lower.tail = FALSE)/sqrt(2)
[1] 2.37741
```

Tukey's HSD for Comparing the 3 Medium (2)

```
sort(mean(fracnormal ~ medium, data = shoot))
       2        1        3
0.581136 0.669318 0.670682
```

Conclusion: For FWER $= 0.05$, Medium 2 is still significantly lower than Medium 1 and 3 as $\bar{y}_{\bullet 1 \bullet} - \bar{y}_{\bullet 2 \bullet} = 0.66932 - 0.58114 = 0.08818$ $>$ HSD ≈ 0.084, even after accounting for the fact that we conducted 3 pairwise comparisons between β_1, β_2, and β_3.