

# Experimental Design and the Analysis of Variance

# Comparing $t > 2$ Groups - Numeric Responses

- Extension of Methods used to Compare 2 Groups
- Independent Samples and Paired Data Designs
- Normal and non-normal data distributions

Data Design	Normal	Non-normal
Independent Samples (CRD)	F-Test 1-Way ANOVA	Kruskal-Wallis Test
Paired Data (RBD)	F-Test 2-Way ANOVA	Friedman's Test

# Completely Randomized Design (CRD)

- Controlled Experiments - Subjects assigned at random to one of the  $t$  treatments to be compared
- Observational Studies - Subjects are sampled from  $t$  existing groups
- Statistical model  $y_{ij}$  is measurement from the  $j^{\text{th}}$  subject from group  $i$ :

$$y_{ij} = \mu + \alpha_i + \varepsilon_{ij} = \mu_i + \varepsilon_{ij}$$

where  $\mu$  is the overall mean,  $\alpha_i$  is the effect of treatment  $i$ ,  $\varepsilon_{ij}$  is a random error, and  $\mu_i$  is the population mean for group  $i$

# 1-Way ANOVA for Normal Data (CRD)

- For each group obtain the mean, standard deviation, and sample size:

$$\bar{y}_{i.} = \frac{\sum_j y_{ij}}{n_i} \quad s_i = \sqrt{\frac{\sum_j (y_{ij} - \bar{y}_{i.})^2}{n_i - 1}}$$

- Obtain the overall mean and sample size

$$N = n_1 + \dots + n_t \quad \bar{y}_{..} = \frac{n_1 \bar{y}_{1.} + \dots + n_t \bar{y}_{t.}}{N} = \frac{\sum_i \sum_j y_{ij}}{N}$$

# Analysis of Variance - Sums of Squares

- Total Variation

$$TSS = \sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{..})^2 \quad df_{Total} = N - 1$$

- Between Group (Sample) Variation

$$SST = \sum_{i=1}^t \sum_{j=1}^{n_i} (\bar{y}_{i.} - \bar{y}_{..})^2 = \sum_{i=1}^t n_i (\bar{y}_{i.} - \bar{y}_{..})^2 \quad df_T = t - 1$$

- Within Group (Sample) Variation

$$SSE = \sum_{i=1}^t \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{i.})^2 = \sum_{i=1}^t (n_i - 1) s_i^2 \quad df_E = N - t$$

$$TSS = SST + SSE \quad df_{Total} = df_T + df_E$$

# Analysis of Variance Table and $F$ -Test

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	$F$
Treatments	$SST$	$t-1$	$MST=SST/(t-1)$	$F=MST/MSE$
Error	$SSE$	$N-t$	$MSE=SSE/(N-t)$	
Total	$TSS$	$N-1$		

- Assumption: All distributions normal with common variance
- $H_0$ : No differences among Group Means ( $\alpha_1 = \dots = \alpha_t = 0$ )
- $H_A$ : Group means are not all equal (Not all  $\alpha_i$  are 0)

$$T.S.: F_{obs} = \frac{MST}{MSE}$$

$$R.R.: F_{obs} \geq F_{\alpha, t-1, N-t} \quad (Table\ 9)$$

$$P - val : P(F \geq F_{obs})$$

# Expected Mean Squares

- Model:  $y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$  with  $\varepsilon_{ij} \sim N(0, \sigma^2)$ ,  $\sum \alpha_i = 0$ :

$$E(MSE) = \sigma^2$$

$$E(MST) = \sigma^2 + \frac{\sum_{i=1}^t n_i \alpha_i^2}{t-1}$$

$$\Rightarrow \frac{E(MST)}{E(MSE)} = \frac{\sigma^2 + \frac{\sum_{i=1}^t n_i \alpha_i^2}{t-1}}{\sigma^2} = 1 + \frac{\sum_{i=1}^t n_i \alpha_i^2}{\sigma^2 (t-1)}$$

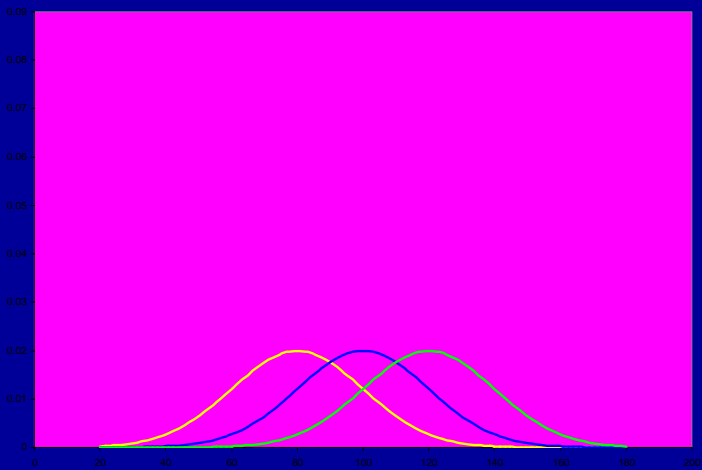
When  $H_0 : \alpha_1 = \dots = \alpha_t = 0$  is true,  $\frac{E(MST)}{E(MSE)} = 1$

otherwise ( $H_a$  is true),  $\frac{E(MST)}{E(MSE)} > 1$

# Expected Mean Squares

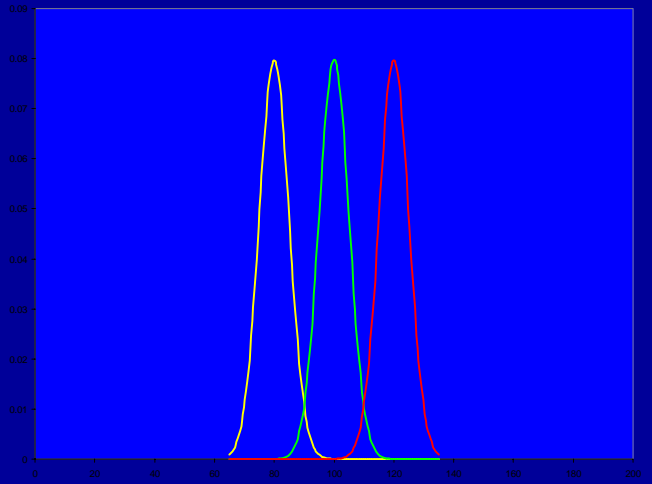
- 3 Factors effect magnitude of  $F$ -statistic (for fixed  $t$ )
  - True group effects ( $\alpha_1, \dots, \alpha_t$ )
  - Group sample sizes ( $n_1, \dots, n_t$ )
  - Within group variance ( $\sigma^2$ )
- $F_{\text{obs}} = MST/MSE$
- When  $H_0$  is true ( $\alpha_1 = \dots = \alpha_t = 0$ ),  $E(MST)/E(MSE) = 1$
- Marginal Effects of each factor (all other factors fixed)
  - As spread in ( $\alpha_1, \dots, \alpha_t$ )  $\uparrow$   $E(MST)/E(MSE) \uparrow$
  - As ( $n_1, \dots, n_t$ )  $\uparrow$   $E(MST)/E(MSE) \uparrow$  (when  $H_0$  false)
  - As  $\sigma^2 \uparrow$   $E(MST)/E(MSE) \downarrow$  (when  $H_0$  false)





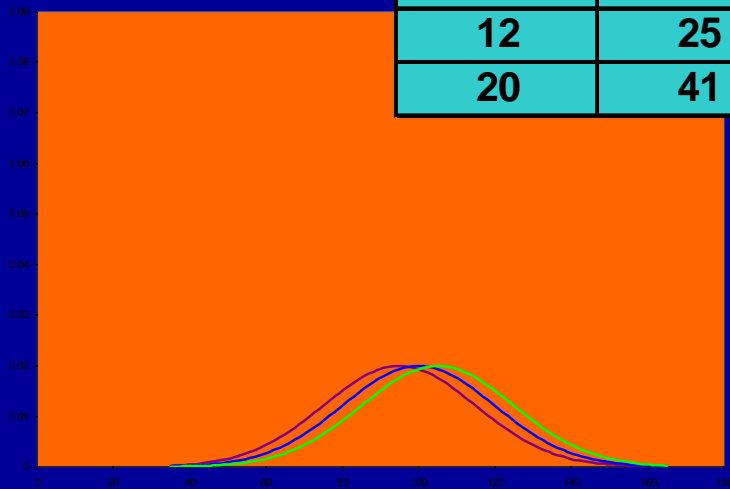
A)  $\mu=100, \tau_1=-20, \tau_2=0, \tau_3=20, \sigma = 20$

$$\frac{E(MST)}{E(MSE)}$$

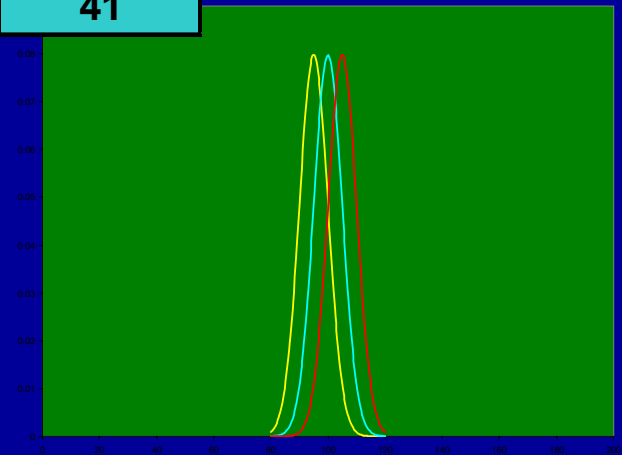


B)  $\mu=100, \tau_1=-20, \tau_2=0, \tau_3=20, \sigma = 5$

n	A	B	C	D
4	9	129	1.5	9
8	17	257	2	17
12	25	385	2.5	25
20	41	641	3.5	41



C)  $\mu=100, \tau_1=-5, \tau_2=0, \tau_3=5, \sigma = 20$



D)  $\mu=100, \tau_1=-5, \tau_2=0, \tau_3=5, \sigma = 5$

# Example - Seasonal Diet Patterns in Ravens

- “Treatments” -  $t = 4$  seasons of year (3 “replicates” each)
  - Winter: November, December, January
  - Spring: February, March, April
  - Summer: May, June, July
  - Fall: August, September, October
- Response ( $Y$ ) - Vegetation (percent of total pellet weight)
- Transformation (For approximate normality):

$$Y' = \arcsin\left(\sqrt{\frac{Y}{100}}\right)$$

# Seasonal Diet Patterns in Ravens - Data/Means

Y	Winter(i=1)	Fall(i=2)	Summer(i=3)	Fall (i=4)
j=1	94.3	80.7	80.5	67.8
j=2	90.3	90.5	74.3	91.8
j=3	83.0	91.8	32.4	89.3

Y'	Winter(i=1)	Fall(i=2)	Summer(i=3)	Fall (i=4)
j=1	1.329721	1.115957	1.113428	0.967390
j=2	1.254080	1.257474	1.039152	1.280374
j=3	1.145808	1.280374	0.605545	1.237554

$$\bar{y}_{1.} = \frac{1.329721 + 1.254080 + 1.145808}{3} = 1.24203$$

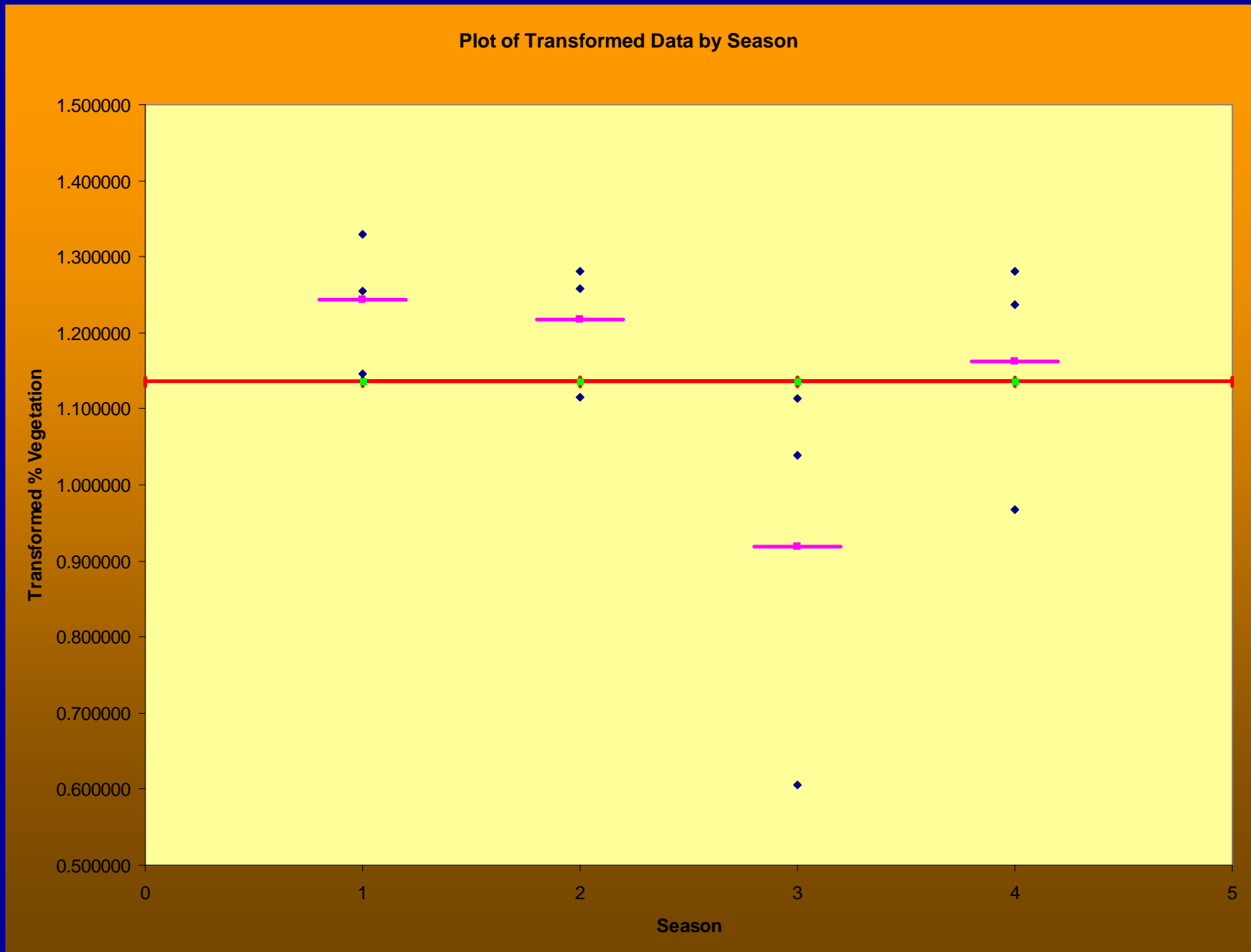
$$\bar{y}_{2.} = \frac{1.115957 + 1.257474 + 1.280374}{3} = 1.217935$$

$$\bar{y}_{3.} = \frac{1.113428 + 1.039152 + 0.605545}{3} = 0.919375$$

$$\bar{y}_{4.} = \frac{0.967390 + 1.280374 + 1.237554}{3} = 1.16773$$

$$\bar{y}_{..} = \frac{1.329721 + \dots + 1.237554}{12} = 1.135572$$

# Seasonal Diet Patterns in Ravens - Data/Mean



# Seasonal Diet Patterns in Ravens - ANOVA

Total Variation :  $(df_{\text{Total}} = 12 - 1 = 11)$

$$TSS = (1.329721 - 1.135572)^2 + \dots + (1.27554 - 1.135572)^2 = 0.438425$$

Between Group Variation :  $(df_T = 4 - 1 = 3)$

$$SST = 3 \left[ (1.24203 - 1.135572)^2 + \dots + (1.161773 - 1.135572)^2 \right] = 0.197387$$

Within Group Variation :  $(df_E = 12 - 4 = 8)$

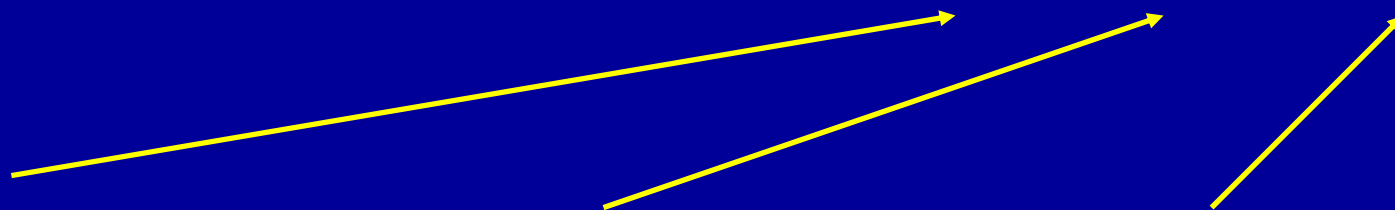
$$SSE = (1.329721 - 1.243203)^2 + \dots + (1.237554 - 1.161773)^2 = 0.241038$$

ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.197387	3	0.065796	2.183752	0.167768	4.06618
Within Groups	0.241038	8	0.03013			
Total	0.438425	11				

Do not conclude that seasons differ with respect to vegetation intake

# Seasonal Diet Patterns in Ravens - Spreadsheet

Month	Season	Y'	Season Mean	Overall Mean	TSS	SST	SSE
NOV	1	1.329721	1.243203	1.135572	0.037694	0.011584	0.007485
DEC	1	1.254080	1.243203	1.135572	0.014044	0.011584	0.000118
JAN	1	1.145808	1.243203	1.135572	0.000105	0.011584	0.009486
FEB	2	1.115957	1.217935	1.135572	0.000385	0.006784	0.010400
MAR	2	1.257474	1.217935	1.135572	0.014860	0.006784	0.001563
APR	2	1.280374	1.217935	1.135572	0.020968	0.006784	0.003899
MAY	3	1.113428	0.919375	1.135572	0.000490	0.046741	0.037657
JUN	3	1.039152	0.919375	1.135572	0.009297	0.046741	0.014346
JUL	3	0.605545	0.919375	1.135572	0.280928	0.046741	0.098489
AUG	4	0.967390	1.161773	1.135572	0.028285	0.000687	0.037785
SEP	4	1.280374	1.161773	1.135572	0.020968	0.000687	0.014066
OCT	4	1.237554	1.161773	1.135572	0.010400	0.000687	0.005743
				Sum	0.438425	0.197387	0.241038



**Total SS**

$(Y' - \text{Overall Mean})^2$

**Between Season SS**

$(\text{Group Mean} - \text{Overall Mean})^2$

**Within Season SS**

$(Y' - \text{Group Mean})^2$

# CRD with Non-Normal Data

## Kruskal-Wallis Test

- Extension of Wilcoxon Rank-Sum Test to  $k > 2$  Groups
- Procedure:
  - Rank the observations across groups from smallest (1) to largest ( $N = n_1 + \dots + n_k$ ), adjusting for ties
  - Compute the rank sums for each group:  $T_1, \dots, T_k$ . Note that  $T_1 + \dots + T_k = N(N+1)/2$

# Kruskal-Wallis Test

- $H_0$ : The  $k$  population distributions are identical ( $\mu_1 = \dots = \mu_k$ )
- $H_A$ : Not all  $k$  distributions are identical (Not all  $\mu_i$  are equal)

$$T.S.: H = \frac{12}{N(N+1)} \sum_{i=1}^k \frac{T_i^2}{n_i} - 3(N+1)$$

$$R.R.: H \geq \chi_{\alpha, k-1}^2$$

$$P\text{-val} : P(\chi^2 \geq H)$$

An adjustment to  $H$  is suggested when there are many ties in the data. Formula is given on page 344 of O&L.



# Example - Seasonal Diet Patterns in Ravens

Month	Season	Y'	Rank
NOV	1	1.329721	12
DEC	1	1.254080	8
JAN	1	1.145808	6
FEB	2	1.115957	5
MAR	2	1.257474	9
APR	2	1.280374	10.5
MAY	3	1.113428	4
JUN	3	1.039152	3
JUL	3	0.605545	1
AUG	4	0.967390	2
SEP	4	1.280374	10.5
OCT	4	1.237554	7

- $T_1 = 12+8+6 = 26$
- $T_2 = 5+9+10.5 = 24.5$
- $T_3 = 4+3+1 = 8$
- $T_4 = 2+10.5+7 = 19.5$

$H_0$  : No seasonal difference       $H_a$  : Seasonal Differences

$$T.S.: H = \frac{12}{12(12+1)} \left[ \frac{(26)^2}{3} + \frac{(24.5)^2}{3} + \frac{(8)^2}{3} + \frac{(19.5)^2}{3} \right] - 3(12+1) = 44.12 - 39 = 5.12$$

$$R.R.(\alpha = 0.05) : H \geq \chi_{.05, 4-1}^2 = 7.815$$

$$P\text{-value} : P(\chi^2 \geq H = 5.12) = .1632$$

# Post-hoc Comparisons of Treatments

- If differences in group means are determined from the  $F$ -test, researchers want to compare pairs of groups. Three popular methods include:
  - Fisher's LSD - Upon rejecting the null hypothesis of no differences in group means, LSD method is equivalent to doing pairwise comparisons among all pairs of groups as in Chapter 6.
  - Tukey's Method - Specifically compares all  $t(t-1)/2$  pairs of groups. Utilizes a special table (Table 11, p. 701).
  - Bonferroni's Method - Adjusts individual comparison error rates so that all conclusions will be correct at desired confidence/significance level. Any number of comparisons can be made. Very general approach can be applied to any inferential problem

# Fisher's Least Significant Difference Procedure

- Protected Version is to only apply method after significant result in overall  $F$ -test
- For each pair of groups, compute the **least significant difference (LSD)** that the sample means need to differ by to conclude the population means are not equal

$$LSD_{ij} = t_{\alpha/2} \sqrt{MSE \left( \frac{1}{n_i} + \frac{1}{n_j} \right)} \quad \text{with df} = N - t$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq LSD_{ij}$

Fisher's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm LSD_{ij}$

# Tukey's $W$ Procedure

- More conservative than Fisher's LSD (minimum significant difference and confidence interval width are higher).
- Derived so that the probability that at least one false difference is detected is  $\alpha$  (experimentwise error rate)

$$W_{ij} = q_{\alpha}(t, \nu) \sqrt{\frac{MSE}{n}} \quad q \text{ given in Table 11, p. 701 with } \nu = N-t$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq W_{ij}$

Tukey's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm W_{ij}$

When the sample sizes are unequal, use  $n = \frac{t}{\frac{1}{n_1} + \dots + \frac{1}{n_t}}$

# Bonferroni's Method (Most General)

- Wish to make  $C$  comparisons of pairs of groups with simultaneous confidence intervals or 2-sided tests
- When all pair of treatments are to be compared,  $C = t(t-1)/2$
- Want the overall confidence level for all intervals to be “correct” to be 95% or the overall type I error rate for all tests to be 0.05
- For confidence intervals, construct  $(1-(0.05/C))100\%$  CIs for the difference in each pair of group means (wider than 95% CIs)
- Conduct each test at  $\alpha=0.05/C$  significance level (rejection region cut-offs more extreme than when  $\alpha=0.05$ )
- Critical  $t$ -values are given in table on class website, we will use notation:  $t_{\alpha/2, C, \nu}$  where  $C=\#\text{Comparisons}$ ,  $\nu = \text{df}$

## Bonferroni's Method (Most General)

$$B_{ij} = t_{\alpha/2, C, \nu} \sqrt{MSE \left( \frac{1}{n_i} + \frac{1}{n_j} \right)}$$

( $t$  given on class website with  $\nu = N-t$ )

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq B_{ij}$

Bonferroni's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm B_{ij}$

# Example - Seasonal Diet Patterns in Ravens

**Note: No differences were found, these calculations are only for demonstration purposes**

$$MSE = 0.03013 \quad n_i = 3 \quad t_{.025,8} = 2.306 \quad q_{.05,t=4,df_E=8} = 4.53 \quad t_{.025,C=6,df_E=8} = 3.479$$

$$LSD_{ij} = 2.306 \sqrt{(0.03013) \left( \frac{1}{3} + \frac{1}{3} \right)} = 0.3268$$

$$W_{ij} = 4.53 \sqrt{(0.03013) \left( \frac{1}{3} \right)} = 0.4540$$

$$B_{ij} = 3.479 \sqrt{(0.03013) \left( \frac{1}{3} + \frac{1}{3} \right)} = 0.4930$$

Comparison(i vs j)	Group i Mean	Group j Mean	Difference
1 vs 2	1.243203	1.217935	0.025267
1 vs 3	1.243203	0.919375	0.323828
1 vs 4	1.243203	1.161773	0.081430
2 vs 3	1.217935	0.919375	0.298560
2 vs 4	1.217935	1.161773	0.056162
3 vs 4	0.919375	1.161773	-0.242398

# Randomized Block Design (RBD)

- $t > 2$  Treatments (groups) to be compared
- $b$  Blocks of homogeneous units are sampled. Blocks can be individual subjects. Blocks are made up of  $t$  subunits
- Subunits within a block receive one treatment. When subjects are blocks, receive treatments in random order.
- Outcome when Treatment  $i$  is assigned to Block  $j$  is labeled  $Y_{ij}$
- Effect of Trt  $i$  is labeled  $\alpha_i$
- Effect of Block  $j$  is labeled  $\beta_j$
- Random error term is labeled  $\varepsilon_{ij}$
- Efficiency gain from removing block-to-block variability from experimental error



# Randomized Complete Block Designs

- Model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \varepsilon_{ij} = \mu_i + \beta_j + \varepsilon_{ij}$$

$$\sum_{i=1}^t \alpha_i = 0 \quad E(\varepsilon_{ij}) = 0 \quad V(\varepsilon_{ij}) = \sigma^2$$

- Test for differences among treatment effects:

- $H_0: \alpha_1 = \dots = \alpha_t = 0$  ( $\mu_1 = \dots = \mu_t$ )
- $H_A: \text{Not all } \alpha_i = 0$  (Not all  $\mu_i$  are equal)

Typically not interested in measuring block effects (although sometimes wish to estimate their variance in the population of blocks). Using Block designs increases efficiency in making inferences on treatment effects

# RBD - ANOVA $F$ -Test (Normal Data)

- Data Structure: ( $t$  Treatments,  $b$  Subjects)

- Mean for Treatment  $i$ :  $\bar{y}_{i.}$
- Mean for Subject (Block)  $j$ :  $\bar{y}_{.j}$
- Overall Mean:  $\bar{y}_{..}$
- Overall sample size:  $N = bt$

- ANOVA: **Treatment**, **Block**, and **Error Sums of Squares**

$$TSS = \sum_{i=1}^t \sum_{j=1}^b (y_{ij} - \bar{y}_{..})^2 \quad df_{Total} = bt - 1$$

$$SST = b \sum_{i=1}^t (\bar{y}_{i.} - \bar{y}_{..})^2 \quad df_T = t - 1$$

$$SSB = t \sum_{j=1}^b (\bar{y}_{.j} - \bar{y}_{..})^2 \quad df_B = b - 1$$

$$SSE = \sum \sum (y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..})^2 = TSS - SST - SSB \quad df_E = (b - 1)(t - 1)$$

# RBD - ANOVA $F$ -Test (Normal Data)

- ANOVA Table:

Source	SS	df	MS	$F$
Treatments	$SST$	$t-1$	$MST = SST/(t-1)$	$F = MST/MSE$
Blocks	$SSB$	$b-1$	$MSB = SSB/(b-1)$	
Error	$SSE$	$(b-1)(t-1)$	$MSE = SSE/[(b-1)(t-1)]$	
Total	$TSS$	$bt-1$		

- $H_0: \alpha_1 = \dots = \alpha_t = 0 \quad (\mu_1 = \dots = \mu_t)$

- $H_A: \text{Not all } \alpha_i = 0 \quad (\text{Not all } \mu_i \text{ are equal})$

$$T.S.: F_{obs} = \frac{MST}{MSE}$$

$$R.R.: F_{obs} \geq F_{\alpha, t-1, (b-1)(t-1)}$$

$$P\text{-val} : P(F \geq F_{obs})$$

# Pairwise Comparison of Treatment Means

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = (b-1)(t-1)$

$$W_{ij} = q_{\alpha}(t, \nu) \sqrt{\frac{MSE}{b}}$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq W_{ij}$

Tukey's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm W_{ij}$

- Bonferroni's Method -  $t$ -values from table on class website with  $\nu = (b-1)(t-1)$  and  $C = t(t-1)/2$

$$B_{ij} = t_{\alpha/2, C, \nu} \sqrt{\frac{2MSE}{b}}$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq B_{ij}$

Bonferroni's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm B_{ij}$

# Expected Mean Squares / Relative Efficiency

- Expected Mean Squares: As with CRD, the Expected Mean Squares for Treatment and Error are functions of the sample sizes ( $b$ , the number of blocks), the true treatment effects ( $\alpha_1, \dots, \alpha_t$ ) and the variance of the random error terms ( $\sigma^2$ )
- By assigning all treatments to units within blocks, error variance is (much) smaller for RBD than CRD (which combines block variation & random error into error term)
- Relative Efficiency of RBD to CRD (how many times as many replicates would be needed for CRD to have as precise of estimates of treatment means as RBD does):

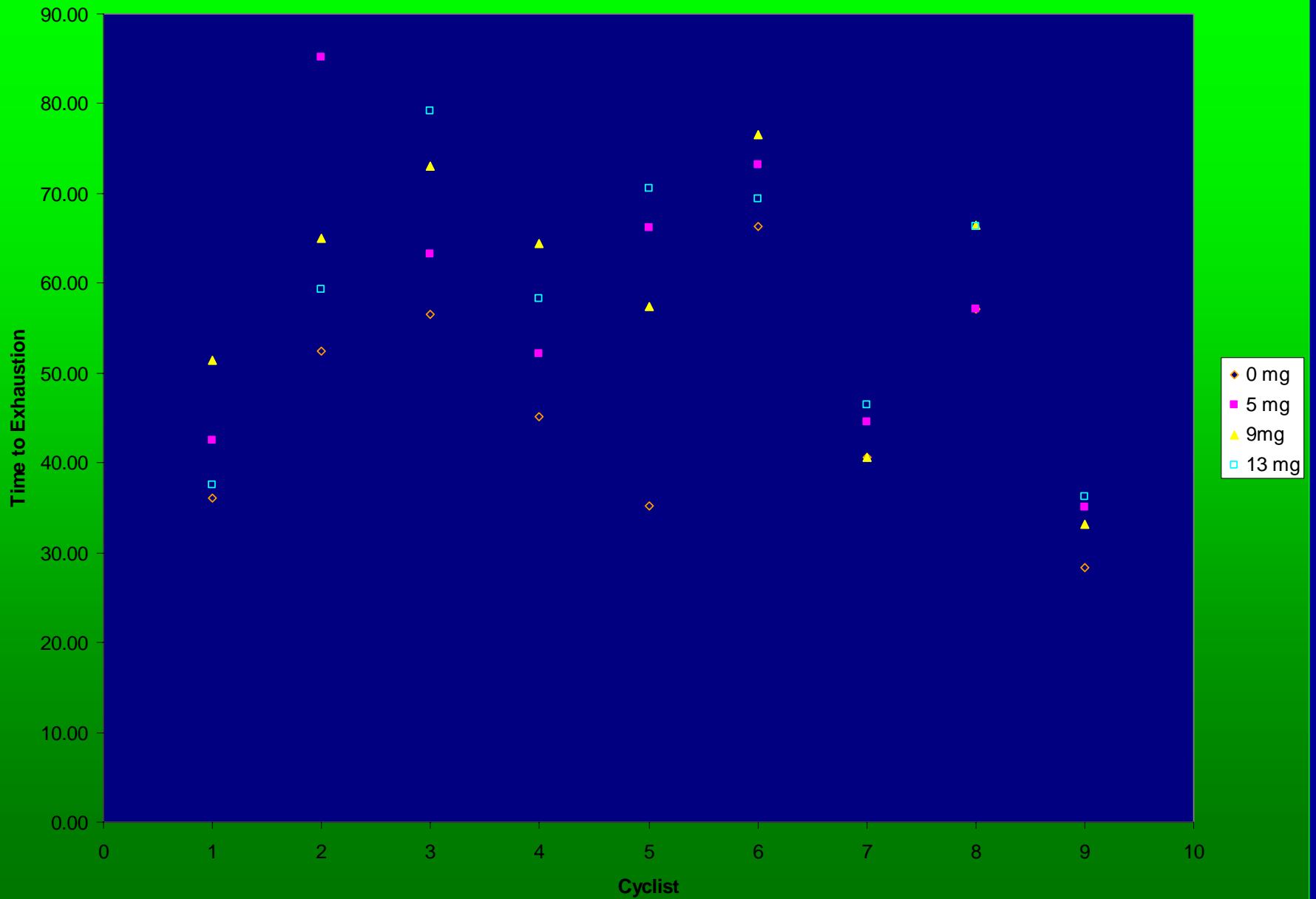
$$RE(RCB, CR) = \frac{MSE_{CR}}{MSE_{RCB}} = \frac{(b-1)MSB + b(t-1)MSE}{(bt-1)MSE}$$

# Example - Caffeine and Endurance

- Treatments:  $t=4$  Doses of Caffeine: 0, 5, 9, 13 *mg*
- Blocks:  $b=9$  Well-conditioned cyclists
- Response:  $y_{ij}$ =Minutes to exhaustion for cyclist  $j$  @ dose  $i$
- Data:

Dose \ Subject	1	2	3	4	5	6	7	8	9
0	36.05	52.47	56.55	45.20	35.25	66.38	40.57	57.15	28.34
5	42.47	85.15	63.20	52.10	66.20	73.25	44.50	57.17	35.05
9	51.50	65.00	73.10	64.40	57.45	76.49	40.55	66.47	33.17
13	37.55	59.30	79.12	58.33	70.54	69.47	46.48	66.35	36.20

Plot of Y versus Subject by Dose



# Example - Caffeine and Endurance

Subject\Dose	0mg	5mg	9mg	13mg	Subj Mea	Subj Dev	Sqr Dev
1	36.05	42.47	51.50	37.55	41.89	-13.34	178.07
2	52.47	85.15	65.00	59.30	65.48	10.24	104.93
3	56.55	63.20	73.10	79.12	67.99	12.76	162.71
4	45.20	52.10	64.40	58.33	55.01	-0.23	0.05
5	35.25	66.20	57.45	70.54	57.36	2.12	4.51
6	66.38	73.25	76.49	69.47	71.40	16.16	261.17
7	40.57	44.50	40.55	46.48	43.03	-12.21	149.12
8	57.15	57.17	66.47	66.35	61.79	6.55	42.88
9	28.34	35.05	33.17	36.20	33.19	-22.05	486.06
<b>Dose Mean</b>	<b>46.44</b>	<b>57.68</b>	<b>58.68</b>	<b>58.15</b>	<b>55.24</b>		<b>1389.50</b>
<b>Dose Dev</b>	<b>-8.80</b>	<b>2.44</b>	<b>3.44</b>	<b>2.91</b>			
<b>Squared Dev</b>	<b>77.38</b>	<b>5.95</b>	<b>11.86</b>	<b>8.48</b>	<b>103.68</b>		
<b>TSS</b>	<b>7752.773</b>						

$$TSS = (36.05 - 55.24)^2 + \dots + (36.20 - 55.24)^2 = 7752.773 \quad df_{Total} = 4(9) - 1 = 35$$

$$SST = 9[(46.44 - 55.24)^2 + \dots + (58.15 - 55.24)^2] = 9(103.68) = 933.12 \quad df_T = 4 - 1 = 3$$

$$SSB = 4[(41.89 - 55.24)^2 + \dots + (33.19 - 55.24)^2] = 4(1389.50) = 5558.00 \quad df_B = 9 - 1 = 8$$

$$SSE = (36.05 - 41.89 - 46.44 + 55.24)^2 + \dots + (36.20 - 33.19 - 58.15 + 55.24)^2 = \\ = TSS - SST - SSB = 7752.773 - 933.12 - 5558 = 1261.653 \quad df_E = (4 - 1)(9 - 1) = 24$$



# Example - Caffeine and Endurance

Source	df	SS	MS	F
Dose	3	933.12	311.04	5.92
Cyclist	8	5558.00	694.75	
Error	24	1261.65	52.57	
Total	35	7752.77		

$H_0$  : No Caffeine Dose Effect ( $\alpha_1 = \dots = \alpha_4 = 0$ )

$H_A$  : Differences Exist Among Doses

$$T.S.: F_{obs} = \frac{MST}{MSE} = \frac{311.04}{52.57} = 5.92$$

$$R.R.(\alpha = 0.05): F_{obs} \geq F_{.05,3,24} = 3.01$$

$P$ -value:  $P(F \geq 5.92) = .0036$  (From EXCEL)

Conclude that true means are not all equal

# Example - Caffeine and Endurance

$$\text{Tukey's } W : q_{.05,4,24} = 3.90 \quad W = 3.90 \sqrt{52.57 \left( \frac{1}{9} \right)} = 9.43$$

$$\text{Bonferroni's } B : t_{.05/2,6,24} = 2.875 \quad B = 2.875 \sqrt{52.57 \left( \frac{2}{9} \right)} = 9.83$$

Doses	High Mean	Low Mean	Difference	Conclude
5mg vs 0mg	57.6767	46.4400	11.2367	$\mu_5 > \mu_0$
9mg vs 0mg	58.6811	46.4400	12.2411	$\mu_9 > \mu_0$
13mg vs 0mg	58.1489	46.4400	11.7089	$\mu_{13} > \mu_0$
9mg vs 5mg	58.6811	57.6767	1.0044	NSD
13mg vs 5mg	58.1489	57.6767	0.4722	NSD
13mg vs 9mg	58.1489	58.6811	-0.5322	NSD

# Example - Caffeine and Endurance

Relative Efficiency of Randomized Block to Completely Randomized Design :

$$t = 4 \quad b = 9 \quad MSB = 694.75 \quad MSE = 52.57$$

$$RE(RCB, CR) = \frac{(b-1)MSB + b(t-1)MSE}{(bt-1)MSE} = \frac{8(694.75) + 9(3)(52.57)}{(9(4)-1)(52.57)} = \frac{6977.39}{1839.95} = 3.79$$

Would have needed 3.79 times as many cyclists per dose to have the same precision on the estimates of mean endurance time.

- $9(3.79) \approx 35$  cyclists per dose
- $4(35) = 140$  total cyclists

# RBD -- Non-Normal Data

## Friedman's Test

- When data are non-normal, test is based on ranks
- Procedure to obtain test statistic:
  - Rank the  $k$  treatments within each block (1=smallest,  $k$ =largest) adjusting for ties
  - Compute rank sums for treatments ( $T_i$ ) across blocks
  - $H_0$ : The  $k$  populations are identical ( $\mu_1 = \dots = \mu_k$ )
  - $H_A$ : Differences exist among the  $k$  group means

$$T.S.: F_r = \frac{12}{bk(k+1)} \sum_{i=1}^k T_i^2 - 3b(k+1)$$

$$R.R.: F_r \geq \chi_{\alpha, k-1}^2$$

$$P\text{-val}: P(\chi^2 \geq F_r)$$

# Example - Caffeine and Endurance

Subject\Dose	0mg	5mg	9mg	13mg	Ranks	0mg	5mg	9mg	13mg
1	36.05	42.47	51.5	37.55		1	3	4	2
2	52.47	85.15	65	59.3		1	4	3	2
3	56.55	63.2	73.1	79.12		1	2	3	4
4	45.2	52.1	64.4	58.33		1	2	4	3
5	35.25	66.2	57.45	70.54		1	3	2	4
6	66.38	73.25	76.49	69.47		1	3	4	2
7	40.57	44.5	40.55	46.48		2	3	1	4
8	57.15	57.17	66.47	66.35		1	2	4	3
9	28.34	35.05	33.17	36.2		1	3	2	4
					Total	10	25	27	28

$H_0$  : No Dose Differences

$H_a$  : Dose Differences Exist

$$T.S.: F_r = \frac{12}{9(4)(4+1)} \left[ (10)^2 + \dots + (28)^2 \right] - 3(9)(4+1) = \frac{26856}{180} - 135 = 14.2$$

$$R.R.(\alpha = 0.05) : F_r \geq \chi_{.05, 4-1}^2 = 7.815$$

$$P\text{-value} : P(\chi^2 \geq 14.2) = .0026 \text{ (From EXCEL)}$$

Conclude Means (Medians) are not all equal

# Latin Square Design

- Design used to compare  $t$  treatments when there are two sources of extraneous variation (types of blocks), each observed at  $t$  levels
- Best suited for analyses when  $t \leq 10$
- Classic Example: Car Tire Comparison
  - Treatments: 4 Brands of tires (A,B,C,D)
  - Extraneous Source 1: Car (1,2,3,4)
  - Extraneous Source 2: Position (Driver Front, Passenger Front, Driver Rear, Passenger Rear)

Car\Position	DF	PF	DR	PR
1	A	B	C	D
2	B	C	D	A
3	C	D	A	B
4	D	A	B	C

# Latin Square Design - Model

- Model ( $t$  treatments, rows, columns,  $N=t^2$ ) :

$$y_{ijk} = \mu + \alpha_k + \beta_i + \gamma_k + \varepsilon_{ijk}$$

$$\mu \equiv \text{Overall Mean} \quad \hat{\mu} = \bar{y}_{...}$$

$$\alpha_k \equiv \text{Effect of Treatment } k \quad \hat{\alpha}_k = \bar{y}_{..k} - \bar{y}_{...}$$

$$\beta_i \equiv \text{Effect due to row } i \quad \hat{\beta}_i = \bar{y}_{i..} - \bar{y}_{...}$$

$$\gamma_j \equiv \text{Effect due to Column } j \quad \hat{\gamma}_j = \bar{y}_{.j.} - \bar{y}_{...}$$

$$\varepsilon_{ijk} \equiv \text{Random Error Term}$$

# Latin Square Design - ANOVA & $F$ -Test

$$\text{Total Sum of Squares : } TSS = \sum_{i=1}^t \sum_{j=1}^t (y_{ijk} - \bar{y}_{...})^2 \quad df = t^2 - 1$$

$$\text{Treatment Sum of Squares } SST = t \sum_{k=1}^t (\bar{y}_{..k} - \bar{y}_{...})^2 \quad df_T = t - 1$$

$$\text{Row Sum of Squares } SSR = t \sum_{i=1}^t (\bar{y}_{i..} - \bar{y}_{...})^2 \quad df_R = t - 1$$

$$\text{Column Sum of Squares } SSC = t \sum_{j=1}^t (\bar{y}_{.j.} - \bar{y}_{...})^2 \quad df_C = t - 1$$

$$\text{Error Sum of Squares } SSE = TSS - SST - SSR - SSC \quad df_E = (t^2 - 1) - 3(t - 1) = (t - 1)(t - 2)$$

- $H_0: \alpha_1 = \dots = \alpha_t = 0$       $H_a: \text{Not all } \alpha_k = 0$
- **TS:**  $F_{\text{obs}} = MST/MSE = (SST/(t-1))/(SSE/((t-1)(t-2)))$
- **RR:**  $F_{\text{obs}} \geq F_{\alpha, t-1, (t-1)(t-2)}$



# Pairwise Comparison of Treatment Means

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = (t-1)(t-2)$

$$W_{ij} = q_{\alpha}(t, \nu) \sqrt{\frac{MSE}{t}}$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq W_{ij}$

Tukey's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm W_{ij}$

- Bonferroni's Method -  $t$ -values from table on class website with  $\nu = (t-1)(t-2)$  and  $C = t(t-1)/2$

$$B_{ij} = t_{\alpha/2, C, \nu} \sqrt{\frac{2MSE}{t}}$$

Conclude  $\mu_i \neq \mu_j$  if  $|\bar{y}_{i.} - \bar{y}_{j.}| \geq B_{ij}$

Bonferroni's Confidence Interval:  $(\bar{y}_{i.} - \bar{y}_{j.}) \pm B_{ij}$

# Expected Mean Squares / Relative Efficiency

- Expected Mean Squares: As with CRD, the Expected Mean Squares for Treatment and Error are functions of the sample sizes ( $t$ , the number of blocks), the true treatment effects ( $\alpha_1, \dots, \alpha_t$ ) and the variance of the random error terms ( $\sigma^2$ )
- By assigning all treatments to units within blocks, error variance is (much) smaller for LS than CRD (which combines block variation & random error into error term)
- Relative Efficiency of LS to CRD (how many times as many replicates would be needed for CRD to have as precise of estimates of treatment means as LS does):

$$RE( LS, CR ) = \frac{MSE_{CR}}{MSE_{LS}} \frac{MSR + MSC + (t - 1)MSE}{(t + 1)MSE}$$

# 2-Way ANOVA

- 2 nominal or ordinal factors are believed to be related to a quantitative response
- Additive Effects: The effects of the levels of each factor do not depend on the levels of the other factor.
- Interaction: The effects of levels of each factor depend on the levels of the other factor
- Notation:  $\mu_{ij}$  is the mean response when factor A is at level  $i$  and Factor B at  $j$

# 2-Way ANOVA - Model

$$y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk} \quad i = 1, \dots, a \quad j = 1, \dots, b \quad k = 1, \dots, r$$

$y_{ijk}$   $\equiv$  Measurement on  $k^{\text{th}}$  unit receiving Factors A at level  $i$ , B at level  $j$

$\mu$   $\equiv$  Overall Mean

$\alpha_i$   $\equiv$  Effect of  $i^{\text{th}}$  level of factor A

$\beta_j$   $\equiv$  Effect of  $j^{\text{th}}$  level of factor B

$\alpha\beta_{ij}$   $\equiv$  Interaction effect when  $i^{\text{th}}$  level of A and  $j^{\text{th}}$  level of B are combined

$\varepsilon_{ijk}$   $\equiv$  Random Error Terms

- Model depends on whether all levels of interest for a factor are included in experiment:
  - **Fixed Effects:** All levels of factors A and B included
  - **Random Effects:** Subset of levels included for factors A and B
  - **Mixed Effects:** One factor has all levels, other factor a subset

# Fixed Effects Model

- Factor A: Effects are fixed constants and sum to 0
- Factor B: Effects are fixed constants and sum to 0
- Interaction: Effects are fixed constants and sum to 0 over all levels of factor B, for each level of factor A, and vice versa
- Error Terms: Random Variables that are assumed to be independent and normally distributed with mean 0, variance  $\sigma_{\varepsilon}^2$

$$\sum_{i=1}^a \alpha_i = 0, \quad \sum_{j=1}^b \beta_j = 0 \quad \sum_{i=1}^a \alpha\beta_{ij} = 0 \quad \forall j \quad \sum_{j=1}^b \alpha\beta_{ij} = 0 \quad \forall i \quad \varepsilon_{ijk} \sim N(0, \sigma_{\varepsilon}^2)$$

# Example - Thalidomide for AIDS

- Response: 28-day weight gain in AIDS patients
- Factor A: Drug: Thalidomide/Placebo
- Factor B: TB Status of Patient: TB<sup>+</sup>/TB<sup>-</sup>
- Subjects: 32 patients (16 TB<sup>+</sup> and 16 TB<sup>-</sup>).  
Random assignment of 8 from each group to each drug). Data:
  - Thalidomide/TB<sup>+</sup>: 9,6,4.5,2,2.5,3,1,1.5
  - Thalidomide/TB<sup>-</sup>: 2.5,3.5,4,1,0.5,4,1.5,2
  - Placebo/TB<sup>+</sup>: 0,1,-1,-2,-3,-3,0.5,-2.5
  - Placebo/TB<sup>-</sup>: -0.5,0,2.5,0.5,-1.5,0,1,3.5

# ANOVA Approach

- Total Variation (*TSS*) is partitioned into 4 components:
  - Factor A: Variation in means among levels of A
  - Factor B: Variation in means among levels of B
  - Interaction: Variation in means among combinations of levels of A and B that are not due to A or B alone
  - Error: Variation among subjects within the same combinations of levels of A and B (Within SS)

# Analysis of Variance

$$\text{Total Variation: } TSS = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^r (y_{ijk} - \bar{y}_{...})^2 \quad df_{Total} = abr - 1$$

$$\text{Factor A Sum of Squares: } SSA = br \sum_{i=1}^a (\bar{y}_{i..} - \bar{y}_{...})^2 \quad df_A = a - 1$$

$$\text{Factor B Sum of Squares: } SSB = ar \sum_{j=1}^b (\bar{y}_{.j.} - \bar{y}_{...})^2 \quad df_B = b - 1$$

$$\text{Interaction Sum of Squares: } SSAB = r \sum_{i=1}^a \sum_{j=1}^b (\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 \quad df_{AB} = (a-1)(b-1)$$

$$\text{Error Sum of Squares: } SSE = \sum_{i=1}^a \sum_{j=1}^b \sum_{k=1}^r (y_{ijk} - \bar{y}_{ij.})^2 \quad df_E = ab(r-1)$$

- $TSS = SSA + SSB + SSAB + SSE$

- $df_{Total} = df_A + df_B + df_{AB} + df_E$



# ANOVA Approach - Fixed Effects

Source	df	SS	MS	F
Factor A	a-1	SSA	$MSA=SSA/(a-1)$	$F_A=MSA/MSE$
Factor B	b-1	SSB	$MSB=SSB/(b-1)$	$F_B=MSB/MSE$
Interaction	$(a-1)(b-1)$	SSAB	$MSAB=SSAB/[(a-1)(b-1)]$	$F_{AB}=MSAB/MSE$
Error	$ab(r-1)$	SSE	$MSE=SSE/[ab(r-1)]$	
Total	$abr-1$	TSS		

- Procedure:

- First test for interaction effects

- If interaction test not significant, test for Factor A and B effects

Test for Interaction :

$$H_0 : \alpha\beta_{11} = \dots = \alpha\beta_{ab} = 0$$

$$H_a : \text{Not all } \alpha\beta_{ij} = 0$$

$$TS : F_{AB} = \frac{MSAB}{MSE}$$

$$RR : F_{AB} \geq F_{\alpha, (a-1)(b-1), ab(r-1)}$$

Test for Factor A

$$H_0 : \alpha_1 = \dots = \alpha_a = 0$$

$$H_a : \text{Not all } \alpha_i = 0$$

$$TS : F_A = \frac{MSA}{MSE}$$

$$RR : F_A \geq F_{\alpha, (a-1), ab(r-1)}$$

Test for Factor B

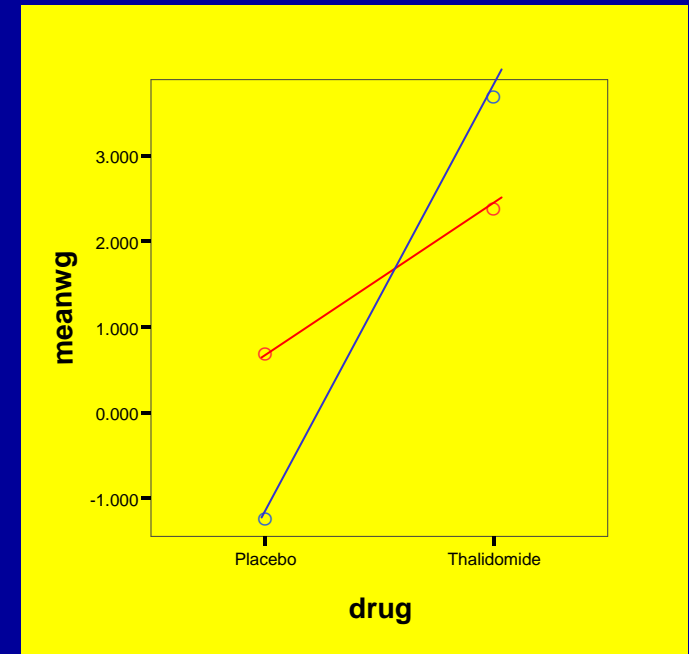
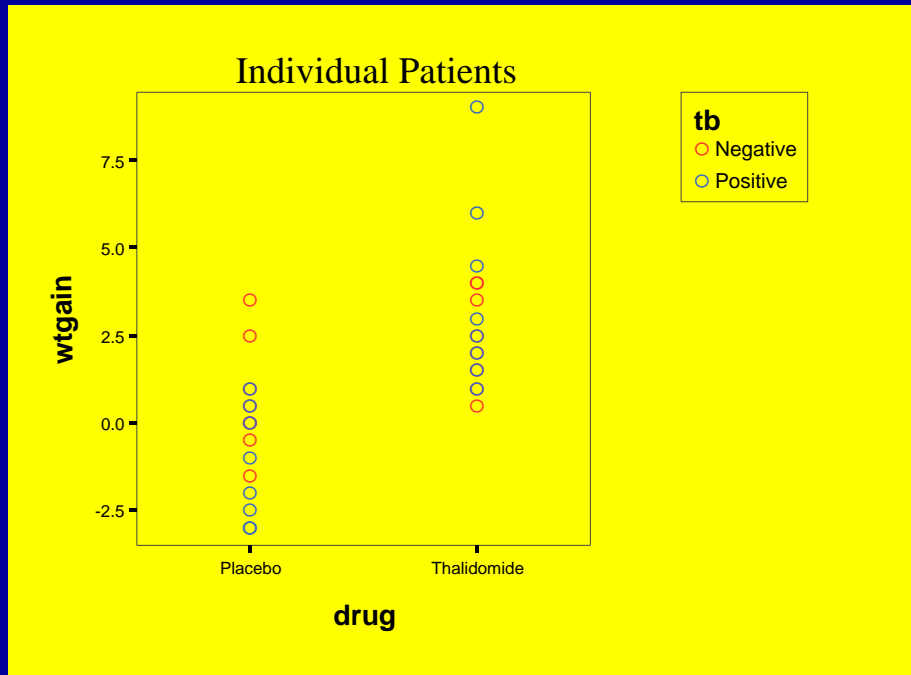
$$H_0 : \beta_1 = \dots = \beta_b = 0$$

$$H_a : \text{Not all } \beta_j = 0$$

$$TS : F_B = \frac{MSB}{MSE}$$

$$RR : F_B \geq F_{\alpha, (b-1), ab(r-1)}$$

# Example - Thalidomide for AIDS



## Report

WTGAIN

GROUP	Mean	N	Std. Deviation
TB+/Thalidomide	3.688	8	2.6984
TB-/Thalidomide	2.375	8	1.3562
TB+/Placebo	-1.250	8	1.6036
TB-/Placebo	.688	8	1.6243
Total	1.375	32	2.6027

# Example - Thalidomide for AIDS

## Tests of Between-Subjects Effects

Dependent Variable: WTGAIN

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	109.688 <sup>a</sup>	3	36.563	10.206	.000
Intercept	60.500	1	60.500	16.887	.000
DRUG	87.781	1	87.781	24.502	.000
TB	.781	1	.781	.218	.644
DRUG * TB	21.125	1	21.125	5.897	.022
Error	100.313	28	3.583		
Total	270.500	32			
Corrected Total	210.000	31			

a. R Squared = .522 (Adjusted R Squared = .471)

- There is a significant Drug\*TB interaction ( $F_{DT}=5.897$ ,  $P=.022$ )
- The Drug effect depends on TB status (and vice versa)

# Comparing Main Effects (No Interaction)

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = ab(r-1)$

$$W_{ij}^A = q_{\alpha}(a, \nu) \sqrt{\frac{MSE}{br}} \quad W_{ij}^B = q_{\alpha}(b, \nu) \sqrt{\frac{MSE}{ar}}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq W_{ij}^A$      $\beta_i \neq \beta_j$  if  $|\bar{y}_{.i.} - \bar{y}_{.j.}| \geq W_{ij}^B$

Tukey's CI:  $(\alpha_i - \alpha_j): (\bar{y}_{i..} - \bar{y}_{j..}) \pm W_{ij}^A$      $(\beta_i - \beta_j): (\bar{y}_{.i.} - \bar{y}_{.j.}) \pm W_{ij}^B$

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = ab(r-1)$

$$B_{ij}^A = t_{\alpha/2, a(a-1)/2, \nu} \sqrt{\frac{2MSE}{br}} \quad B_{ij}^B = t_{\alpha/2, b(b-1)/2, \nu} \sqrt{\frac{2MSE}{ar}}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq B_{ij}^A$      $\beta_i \neq \beta_j$  if  $|\bar{y}_{.i.} - \bar{y}_{.j.}| \geq B_{ij}^B$

Bonferroni's CI:  $(\alpha_i - \alpha_j): (\bar{y}_{i..} - \bar{y}_{j..}) \pm B_{ij}^A$      $(\beta_i - \beta_j): (\bar{y}_{.i.} - \bar{y}_{.j.}) \pm B_{ij}^B$

# Comparing Main Effects (Interaction)

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = ab(r-1)$

$$W_{ij}^A = q_{\alpha}(a, \nu) \sqrt{\frac{MSE}{r}}$$

Within  $k^{\text{th}}$  level of Factor B, Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{ik.} - \bar{y}_{jk.}| \geq W_{ij}^A$

Tukey's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{ik.} - \bar{y}_{jk.}) \pm W_{ij}^A$  Similar for Factor B in A

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = ab(r-1)$

$$B_{ij}^A = t_{\alpha/2, a(a-1)/2, \nu} \sqrt{\frac{2MSE}{r}}$$

Within  $k^{\text{th}}$  level of B, Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{ik.} - \bar{y}_{jk.}| \geq B_{ij}^A$

Bonferroni's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{ik.} - \bar{y}_{jk.}) \pm B_{ij}^A$

# Miscellaneous Topics

- 2-Factor ANOVA can be conducted in a Randomized Block Design, where each block is made up of  $ab$  experimental units. Analysis is direct extension of RBD with 1-factor ANOVA
- Factorial Experiments can be conducted with any number of factors. Higher order interactions can be formed (for instance, the  $AB$  interaction effects may differ for various levels of factor  $C$ ).
- When experiments are not balanced, calculations are immensely messier and you must use statistical software packages for calculations

# Mixed Effects Models

- Assume:
  - Factor A Fixed (All levels of interest in study)  
 $\alpha_1 + \alpha_2 + \dots + \alpha_\alpha = 0$
  - Factor B Random (Sample of levels used in study)  
 $\beta_j \sim N(0, \sigma_b^2)$  (Independent)
  - AB Interaction terms Random  
 $(\alpha\beta)_{ij} \sim N(0, \sigma_{ab}^2)$  (Independent)
- Analysis of Variance is computed exactly as in Fixed Effects case (Sums of Squares, df's, MS's)
- Error terms for tests change (See next slide).

# ANOVA Approach – Mixed Effects

Source	df	SS	MS	F
Factor A	a-1	SSA	MSA=SSA/(a-1)	$F_A = MSA/MSAB$
Factor B	b-1	SSB	MSB=SSB/(b-1)	$F_B = MSB/MSAB$
Interaction	(a-1)(b-1)	SSAB	MSAB=SSAB/[(a-1)(b-1)]	$F_{AB} = MSAB/MSE$
Error	ab(r-1)	SSE	MSE=SSE/[ab(n-1)]	
Total	abr-1	TSS		

- Procedure:

- First test for interaction effects

- If interaction test not significant, test for Factor A and B effects

Test for Interaction :

$$H_0 : \sigma_{ab}^2 = 0$$

$$H_a : \sigma_{ab}^2 > 0$$

$$TS : F_{AB} = \frac{MSAB}{MSE}$$

$$RR : F_{AB} \geq F_{\alpha, (a-1)(b-1), ab(r-1)}$$

Test for Factor A

$$H_0 : \alpha_1 = \dots = \alpha_a = 0$$

$$H_a : \text{Not all } \alpha_i = 0$$

$$TS : F_A = \frac{MSA}{MSAB}$$

$$RR : F_A \geq F_{\alpha, (a-1), (a-1)(b-1)}$$

Test for Factor B

$$H_0 : \sigma_b^2 = 0$$

$$H_a : \sigma_b^2 > 0$$

$$TS : F_B = \frac{MSB}{MSAB}$$

$$RR : F_B \geq F_{\alpha, (b-1), (a-1)(b-1)}$$



# Comparing Main Effects for A (No Interaction)

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = (a-1)(b-1)$

$$W_{ij}^A = q_{\alpha}(a, \nu) \sqrt{\frac{MSAB}{br}}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq W_{ij}^A$

Tukey's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm W_{ij}^A$

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = (a-1)(b-1)$

$$B_{ij}^A = t_{\alpha/2, a(a-1)/2, \nu} \sqrt{\frac{2MSAB}{br}}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq B_{ij}^A$

Bonferroni's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm B_{ij}^A$

# Random Effects Models

- Assume:
  - Factor A Random (Sample of levels used in study)  
 $\alpha_i \sim N(0, \sigma_a^2)$  (Independent)
  - Factor B Random (Sample of levels used in study)  
 $\beta_j \sim N(0, \sigma_b^2)$  (Independent)
  - AB Interaction terms Random  
 $(\alpha\beta)_{ij} \sim N(0, \sigma_{ab}^2)$  (Independent)
- Analysis of Variance is computed exactly as in Fixed Effects case (Sums of Squares, df's, MS's)
- Error terms for tests change (See next slide).

# ANOVA Approach – Mixed Effects

Source	df	SS	MS	F
Factor A	a-1	SSA	$MSA=SSA/(a-1)$	$F_A=MSA/MSAB$
Factor B	b-1	SSB	$MSB=SSB/(b-1)$	$F_B=MSB/MSAB$
Interaction	$(a-1)(b-1)$	SSAB	$MSAB=SSAB/[(a-1)(b-1)]$	$F_{AB}=MSAB/MSE$
Error	$ab(n-1)$	SSE	$MSE=SSE/[ab(n-1)]$	
Total	$abn-1$	TSS		

- Procedure:

- First test for interaction effects

- If interaction test not significant, test for Factor A and B effects

Test for Interaction :

$$H_0 : \sigma_{ab}^2 = 0$$

$$H_a : \sigma_{ab}^2 > 0$$

$$TS : F_{AB} = \frac{MSAB}{MSE}$$

$$RR : F_{AB} \geq F_{\alpha, (a-1)(b-1), ab(n-1)}$$

Test for Factor A

$$H_0 : \sigma_a^2 = 0$$

$$H_a : \sigma_a^2 > 0$$

$$TS : F_A = \frac{MSA}{MSAB}$$

$$RR : F_A \geq F_{\alpha, (a-1), (a-1)(b-1)}$$

Test for Factor B

$$H_0 : \sigma_b^2 = 0$$

$$H_a : \sigma_b^2 > 0$$

$$TS : F_B = \frac{MSB}{MSAB}$$

$$RR : F_B \geq F_{\alpha, (b-1), (a-1)(b-1)}$$

# Nested Designs

- Designs where levels of one factor are nested (as opposed to crossed) wrt other factor
- Examples Include:
  - Classrooms nested within schools
  - Litters nested within Feed Varieties
  - Hair swatches nested within shampoo types
  - Swamps of varying sizes (e.g. large, medium, small)
  - Restaurants nested within national chains

# Nested Design - Model

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \varepsilon_{ijk} \quad i = 1, \dots, a \quad j = 1, \dots, b_i \quad k = 1, \dots, r$$

where :

$Y_{ijk}$   $\equiv$  Response for  $k^{\text{th}}$  rep of Factor A at  $i^{\text{th}}$  level, B at  $j^{\text{th}}$  level within A

$\mu$   $\equiv$  Overall Mean

$\alpha_i$   $\equiv$  Effect of  $i^{\text{th}}$  level of A (Fixed or Random)

$\beta_{j(i)}$   $\equiv$  Effect of  $j^{\text{th}}$  level of B within  $i^{\text{th}}$  level of A (Fixed or Random)

$\varepsilon_{ijk}$   $\equiv$  Random error term for  $k^{\text{th}}$  rep when A is at  $i$ , B is at  $j(i)$

# Nested Design - ANOVA

Total Variation :

$$TSS = \sum_{i=1}^a \sum_{j=1}^{b_i} \sum_{k=1}^r (Y_{ijk} - \bar{Y}_{...})^2 \quad df_{Total} = r \sum_{i=1}^a b_i - 1$$

Factor A :

$$SSA = r \sum_{i=1}^a b_i (\bar{Y}_{i..} - \bar{Y}_{...})^2 \quad df_A = a - 1$$

Factor B Nested Within A

$$SSB(A) = r \sum_{i=1}^a \sum_{j=1}^{b_i} (\bar{Y}_{ij.} - \bar{Y}_{i..})^2 \quad df_{B(A)} = \sum_{i=1}^a b_i - a$$

Error :

$$SSE = \sum_{i=1}^a \sum_{j=1}^{b_i} \sum_{k=1}^r (Y_{ijk} - \bar{Y}_{ij.})^2 \quad df_E = (r - 1) \sum_{i=1}^a b_i$$

# Factors A and B Fixed

$$\sum_{i=1}^a \alpha_i = 0 \quad \sum_{j=1}^{b_i} \beta_{j(i)} = 0 \quad i = 1, \dots, a \quad \varepsilon_{ijk} \sim N(0, \sigma^2)$$

Tests for Differences Among Factor A Effects

$$H_0 : \alpha_1 = \dots = \alpha_a = 0 \quad H_A : \text{Not all } \alpha_i = 0$$

$$\text{Test Statistic : } F_A = \frac{MSA}{MSE} \quad \text{P - value : } P(F \geq F_A)$$

$$\text{Rejection Region : } F_A \geq F_{\alpha, a-1, (r-1)\sum b_i}$$

Tests for Differences Among Factor B Effects

$$H_0 : \beta_{j(i)} = 0 \quad \forall i, j \quad H_A : \text{Not all } \beta_{j(i)} = 0$$

$$\text{Test Statistic : } F_{B(A)} = \frac{MSB(A)}{MSE} \quad \text{P - value : } P(F \geq F_{B(A)})$$

$$\text{Rejection Region : } F_{B(A)} \geq F_{\alpha, \sum b_i - a, (r-1)\sum b_i}$$

# Comparing Main Effects for A

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = (r-1)\Sigma b_i$

$$W_{ij}^A = q_{\alpha}(a, \nu) \sqrt{\frac{MSE}{2} \left( \frac{1}{rb_i} + \frac{1}{rb_j} \right)}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq W_{ij}^A$

Tukey's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm W_{ij}^A$

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = (r-1)\Sigma b_i$

$$B_{ij}^A = t_{\alpha/2, a(a-1)/2, \nu} \sqrt{MSE \left( \frac{1}{rb_i} + \frac{1}{rb_j} \right)}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq B_{ij}^A$

Bonferroni's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm B_{ij}^A$



# Comparing Effects for Factor B Within A

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = (r-1)\Sigma b_i$

$$W_{ij(k)}^B = q_{\alpha}(b_k, \nu) \sqrt{\frac{MSE}{r}}$$

Conclude:  $\beta_{i(k)} \neq \beta_{j(k)}$  if  $|\bar{y}_{ki.} - \bar{y}_{kj.}| \geq W_{ij(k)}^B$

Tukey's CI:  $(\beta_{i(k)} - \beta_{j(k)}) : (\bar{y}_{ki.} - \bar{y}_{kj.}) \pm W_{ij(k)}^B$

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = (r-1)\Sigma b_i$

$$B_{ij(k)}^B = t_{\alpha/2, b_k(b_k-1)/2, \nu} \sqrt{MSE \left( \frac{2}{r} \right)}$$

Conclude:  $\beta_{i(k)} \neq \beta_{j(k)}$  if  $|\bar{y}_{ki.} - \bar{y}_{kj.}| \geq B_{ij(k)}^B$

Bonferroni's CI:  $(\beta_{i(k)} - \beta_{j(k)}) : (\bar{y}_{ki.} - \bar{y}_{kj.}) \pm B_{ij(k)}^B$

# Factor A Fixed and B Random

$$\sum_{i=1}^a \alpha_i = 0 \quad \beta_{j(i)} \sim N(0, \sigma_b^2) \quad \varepsilon_{ijk} \sim N(0, \sigma^2)$$

Tests for Differences Among Factor A Effects

$$H_0 : \alpha_1 = \dots = \alpha_a = 0 \quad H_A : \text{Not all } \alpha_i = 0$$

$$\text{Test Statistic : } F_A = \frac{MSA}{MSB(A)} \quad \text{P - value : } P(F \geq F_A)$$

$$\text{Rejection Region : } F_A \geq F_{\alpha, a-1, \sum b_i - a}$$

Tests for Differences Among Factor B Effects

$$H_0 : \sigma_b^2 = 0 \quad \forall i, j \quad H_A : \sigma_b^2 > 0$$

$$\text{Test Statistic : } F_{B(A)} = \frac{MSB(A)}{MSE} \quad \text{P - value : } P(F \geq F_{B(A)})$$

$$\text{Rejection Region : } F_{B(A)} \geq F_{\alpha, \sum b_i - a, (r-1) \sum b_i}$$

# Comparing Main Effects for A (B Random)

- Tukey's Method-  $q$  in Studentized Range Table with  $\nu = \Sigma b_i - a$

$$W_{ij}^A = q_{\alpha}(a, \nu) \sqrt{\frac{MSAB}{2} \left( \frac{1}{rb_i} + \frac{1}{rb_j} \right)}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq W_{ij}^A$

Tukey's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm W_{ij}^A$

- Bonferroni's Method -  $t$ -values in Bonferroni table with  $\nu = \Sigma b_i - a$

$$B_{ij}^A = t_{\alpha/2, a(a-1)/2, \nu} \sqrt{MSAB \left( \frac{1}{rb_i} + \frac{1}{rb_j} \right)}$$

Conclude:  $\alpha_i \neq \alpha_j$  if  $|\bar{y}_{i..} - \bar{y}_{j..}| \geq B_{ij}^A$

Bonferroni's CI:  $(\alpha_i - \alpha_j) : (\bar{y}_{i..} - \bar{y}_{j..}) \pm B_{ij}^A$

# Factors A and B Random

$$\alpha_i \sim N(0, \sigma_a^2) \quad \beta_{j(i)} \sim N(0, \sigma_b^2) \quad \varepsilon_{ijk} \sim N(0, \sigma^2)$$

Tests for Differences Among Factor A Effects

$$H_0 : \sigma_a^2 = 0 \quad H_A : \sigma_a^2 > 0$$

$$\text{Test Statistic : } F_A = \frac{MSA}{MSB(A)} \quad \text{P - value : } P(F \geq F_A)$$

$$\text{Rejection Region : } F_A \geq F_{\alpha, a-1, \sum b_i - a}$$

Tests for Differences Among Factor B Effects

$$H_0 : \sigma_b^2 = 0 \quad \forall i, j \quad H_A : \sigma_b^2 > 0$$

$$\text{Test Statistic : } F_{B(A)} = \frac{MSB(A)}{MSE} \quad \text{P - value : } P(F \geq F_{B(A)})$$

$$\text{Rejection Region : } F_{B(A)} \geq F_{\alpha, \sum b_i - a, (r-1) \sum b_i}$$

# Elements of Split-Plot Designs

- Split-Plot Experiment: Factorial design with at least 2 factors, where experimental units wrt factors differ in “size” or “observational points”.
- Whole plot: Largest experimental unit
- Whole Plot Factor: Factor that has levels assigned to whole plots. Can be extended to 2 or more factors
- Subplot: Experimental units that the whole plot is split into (where observations are made)
- Subplot Factor: Factor that has levels assigned to subplots
- Blocks: Aggregates of whole plots that receive all levels of whole plot factor

# Split Plot Design

Block 1	Block 2	Block 3	Block 4
A=1, B=1	A=1, B=1	A=1, B=1	A=1, B=1
A=1, B=2	A=1, B=2	A=1, B=2	A=1, B=2
A=1, B=3	A=1, B=3	A=1, B=3	A=1, B=3
A=1, B=4	A=1, B=4	A=1, B=4	A=1, B=4
A=2, B=1	A=2, B=1	A=2, B=1	A=2, B=1
A=2, B=2	A=2, B=2	A=2, B=2	A=2, B=2
A=2, B=3	A=2, B=3	A=2, B=3	A=2, B=3
A=2, B=4	A=2, B=4	A=2, B=4	A=2, B=4
A=3, B=1	A=3, B=1	A=3, B=1	A=3, B=1
A=3, B=2	A=3, B=2	A=3, B=2	A=3, B=2
A=3, B=3	A=3, B=3	A=3, B=3	A=3, B=3
A=3, B=4	A=3, B=4	A=3, B=4	A=3, B=4

**Note:** Within each block we would assign at random the 3 levels of A to the whole plots and the 4 levels of B to the subplots within whole plots

# Examples

- **Agriculture:** Varieties of a crop or gas may need to be grown in large areas, while varieties of fertilizer or varying growth periods may be observed in subsets of the area.
- **Engineering:** May need long heating periods for a process and may be able to compare several formulations of a by-product within each level of the heating factor.
- **Behavioral Sciences:** Many studies involve repeated measurements on the same subjects and are analyzed as a split-plot (See Repeated Measures lecture)

# Design Structure

- Blocks:  $b$  groups of experimental units to be exposed to all combinations of whole plot and subplot factors
- Whole plots:  $a$  experimental units to which the whole plot factor levels will be assigned to at random within blocks
- Subplots:  $c$  subunits within whole plots to which the subplot factor levels will be assigned to at random.
- Fully balanced experiment will have  $n=abc$  observations



# Data Elements (Fixed Factors, Random Blocks)

- $Y_{ijk}$ : Observation from wpt  $i$ , block  $j$ , and spt  $k$
- $\mu$ : Overall mean level
- $\alpha_i$ : Effect of  $i^{\text{th}}$  level of whole plot factor (Fixed)
- $b_j$ : Effect of  $j^{\text{th}}$  block (Random)
- $(ab)_{ij}$ : Random error corresponding to whole plot elements in block  $j$  where wpt  $i$  is applied
- $\gamma_k$ : Effect of  $k^{\text{th}}$  level of subplot factor (Fixed)
- $(\alpha\gamma)_{ik}$ : Interaction btwn wpt  $i$  and spt  $k$
- $(bc)_{jk}$ : Interaction btwn block  $j$  and spt  $k$  (often set to 0)
- $\varepsilon_{ijk}$ : Random Error =  $(bc)_{jk} + (abc)_{ijk}$
- Note that if block/spt interaction is assumed to be 0,  $\varepsilon$  represents the block/spt within wpt interaction

# Model and Common Assumptions

- $Y_{ijk} = \mu + \alpha_i + b_j + (ab)_{ij} + \gamma_k + (\alpha\gamma)_{ik} + \varepsilon_{ijk}$

$$\sum_{i=1}^a \alpha_i = 0$$

$$b_j \sim NID(0, \sigma_b^2)$$

$$(ab)_{ij} \sim NID(0, \sigma_{ab}^2)$$

$$\sum_{k=1}^c \gamma_k = 0$$

$$\sum_{i=1}^a (\alpha\gamma)_{ik} = \sum_{k=1}^c (\alpha\gamma)_{ik} = 0$$

$$\varepsilon_{ijk} \sim NID(0, \sigma_\varepsilon^2)$$

$$COV(b_j, (ab)_{ij}) = COV(b_j, \varepsilon_{ijk}) = COV((ab)_{ij}, \varepsilon_{ijk}) = 0$$

# Tests for Fixed Effects

Whole Plot Trt Effects :  $H_0 : \alpha_1 = \dots = \alpha_a = 0$

$$\text{Test Statistic} : F_{WP} = \frac{MS_{WP}}{MS_{BLOCK * WP}}$$

$$P_{WP} = P(F \geq F_{WP} \mid F \sim F_{a-1, (a-1)(b-1)})$$

Subplot Trt Effects :  $H_0 : \gamma_1 = \dots = \gamma_c = 0$

$$\text{Test Statistic} : F_{SP} = \frac{MS_{SP}}{MS_{ERROR}}$$

$$P_{SP} = P(F \geq F_{SP} \mid F \sim F_{c-1, a(b-1)(c-1)})$$

WP  $\times$  SP Interactio n :  $H_0 : (\alpha\gamma)_{ik} = 0 \forall i, k$

$$\text{Test Statistic} : F_{WP \times SP} = \frac{MS_{WP \times SP}}{MS_{ERROR}}$$

$$P_{WP \times SP} = P(F \geq F_{WP \times SP} \mid F \sim F_{(a-1)(c-1), a(b-1)(c-1)})$$

# Comparing Factor Levels

Whole Plot Factor Levels:

$$95\% \text{ CI for } (\alpha_i - \alpha_{i'}) : (\bar{Y}_{i..} - \bar{Y}_{i'..}) \pm t \sqrt{\frac{2MS_{BLOCK \times WP}}{bc}}$$

Sub Plot Factor Levels:

$$95\% \text{ CI for } (\gamma_k - \gamma_{k'}) : (\bar{Y}_{..k} - \bar{Y}_{..k'}) \pm t \sqrt{\frac{2MS_{ERROR}}{ab}}$$

Sub Plot Effects Within same whole plot (Interaction):

$$95\% \text{ CI for } (\gamma_k - \gamma_{k'}) + ((\alpha\gamma)_{ik} - (\alpha\gamma)_{ik'}) : (\bar{Y}_{i.k} - \bar{Y}_{i.k'}) \pm t \sqrt{\frac{2MS_{ERROR}}{b}}$$

Whole Plot Effects within same sub plot (Interaction):

$$(\bar{Y}_{i.k} - \bar{Y}_{i'.k}) \pm t \sqrt{\frac{2[MS_{BLOCK \times WP} + (c-1)MS_{ERROR}]}{bc}} \quad (\text{df given below})$$

$$\hat{v} = \frac{[(c-1)MS_{ERROR} + MS_{BLOCK \times WP}]^2}{\left[ \frac{[(c-1)MS_{ERROR}]^2}{a(b-1)(c-1)} + \frac{[MS_{BLOCK \times WP}]^2}{(a-1)(b-1)} \right]}$$

# Repeated Measures Designs

- $a$  Treatments/Conditions to compare
- $N$  subjects to be included in study (each subject will receive only one treatment)
  - $n$  subjects receive trt  $i$ :  $an = N$
- $t$  time periods of data will be obtained
- Effects of trt, time and trtxtime interaction of primary interest.
  - Between Subject Factor: Treatment
  - Within Subject Factors: Time, TrtxTime

# Model

$$Y_{ijk} = \mu + \alpha_i + b_{j(i)} + \tau_k + (\alpha\tau)_{ik} + \varepsilon_{ijk}$$

$\mu \equiv$  overall mean

$\alpha_i \equiv$  effect of trt  $i$   $\sum_{i=1}^a \alpha_i = 0$

$b_{j(i)} \equiv$  effect of  $j^{\text{th}}$  subject in trt  $i$   $b_{j(i)} \sim NID(0, \sigma_b^2)$

$\tau_k \equiv$  effect of  $k^{\text{th}}$  time period  $\sum_{k=1}^t \tau_k = 0$

$(\alpha\tau)_{ik} \equiv$  interaction between trt  $i$  and time  $k$   $\sum_{i=1}^a (\alpha\tau)_{ik} = \sum_{k=1}^t (\alpha\tau)_{ik} = 0$

$\varepsilon_{ijk} \equiv$  random error term  $\varepsilon_{ijk} \sim NID(0, \sigma_\varepsilon^2)$

Note the random error term is actually the interaction between subjects (within treatments) and time

# Tests for Fixed Effects

Treatment Effects :  $H_0 : \alpha_1 = \dots = \alpha_a = 0$

$$\text{Test Statistic} : F_{TRTS} = \frac{MS_{TRTS}}{MS_{SUBJECTS (TRTS)}}$$

$$P_{TRTS} = P(F \geq F_{TRTS} \mid F \sim F_{a-1, a(n-1)})$$

Time Effects :  $H_0 : \tau_1 = \dots = \tau_t = 0$

$$\text{Test Statistic} : F_{TIME} = \frac{MS_{TIME}}{MS_{ERROR}}$$

$$P_{TIME} = P(F \geq F_{TIME} \mid F \sim F_{t-1, a(n-1)(t-1)})$$

Treatment/ Time Interaction :  $H_0 : (\alpha\tau)_{ik} = 0 \forall i, k$

$$\text{Test Statistic} : F_{TRT \times TIME} = \frac{MS_{TRT \times TIME}}{MS_{ERROR}}$$

$$P_{TRT \times TIME} = P(F \geq F_{TRT \times TIME} \mid F \sim F_{(a-1)(t-1), a(n-1)(t-1)})$$

# Comparing Factor Levels

Comparing Treatment Levels:

$$95\% \text{ CI for } \alpha_i - \alpha_{i'} : \left( \bar{Y}_{i..} - \bar{Y}_{i'..} \right) \pm t \sqrt{\frac{2MS_{SUBJECTS(TRTS)}}{nt}}$$

Comparing Time Levels:

$$95\% \text{ CI for } \tau_k - \tau_{k'} : \left( \bar{Y}_{..k} - \bar{Y}_{..k'} \right) \pm t \sqrt{\frac{2MS_{ERROR}}{an}}$$

Comparing Treatment Levels Within Time Levels:

$$\left( \bar{Y}_{i.k} - \bar{Y}_{i'.k} \right) \pm t \sqrt{\frac{2 \left( MS_{SUBJECTS(TRTS)} + (t-1)MS_{ERROR} \right)}{nt}}$$

with approximate df :

$$\hat{\nu} = \frac{\left[ (t-1)MS_{ERROR} + MS_{SUBJECT(TRT)} \right]^2}{\left[ \frac{\left[ (t-1)MS_{ERROR} \right]^2}{a(n-1)(t-1)} + \frac{\left[ MS_{SUBJECT(TRT)} \right]^2}{a(n-1)} \right]}$$