Topic 5: Means Separation (Multiple Comparisons)

Basic concepts

In ANOVA, the null hypothesis that is tested is always that all means are equal. If the F statistic is not significant, we fail to reject H_0 and there is nothing more to do, except possibly to redo the experiment, taking measures to make it more sensitive. If H_0 *is* rejected, then we conclude that at least one mean is significantly different from at least one other mean. The ANOVA itself gives no indication as to which means are significantly different. If there are only two treatments, there is no problem, of course; but if there are more than two treatments, the problem emerges of needing to determine which means are significantly different. This is the process of mean separation.

Mean separation takes two general forms:

1. Planned, single degree of freedom F tests (orthogonal contrasts, last topic)
2. Multiple comparison tests that are suggested by the data itself (this topic)

Of these two methods, orthogonal F tests are preferred because they are more powerful than multiple comparison tests (i.e. they are more sensitive to differences than are multiple comparison tests). As you saw in the last topic, however, contrasts are not always appropriate because they must satisfy a number of strict constraints:

1. Contrasts are planned comparisons, so the researcher must have *a priori* knowledge about which comparisons are most interesting. This prior knowledge, in fact, determines the treatment structure of the experiment.
2. The set of contrasts must be orthogonal.
3. The researcher is limited to making, at most, $(t - 1)$ comparisons.

Very often, however, there is no such prior knowledge. The treatment levels do not fall into meaningful groups, and the researcher is left with no choice but to carry out a sequence of multiple, unconstrained comparisons for the purpose of ranking and discriminating means. The different methods of multiple comparisons allow the researcher to do just that. There are many such methods, the details of which form the bulk of this topic, but generally speaking each involves more than one comparison among three or more means and is particularly useful in those experiments where there are no particular relationships among the treatment means.

Error rates

Selection of the most appropriate multiple comparison test is heavily influenced by the ***error rate***. Recall that a Type I error occurs when one incorrectly rejects a true null hypothesis H_0. The **Type I error rate** is the fraction of times a Type I error is made. In a single comparison (imagine a simple t test), this is the value α. When comparing three or more treatment means, however, there are at least two different rates of Type I error:

1. **Comparison-wise Type I error rate (CER)**
   This is the number of Type I errors, divided by the total number of comparisons. For a single comparison, CER = α / 1 = α.

2. **Experiment-wise Type I error rate (EER)**
   This is the number of experiments in which **at least** one Type I error occurs, divided by the total number of experiments.

Suppose an experimenter conducts an experiment with 5 treatment levels. In such an experiment, there is a total of 10 possible pairwise comparisons that can be made:

$$\text{Total possible pairwise comparisons (p)} = \frac{t(t-1)}{2}$$

For t = 5, p = (1/2)*(5*4) = 10
i.e. T_1 vs. T_2,T_3,T_4,T_5; T_2 vs. T_3,T_4,T_5; T_3 vs. T_4,T_5; T_4 vs. T_5

Suppose there are no true differences among the treatments (i.e. H_0 is true) and that in the experiments, one Type I error is made. Then the CER for the experiment is:

CER = (1 Type I error) / (10 comparisons) = 0.1 or 10%

And the EER is:

EER = (1 experiment with a Type I error) / (1 experiment) = 1 or 100%

The EER is the probability of making at least one Type I error in the experiment. As the number of means (and therefore the number of possible comparisons) increases, the chance of making at least one Type I error approaches 1. To preserve a low experiment-wise error rate, then, the comparison-wise error rate must be held extremely low. Conversely, to maintain a reasonable comparison-wise error rate, the experiment-wise error rate will inflate.

The relative importance of controlling these two Type I error rates depends on the objectives of the study, and different multiple comparison procedures have been developed based on different philosophies of controlling these two kinds of error. In situations where incorrectly rejecting one comparison may jeopardize the entire experiment or where the consequence of incorrectly rejecting one comparison is as serious as incorrectly rejecting a number of comparisons, the control of experiment-wise error rate is more important. On the other hand, when one erroneous conclusion will not affect other inferences in an experiment, the comparison-wise error rate is more pertinent.

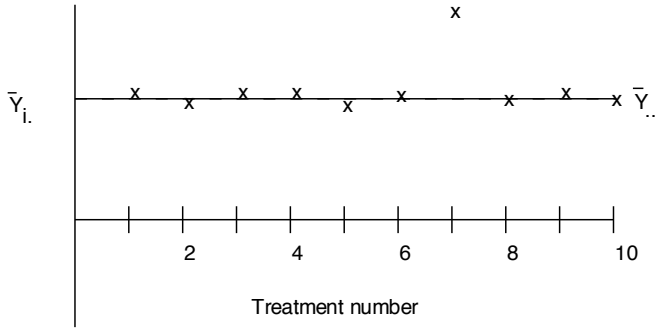
The experiment-wise error rate is always larger than the comparison-wise error rate. It is difficult to compute the exact experiment-wise error rate because, for a given set of data, Type I errors are not independent. But it *is* possible to compute an upper bound for the EER by assuming that the probability of a Type I error for any single comparison is α and is independent of all other comparisons. In that case:

Upper bound EER = $1 - (1 - \alpha)^p$ where $p = \frac{t(t-1)}{2}$, as before

So, for 10 treatments and $\alpha = 0.05$, the upper bound of the EER is 0.9 (90%):

$$EER = 1 - (1 - 0.05)^{45} = 0.90$$

The situation is more complicated than this, however. Suppose there are 10 treatments and one shows a significant effect while the other 9 are approximately equal. Such a situation is indicated graphically below:



A simple ANOVA will probably reject H_0, so the experimenter will want to determine which specific means are different. Even though one mean is truly different, there is still a chance of making a Type I error in each pairwise comparison among the 9 similar treatments. An upper bound on this probability is computed by setting t = 9 in the above formula, giving a result of 0.84. That is, the experimenter will incorrectly conclude that two truly similar effects are actually different **84% of the time**. This is called the experiment-wise error rate under a partial null hypothesis, the partial null hypothesis in this case being that the subset of nine treatment means are all equal to one another.

So we can distinguish between the EER under the complete null hypothesis, in which all treatment means are equal, and the EER under a partial null hypothesis, in which some means are equal but some differ. Because of this fact, error rates can be divided into the following four categories:

CER = comparison-wise error rate
EERC = experiment-wise error rate under a complete null hypothesis (standard EER)
EERP = experiment-wise error rate under a partial null hypothesis
MEER = maximum experiment-wise error rate under any complete or partial null hypothesis.

Multiple comparisons tests

Statistical methods for making two or more inferences while controlling cumulative Type I error rates are called ***simultaneous inference methods***. The material in this section is based primarily on ST&D chapter 8. The basic techniques of multiple comparisons fall into two groups:

1. Fixed-range tests: Those which provide confidence intervals and tests of hypotheses
2. Multiple-range tests: Those which provide only tests of hypotheses

To illustrate the various procedures, we will use the data from two separate experiments, one with equal replications (Table 5.1) and one with unequal replications (Table 5.3). The ANOVAs for these experiments are given in Tables 5.2 and 5.4, respectively.

Table 5.1 *Equal replications*. Results (mg shoot dry weight) of an experiment (CRD) to determine the effect of seed treatment by different acids on the early growth of rice seedlings.

Treatment	Replications					Mean
Control	4.23	4.38	4.10	3.99	4.25	4.19
HCl	3.85	3.78	3.91	3.94	3.86	3.87
Propionic	3.75	3.65	3.82	3.69	3.73	3.73
Butyric	3.66	3.67	3.62	3.54	3.71	3.64

t = 4, r = 5, overall mean = 3.86

Table 5.2 ANOVA of data in Table 5.1

Source	df	SS	MS	F
Total	19	1.0113		
Treatment	3	0.8738	0.2912	33.87
Error	16	0.1376	0.0086	

Table 5.3 *Unequal replications*. Results (lbs/animal·day) of an experiment (CRD) to determine the effect of different forage genotypes on animal weight gain.

Treatment	Replications (Animals)								r	Mean
Control	1.21	1.19	1.17	1.23	1.29	1.14			6	1.205
Feed-A	1.34	1.41	1.38	1.29	1.36	1.42	1.37	1.32	8	1.361
Feed-B	1.45	1.45	1.51	1.39	1.44				5	1.448
Feed-C	1.31	1.32	1.28	1.35	1.41	1.27	1.37		7	1.330
							Overall		26	1.336

Table 5.4 ANOVA of data in Table 5.3

Source	df	SS	MS	F
Total	25	0.2202		
Treatment	3	0.1709	0.05696	25.41
Error	22	0.0493	0.00224	

Fixed-range tests

These tests provide a single range for making all possible pairwise comparisons in experiments with equal replications across treatment groups (i.e. in balanced designs). Many fixed-range procedures are available, and considerable controversy exists as to which procedure is most appropriate. We will present four commonly used procedures, moving from the less conservative to the more conservative: LSD, Dunnett, Tukey, and Scheffe.

The repeated t-test (least significant difference: LSD)

One of the oldest, simplest, and most widely misused multiple pairwise comparison tests is the least significant difference (LSD) test. The LSD is based on the t-test (ST&D 101); in fact, it is simply a sequence of many t-tests. Recall the formula for the t statistic:

$$t = \frac{\overline{Y}_{(r)} - \mu}{s_{\overline{Y}(r)}} \quad \text{where} \quad s_{\overline{Y}(r)} = \frac{s}{\sqrt{r}}$$

This t statistic is distributed according to a t distribution with (r – 1) degrees of freedom. The LSD test declares the difference between means $\overline{Y}_i$ and $\overline{Y}_j$ of treatments T_i and T_j to be significant when:

$$|\overline{Y}_i - \overline{Y}_j| > \text{LSD, where}$$

$$LSD = t_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\left(\frac{1}{r_1} + \frac{1}{r_2}\right)} \quad \text{for unequal r}$$

$$LSD = t_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\,\frac{2}{r}} \quad \text{for equal r}$$

The above LSD statistic is called the *studentized range statistic*. As usual, the mean square error (MSE) is the pooled error variance (i.e. weighted average of the within-treatment variances). The argument of the square root is called the standard error of the difference, or SED.

As an example, let's perform the calculations for Table 5.1. Note that the significance level selected for pairwise comparisons does not have to conform to the significance level of the overall F test. To compare procedures across the examples to come, we will use a standard $\alpha = 0.05$. From Table 5.2, MSE = 0.0086 with 16 df.

$$LSD = t_{\frac{\alpha}{2},df_{MSE}}\sqrt{MSE\frac{2}{r}} = 2.120\sqrt{0.0086\frac{2}{5}} = 0.1243$$

So, if the absolute difference between any two treatment means is more than 0.1243, the treatments are said to be significantly different at the 5% confidence level. As the number of treatments increases, it becomes more and more difficult, just from a logistical point of view, to identify those pairs of treatments that are significantly different. A systematic procedure for comparison and ranking begins by arranging the means in descending or ascending order as shown below:

Control	4.19
HCl	3.87
Propionic	3.73
Butyric	3.64

Once the means are so arranged, compare the largest with the smallest mean. If these two means are significantly different, compare the next largest mean with the smallest. Repeat this process until a non-significant difference is found. Label these two and any means in between with a common lower case letter by each mean. Repeat the process with the next smallest mean, etc. Ultimately, you will arrive at a mean separation table like the one shown below:

Treatment	Mean	LSD
Control	4.19	a
HCl	3.87	b
Propionic	3.73	c
Butyric	3.64	c

Pairs of treatments that are not significantly different from one another share the same letter. For the above example, we draw the following conclusions at the 5% confidence level:

All acids reduced shoot growth.
The reduction was more severe with butyric and propionic acid than with HC1.
We do not have evidence to conclude that propionic acid is different in its effect than butyric acid.

When all the treatments are equally replicated, note that only one LSD value is required to test all six possible pairwise comparisons between treatment means. This is not true in cases of unequal replication, where different LSD values must be calculated for each comparison involving different numbers of replications.

For the second data set (Table 5.3), we find the 5% LSD for comparing the control with Feed B to be:

$$LSD = t_{\frac{\alpha}{2},df_{MSE}}\sqrt{MSE\left(\frac{1}{r_1}+\frac{1}{r_2}\right)} = 2.074\sqrt{0.00224\left(\frac{1}{6}+\frac{1}{5}\right)} = 0.0594$$

The other required LSD's are:

A vs. Control = 0.0531
A vs. B = 0.0560
A vs. C = 0.0509
B vs. C = 0.0575
C vs. Control = 0.0546

Using these values, we can construct a mean separation table:

Treatment	Mean	LSD
Feed B	1.45	a
Feed A	1.36	b
Feed C	1.33	b
Control	1.20	c

Thus, at the 5% level, we conclude all feeds cause significantly greater weight gain than the control. Feed B causes the highest weight gain; Feeds A and C are equally effective.

One advantage of the LSD procedure is its ease of application. Additionally, it is easily used to construct confidence intervals for mean differences. The $(1 - \alpha)$ confidence limits of the quantity $(\mu_A - \mu_B)$ are given by:

$$(1 - \alpha) \text{ CI for } (\mu_A - \mu_B) = (\bar{Y}_A - \bar{Y}_B) \pm \text{LSD}$$

Because fewer comparisons would be involved, the LSD test would be much safer if the means to be compared were selected *in advance* of the experiment; although hardly anyone ever does this. The test is primarily intended for use when there is no predetermined structure to the treatments. If a large number of means are to be compared and the ones compared are selected *after* the ANOVA and the comparisons target those means with most different values, the actual error rate will be much higher than predicted.

The LSD test is the only test for which the comparison-wise error rate equals α. This is often regarded as too liberal (i.e. too ready to reject H_0). It has been suggested that the EEER can be maintained at α by performing the overall ANOVA test at the α level and making further comparisons *if and only if* the F test is significant (**Fisher's Protected LSD test**). However, it was then demonstrated that this assertion is false if there are more than three means. In those cases, a preliminary F test controls only the EERC, not the EERP.

Another way some people try to account for the error-rate issue inherent in multiple comparisons within a method like LSD, which does not control EER, is to simply adjust the CER. There are many such adjusting methods, but probably the most common one is the Bonferroni Correction. To implement the Bonferroni Correction, one simply reduces the comparison-wise Type I error rate by dividing it by the number of hypotheses to be tested. For example, an experiment with 5 treatment levels has a total of 5*4/2 = 10 possible pairwise comparisons to make (i.e. 10 hypotheses). In this case, the CER would be adjusted by dividing the chosen Type I error rate by 10 (e.g. 0.05/10 = 0.005); and it is this reduced Type I error rate ($\alpha_{Bon} = 0.005$) that would be used for all pairwise comparisons. By reducing the CER, one loses power for any given test but helps control the EER. In this example, the upper bound of the EER is found to be:

$$EER = 1 - (1 - 0.005)^{10} = 0.489$$

Not bad. Once criticism of Bonferroni is that it is a conservative correction, seen both in the theoretical upper bound of the EER above (< 0.05) and in the table below (this upper bound falls as more treatment levels are considered).

Treatment Levels (t)	Pairwise Comparisons	α_{Bon}	UpBound EER
5	10	0.00500	0.04889
6	15	0.00333	0.04885
7	21	0.00238	0.04883
8	28	0.00179	0.04881
9	36	0.00139	0.04880
10	45	0.00111	0.04880
11	55	0.00091	0.04879
12	66	0.00076	0.04879
13	78	0.00064	0.04879
14	91	0.00055	0.04878
15	105	0.00048	0.04878
16	120	0.00042	0.04878
17	136	0.00037	0.04878
18	153	0.00033	0.04878
19	171	0.00029	0.04878
20	190	0.00026	0.04878

Dunnett's Method

In certain experiments, one may desire only to compare a control with each of the other treatments, such as comparing a standard product with several new ones. Dunnett's method performs such an analysis while holding the maximum experimentwise error rate under any complete or partial null hypothesis (MEER) to a level not exceeding the stated α. In this method, a t* value is calculated for each comparison. This tabular t* value for determining statistical significance, however, is not the Student's t but a special t* given in Appendix Tables A-9a and A-9b (ST&D 624-625). Let $\overline{Y}_0$ represent the control mean with r_0 replications; then:

$$DLSD = t^*_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\left(\frac{1}{r_1} + \frac{1}{r_2}\right)} \quad \text{for unequal r } (r_0 \neq r_i)$$

$$DLSD = t^*_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\frac{2}{r}} \quad \text{for equal r } (r_0 = r_i)$$

For the seed treatment experiment, MSE = 0.0086 with 16 df and the number of comparisons (p) = 3. By Table A-19b, $t^*_{\frac{\alpha}{2},16} = 2.59$.

$$DLSD = t^*_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\frac{2}{r}} = 2.59\sqrt{0.0086\frac{2}{5}} = 0.1519$$

(**Note that DLSD = 0.1519 > LSD = 0.1243**)

This provides the least significant difference between a control and any other treatment. Note that the smallest difference between the control and any acid treatment is:

$$\text{Control - HC1} = 4.19 - 3.87 = 0.32$$

Since this difference is larger than DLSD, it is significant; and all other differences, being larger, are also significant. The 95% simultaneous confidence intervals for all three differences take the form:

$$(1 - \alpha)\ \text{CI for } (\mu_0 - \mu_i) = (\overline{Y}_0 - \overline{Y}_i) \pm \text{DLSD}$$

The limits of these differences are:

Control – Butyric = 0.32 ± 0.15
Control – HC1 = 0.46 ± 0.15
Control – Propionic = 0.55 ± 0.15

We have 95% confidence that the 3 true differences fall **simultaneously** within the above ranges.

When treatments are not equally replicated, as in the feed ration experiment, there are different DLSD values for each of the comparisons. To compare the control with Feed-C, first note that $t^*_{0.025,22} = 2.517$ (from SAS; by Table A-9b, t* is 2.54 or 2.51 for 20 and 24 df, respectively):

$$DLSD = t^*_{\frac{\alpha}{2},df_{MSE}} \sqrt{MSE\left(\frac{1}{r_0}+\frac{1}{r_1}\right)} = 2.517\sqrt{0.00224\left(\frac{1}{6}+\frac{1}{7}\right)} = 0.0663$$

Since $|\overline{Y}_0 - \overline{Y}_C| = 0.125$ is larger than 0.06627, the difference is significant. All other differences with the control, being larger than this, are also significant.

Tukey's w procedure

Tukey's test was designed specifically for **pairwise comparisons**. This test, sometimes called the "honestly significant difference" (HSD) test, controls the MEER *when the sample sizes are equal*. Instead of t or t*, it uses the statistic $q_{\alpha,p,df_{MSE}}$ that is obtained from Table A-8. The Tukey critical values are larger than those of Dunnett because the Tukey family of contrasts is larger (all possible pairs of means instead of just comparisons to a control). The critical difference in this method is labeled *w*:

$$w = q_{\alpha,p,df_{MSE}} \sqrt{\frac{MSE}{2}\left(\frac{1}{r_1}+\frac{1}{r_2}\right)} \quad \text{for unequal r}$$

$$w = q_{\alpha,p,df_{MSE}} \sqrt{\frac{MSE}{r}} \quad \text{for equal r}$$

Aside from the new critical value, things look basically the same as before, except notice that here we do not multiply MSE by a factor of 2 *because Table A-8 (ST&D) already includes the factor √2 in its values*. For example, for p = 2, df = ∞ (equivalent to the standard normal distribution Z), and α= 5%, the critical value is 2.77, which is equal to 1.96 * √2.

Considering the seed treatment data, $q_{0.05,4,16} = 4.05$; and:

$$w = q_{\alpha,p,df_{MSE}} \sqrt{\frac{MSE}{r}} = 4.05\sqrt{\frac{0.0086}{5}} = 0.1680$$

(**Note that w = 0.1680 > DLSD = 0.1519 > LSD = 0.1243**)

By this method, the means separation table looks like:

Treatment	Mean	*w*	
Control	4.19	a	
HCl	3.87	b	
Propionic	3.73	b	c
Butyric	3.64		c

Like the LSD and Dunnett's methods, this test detects significant differences between the control and all other treatments. But unlike with the LSD method, it detects no significant differences between the HCl and Propionic treatments.

For unequal r, as in the feeding experiment, the contrast between the Control and Feed-C would be tested using:

$$w = q_{\alpha,p,df_{MSE}}\sqrt{\frac{MSE}{2}\left(\frac{1}{r_{Cont}}+\frac{1}{r_C}\right)} = 3.93\sqrt{\frac{0.00224}{2}\left(\frac{1}{6}+\frac{1}{7}\right)} = 0.0732$$

Since $|\overline{Y}_{Cont} - \overline{Y}_C| = 0.125$ is larger than 0.0731, it is significant. As in the LSD, the only pairwise comparison that is not significant is that between Feed C ($Y_C = 1.330$) and Feed A ($Y_A = 1.361$).

Scheffe's F test for pairwise comparisons

Scheffe's test is compatible with the overall ANOVA F test in the sense that it never declares a contrast significant if the overall F test is nonsignificant. Scheffe's test controls the MEER for **ANY** set of contrasts. This includes *all possible pairwise and group comparisons*. Since this procedure controls MEER while allowing for a larger number of comparisons, it is less sensitive (i.e. more conservative) than other multiple comparison procedures.

The Scheffe critical difference (SCD) has a similar structure as that described for previous tests, scaling the critical F value for its statistic:

$$SCD = \sqrt{df_{Trt}F_{\alpha,df_{Trt},df_{MSE}}}\sqrt{MSE\left(\frac{1}{r_1}+\frac{1}{r_2}\right)} \quad \text{for unequal r}$$

$$SCD = \sqrt{df_{Trt}F_{\alpha,df_{Trt},df_{MSE}}}\sqrt{MSE\frac{2}{r}} \quad \text{for equal r}$$

For the seed treatment data, MSE = 0.0086, $df_{Trt} = 3$, $df_{MSE} = 16$, and r = 5:

$$SCD = \sqrt{df_{Trt} F_{\alpha, df_{Trt}, df_{MSE}}} \sqrt{MSE \frac{2}{r}} = \sqrt{3(3.24)0.0086 \frac{2}{5}} = 0.1829$$

(**Note that SCD = 0.1829 > w = 0.1680 > DLSD = 0.1519 > LSD = 0.1243**)

Again, if the difference between a pair of means is greater than SCD, that difference will be declared significant at the given α level, *while holding MEER below α.* The table of means separations:

Treatment	Mean	F_s	
Control	4.19	a	
HCl	3.87	b	
Propionic	3.73	b	c
Butyric	3.64		c

When the means to be compared are not based on equal replications, a different SCD is required for each comparison. For the animal feed experiment, critical difference for the contrast between the Control and Feed-C is:

$$SCD = \sqrt{df_{Trt} F_{\alpha, df_{Trt}, df_{MSE}}} \sqrt{MSE\left(\frac{1}{r_1} + \frac{1}{r_2}\right)} = \sqrt{3(3.05)0.00224\left(\frac{1}{6} + \frac{1}{7}\right)} = 0.0796$$

Since $|\bar{Y}_{Cont} - \bar{Y}_C| = 0.125$ is larger than 0.0796, it is significant. Scheffe's procedure is also readily used for interval estimation:

$$(1 - \alpha) \text{ CI for } (\mu_0 - \mu_i) = (\bar{Y}_0 - \bar{Y}_i) \pm \text{SCD}$$

The resulting intervals are **simultaneous** in that the probability is at least (1 – α) that all of them are true simultaneously.

Scheffe's F test for group comparisons

The most important use of Scheffe's test is for arbitrary **comparisons among groups of means**. We use the word "arbitrary" here because, unlike the group comparisons using contrasts, group comparisons using Scheffe's test do not have to be orthogonal, nor are they limited to (t – 1) questions. If you are interested only in testing the differences between all pairs of means, the Scheffe method is not the best choice; Tukey's is better because it is more sensitive while controlling MEER. But if you want to "mine" your data by making all possible comparisons (pairwise and group comparisons) while still controlling MEER, Scheffe's is the way to go.

To make comparisons among groups of means, you first define a contrast, as in Topic 4:

$$Q = \sum_{i=1}^{t} c_i \bar{Y}_i \text{, with the constraint that } \sum_{i=1}^{t} c_i = 0 \text{ (or } \sum_{i=1}^{t} r_i c_i = 0 \text{ for unequal r)}$$

We will reject the null hypothesis (H_0) that the contrast Q = 0 if the absolute value of Q is larger than some critical value F_S. This is the general form for Scheffe's test:

$$\text{Critical value } F_S = \sqrt{df_{Trt} F_{\alpha, df_{Trt}, df_{MSE}}} \sqrt{MSE \sum_{i=1}^{t} \frac{c_i^2}{r_i}}$$

Note that the previous expressions for Scheffe pairwise comparisons are for the particular contrast 1 vs. -1. If we wish to compare the control to the average of the three acid treatments, the contrast coefficients are (+3, –1, –1, –1). In this case, Q is:

$$Q = \sum_{i=1}^{t} c_i \bar{Y}_i = 4.190(3) + 3.868(-1) + 3.728(-1) + 3.640(-1) = 1.334$$

The critical Fs value for this contrast is:

$$F_S = \sqrt{df_{Trt} F_{\alpha, df_{Trt}, df_{MSE}}} \sqrt{MSE \sum_{i=1}^{t} \frac{c_i^2}{r_i}} = \sqrt{3(3.24)0.0086 \frac{3^2 + (-1)^2 + (-1)^2 + (-1)^2}{5}} = 0.4479$$

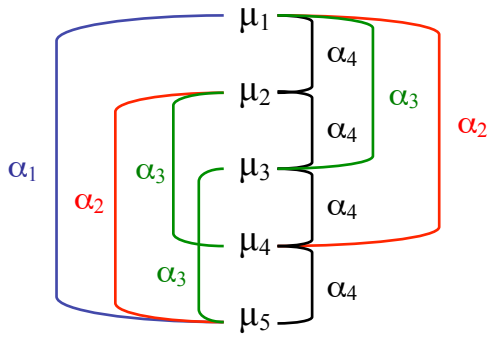
Since $|Q| = 1.334 > 0.4479 = F_S$, we reject H_0. The average of the control (4.190 mg) is significantly different from the average of the three acid treatments (3.745 mg).

Again, with Scheffe's method, you can test any conceivable set of contrasts, even if they number more than (t – 1) questions and are not orthogonal. The price you pay for this freedom, however, is very low sensitivity. Scheffe's is the most conservative method of comparing means; so if Scheffe's declares a difference to be real, you can believe it.

Multiple-stage tests

The methods discussed so far are all "fixed-range" tests, so called because they use a single, fixed value to test hypotheses and build simultaneous confidence intervals. If one forfeits the ability to build simultaneous confidence intervals with a single value, it is possible to obtain simultaneous hypothesis tests of greater power using multiple-stage tests (MSTs). MSTs come in both step-up (first comparing closest means, then more distant means) and step-down varieties (the reverse); but it is the step-down methods which are more widely used. The best known MSTs are the Duncan and the Student-Newman-Keuls (SNK) methods. Both use the studentized range statistic (q) and also go by the name **multiple range** tests. With means arranged from the lowest to the highest, a multiple-range test provides critical distances or ranges that become smaller as the pairwise means to be compared become closer together in the array. Such a strategy allows the researcher to allocate test sensitivity where it is most needed, in discriminating neighboring means.

The general strategy behind step-down MSTs is this: First, the maximum and minimum means are compared pairwise using some significance level α_1. If this H_0 is accepted, the procedure stops. Otherwise, the analysis continues by comparing pairwise the two sets of next-most-extreme means (i.e. μ_1 vs. μ_{t-1}, and μ_2 vs. μ_t) using some significance level $\alpha_2 > \alpha_1$. This process is repeated with closer and closer pairs of means until one reaches the set of (t – 1) pairs of adjacent means, compared pairwise using some significance level α_{t-1}.



Graphical depiction of the general strategy of step-down MSTs. In this figure, the means are arranged highest (μ_1) to lowest (μ_5). The significance levels of each of the 10 possible pairwise comparisions are indicated by the alphas, where $\alpha_1 < \alpha_2 < \alpha_3 < \ldots < \alpha_{t-1}$.

Two other general comments: Because MSTs are result-guided (i.e. they require the researcher to make decisions along the way, such as to continue analysis or to stop analysis), the true error rates are difficult to pin down. Also, multiple range tests should *only* be used with balanced designs since they are inefficient (and can give very strange results) with unbalanced ones.

Duncan's multiple range test

The idea behind Duncan's test is that as the number of means under test increases, the smaller is the probability that they will all be alike. In this test, "confidence" is replaced by the concept of "protection levels" against committing Type I errors at the various stages of testing. So if a difference is detected at one level of the test, the researcher is justified in separating means at a finer resolution with less protection (i.e. with a higher α).

Imagine the treatment means are ranked in order from highest to lowest. As the test progresses, Duncan's method uses a variable significance level (α_{p-1}) depending on the number of means involved:

$$\alpha_{p-1} = 1 - (1 - \alpha)^{p-1}$$

where p = 2,...,t is the number of means to be compared at that significance level (refer to diagram above). For example, consider a set of five treatment means.

For the extreme means, p = 2 and $\alpha_1 = 0.05$;
For the next group, p = 3 and $\alpha_2 = 0.098$;
For the next group, p = 4 and $\alpha_3 = 0.143$; and finally,
For the set of adjacent means, p = 5 and $\alpha_4 = 0.185$

Despite the level of protection offered at each stage, the experiment-wise Type I error rate (MEER) of this test is uncontrolled. The higher power of Duncan's method compared to Tukey's is, in fact, due to its higher Type I error rate (Einot and Gabriel 1975).

Duncan's operating characteristics are actually quite similar to those of Fisher's unprotected LSD at level α (for p = 2, the Duncan critical value is the same as that for LSD). Since the LSD is easier to compute, easier to explain, and applicable to unequal sample sizes, Duncan's method is not recommended by SAS. Duncan's test used to be the most popular method of means separations, but today many journals no longer accept it.

To compute Duncan critical ranges (R_p), use the following expression, plugging in the appropriate values of the Studentized range statistic (q_α):

$$R_p = q_{\alpha_{p-1},p,df_{MSE}} \sqrt{\frac{MSE}{r}}$$

[Note: Due to the non-standard significance levels involved, Table A-7 (ST&D) will not suffice for this exercise. You will need to consult an online calculator in this case; for example, see: http://onlinestatbook.com/calculators/tukeycdf.html]

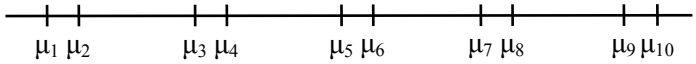
For the seed treatment data:

p	2	3	4
$q_{\alpha_{p-1},p,16}$	3.00	3.15	3.23
$\mathbf{R_p}$	0.124	0.131	0.134

The Student-Newman-Keuls (SNK) test

The Student-Newman-Keuls (SNK) is more conservative than Duncan's in that the Type I error rate is smaller. This is because SNK simply uses α as the significance level at all stages of testing, again stopping the analysis at the highest level of non-significance. Because α is lower than Duncan's variable significance values, the power of SNK is generally lower than that of Duncan's test. It is often accepted by journals that do not accept Duncan's test.
So, if the distance between the maximum and the minimum means is significant, SNK continues to the next finer comparison (e.g. between the minimum and the one before the maximum). If no difference is detected, the test stops.

While the SNK test controls EERC at the α level, it behaves poorly in terms of the EERP and MEER (Einot and Gabriel 1975). To see this, consider ten population means that cluster in five pairs such that means within pairs are equal but there are large differences among pairs:



In such a case, all subset homogeneity hypotheses for three or more means are rejected. The SNK method then comes down to five independent tests, one for each pair, each conducted at the α level. The probability of at least one false rejection is:

$$1 - (1 - \alpha)5 = 0.23$$

As the number of means increases, the MEER approaches 1. For this reason, the SNK method is not recommended by SAS. To find the critical range (W_p) at each level of the analysis, the procedure is similar to Duncan's, except that the significance level is α throughout:

$$W_p = q_{\alpha,p,df_{MSE}} \sqrt{\frac{MSE}{r}}$$

For unequal r, use the same correction as in Tukey (5.3.1.3). For the seed treatment data:

p	2	3	4
$q_{0.05,p,16}$	3.00	3.65	4.05
$\mathbf{R_p}$	0.124	0.151	0.168

Because α is a standard value, Table A-8 (ST&D) provides all the necessary critical values. Note in this case that for p = t (i.e. when comparing adjacent means) W_p = Tukey's *w*. For p = 2, W_p = LSD.

Treatment	Mean	W_p
Control	4.19	a
HCl	3.87	b
Propionic	3.73	c
Butyric	3.64	c

The REGWQ method

A variety of MSTs that control MEER have been proposed, but these methods are not as well known as those of Duncan and SNK. An approach developed by Ryan, Einot, Gabriel, and Welsh (REGW) sets:

$$\alpha_{p-1} = 1 - (1 - \alpha)^{p/t} \text{ for } p < (t - 1) \quad \text{and} \quad \alpha_{p-1} = \alpha \text{ for } p \geq (t - 1)$$

The REGWQ method performs the comparisons using a range test. This method appears to be among the most powerful step-down multiple range tests and is recommended by the developers of the statistical software package SAS for *equal replication* (i.e. balanced designs).

Assuming the sample means have been arranged in descending order from $\bar{Y}_1$ to $\bar{Y}_t$, the homogeneity of means $\bar{Y}_i,\ldots,\bar{Y}_j$, with i < j, is rejected by REGWQ if:

$$| \bar{Y}_i - \bar{Y}_j | > q_{\alpha_{p-1},p,df_{MSE}} \sqrt{\frac{MSE}{r}}$$

For the seed treatment data:

p	2	3	4
α_{p-1}	0.025	0.05	0.05
$q_{0.05,p,16}$	3.49	3.65	4.05
$\mathbf{R_p}$	0.145	0.151	0.168

For p = t and p = t – 1, the critical value is as in SNK, but it is larger for p < t – 1. Note that the difference between HCl and propionic is declared significant with SNK but not significant with REGWQ (3.87 - 3.73 < 0.145).

Treatment	Mean	*REGWQ*	
Control	4.19	a	
HCl	3.87	b	
Propionic	3.73	b	c
Butyric	3.64		c

Conclusions and recommendations

There are at least twenty other parametric procedures available for multiple comparisons, not to mention the many non-parametric and multivariate methods. There is no consensus as to which is the most appropriate procedure to recommend to all users, and comparisons upon them generally reduce to a consideration of how individual methods control the two kinds of Type I error, CER and MEER. All this is to say that the difference in performance between any two procedures is likely due to the different underlying philosophies of Type I error control rather than to specific techniques. To a large extent, the choice of a procedure is subjective and will hinge on a choice between a comparison-wise error rate (such as LSD) and an experiment-wise error rate (such as Tukey and Scheffe's test).

Some suggested rules of thumb:

1. **When in doubt, use Tukey.** Tukey's method is a good general technique for carrying out all pairwise comparisons, enabling you to rank means and put them into significance groups, while controlling MEER.

2. Use Dunnett's (more powerful than Tukey's) if you only wish to compare each treatment level to a control.

3. Use Scheffe's if you wish to test a set of non-orthogonal group comparisons *OR* if you wish to carry out group comparisons *in addition to* all possible pairwise comparisons. MEER will be controlled in both cases.

One final point to note is that severely unbalanced designs can yield very strange results, regardless of means separation method. To illustrate this, consider the example in your lecture notes. In this example, an experiment with four treatments (A, B, C, and D) has responses in the order A > B > C > D. A and D each have 2 replications, while B and C each have 11. The strange result: The extreme means (A and D) are found to be not significantly different, but the intermediate means (B and C) are!