Statistics

Analysis of Variance (ANOVA)

February 24, 2010

Jobayer Hossain, Ph.D. & Tim Bunnell, Ph.D.

Nemours Bioinformatics Core Facility



Class Objectives -- You will Learn:

- Experimental design terminologies
- Analysis of variance (ANOVA) and the situation it is applicable
- One-way ANOVA
- Multiple comparisons
- How to do ANOVA with SPSS



- An Experimental Unit is the entity on which measurement or an observation is made. For example, subjects are experimental units in most clinical studies.
- Homogeneous Experimental Units: Units that are as uniform as possible on all characteristics that could affect the response.
- Randomization is the process of assigning experimental units randomly to different experimental groups. It is the most reliable method of creating homogeneous treatment groups, without involving potential biases or judgments.



- Replication is the repetition of an entire experiment or portion of an experiment under two or more sets of conditions.
- Replication reduces variability in experimental results and increases the significance and the confidence level with which a researcher can draw conclusions about an experimental factor
- To establish the significance of an experimental result, *replication*, the repetition of an experiment on a large group of subjects, is required.
 - If a treatment is truly effective, the average effect of replicated experimental units will reflect it.
 - If it is not effective, then the few members of the experimental units who
 may have reacted to the treatment will be negated by the large numbers
 of subjects who were unaffected by it.



 A Block is a group of homogeneous experimental units. For example, if an investigator had reason to believe that response of the medication differs significantly with age difference, he might choose to first divide the experimental subjects into age groups, such as under 5 years old, 5-10 years old, and over 10 years old etc to acquire homogeneity of response of the medication within age group.



- A Factor is a controllable independent variable that is being investigated to determine its effect on a response. E.g. treatment group is a factor.
- Factors can be fixed or random
 - Fixed -- the factor can take on a finite number of levels and these are the only levels of interest e.g. treatment group in our data set is a fixed factor.
 - Random -- the factor can take on a wide range of values and one wants to generalize from specific values to all possible values e.g. subjects in the data set can be a random factor.
- Each specific value of a factor is called a level. E.g. treatment group:
 A, B, and placebo. Then all these are three levels.



- A covariate is an independent variable not manipulated by the
 experimenter but still affecting the response. E.g. in many
 clinical experiments, the demographic variables such as race,
 gender, age may influence the response variable significantly
 even though these are not the variables of interest of the study.
 These variables are termed as covariate.
- Effect is the change in the average response between two
 factor levels. That is, factor effect = average response at one
 level average response at a second level.



• **Interaction** is the joint factor effects in which the effect of one factor depends on the levels of the other factors.

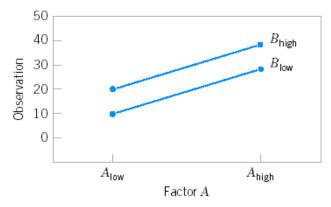


Figure 7-1 An interaction plot of a factorial experiment, no interaction.

No interaction effect of factor A and B

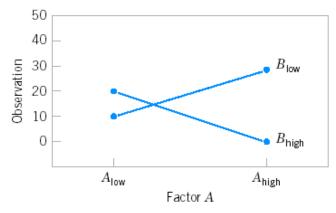


Figure 7-2 An interaction plot of a factorial experiment, with interaction.

Interaction effect of factor A and B



The basic ANOVA situation

- Type of variables: Quantitative response and Categorical (factor) predictors (independent variable).
- Main Question: Are mean response measures of different groups equal?
- One categorical variable with only 2 levels (groups):
 - 2-sample t-test
- One categorical variable with more than two levels (groups):
 - One way ANOVA
- Two or more categorical variable, each with at least two or more levels (groups) of each:
 - Factorial ANOVA



Analysis of variance (ANOVA)

- The analysis of variance (ANOVA) is a technique of decomposing the total variability of a response variable into:
 - Variability due to the experimental factor(s) and...
 - Variability due to error (i.e., factors that are not accounted for in the experimental design).
- The basic purpose of ANOVA is to test the equality of several means.
- A fixed effect model includes only fixed factors in the model.
- A random effect model includes only random factors in the model.
- A mixed effect model includes both fixed and random factors in the model.



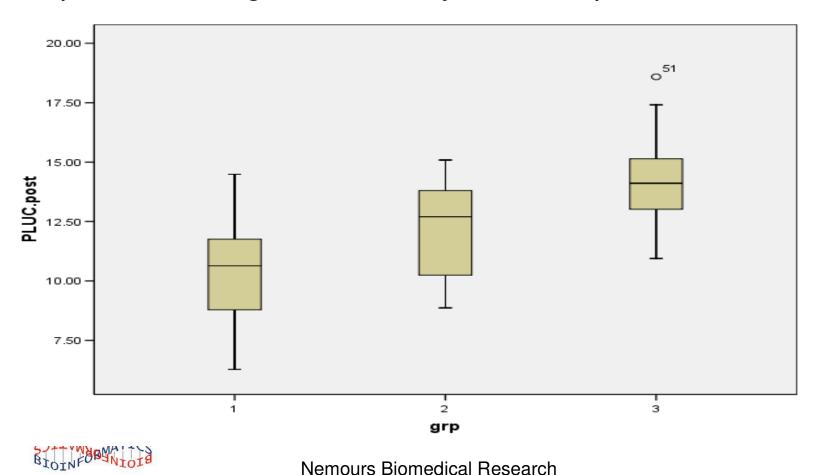
Analysis of variance (ANOVA)

- ☐ Graphical investigation:
 - side-by-side box plots
 - multiple histograms



Analysis of variance (ANOVA)

Graphical Investigation: Side by Side Boxplots



- One factor of k levels or groups. E.g., 3 treatment groups in our default data
- Total variation of observations (SST) can be split in two components:
 variation between groups (SSG) and variation within groups (SSE).
- Variation between groups is due to the difference in different groups.
 E.g. different treatment groups or different doses of the same treatment.
- Variation within groups is the inherent variation among the observations within each group.
- Completely randomized design (CRD) is an example of one-way analysis of variance.



Model:

- $y_{ij} = \mu + a_i + e_{ij}$
- Where y_{ii} is the ith observation of the jth group
- a_i is the effect of the ith group
- $-\mu$ is the grand mean and e_{ii} is the error.

Assumptions:

- Observations y_{ij} are independent.
- e_{ij} are normally distributed with mean zero and constant standard deviation.
- The second assumption implies that response variable for each group is normal (Check using q-q plot, histogram, or test for normality) and standard deviations for all groups are equal (rule of thumb: ratio of largest to smallest are approximately 2:1).



Hypothesis:

H_o: Means of all groups are equal.

H_a: At least one of them is not equal to other.

- doesn't say how or which ones differ.
- Can follow up with "multiple comparisons"
- ANOVA Table for one way classified data

Sources of Variation	Sum of Squares	df	Mean Sum of Squares	F-Ratio
Group	SSG	k-1	MSG=SSG/k-1	F=MSG/MSE
Error	SSE	n-k	MSE=SSE/n-k	
Total	SST	n-1		

Note: Large F means that MSG is large compared to MSE



- Significance of the differences between the groups depends on
 - the difference in the means
 - the standard deviations of each group
 - the sample sizes
 - A useful web www.psych.utah.edu/stat/introstats/anovaflash.html
- ANOVA determines P-value from the F statistic



Multiple comparisons

- If the F test is significant in ANOVA table, then we intend to find the pairs of groups are significantly different. Following are the commonly used procedures:
 - Fisher's Least Significant Difference (LSD)
 - Tukey's HSD method
 - Bonferroni's method
 - Scheffe's method
 - Dunn's multiple-comparison procedure
 - Dunnett's Procedure



SPSS demonstration: One Way ANOVA

Analyze -> Compare Means -> One-Way ANOVA->
 select a dependent variable (e.g. PLUC.post) and a
 Factor (e.g. grp) and then select other output options. For
 Multiple comparisons, click on Post Hoc and select a
 suitable test.



Thank you

