#### **Comparing Several Means**



Some slides from R. Pruim

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#### The Dating World of Swordtail Fish

- In some species of swordtail fish, males develop brightly coloured swordtails
- Southern Platyfish do not
- Want to know: will female Southern Platyfish prefer males with artificial brightly-coloured swordtails?
  - If they do, that's evidence that males in other species evolved as a result of female preference
- Experiment: multiple pair of males, one with a transparent artificial tail, one with a bright yellow artificial swordtail. Measure the percentage of time the female spends courting with the male with the yellow tail. There are 84 females in total.

## Platyfish

- Eventually, we would like to know whether females spent more time with the yellow-swordtailed males. But we would like to first investigate whether there is anything else going on in the data that might affect our conclusions
- Question: Do the (means of) the quantitative variables depend on which group (given by categorical variable) the individual is in?
- (the fish, in R)

## Computing Group Means with Linear Regression

- Fit a linear regression:
- $Y \sim a_0 + a_{g_1}I_{g_1} + a_{g_2}I_{g_2} + \dots + a_{g_N}I_{g_N}$
- *Y*: the percentage of time the female spends with the yellow-tailed male
- $I_{gk}$ : 1 if the case involves Group k, 0 otherwise
- Regression:
  - Minimize  $\sum_{i} (Y_i (a_0 + a_{g_1}I_{i,g_1} + a_{g_2}I_{i,g_2} + \dots + a_{g_N}I_{i,g_N}))^2$

## Computing Group Means with Linear Regression

• 
$$\sum_{i} \left( Y_{i} - (a_{0} + a_{g1}I_{i,g1} + a_{g2}I_{i,g2} + \dots + a_{gN}I_{i,gN}) \right)^{2}$$
  
=  $\sum_{group} \sum_{i \in group} \left( Y_{i} - a_{group} \right)^{2}$ 

•  $\sum_{i \in group} (Y_i - a_{group})^2$  is minimized when  $a_{group} = ?$  (show how to do this)

# Computing Group Means with Linear Regression

• 
$$\left(\sum_{i \in group} (Y_i - a_{group})^2\right)' = 0$$
  
 $-2 \sum_{i \in group} (Y_i - a_{group}) = 0$   
 $\sum_{i \in group} Y_i = \sum_{i \in group} a_{group}$   
 $a_{group} = \frac{\sum_{i \in group} Y_i}{N_{group}}$ 

## Computing the Means with R

• (in R)

# Are the Pairs Different from Each Other?

- If we had just two pairs in which we're interested, we could simply use a t-Test
  - Estimate the pooled variance from the entire dataset

• 
$$s_p^2 = \frac{(N_{g_1}-1)s_1^2 + \dots + (N_{g_N}-1)s_N^2}{(N_{g_1}-1) + \dots + (N_{g_N}-1)} ((N_{g_1}-1) + \dots + (N_{g_N}-1) d.f.)$$
  
•  $\frac{mean_{g_1}-mean_{g_2}}{s_p\sqrt{\frac{1}{N_{g_1}} + \frac{1}{N_{g_2}}}} \sim t((N_{g_1}-1) + \dots + (N_{g_N}-1))$ 

• But we're interested in whether *any* pair is different from *any other pair* 

#### ANOVA

- Null Hypothesis: the means of all the groups are equal
- Notation:
  - N: number of individuals/observation all together
  - $\overline{X}$ : mean for entire data set is
- Group i:
  - N<sub>i</sub>: number of individuals in group *i*
  - $X_{ij}$ : value for individual *j* in group *i*
  - $\overline{X}_i$ : mean for group *i*

### ANOVA: Idea

- If all the group means are the same, the average variation within the groups should be almost as large as the average variation within the entire dataset (why almost?)
- Variation BETWEEN groups:
  - For each data value look at the difference between its group mean and the overall mean:  $\sum_i N_i (\overline{X_i} \overline{X})^2$
- Variation WITHIN groups:
  - For each data value look at the difference between the value and the group mean:  $\sum_{i} \sum_{j} (X_{ij} \overline{X}_{i})^{2}$

#### ANOVA: Idea

- SSReg (Regression Sum of Squares, variation across groups) :  $\sum_i N_i (\overline{X_i} \overline{X})^2$  (d.f.: Ngroups-1)
- RSS (Residual Sum of Squares, variation within groups):  $\sum_{i} \sum_{j} (X_{ij} \overline{X}_{i})^{2}$  (d.f.: Npoints-Ngroups)
- Compute the ratio of the averages:

• 
$$F = \frac{\sum_{i} N_{i}(\overline{X_{i}} - \overline{X})^{2}}{Ngroups - 1} / \frac{\sum_{i} \sum_{j} (X_{ij} - \overline{X}_{i})^{2}}{Npoints - Ngroups}$$

#### ANOVA: Idea

• 
$$F = \frac{\sum_{i} (\overline{X_{i}} - \overline{X})^{2}}{Ngroups - 1} / \frac{\sum_{i} \sum_{j} (X_{ij} - \overline{X}_{i})^{2}}{Npoints - ngroups}$$

- If "average" between-group variation is not larger than "average" within-group variation (i.e., the Null Hypothesis is true),  $F\,\approx\,1$
- If between-group variation is larger than within-group variation (i.e., the means for the different groups are different), F > 1

• 
$$\frac{\sum_{i} N_{i}(\overline{X_{i}} - \overline{X})^{2}}{\sigma^{2}} \sim \chi^{2}(Ngroups - 1)$$
  
• 
$$\frac{\sum_{ij}(X_{ij} - \overline{X_{i}})^{2}}{\sigma^{2}} \sim \chi^{2}(Npoints - Ngroups)$$

• *F*~*F*(*Ngroups* – 1, *Npoints* – *Ngroups*)

### The F distribution

• If 
$$W_1 \sim \chi^2(k_1)$$
 and  $W_2 \sim \chi^2(k_2)$ , then  
 $F = \frac{W_1}{W_2} \sim F(k_1, k_2)$ 

#### ANOVA: the model

• Constant variance  $\sigma^2$ , (possibly) different means  $\mu_i$  for the different groups

 $X_{ij} \sim N(\mu_i, \sigma^2)$ 

• Null Hypothesis:  $\mu_1 = \mu_2 = \cdots = \mu_{Ngroups}$ 

• F statistic: 
$$F = \frac{\sum_{i} N_{i}(\overline{X_{i}} - \overline{X})^{2}}{Ngroups - 1} / \frac{\sum_{i} \sum_{j} (X_{ij} - \overline{X}_{i})^{2}}{Npoints - ngroups}$$

- F-test:  $P_{\mu_1=\cdots=\mu_{Ngroups}}$  (F > f)
  - If the Null Hypothesis is true,

$$F \sim F(Ngroups - 1, Npoints - Ngroups)$$









#### Pairwise t-Tests

- Suppose we find (using an F-test) that there are differences between the different means. That still doesn't tell us what the differences are
- Naively, we can run a t-Test for every pair of groups
- (in R)

#### Problem with Multiple Comparisons

- If we are computing a p-value and the Null Hypothesis is true, we'd get a false positive 5% of the time (1 time out of 20)
  - False positive: p-value<.05, but the Null Hypothesis is true
- If we are computing 20 p-values and the Null Hypothesis is true, what percent of the time will we get at least one false positive?

#### Problem with Multiple Comparisons

• If we are computing 20 p-values and the Null Hypothesis is true, what percent of the time will we get at least one false positive?

 $1 - (1 - 0.05)^{20} \approx 64\%$ 

- If we have 7 groups, and compare each mean to each other mean, how many comparisons do we make?
  - (Show in R)

#### Problem with Multiple Comparisons

• N variables to do pairwise comparison on:

$$\binom{N}{2} = N(N-1)/2$$
 comparisons

- Intuition:
  - See the table in R
  - For each coefficient (N) of them, compare it to every other (N-1): N(N-1) comparisons. But we compared each pair twice, so divide by two: N(N-1)/2

### Bonferroni correction

- Boole's inequality: the probability of any one of the events  $E_1, E_2, \ldots, E_n$  happening is smaller than  $\sum_i P(E_i)$ :
  - $P(\bigcup_i E_i) \leq \sum_i P(E_i)$
  - Idea: the probability is largest when the events are mutually exclusive, in which case the probability is  $\sum_i P(E_i)$

• 
$$P\left(\bigcup_{i=1\dots n}^{n}\left(p_{i}\leq\frac{\alpha}{n}\right)\right)\leq\sum_{i=1}^{n}P\left(p_{i}\leq\frac{\alpha}{n}\right)=\frac{n\alpha}{n}=\alpha$$

### Bonferroni correction

- If we want the *familywise* p-value threshold to be  $\alpha$ , make the individual p-value threshold be  $\frac{\alpha}{n}$ , where *n* is the number of groups
- Generally, *very* conservative
  - Why?

Tukey's Honest Significant Differences (HSD)

- Tukey's HSD is a method of adjusting the SE estimate based on the range of the data
  - Not as conservative as using the Bonferroni correction

#### Confidence Intervals -- Bonferroni

- If the statistic is t-distributed:  $\hat{\theta} \pm t_{df,1-\frac{\alpha}{k}} \cdot SE(\hat{\theta})$
- (In R)

# Summary: F-test and Pairwise Comparisons

- Assuming (and checking) normal distributions with constant variance in different groups:
  - Run F-test to see if any of the means are different
  - Can follow up and check pairwise differences
- If you have a hypothesis about which group means are different *ahead of time*, that's like running multiple studies
  - Some of your multiple studies might be wrong, of course
  - Still, okay not to adjust as long as you report that you had lots of hypotheses about which means might be different
    - Of course, if you have lots of hypotheses, people might think you're a little bit scatterbrained