## I. Introduction to Two Factor ANOVA

- A. Factorial analysis of variance
- B. No good general nonparametric tests
- C. Experimental set-up:
  - factor A, w/ a levels
  - factor B, w/ *b* levels
  - *n* = number of replicates for each combination of factors equal replication = <u>balanced</u> experimental design
  - total # measurements = abn

## D. Hypotheses:

- H<sub>0</sub>: No effect of factor A on response variable
- H<sub>A</sub>: Factor A does have an effect on response variable
- H<sub>0</sub>: No effect of factor B on response variable
- H<sub>A</sub>: Factor B does have an effect on response variable
- H<sub>0</sub>: No effect of interaction between factors A & B on response variable
- HA: Interaction between factors A & B does have an effect on response variable
- E. Notation
  - 1. individual measurements:  $X_{ijl} = l^{th}$  measurement in j<sup>th</sup> level of B & i<sup>th</sup> level of A  $1^{st}$  subscript = factor A  $2^{nd}$  subscript = factor B  $3^{rd}$  subscript = replicate
  - 2. means: 4 kinds of means:
    - cell means (cell ij):  $\overline{X}_{ij}$

level means of factor A:  $\overline{X}_{i}$ , e.g.,  $\overline{X}_{2}$ .

level means of factor B:  $\overline{X}_{.i}$ , e.g.,  $\overline{X}_{.i}$ 

grand mean:  $\overline{X}$ 

3. Summation: 
$$\sum_{i} X_{i} \qquad \sum_{i} \sum_{j} \sum_{l} X_{ijl} = \sum_{i=1}^{a} \left[ \sum_{j=1}^{b} \left( \sum_{l=1}^{n} X_{ijl} \right) \right]$$

# II. Linear Model for Two-Factor ANOVA

 $\begin{aligned} X_{ijl} &= \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijl} \\ \text{where:} \quad \varepsilon_{ijl} \sim N(0, \sigma^2) \quad \text{and independent of each other} \\ H_0: \quad \alpha_i &= 0 \\ H_0: \quad \beta_i &= 0 \\ H_0: \quad (\alpha\beta)_{ij} &= 0, \quad \forall ij \end{aligned}$ 

## III. Calculations:

A. Sums of Squares & Degrees of Freedom

total: total SS = 
$$\sum_{i=1}^{a} \sum_{j=1}^{b} \sum_{l=1}^{n} (X_{ijl} - \overline{X})^2$$
 total DF =  $N - 1$   
cells: cells SS =  $\sum_{i=1}^{a} \sum_{j=1}^{b} n(\overline{X}_{ij} - \overline{X})^2$  cells DF =  $ab - 1$ 

#### **III.** Calculations (continued):

$$\underline{\text{within-cells (error)}}: \text{ error SS} = \sum_{i=1}^{a} \sum_{j=1}^{b} \left[ \sum_{l=1}^{n} (X_{ijl} - \overline{X}_{ij})^2 \right]$$

$$\text{ error DF} = ab(n-1) = \text{ total DF} - \text{ cells DF}$$

$$\underline{\text{factor A}}: \text{ factor } A \text{ SS} = bn \sum_{i=1}^{a} (\overline{X}_{i\cdot} - \overline{X})^2 \qquad \text{ factor } A \text{ DF} = a - 1$$

$$\underline{\text{factor B}}: \text{ factor } B \text{ SS} = an \sum_{j=1}^{b} (\overline{X}_{\cdot j} - \overline{X})^2 \qquad \text{ factor } B \text{ DF} = b - 1$$

$$\underline{\text{interaction}} \text{ between A \& B}:$$

$$A \times B \text{ interaction SS} = \text{ cells SS} - \text{ factor } A \text{ SS} - \text{ factor } B \text{ SS}$$

$$A \times B \text{ interaction DF} = \text{ cells DF} - \text{ factor } A \text{ DF} = a - 1$$

$$\text{ or: } A \times B \text{ interaction DF} = (\text{ factor } A \text{ DF})(\text{ factor } B \text{ DF}) = (a-1)(b-1)$$

- B. Mean Square = SS / DF
- C. Machine formulas: see Zar, table 12.2

### IV. Testing Hypotheses & Models of Two-Factor ANOVA

- A. Model I ANOVA: both factors fixed
  - test each of 3 null hypotheses (A, B, interaction) independently:
  - significant interaction => difference among levels of a factor not constant w/ other factor => do not interpret factor effect if ∃ significant interaction effect
  - Hypothesis testing w/ both factors fixed:

all tests against within-cells MS (or error MS)

• Factor A: 
$$\frac{\text{factor } A \text{ MS}}{\text{error } \text{MS}} \sim F_{(a-1),ab(n-1)}$$
  
• Factor B:  $\frac{\text{factor } B \text{ MS}}{\text{error } \text{MS}} \sim F_{(b-1),ab(n-1)}$ 

- Interaction:  $\frac{A \times B \text{ MS}}{\text{error MS}} \sim F_{(a-1)(b-1),ab(n-1)}$
- B. Model II ANOVA: both factors random:
  - test Factor A & B against interaction MS,
  - test interaction against within-cells MS (error MS)
    - Factor A:  $\frac{\text{factor } A \text{ MS}}{A \times B \text{ MS}} \sim F_{(a-1),(a-1)(b-1)}$
    - Factor B:  $\frac{\text{factor } B \text{ MS}}{A \times B \text{ MS}} \sim F_{(b-1),(a-1)(b-1)}$
    - Interaction:  $\frac{A \times B \text{ MS}}{\text{error MS}} \sim F_{(a-1)(b-1),ab(n-1)}$

- C. Model III ANOVA (or mixed model ANOVA): one factor fixed, other random  $\rightarrow$  e.g., for A fixed, B random:
  - test fixed factor (A) against interaction MS,
  - test random factor (B) and interaction against within-cells MS (error MS)

• Factor A: 
$$\frac{\text{factor } A \text{ MS}}{A \times B \text{ MS}} \sim F_{(a-1),(a-1)(b-1)}$$
  
factor B MS

• Factor B: 
$$\frac{\text{factor } B \text{ MS}}{\text{error } \text{MS}} \sim F_{(b-1),ab(n-1)}$$

• Interaction: 
$$\frac{A \times B \text{ MS}}{\text{error MS}} \sim F_{(a-1)(b-1),ab(n-1)}$$

### V. Multiple Comparisons Testing

- A. If two factor ANOVA => significant differences among levels of factor,
  - use multiple comparison test, Zar chapter 11 (e.g., Tukey test)
  - $s^2$  = within-cells MS
  - v = within-cells DF
  - n = total # data per level, e.g., for factor A, n = bn
- B. If significant interaction between factors,

DO NOT compare means of levels of a factor instead, do multiple comparison test among <u>cell means</u>

### VI. Confidence Limits of Means

same procedure as for single-factor ANOVA