

## 28 Lecture 28: April 14

### Last time

- Analysis of Variance (JF chapter 8)
- one-way anova

### Today

- No class Wednesday
- Friday presentation
- two-way anova

### One-way ANOVA example

The following data come from study investigating binding fraction for several antibiotics using  $n = 20$  bovine serum samples:

Antibiotic	Binding Percentage	Sample mean
Penicillin G	29.6 24.3 28.5 32.0	28.6
Tetracyclin	27.3 32.6 30.8 34.8	31.4
Streptomycin	5.8 6.2 11.0 8.3	7.8
Erythromycin	21.6 17.4 18.3 19	19.1
Chloramphenicol	29.2 32.8 25.0 24.2	27.8

Question: Are the population means for these 5 treatments plausibly equal?

*Answer:*

How do we obtain standard errors of parameter estimates? (HW)

### Two-Way ANOVA

The inclusion of a second factor permits us to model and test partial relationships, as well as to introduce interactions. Let's take a look at the patterns of relationship that can occur when a quantitative response variable is classified by two factors.

#### Patterns of Means in the two-way classification

Consider the following table:

	$C_1$	$C_2$	$\dots$	$C_c$	
$R_1$	$\mu_{11}$	$\mu_{12}$	$\dots$	$\mu_{1c}$	$\mu_{1\cdot}$
$R_2$	$\mu_{21}$	$\mu_{22}$	$\dots$	$\mu_{2c}$	$\mu_{2\cdot}$
$\vdots$	$\vdots$	$\vdots$		$\vdots$	$\vdots$
$R_r$	$\mu_{r1}$	$\mu_{r2}$	$\dots$	$\mu_{rc}$	$\mu_{r\cdot}$
	$\mu_{\cdot 1}$	$\mu_{\cdot 2}$	$\dots$	$\mu_{\cdot c}$	$\mu_{\cdot\cdot}$

The factors,  $R$  and  $C$  (for “rows” and “columns” of the table of means), have  $r$  and  $c$  categories, respectively. The factor categories are denoted  $R_j$  and  $C_k$ . Within each cell of the design - that is, for each combination of categories  $\{R_j, C_k\}$  of the two factors - there is a population cell mean  $\mu_{jk}$  for the response variable. Extending the dot notation, we have

$$\mu_{j\cdot} \equiv \frac{\sum_{k=1}^c \mu_{jk}}{c}$$

is the marginal mean of the response variable in row  $j$ .

$$\mu_{\cdot k} \equiv \frac{\sum_{j=1}^r \mu_{jk}}{r}$$

is the marginal mean in column  $k$ . And

$$\mu_{\cdot\cdot} \equiv \frac{\sum_j \sum_k \mu_{jk}}{r \times c}$$

is the grand mean.

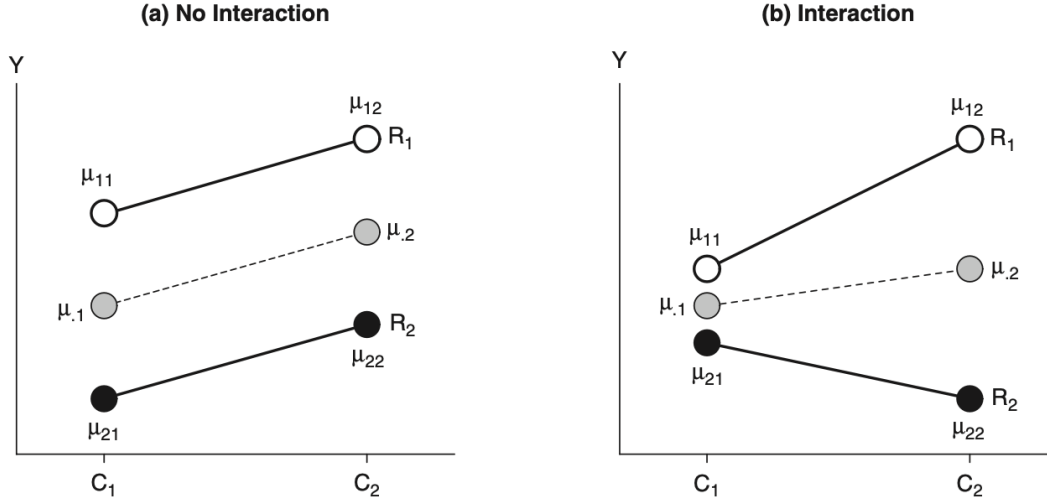


Figure 28.1: Interaction in the two-way classification. In (a), the parallel profiles of means (given by the white and black circles connected by solid lines) indicate that  $R$  and  $C$  do not interact in affecting  $Y$ . The  $R$ -effect – that is, the difference between the two profiles – is the same at both  $C_1$  and  $C_2$ . Likewise, the  $C$ -effect – that is, the rise in the line from  $C_1$  to  $C_2$  – is the same for both profiles. In (b), the  $R$ -effect differs at the two categories of  $C$ , and the  $C$ -effect differs at the two categories of  $R$ :  $R$  and  $C$  interact in affecting  $Y$ . In both graphs, the column marginal means  $\mu_{.1}$  and  $\mu_{.2}$  are shown as averages of the cell means in each column (represented by the gray circles connected by broken lines). JF Figure 8.2.

### Two-way ANOVA model

The two-way ANOVA model, suitably defined, provides a convenient means for testing the hypotheses concerning interactions and main effects. The model is

$$Y_{ijk} = \mu + \alpha_j + \beta_k + \gamma_{jk} + \epsilon_{ijk}$$

where  $Y_{ijk}$  is the  $i$ th observation in row  $j$ , column  $k$  of the  $RC$  table;  $\mu$  is the general mean of  $Y$ ;  $\alpha_j$  and  $\beta_k$  are the main-effect parameters;  $\gamma_{jk}$  are interaction effect parameters; and  $\epsilon_{ijk}$  are errors satisfying the usual linear-model assumptions (i.e.  $\epsilon_{ijk} \stackrel{iid}{\sim} \mathcal{N}(0, \sigma^2)$ ). By taking expectations, we have

$$\mu_{jk} \equiv E(Y_{ijk}) = \mu + \alpha_j + \beta_k + \gamma_{jk}$$

We have  $r \times c$  population cell means with  $1 + r + c + r \times c$  model parameters. Similar to one-way ANOVA model, we add in additional constraints to make the model identifiable.

$$\begin{aligned}\sum_{j=1}^r \alpha_j &= 0 \\ \sum_{k=1}^c \beta_k &= 0 \\ \sum_{j=1}^r \gamma_{jk} &= 0 \quad \text{for all } k = 1, \dots, c \\ \sum_{k=1}^c \gamma_{jk} &= 0 \quad \text{for all } j = 1, \dots, r\end{aligned}$$

The constraints produce the following solution for model parameters in terms of population cell and marginal means (and we add a hat for their estimates using the sample means):

$$\begin{aligned}\hat{\mu} &= \tilde{\mu}_{..} \\ \hat{\alpha}_j &= \tilde{\mu}_{j.} - \tilde{\mu}_{..} \\ \hat{\beta}_k &= \tilde{\mu}_{.k} - \tilde{\mu}_{..} \\ \hat{\gamma}_{jk} &= \tilde{\mu}_{jk} - \hat{\mu} - \hat{\alpha}_j - \hat{\beta}_k \\ &= \tilde{\mu}_{jk} - \tilde{\mu}_{j.} - \tilde{\mu}_{.k} + \tilde{\mu}_{..}\end{aligned}$$

## Hypotheses with two-way ANOVA

Some interesting hypotheses:

1. Are the cell means all equal? (Equivalent to one-factor ANOVA's "overall F-test")  
 $H_0 : \mu_{11} = \mu_{12} = \dots = \mu_{rc}$  vs.  $H_a : \text{At least two } \mu_{ij} \text{ differ}$
2. Are the marginal means for row main effect equal?  
 $H_0 : \mu_{1.} = \mu_{2.} = \dots = \mu_{r.}$  vs  $H_a : \text{At least two } \mu_{j.} \text{ differ}$   
which is equivalent as testing for no row main effects  $H_0 : \text{all } \alpha_j = 0$  (why?)
3. Are the marginal means for column main effect equal?  
 $H_0 : \mu_{.1} = \mu_{.2} = \dots = \mu_{.c}$  vs  $H_a : \text{At least two } \mu_{.k} \text{ differ}$
4. Do the factors interact? In other words, does effect of one factor depend on the other factor?  $H_0 : \mu_{ij} = \mu_{..} + (\mu_{i.} - \mu_{..}) + (\mu_{.j} - \mu_{..})$  vs  $H_a : \text{At least one } \mu_{ij} \neq \mu_{..} + (\mu_{i.} - \mu_{..}) + (\mu_{.j} - \mu_{..})$   
The null hypothesis is also equivalent as  $H_0 : \text{all } \gamma_{jk} = 0$ .

## Testing hypotheses in two-way ANOVA

We follow the notations of JF for incremental sums of squares in ANOVA:

$$\begin{aligned}
 \mathbf{SS}(\gamma|\alpha, \beta) &= \mathbf{SS}(\alpha, \beta, \gamma) - \mathbf{SS}(\alpha, \beta) \\
 \mathbf{SS}(\alpha|\beta, \gamma) &= \mathbf{SS}(\alpha, \beta, \gamma) - \mathbf{SS}(\beta, \gamma) \\
 \mathbf{SS}(\beta|\alpha, \gamma) &= \mathbf{SS}(\alpha, \beta, \gamma) - \mathbf{SS}(\alpha, \gamma) \\
 \mathbf{SS}(\alpha|\beta) &= \mathbf{SS}(\alpha, \beta) - \mathbf{SS}(\beta) \\
 \mathbf{SS}(\beta|\alpha) &= \mathbf{SS}(\alpha, \beta) - \mathbf{SS}(\alpha)
 \end{aligned}$$

where  $\mathbf{SS}(\alpha, \beta, \gamma)$  denotes the regression sum of squares for the full model which includes both sets of main effects and the interaction.  $\mathbf{SS}(\alpha, \beta)$  denotes the regression sum of squares for the no-interaction model and  $\mathbf{SS}(\alpha, \gamma)$  denotes the regression for the model that omits the column main-effect regressors. Note that the last model violates the principle of marginality because it includes the interaction regressors but omits the column main effects. However, it is useful for constructing the incremental sum of squares for testing the column main effects.

*Additional readings:* [Notes on 3 types of Sum of Squares](#) by Dr. Nancy Reid (attached on canvas site).

We now have the two-way ANOVA table

Table 1: Two-way ANOVA table

Source	Sum of Squares	df	$H_0$
R	$\mathbf{SS}(\alpha \beta, \gamma)$	$r - 1$	all $\alpha_j = 0$
	$\mathbf{SS}(\alpha \beta)$	$r - 1$	all $\alpha_j = 0 \mid$ all $\gamma_{jk} = 0$
C	$\mathbf{SS}(\beta \alpha, \gamma)$	$c - 1$	all $\beta_k = 0$
	$\mathbf{SS}(\beta \alpha)$	$c - 1$	all $\beta_k = 0 \mid$ all $\gamma_{jk} = 0$
RC	$\mathbf{SS}(\gamma \alpha, \beta)$	$(r - 1)(c - 1)$	all $\gamma_{jk} = 0$
Residuals	$\mathbf{TSS} - \mathbf{SS}(\alpha, \beta, \gamma)$	$n - rc$	
Total	$\mathbf{TSS}$	$n - 1$	

where the residual sum of squares

$$RSS = \sum_i \sum_j \sum_k (Y_{ijk} - \bar{Y}_{jk})^2$$

When testing for the hypothesis, use the corresponding SS and df together with the residual SS and df to construct the  $F$ -statistic.

$$F = \frac{SS/df}{RSS/df_{residual}}$$

There are two reasonable procedures for testing main-effect hypotheses in two-way ANOVA:

1. Tests based on  $\mathbf{SS}(\alpha|\beta, \gamma)$  and  $\mathbf{SS}(\beta|\alpha, \gamma)$  (“type III” tests) employ models that violate the principle of marginality, but the tests are valid whether or not interactions are present.
2. Tests based on  $\mathbf{SS}(\alpha|\beta)$  and  $\mathbf{SS}(\beta|\alpha)$  (“type II” tests) conform to the principle of marginality but are valid only if interactions are absent, in which case they are maximally powerful.

Some more jargon:

- Experimental unit (EU): entity to which experimental treatment is assigned.  
For example, Assign fertilizer treatment to fields. Fields = EU.
- Measurement unit (MU): entity that is measured.  
For example, Measure yields at several subplots within each field. MU: subplot
- Treatment structure: describes how different experimental factors are combined to generate treatments.  
For example, Fertilizers: A, B, C; Irrigation: High, Low.
- Randomization structure: how treatments are assigned to EUs.
- Simplest treatment structure: single experimental factor with multiple levels. Ex. Fertilizers A vs B vs C.
- Simplest randomization structure: Completely randomized design – Experimental treatments assigned to EUs entirely at random.

#### Example: Honeybee data

Entomologist records energy expended ( $y$ ) by  $N = 27$  honeybees at  $a = 3$  temperature (A) levels ( $20, 30, 40^\circ C$ ) consuming liquids with  $b = 3$  levels of sucrose concentration ( $B$ ) ( $20\%, 40\%, 60\%$ ) in a balanced, completely randomized crossed  $3 \times 3$  design.

Temp	Suc	Sample		
20	20	3.1	3.7	4.7
20	40	5.5	6.7	7.3
20	60	7.9	9.2	9.3
30	20	6	6.9	7.5
30	40	11.5	12.9	13.4
30	60	17.5	15.8	14.7
40	20	7.7	8.3	9.5
40	40	15.7	14.3	15.9
40	60	19.1	18.0	19.9

1. What is the experimental unit?
2. What is the treatment structure?
3. Finish the table below

Source	df
A	
B	
$A \times B$	
Residual	
Total	

*Answer:*

4. Consider the model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}$$

where  $i = 1, 2, \dots, a$ ,  $j = 1, 2, \dots, b$  and  $k = 1, 2, \dots, n$  for a balanced design.

Deviation:

- total:  $y_{ijk} - \bar{y}_{+++}$
- due to level  $i$  of factor A:  $\bar{y}_{i++} - \bar{y}_{+++}$

- due to level  $j$  of factor B:  $\bar{y}_{+j+} - \bar{y}_{+++}$
- due to levels  $i$  of factor A and  $j$  of factor B after subtracting main effects:

$$\bar{y}_{ij+} - \bar{y}_{+++} - (\bar{y}_{i++} - \bar{y}_{+++}) - (\bar{y}_{+j+} - \bar{y}_{+++}) = \bar{y}_{ij+} - \bar{y}_{i++} - \bar{y}_{+j+} + \bar{y}_{+++}$$

Use the following equations to calculate the Sum of Squares and fill out the ANOVA table.

$$\begin{aligned} SS[Total] &= \sum_i \sum_j \sum_k (y_{ijk} - \bar{y}_{+++})^2 \\ SS[A] &= \sum_i \sum_j \sum_k (\bar{y}_{i++} - \bar{y}_{+++})^2 \\ SS[B] &= \sum_i \sum_j \sum_k (\bar{y}_{+j+} - \bar{y}_{+++})^2 \\ SS[AB] &= \sum_i \sum_j \sum_k (\bar{y}_{ij+} - \bar{y}_{i++} - \bar{y}_{+j+} + \bar{y}_{+++})^2 \\ SS[E] &= \sum_i \sum_j \sum_k (\bar{y}_{ijk} - \bar{y}_{ij+})^2 \end{aligned}$$

where

$$\begin{aligned} \bar{y}_{ij+} &= \frac{1}{n} \sum_k y_{ijk} \\ \bar{y}_{i++} &= \frac{1}{b} \sum_j \bar{y}_{ij+} = \frac{1}{bn} \sum_j \sum_k y_{ijk} \\ \bar{y}_{+j+} &= \frac{1}{a} \sum_i \bar{y}_{ij+} = \frac{1}{an} \sum_i \sum_k y_{ijk} \\ \bar{y}_{+++} &= \frac{1}{a} \sum_i \bar{y}_{i++} = \frac{1}{b} \sum_j \bar{y}_{+j+} \\ &= \frac{1}{abn} \sum_i \sum_j \sum_k y_{ijk} \end{aligned}$$

Source	df	Sum of Squares	Mean Square	F
A				
B				
$A \times B$				
Residual				
Total				

*Answer:*



### A three-factor example

In a balanced, complete, crossed design,  $N = 36$  shrimp were randomized to  $abc = 12$  treatment combinations from the factors below:

- A1: Temperature at  $25^{\circ}C$
- A2: Temperature at  $35^{\circ}C$
- B1: Density of shrimp population at 80 shrimp/40l
- B2: Density of shrimp population at 160 shrimp/40l
- C1: Salinity at 10 units
- C2: Salinity at 25 units
- C3: Salinity at 40 units

The response variable of interest is weight gain  $Y_{ijkl}$  after four weeks.

### Three-way ANOVA model

$$\begin{aligned} Y_{ijkl} &= \mu + \alpha_i + \beta_j + \gamma_k \\ &\quad + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} \\ &\quad + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl} \\ i &= 1, 2 \\ j &= 1, 2 \\ k &= 1, 2, 3 \\ l &= 1, 2, 3 \\ \epsilon_{ijkl} &\stackrel{iid}{\sim} \mathcal{N}(0, \sigma^2) \end{aligned}$$

Many constraints such as (over one dimension):

$$\begin{aligned} \sum_i \alpha_i &= 0 \\ \sum_i (\alpha\beta)_{ij} &= \sum_j (\alpha\beta)_{ij} = 0 \quad \text{for all } i, j \\ \sum_i (\alpha\beta\gamma)_{ijk} &= \sum_j (\alpha\beta\gamma)_{ijk} = \sum_k (\alpha\beta\gamma)_{ijk} = 0 \quad \text{for all } i, j, k \end{aligned}$$

Now, please finish the table below

Source	df
A	
B	
C	
$A \times B$	
$A \times C$	
$B \times C$	
$A \times B \times C$	
Residual	
Total	

*Answer:*

The three-way ANOVA model includes parameters for

- Main effects:  $\alpha_i$ ,  $\beta_j$  and  $\gamma_k$ .
- Two-way interactions between each pair of factors:  $(\alpha\beta)_{ij}$ ,  $(\alpha\gamma)_{ik}$  and  $(\beta\gamma)_{jk}$ .
- Three-way interaction among all three factors:  $(\alpha\beta\gamma)_{ijk}$ .

Readings:

1. JF 8.3.1 on parameter estimates and hypothesis testing for three-way ANOVA model.
2. JF 8.3.2 on Higher-order classifications.

## Analysis of Covariance

Analysis of covariance (ANCOVA) is a term used to describe linear models that contain both qualitative and quantitative explanatory variables. The method is, therefore, equivalent to dummy-variable regression, discussed in the previous lectures, although the ANCOVA model is parametrized differently from the dummy-regression model.

Covariate is a variable known to affect the response that

1. differs among EUs
2. reflects differences that exist independently of experimental treatment.

## A nutrition example

A nutrition scientist conducted an experiment to evaluate the effects of four vitamin supplements on the weight gain of laboratory animals. The experiment was conducted in a completely randomized design with  $N = 20$  animals randomized to  $a = 4$  supplement groups, each with sample size  $n \equiv 5$ . The response variable of interest is weight gain, but calorie intake  $z$  was measured simultaneously.

Diet	$y(g)$	Diet	$y$	Diet	$y$	Diet	$y$
1	48	2	65	3	79	4	59
1	67	2	49	3	52	4	50
1	78	2	37	3	63	4	59
1	69	2	75	3	65	4	42
1	53	2	63	3	67	4	34
1	$\bar{y}_{1+} = 63$	2	$\bar{y}_{2+} = 57.8$	3	$\bar{y}_{3+} = 65.2$	4	$\bar{y}_{4+} = 48.8$
1	$s_1 = 12.3$	2	$s_2 = 14.9$	3	$s_3 = 9.7$	4	$s_4 = 10.9$

Question: Is there evidence of a vitamin supplement effect?

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Diet	3	797.8	265.9	1.823	0.184
Residuals	16	2334.4	145.9		

But calorie intake  $z$  was measured simultaneously:

Diet	$y(g)$	$z$	Diet	$y$	$z$	Diet	$y$	$z$	Diet	$y$	$z$
1	48	350	2	65	400	3	79	510	4	59	530
1	67	440	2	49	450	3	52	410	4	50	520
1	78	440	2	37	370	3	63	470	4	59	520
1	69	510	2	75	530	3	65	470	4	42	510
1	53	470	2	63	420	3	67	480	4	34	430

Question: How and why could these new data be incorporated into analysis?

Answer: ANCOVA can be used to reduce unexplained variation.

ANCOVA model,

$$y_{ij} = \mu + \alpha_i + \beta z_{ij} + \epsilon_{ij}$$

where  $\mu$  is the reference level,  $\alpha_i$  is the main effect of treatment,  $\beta$  is the partial regression coefficient, and  $\epsilon_{ij} \stackrel{iid}{\sim} \mathcal{N}(0, \sigma^2)$ . The model is equivalent as the dummy-variable regression model,

$$Y_i = \beta_0 + \beta_1 x_{i1} + \beta_2 x_{i2} + \beta_3 x_{i3} + \beta_z z_i + \epsilon_i \quad \text{for } i = 1, \dots, 20$$

Finish the table below

Source	df
Diet	
Covariate	1
Residual	
Total	

*Answer:*

To test for difference among treatments. The null hypothesis in terms of  $\alpha_i$  is

$H_0 : \alpha_1 = \alpha_2 = \dots = \alpha_4 = 0$  v.s.  $H_a : \text{at least one } \alpha_i \neq 0$

And the null hypothesis in terms of  $\beta_i$  is

$H_0 : \beta_1 = \beta_2 = \beta_3 = 0$  v.s.  $H_a : \text{at least one } \beta_i \neq 0$

Question: which two models do we compare when testing the above null hypothesis?

*Answer:*