# 34 Lecture 34: April 19

### Last time

- hypothesis test and confidence intervals for one-way random-effects model
- review of one-way random effects ANOVA model

## Today

- HW3 deadline extended to Tuesday 04/18 midnight.
- nested design
- Two-factor designs

#### Additional reference

Course notes by Dr. Jason Osborne. Lecture notes from Lukas Meier on ANOVA using R

## Nested design

Factor B is <u>nested</u> in factor A if there is a new set of levels of factor B for every different level of factor A.

To illustrate the concept of nested design, we consider the "Pastes" data set in "lme4" package in R. The strength of a chemical paste product was measured for a total of 60 samples coming from 10 randomly selected delivery batches each containing 3 randomly selected casks. Hence, two samples were taken from each cask. We want to check what part of the variability of strength is due to batch and cask.

Let  $Y_{ijk}$  be the strength of the kth sample of cask j in batch i. We can use the model

$$Y_{ijk} = \mu + A_i + B_{j(i)} + \epsilon_{ijk}$$

where  $A_i$  is the random effect of batch and  $B_{j(i)}$  is the random effect of cask **within** batch. Note the special notation  $B_{j(i)}$  emphasizes that cask is nested in batch.

# Two-factor designs with factors that are fixed/random and nested/crossed

There are in total six types of two-factor models with fixed/random effects factors that are either crossed or nested.

1. 
$$Y_{ijk} = \mu + A_i + B_j + (AB)_{ij} + \epsilon_{ijk}$$
 crossed/random  
2.  $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{ijk}$  nested/fixed  
3.  $Y_{ijk} = \mu + A_i + B_{j(i)} + \epsilon_{ijk}$  nested/random  
4.  $Y_{ijk} = \mu + \alpha_i + B_j + (\alpha B)_{ij} + \epsilon_{ijk}$  crossed/mixed  
5.  $Y_{ijk} = \mu + \alpha_i + B_{j(i)} + \epsilon_{ijk}$  nested/mixed  
6.  $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \beta)_{ij} + \epsilon_{ijk}$  crossed/fixed

In the models above,

- GREEK symbols parameterized FIXED, unknown treatment means (except for the error term)
- CAPITAL letters represent RANDOM effects (including the error term)
- for Model 1,  $A_i, B_j, (AB)_{ij}$  are all independent
- for Model 2,  $\sum \alpha_i = \sum_j \beta_{j(i)} \equiv 0$
- for Model 3,  $A_i, B_{i(i)}$  are all independent
- for Model 4,  $\sum \alpha_i = 0$  and  $B_j$ ,  $(\alpha B)_{ij}$  are all independent
- for Model 5,  $\sum \alpha_i = 0$
- for Model 6,  $\sum \alpha_i = \sum \beta_j = \sum_i (\alpha \beta)_{ij} = \sum_j (\alpha \beta)_{ij} \equiv 0$

Now let's consider the following 6 examples from Dr. Osborne's notes.

- 1. Entomologist records energy expended (y) by N=27 honeybees
  - at three TEMPERATURES (20, 30,  $40^{\circ}C$ )
  - consuming three levels of SUCROSE (20%, 40%, 60%)

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

- First factor:
- Second factor:
- Fixed or random?
- Crossed or nested?
- Model:  $Y_{ijk} = \mu + \epsilon_{ijk}$

Answer:

- 2. Experiment to study effect of drug and method of administration on fasting blood sugar in a random sample of N=18 diabetic patients.
  - First factor is drug: brand I tablet, brand II tablet, insulin injection
  - Second factor is type of administration (see table)

Drug $(i)$	Type of Administration $(j)$	Mean $\bar{y}_{j(i)}$	Variance $s_{j(i)}^2$
(i=1) Brand I tablet	$(j=1)\ 30mg\times 1$	15.7	6.3
	$(j=2)\ 15mg\times 1$	19.7	9.3
(i=2) Brand II tablet	$(j=1)\ 20mg\times 1$	20	1
	$(j=2)\ 10mg\times 1$	17.3	6.3
(i=3) Brand I tablet	(j=1) before breakfast	28	4
	(j=2) before supper	33	9

• First factor:

- Second factor:
- Fixed or random?
- Crossed or nested?
- Model:  $Y_{ijk} = \mu + \epsilon_{ijk}$

Answer:

3. An experiment is conducted to determine variability among laboratories (interlaboratory differences) in their assessment of bacterial concentration in milk after pasteurization. Milk w/ various degrees of contamination was tested by randomly drawing four samples of milk from a collection of cartons at various stages of spoilage. Y is colony-forming units/ $\mu l$ . Labs think they are receiving 8 independent samples.

	Sample				
Lab	1	2	3	4	
1	2200	3000	210	270	
	2200	2900	200	260	
2	2600	3600	290	360	
	2500	3500	240	380	
3	1900	2500	160	230	
	2100	2200	200	230	
4	2600	2800	330	350	
	4300	1800	340	290	
5	4000	4800	370	500	
	3900	4800	340	480	

- First factor:
- Second factor:
- Fixed or random?
- Crossed or nested?
- Model:  $Y_{ijk} = \mu + \epsilon_{ijk}$

Answer:

4. An experiment measures Campylobacter counts in N=120 chickens in a processing plant, at four locations, over three days. Means (std) for n=10 chickens sampled at each location tabulated below:

	Sample						
	Before	After	After	After			
Day	Washer	Washer	mic. rinse	chill tank			
1	70070.00	48310.00	12020.00	11790.00			
	(79034.49)	(34166.80)	(3807.24)	(7832.05)			
2	75890.00	52020.00	8090.00	8690.00			
	(74551.32)	(17686.27)	(4848.01)	(5526.19)			
3	95260.00	33170.00	6200.00	8370.00			
	(03176.00)	(22259.08)	(5028.81)	(5720.15)			

- First factor:
- Second factor:
- Fixed or random?
- Crossed or nested?
- Model:  $Y_{ijk} = \mu + \epsilon_{ijk}$

Answer:

5. An experiment to assess the variability of a particular acid among plants and among leaves of plants:

Plant $i$		1			2			3			4	
Leaf $j$	1	2	3	1	2	3	1	2	3	1	2	3
k = 1	11.2	16.5	18.3	14.1	19.0	11.9	15.3	19.5	16.5	7.3	8.9	11.3
k = 2	11.6	16.8	18.7	13.8	18.5	12.4	15.9	20.1	17.2	7.8	9.4	10.9
k = 3	12.0	16.1	19.0	14.2	18.2	12.0	16.0	19.3	16.9	7.0	9.3	10.5

- First factor:
- Second factor:
- Fixed or random?

• Crossed or nested?

• Model: 
$$Y_{ijk} = \mu + \epsilon_{ijk}$$

Answer:

6. Plant heights from 20 pots randomized to 10 treatment combinations

Treatment	Dark	Source	Intensity	Pot	Seedling 1	Seedling 2
DD	1	D	D	1	32.94	35.98
DD	1	D	D	2	34.76	32.40
AL	0	A	L	1	30.55	32.64
AL	0	A	L	2	32.37	32.04
AH	0	A	Н	1	31.23	31.09
AH	0	A	Н	2	30.62	30.42
BL	0	В	L	1	34.41	34.88
BL	0	В	L	2	34.07	33.87
ВН	0	В	Н	1	35.61	35.00
ВН	0	В	Н	2	35.65	32.91

• First factor:

• Second factor:

• Fixed or random?

• Crossed or nested?

• Model:  $Y_{ijk} = \mu + \epsilon_{ijk}$ 

Answer:

Tables of expected mean squares (EMS)

When factors A and B are CROSSED, and no sum-to-zero assumptions are made on random effects, expected means associated with sums of squares are given in the table below:

Source	df	A, B fixed	A, B random	A fixed $B$ random
$\overline{A}$	a-1	$\sigma^2 + nb\psi_A^2$	$\sigma^2 + nb\sigma_A^2 + n\sigma_{AB}^2$	$\sigma^2 + nb\psi_A^2 + n\sigma_{\alpha B}^2$
B	b-1	$\sigma^2 + na\psi_B^2$	$\sigma^2 + na\sigma_B^2 + n\sigma_{AB}^2$	$\sigma^2 + na\sigma_B^2 + n\sigma_{\alpha B}^2$
AB	(a-1)(b-1)	$\sigma^2 + n\psi_{AB}^2$	$\sigma^2 + n\sigma_{AB}^2$	$\sigma^2 + n\sigma_{\alpha B}^2$
Error	ab(n-1)	$\sigma^2$	$\sigma^2$	$\sigma^2$

When factor B is NESTED in factor A, expected means associated with sums of squares are given in the table below:

Source	df	A, B fixed	A, B random	A fixed $B$ random
$\overline{A}$	a-1	$\sigma^2 + nb\psi_A^2$	$\sigma^2 + nb\sigma_A^2 + n\sigma_{B(A)}^2$	$\sigma^2 + nb\psi_A^2 + n\sigma_{B(A)}^2$
B(A)	a(b-1)	$\sigma^2 + n\psi_{B(A)}^2$	$\sigma^2 + n\sigma_{B(A)}^2$	$\sigma^2 + n\psi_{B(A)}^2$
Error	ab(n-1)	$\sigma^2$	$\sigma^2$	$\sigma^2$

where  $\psi^2$  and  $\sigma^2$  values are defined below

$$\psi_A^2 = \frac{1}{a-1} \sum_{1}^{a} \alpha_i^2 \quad \text{effect size of factor } A$$

$$\psi_B^2 = \frac{1}{b-1} \sum_{1}^{b} \beta_i^2 \quad \text{effect size of factor } B$$

$$\psi_{AB}^2 = \frac{1}{(a-1)(b-1)} \sum_{i=1}^{a} \sum_{j=1}^{b} (\alpha \beta)_{ij}^2 \quad \text{effect size of interaction}$$

$$\psi_{B(A)}^2 = \frac{1}{a(b-1)} \sum_{i=1}^{a} \sum_{j=1}^{b} \beta_{j(i)}^2 \quad \text{effect size of factor } B$$

$$\sigma_A^2 = \text{Var}(A_i) \quad \text{variance component for factor } A$$

$$\sigma_B^2 = \text{Var}(B_i) \quad \text{variance component for factor } B$$

$$\sigma_{AB}^2 = \text{Var}(AB)_{ij} \quad \text{variance component for interaction}$$

$$\sigma_{B(A)}^2 = \text{Var}(B_{j(i)}) \quad \text{variance component for factor } B$$

$$\sigma^2 = \text{Var}(E_{ijk}) \quad \text{error variance}$$

The term effect size is often used in power considerations and sometimes involves division by  $\sigma^2$ .

Using expected mean squares to analyze data in mixed-effects models

F-tests and estimating variance components.

- 1. To test for interaction effect, use  $F_{AB} = \frac{MS[AB]}{MS[E]}$
- 2. To test for main effect of A, use  $F_A = \frac{MS[A]}{MS[AB]}$
- 3. To test for main effect of B, use  $F_B = \frac{MS[B]}{MS[AB]}$

Note the departure from fixed-effects analysis, where MS[E] is always used in the denominator.

The estimated variance components satisfy the system of equations by equate (observed) mean squares to their expected values.

For example, for a 2 factor crossed, random-effects model

$$MS[E] = \hat{\sigma}^2$$

$$MS[AB] = \hat{\sigma}^2 + n\hat{\sigma}_{AB}^2$$

$$MS[A] = \hat{\sigma}^2 + nb\hat{\sigma}_A^2 + n\hat{\sigma}_{AB}^2$$

$$MS[B] = \hat{\sigma}^2 + na\hat{\sigma}_B^2 + n\hat{\sigma}_{AB}^2$$

### Analysis of variance in nested designs

Consider a two-factor design in which factor B is nested in factor A. Let  $Y_{ijk}$  denote the  $k^{th}$  response at level j of factor B within level i of factor A. A model:

$$Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \epsilon_{ijk}$$

for i = 1, 2, ..., a,  $j = 1, 2, ..., b_i$ , k = 1, 2, ..., n SS[Tot] can be broken down into components reflecting variability due to A, B(A) and variability not due to either factor (SS[E]): SS[Tot] = SS[A] + SS[B(A)] + SS[E]

$$SS[Tot] = \sum_{i} \sum_{j} \sum_{k} (y_{ijk} - \bar{y}_{+++})^{2}$$

$$SS[A] = \sum_{i} \sum_{j} \sum_{k} (\bar{y}_{i++} - \bar{y}_{+++})^{2}$$

$$SS[B(A)] = \sum_{i} \sum_{j} \sum_{k} (\bar{y}_{ij+} - \bar{y}_{i++})^{2}$$

$$SS[E] = \sum_{i} \sum_{j} \sum_{k} (y_{ijk} - \bar{y}_{ij+})^{2}$$

The ANOVA table looks like

Source	df	Sum of Squares	Mean Square	$\mathbf{F}$
$\overline{A}$	a-1	SS[A]	$MS[A] = \frac{SS[A]}{a-1}$	$F_A = \frac{MS[A]}{MS[E]}$
B(A)	$\sum_{i}(b_{i}-1)$	SS[B(A)]	$MS[B(A)] = \frac{SS[B(A)]}{\sum_{i}(b_i - 1)}$	$F_{B(A)} = \frac{MS[B(A)]}{MS[E]}$
Error	$N - \sum b_i$	SS[E]	$MS[E] = \frac{SS[E]}{N - \sum b_i}$	
Total	N-1	SS[Tot]		

And with random-effect, the test statistic becomes

Test for	A, B(A) fixed	A fixed, $B(A)$ random	A, B(A) random
Factor $A$	MS[A]/MS[E]	MS[A]/MS[B(A)]	MS[A]/MS[B(A)]
Factor $B(A)$	MS[B(A)]/MS[E]	MS[B(A)]/MS[E]	MS[B(A)]/MS[E]