- Levels are a random sample
- Variability between levels is of interest
- Nested vs. crossed factors

Serum measurements of blood samples.

Model:

$$Y_{ij} = \mu + a_i + \epsilon_{ij}, \qquad i = 1, \dots, I; j = 1, \dots, J$$

 $a_i$  random effect of sample *i*,  $a_i \sim \mathcal{N}(0, \sigma_a^2)$ ,  $\epsilon_{ij}$  error of *j*th measurement of sample *i*,  $\epsilon_i \sim \mathcal{N}(0, \sigma_e^2)$ ,  $a_i$  and  $\epsilon_{ij}$  are all independent.

$$Var(Y_{ij}) = Var(a_i + \epsilon_{ij}) = \sigma_a^2 + \sigma_e^2, \quad Cov(Y_{ij}, Y_{ij'}) = \sigma_a^2$$

The variance of  $Y_{ij}$  consists of two components. Such models are also called variance components models.

## Illustration



## Anova Table

$$H_0: \sigma_a^2 = 0 \qquad H_A: \sigma_a^2 > 0$$
Source SS df MS=SS/df
Sample  $SS_a = \sum \sum (y_{i.} - y_{..})^2 \quad I - 1 \qquad MS_a$ 
Residual  $SS_{res} = \sum \sum (y_{ij} - y_{i.})^2 \quad N - I \qquad MS_{res}$ 
Total  $SS_{tot} = \sum \sum (y_{ij} - y_{..})^2 \quad N - 1$ 

$$E(MS_{res}) = \sigma_e^2, \qquad E(MS_a) = J\sigma_a^2 + \sigma_e^2$$

Can use  $F = MS_a/MS_{res}$  to test H<sub>0</sub>.

$$\hat{\sigma}_{e}^{2} = MS_{res}$$

$$\hat{\sigma}_{a}^{2} = (MS_{a} - MS_{res})/J \quad \text{can be negative!}$$

$$\hat{\mu} = y_{..} \quad \text{with } Var(\hat{\mu}) = \frac{1}{I}(\sigma_{a}^{2} + \sigma_{e}^{2}/J)$$

Either Maximum Likelihood estimators or  $\hat{\sigma}_a^2 \ge 0$ 

$$Y_{ijk} = \mu + a_i + b_j + \epsilon_{ijk}$$

 $a_i$  random effect of lab i,  $a_i \sim \mathcal{N}(0, \sigma_a^2)$ ,  $b_j$  random effect of sample j,  $b_j \sim \mathcal{N}(0, \sigma_b^2)$ ,  $\epsilon_{ijk}$  measurement error,  $\epsilon_{ijk} \sim \mathcal{N}(0, \sigma_e^2)$ , all random variables are independent.

Source	df	E(MS)	F
Lab	I-1	$\sigma_e^2 + JK\sigma_a^2$	$MS_a/MS_{res}$
Sample	J-1	$\sigma_e^2 + IK\sigma_h^2$	$MS_b/MS_{res}$
Residual	$\ll$ diff $\gg$	$\sigma_e^2$	
Total	IJK - 1		

## Model with Interaction Lab:Sample

Source	E(MS)	$H_0$	F
Lab	$\sigma_e^2 + JK\sigma_a^2 + K\sigma_{ab}^2$	$\sigma_a^2 = 0$	$MS_a/MS_{ab}$
Sample	$\sigma_e^2 + IK\sigma_b^2 + K\sigma_{ab}^2$	$\sigma_b^2 = 0$	$MS_b/MS_{ab}$
Lab : Sample	$\sigma_e^2 + K\sigma_{ab}^2$	$\sigma_{ab}^2 = 0$	$MS_{ab}/MS_{res}$
Residual	$\sigma_e^2$		

 $\begin{array}{ll} H_0: \sigma_a^2 = 0 & \text{Test statistic: } F = MS_a/MS_{ab} \\ H_0: \sigma_a^2 = \sigma_{ab}^2 = 0 & \text{Test statistic: } F = MS_a/MS_{res} \end{array}$ 

Factors A and B are called crossed if every level of B occurs with every level of A. A factorial design involves crossed factors.

	Factor A					
Factor B	1	2	3	4		
1	XX	XX	XX	XX		
2	XX	XX	XX	xx		
3	XX	XX	XX	xx		

Factors A and B are called **nested** if there are different levels of B within each level of A. B is nested within A in the following layout.

Α	1			2	3			4				
В	1	2	3	4	5	6	7	8	9	10	11	12
	XX											

Designs with nested factors are called nested designs or hierarchical designs.



The factor Sample is nested within Lab.

Model for a two-stage nested design:

$$Y_{ijk} = \mu + a_i + b_{j(i)} + \epsilon_{k(ij)}, \qquad i = 1, ..., I; j = 1, ..., J; k = 1, ..., K$$

The subscript j(i) indicates that the *j*th level of factor B is nested within the *i*th level of factor A.

Question: Is an interaction term important?

Decomposition of sum of squares:

$$y_{ijk} - y_{...} = y_{i..} - y_{...} + y_{ij.} - y_{i..} + y_{ijk} - y_{ij.}$$

$$SS_{tot} = SS_A + SS_{B(A)} + SS_{res}$$
.

Source	df	E(MS)
Lab	I-1	$\sigma_e^2 + K\sigma_b^2 + JK\sigma_a^2$
Sample	I(J-1)	$\sigma_e^2 + K \sigma_b^2$
Residual	"diff"	$\sigma_e^2$
Total	IJK - 1	

Effect of milling on moisture content. 3 samples of 100g from 5 batches were milled. From each sample 10g are measured three times.

	sample								
batch	1			2			3		
1	9.3	9.2	8.8	8.6	8.7	9.9	8.9	8.7	8.5
2	8.0	8.2	9.2	9.7	9.4	8.2	9.3	9.5	9.4
3	11.0	10.7	9.9	9.3	13.9	9.2	9.2	10.9	9.7
4	10.1	10.2	9.9	8.6	9.4	8.3	8.3	9.9	9.5
5	12.0	9.3	10.8	12.2	9.6	11.7	11.4	9.8	12.4

- > mod1=aov(moisture~batch + sample%in%batch)
- > summary(mod1)

 Df Sum Sq Mean Sq F value
 Pr(>F)

 batch
 4 30.928
 7.7320
 7.0390
 0.0004027 \*\*\*

 batch:sample
 10
 5.911
 0.5911
 0.5381
 0.8491520

 Residuals
 30
 32.953
 1.0984

$$\hat{\sigma}_{e}^{2} = 1.0984$$
  $MS_{res}$   
 $\hat{\sigma}_{s}^{2} = (0.5911 - 1.0984)/3 = 0$   $(MS_{s} - MS_{res})/K$   
 $\hat{\sigma}_{b}^{2} = (7.732 - 1.0984)/9 = 0.737$   $(MS_{b} - MS_{s})/JK$ 

## Linear mixed-effects model fit

```
> library(nlme)
> summary(lme(moisture~1,random=~1|batch/sample))
Random effects:
Formula: ~1 | batch
        (Intercept)
StdDev: 0.8666916
 Formula: ~1 | sample %in% batch
         (Intercept) Residual
StdDev: 3.783493e-05 0.9857034
Number of Observations: 45
Number of Groups: batch sample %in% batch
                    5
                                      15
```