Multivariate Statistics in Ecology and Quantitative Genetics **1. ANalysis Of VAriance (ANOVA)** 

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http://evol.bio.lmu.de/StatGen.html

17. Mai 2010

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ANOVA and F-Test

Kruskal-Walis Test



ANOVA and F-Test

## Are the group means significantly different?

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#### Or does this look like random deviations?

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Or does this look like random deviations?

This depends on the ratio of the varibility of group means and the variability within the groups.

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Or does this look like random deviations?

This depends on the ratio of the varibility of group means and the variability within the groups. The analysis of variance (ANOVA) quantifies this ratio and its significance.

### Blood-clotting time in rats under 4 different treatments

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group	observation							
1	62	60	63	59				
2	63	67	71	64	65	66		
3	68	66	71	67	68	68		
4	56	62	60	61	63	64	63	59

#### Blood-clotting time in rats under 4 different treatments

group	observation							
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2	63	67	71	64	65	66		
3	68	66	71	67	68	68		
4	56	62	60	61	63	64	63	59

global mean  $\overline{x}_{..} = 64$ ,

group means  $\overline{x}_{1.} = 61$ ,  $\overline{x}_{2.} = 66$ ,  $\overline{x}_{3.} = 68$ ,  $\overline{x}_{4.} = 61$ .

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Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{i}$ .	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	$(61 - 61)^2$	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

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global mean  $\overline{x}_{..} = 64$ , group means  $\overline{x}_{1.} = 61$ ,  $\overline{x}_{2.} = 66$ ,  $\overline{x}_{3.} = 68$ ,  $\overline{x}_{4.} = 61$ .

Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	(61 – 61) <sup>2</sup>	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

global mean  $\overline{x}_{..} = 64$ ,

group means  $\overline{x}_{1.} = 61$ ,  $\overline{x}_{2.} = 66$ ,  $\overline{x}_{3.} = 68$ ,  $\overline{x}_{4.} = 61$ .

The red Differences (unsquared) are the *residuals*: they are the residual variability which is not explained by the model.

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Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	$(63 - 61)^2$	(59 – 61) <sup>2</sup>				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	(71 – 66) <sup>2</sup>	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	$(61 - 61)^2$	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

global mean  $\overline{x}_{..} = 64$ ,

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Sums of squares within groups:

 $ss_{\rm within} = 112,$ 

Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observatio	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	(61 – 61) <sup>2</sup>	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

global mean  $\overline{x}_{..} = 64$ ,

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Sums of squares within groups:

 $ss_{\rm within} = 112$ , 20 degrees of freedom (df)

Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	(61 – 61) <sup>2</sup>	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

global mean  $\overline{x}_{..} = 64$ ,

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Sums of squares within groups:

 $ss_{\rm within} = 112, 20$  degrees of freedom (df)

Sums of squares between groups:

 $\textit{ss}_{\rm betw} = 4 \cdot (61 - 64)^2 + 6 \cdot (66 - 64)^2 + 6 \cdot (68 - 64)^2 + 8 \cdot (61 - 64)^2 = 228,$ 

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Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	(63 – 61) <sup>2</sup>	(59 – 61) <sup>2</sup>				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
4	61	56	62	60	61	63	64	63	59
		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	(61 – 61) <sup>2</sup>	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

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Sums of squares within groups:

 $ss_{\rm within} = 112, 20$  degrees of freedom (df)

Sums of squares between groups:

 $ss_{betw} = 4 \cdot (61 - 64)^2 + 6 \cdot (66 - 64)^2 + 6 \cdot (68 - 64)^2 + 8 \cdot (61 - 64)^2 = 228,$ 3 degrees of freedom (df)

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Blood-clotting times in rats under 4 different treatments

gr.	$\overline{X}_{j.}$	observation	ns						
1	61	62	60	63	59				
		$(62 - 61)^2$	$(60 - 61)^2$	(63 – 61) <sup>2</sup>	(59 – 61) <sup>2</sup>				
2	66	63	67	71	64	65	66		
		$(63 - 66)^2$	$(67 - 66)^2$	$(71 - 66)^2$	$(64 - 66)^2$	$(65 - 66)^2$	$(66 - 66)^2$		
3	68	68	66	71	67	68	68		
		$(68 - 68)^2$	$(66 - 68)^2$	(71 – 68) <sup>2</sup>	$(67 - 68)^2$	$(68 - 68)^2$	$(68 - 68)^2$		
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		$(56 - 61)^2$	$(62 - 61)^2$	$(60 - 61)^2$	(61 – 61) <sup>2</sup>	$(63 - 61)^2$	$(64 - 61)^2$	$(63 - 61)^2$	$(59 - 61)^2$

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Sums of squares within groups:

 $ss_{\rm within} = 112$ , 20 degrees of freedom (df)

Sums of squares between groups:

 $ss_{betw} = 4 \cdot (61 - 64)^2 + 6 \cdot (66 - 64)^2 + 6 \cdot (68 - 64)^2 + 8 \cdot (61 - 64)^2 = 228,$ 3 degrees of freedom (df)

$$F = \frac{ss_{\rm betw}/3}{ss_{\rm within}/20} = \frac{76}{5.6} = 13.57$$

Example: Blood-clotting times in rats under 4 different treatments.

### ANOVA table ("ANalysis Of VAriance")

	df	sum of squares (ss)	mean of (ss/df)	sum squares	F value
groups	3	228		76	13.57
residuals	20	112		5.6	

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Under the Null-Hypothesis  $H_0$  "the group means are equal" (and assuming independent, normally distributed observations) is *F* Fisher-distributed with 3 and 20 degrees of freedom, and  $p = \text{Fisher}_{3,20}([13.57,\infty)) \le 5 \cdot 10^{-5}$ .

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residuals	20	112		5.6	

Under the Null-Hypothesis  $H_0$  "the group means are equal" (and assuming independent, normally distributed observations) is *F* Fisher-distributed with 3 and 20 degrees of freedom, and  $p = \text{Fisher}_{3,20}([13.57,\infty)) \le 5 \cdot 10^{-5}$ . Thus, we can reject  $H_0$ .

ANOVA and F-Test



# Sir Ronald Aylmer Fisher, 1890–1962

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### $n = n_1 + n_2 + \dots + n_l$ obersvations in *l* groups, $X_{ij} = j$ -th observation in *i*-th group, $j = 1, \dots, n_i$ .

 $n = n_1 + n_2 + \dots + n_i$  observations in *I* groups,

 $X_{ij} = j$ -th observation in *i*-th group,  $j = 1, \ldots, n_i$ .

Model assumption:  $X_{ij} = \mu_i + \varepsilon_{ij}$ , with independent, normally distributed  $\varepsilon_{ij}$ ,  $\mathbb{E}[\varepsilon_{ij}] = 0$ ,  $\operatorname{Var}[\varepsilon_{ij}] = \sigma^2$ 

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 $n = n_1 + n_2 + \cdots + n_l$  obersvations in *l* groups,

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$$\overline{X}_{..} = rac{1}{n} \sum_{i=1}^{l} \sum_{j=1}^{n_i} X_{ij}$$
 (empirical) "global mean"  
 $\overline{X}_{i.} = rac{1}{n_i} \sum_{j=1}^{n_i} X_{ij}$  (empirical) mean of group *i*

 $n = n_1 + n_2 + \cdots + n_l$  obersvations in *l* groups,

 $X_{ij} = j$ -th observation in *i*-th group,  $j = 1, \ldots, n_i$ .

Model assumption:  $X_{ij} = \mu_i + \varepsilon_{ij}$ , with independent, normally distributed  $\varepsilon_{ij}$ ,  $\mathbb{E}[\varepsilon_{ij}] = 0$ ,  $\operatorname{Var}[\varepsilon_{ij}] = \sigma^2$ ( $\mu_i$  is the "true" mean within group *i*.)

$$\overline{X}_{..} = rac{1}{n} \sum_{i=1}^{l} \sum_{j=1}^{n_i} X_{ij}$$
 (empirical) "global mean"  
 $\overline{X}_{j.} = rac{1}{n_i} \sum_{j=1}^{n_i} X_{ij}$  (empirical) mean of group *i*

 $egin{aligned} SS_{ ext{within}} &= \sum\limits_{i=1}^{l} \sum\limits_{j=1}^{n_i} (X_{ij} - \overline{X}_{i.})^2 \ SS_{ ext{betw}} &= \sum\limits_{i=1}^{l} n_i (\overline{X}_{i.} - \overline{X}_{..})^2 \end{aligned}$ 

sum of squares within the groups, n - I degrees of freedom

sum of squares between the groups, I - 1 degrees of freedom

 $n = n_1 + n_2 + \cdots + n_l$  observations in *l* groups,

 $X_{ij} = j$ -th observation in *i*-th group,  $j = 1, \ldots, n_i$ .

Model assumption:  $X_{ij} = \mu_i + \varepsilon_{ij}$ , with independent, normally distributed  $\varepsilon_{ij}$ ,  $\mathbb{E}[\varepsilon_{ij}] = 0$ ,  $\operatorname{Var}[\varepsilon_{ij}] = \sigma^2$ ( $\mu_i$  is the "true" mean within group *i*.)

$$\overline{X}_{..} = rac{1}{n} \sum_{i=1}^{l} \sum_{j=1}^{n_i} X_{ij}$$
 (empirical) "global mean"  
 $\overline{X}_{j.} = rac{1}{n_i} \sum_{j=1}^{n_i} X_{ij}$  (empirical) mean of group *i*

$$SS_{ ext{within}} = \sum_{i=1}^{l} \sum_{j=1}^{n_i} (X_{ij} - \overline{X}_{i.})^2$$

sum of squares within the groups, n - I degrees of freedom

$$SS_{\text{betw}} = \sum_{i=1}^{l} n_i (\overline{X}_{i\cdot} - \overline{X}_{\cdot\cdot})^2$$

sum of squares between the groups, I - 1 degrees of freedom

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$$F = rac{SS_{
m betw}/(I-1)}{SS_{
m within}/(n-I)}$$

 $X_{ij} = j$ -th observation *i*-th group,  $j = 1, ..., n_i$ , Model assumption:  $X_{ij} = \mu_i + \varepsilon_{ij}$ .  $\mathbb{E}[\varepsilon_{ij}] = 0$ ,  $\operatorname{Var}[\varepsilon_{ij}] = \sigma^2$ 

$$SS_{ ext{within}} = \sum_{i=1}^{l} \sum_{j=1}^{n_i} (X_{ij} - \overline{X}_{i\cdot})^2$$

sum of squares within groups, n - I degrees of feedom

 $SS_{\text{betw}} = \sum_{i=1}^{l} n_i (\overline{X}_{i.} - \overline{X}_{..})^2$  sum of squares between groups, l - 1 degrees of freedom

$$F = rac{SS_{
m betw}/(I-1)}{SS_{
m within}/(n-I)}$$

Under the hypothesis  $H_0: \mu_1 = \cdots = \mu_I$  ("all  $\mu_i$  are equal") *F* is Fisher-distributed with I - 1 and n - I degrees of freedom (no matter what the true joint value of  $\mu_i$  is).

*F*-Test: We reject  $H_0$  on the level of significance  $\alpha$  if  $F \ge q_{\alpha}$ , whereas  $q_{\alpha}$  is the  $(1 - \alpha)$ -quantile of the Fisher-distribution with I - 1 and n - I degrees of freedom.

Kruskal-Walis Test

### The statistic of the Kruskal-Wallis test

$$\left[\frac{12}{\sum^{a} n_{i}\left(\sum^{a} n_{i}+1\right)} \sum^{a} \frac{\left(\sum^{n_{i}} R\right)_{i}^{2}}{n_{i}}\right] - 3 \cdot \left(\sum^{a} n_{i}+1\right)$$

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Kruskal-Walis Test

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$$\left[\frac{12}{\sum^{a} n_{i}\left(\sum^{a} n_{i}+1\right)} \sum^{a} \frac{\left(\sum^{n_{i}} R\right)_{i}^{2}}{n_{i}}\right] - 3 \cdot \left(\sum^{a} n_{i}+1\right)$$

Explanations are given verbally in the lecture and in Sokal and Rohlf (1995) *Biometry*, 3rd ed., on pp. 423–426.

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