Two-Way ANOVA
(Ch 29 De Veaux, Velleman & Bock)

- A method for determining the effect of two sources of variation on a continuous response.
- The effect of one factor may differ depending on the level of the other factor - this is called *interaction*.

In a two way **factorial design**

- two factors affect the response
- each level of one factor is used in combination with every level of the other factor
- a set of homogeneous experimental units (eg subjects) are randomly allocated to the 2-factor treatment combinations

Example: The effect of lethal histamine shock on the guinea pig thymus was studied to determine if changes in the thymus correspond to pathological changes observed in SIDS victims. Results below are the medullar blood vessel surface (mm^2/mm^3). (Baak & Huber, 1974; Van Belle & Fisher, 1993)

	Control					Histamine Shock				
Female	6.4	6.2	6.9	6.9	5.4	8.4	10.2	6.2	5.4	5.5
	7.5	6.1	7.3	5.9	6.8	7.3	5.2	5.1	5.7	9.8
Male	4.3	7.5	5.2	4.9	5.7	7.5	6.7	5.7	4.9	6.8
	4.3	6.4	6.2	5.0	5.0	6.6	6.9	11.8	6.7	9.0

- Averaging over the animals within each cell

	Control	Histamine Shock	Difference
Female	6.54	6.88	.34
Male	5.45	7.26	1.81

Questions

1. Is there an effect of histamine shock?
2. Is there a difference between the sexes?
3. Is the effect of histamine shock different for males and females?

- This is a factorial experiment with factors *sex* and *shock*.

The ANOVA table contains lines for each factor, and for the interaction.

Source	Sum of Squares	Degrees of Freedom	Mean Square	F	P
Sex	1.260	1	1.260	.566	.456
Shock	11.556	1	11.556	5.195	.029
Shock $\times$ Sex	5.402	1	5.402	2.428	.128
Residual	80.089	36	2.225		
Total	98.308	39			

Notes:

- "Residual" is synonymous with "Error"
- Shock $\times$ Sex means the "Interaction" of Shock and Sex.
- We conclude:
  1. That the interaction between shock and sex is not significant.
  2. That there is strong evidence of a shock effect.
  3. That there was no significant difference between the sexes.

The general case

- Call the two factors A and B, and let a and b be the number of levels of each.
- The response is modeled as the sum of several terms

$$y = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon$$

  where
  - α_i is the effect (additive contribution) of level i of factor A
  - β_j is the effect of level j of factor B
  - $(\alpha\beta)_{ij}$ is the interaction contribution for level i of factor A and level j of factor B
  - ϵ is the random deviation of the response about its mean
- We usually assume ϵ is normally distributed with mean 0 and constant variance σ^2, and that the different deviations are independent of each other.

- Interaction occurs when the effect of one factor is different for different levels of the other factor. **What does this mean graphically?**

- In the table below, the effect of factor B is 10 regardless of whether factor A is at the low (L) or high (H) level, so there is no interaction.

		B	
		L	H
A	L	10	20
	H	15	25

- The following tables illustrate interaction.

		B	
		L	H
A	L	10	20
	H	15	5

		B	
		L	H
A	L	10	20
	H	10	10

Things to remember:

- **Additivity relationship for the total sum of squares.**

$$TSS = SSA + SSB + SSAB + SSE$$

- There are complicated formulae for each of the sums of squares.
- **Additivity relationships for the total degrees of freedom.**

$$n - 1 = a - 1 + b - 1 + (a - 1)(b - 1) + (n - ab)$$

- **If there are r replicate observations in each cell, then $n = rab$** and
  - **the total degrees of freedom is** $rab - 1$
  - **the degrees of freedom for each factor is the number of levels minus 1,** ie $df_A = a - 1$, $df_B = b - 1$
  - **the interaction degrees of freedom is the product of the degrees of freedom for the two factors,** $(a - 1)(b - 1)$
  - **the residual degrees of freedom is** $ab(r - 1)$
- **When there is only one observation in each cell (**$r = 1$**) the residual degrees of freedom is zero, in this case**
  - **there is no way to test for the presence of interaction**
  - **and** SS_{AB} **is then used in place of** SSE
- The results are usually presented in an ANOVA table.

Source	Sum of Squares	DF	Mean Square	F	P
A	SS_A	$a - 1$	MS_A	MS_A/MSE	
B	SS_B	$b - 1$	MS_B	MS_B/MSE	
$A \times B$	SS_{AB}	$(a - 1)(b - 1)$	MS_{AB}	MS_{AB}/MSE	
Residual	SSE	$ab(r - 1)$	MSE		
Total	TSS	$rab - 1$			

- **The mean squares are the sums of squares divided by their degrees of freedom.**
- MSE **is an estimate of the variance of the deviations,** σ^2**.**
- **The** F **statistics standardize the contribution of the factor (i.e. its sum of squares) so that comparison with the** F **distribution is possible.**
- The hypotheses are
  1. for interaction
     H_0 : there is no interaction
     H_a : there is interaction
  2. for factor A
     H_0 : there is no effect of factor A
     H_a : there is an effect of factor A
  3. for factor B
     H_0 : there is no effect of factor B
     H_a : there is an effect of factor B
- **There are p-values for each of the three hypothesis tests.**
  - The p-value for the test of no interaction is $P(F_{(a-1)(b-1),ab(r-1)} \geq F_{obs})$ where $F_{obs} = MS_{AB}/MSE$.
  - The p-value for the test of no effect of factor A is is $P(F_{(a-1),ab(r-1)} \geq F_{obs})$ where $F_{obs} = MS_A/MSE$.
  - The p-value for the test of no effect of factor B is is $P(F_{(b-1),ab(r-1)} \geq F_{obs})$ where $F_{obs} = MS_B/MSE$.
  - **Note that for each test, the denominator degrees of freedom is the degrees of freedom for error, and the numerator degrees of freedom is the degrees of freedom for that term (line) in the ANOVA table.**
- **In a factorial experiment, you must test for interactions first**
  - **if the interaction is significant** this means that both factors have an effect, and that their effects differ depending on the level of the other factor, so **it does not make sense to test for the main effects of the individual factors**

Multiple comparisons:

- When a significant result is found, one usually wants to determine the source of the difference by comparing means.
- An adjustment like the Bonferroni correction should be used to ensure that the overall probability of a type one error is not inflated.
- **You won't be asked to do carry out multiple comparisons procedures for two way ANOVA.**

References

- Baak, J.P.A and Huber, J. (1974) in SIDS 1974, Proceedings of the Francis E. Camps International Symposium of Sudden and Unexpected Deaths in infacy, R.R. Robertson (ed.). Canadian Foundation for the study of Infant Death, Toronto, Ontario, Canada.

Experimental Designs
In a two way **factorial design**

- two factors affect the response
- each level of one factor is used in combination with every level of the other factor
- a set of homogeneous experimental units (eg subjects) are randomly allocated to the 2-factor treatment combinations

In a **randomized block design**:

- groups of experimental units which are similar form blocks, e.g. animal litters, tissues
- each treatment is each applied to experimental units within each block
- within-block comparisons among treatments are combined across blocks
- we assume the treatment differences are the same within each block, i.e. that there is no interaction
- this is an extension of the paired two-sample design
- can be used with repeated observations on people

Our focus has been be on the factorial design.
In the histamine shock example, one could argue that males and females represent different homogeneous groups of experimental units, so that this was a randomized block design. However, the researchers were specifically interested in whether the effect was different for the two sexes, and so we must allow for an interaction effect. Only the factorial design allows the effect of one factor to differ between levels of the other factor.