Lecture 19: Two-Way ANOVA

GENOME 560

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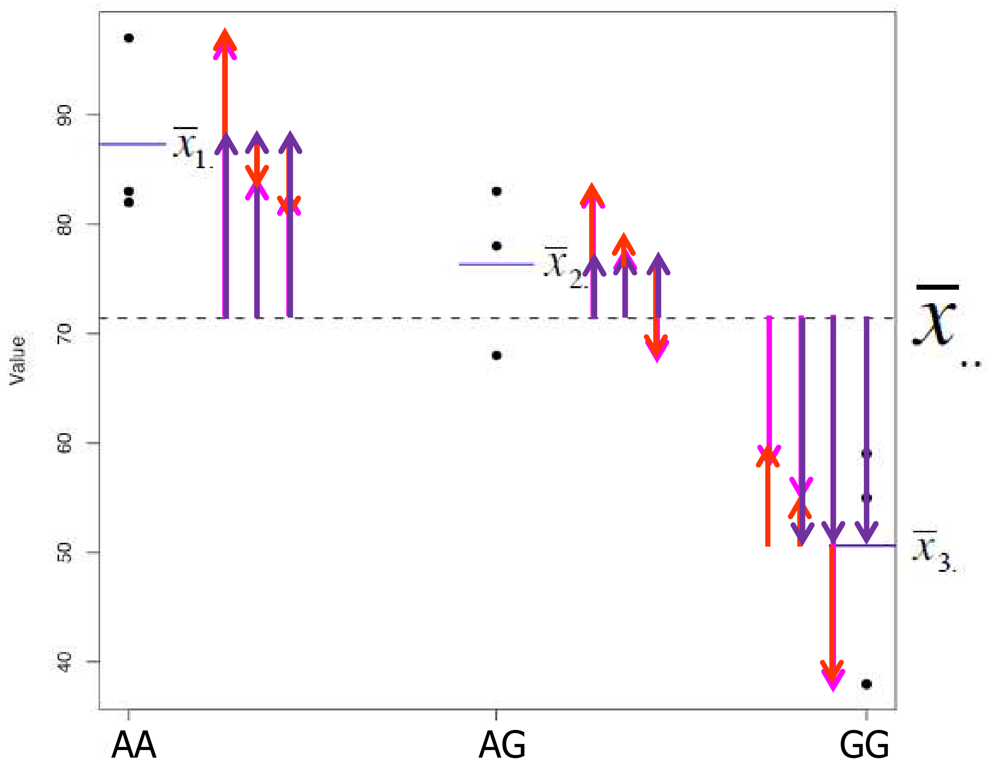
Review of Last Lecture

- Total variation can be partitioned into between-group variation and within-group variation

$$SST = SST_G + SST_E$$

$$\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij} - \bar{x}_{..})^2 \qquad \sum_{i=1}^{K} n_i \bullet (\bar{x}_{i.} - \bar{x}_{..})^2 \qquad \sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij} - \bar{x}_{i.})^2$$



x_ij **:** *j*th observation in the *i*th genotype group

x_{11} x_{12} x_{13}

AA: 82, 83, 97 → *average* → $\bar{x}_{1.}$

AG: 83, 78, 68 → *average* → $\bar{x}_{2.}$

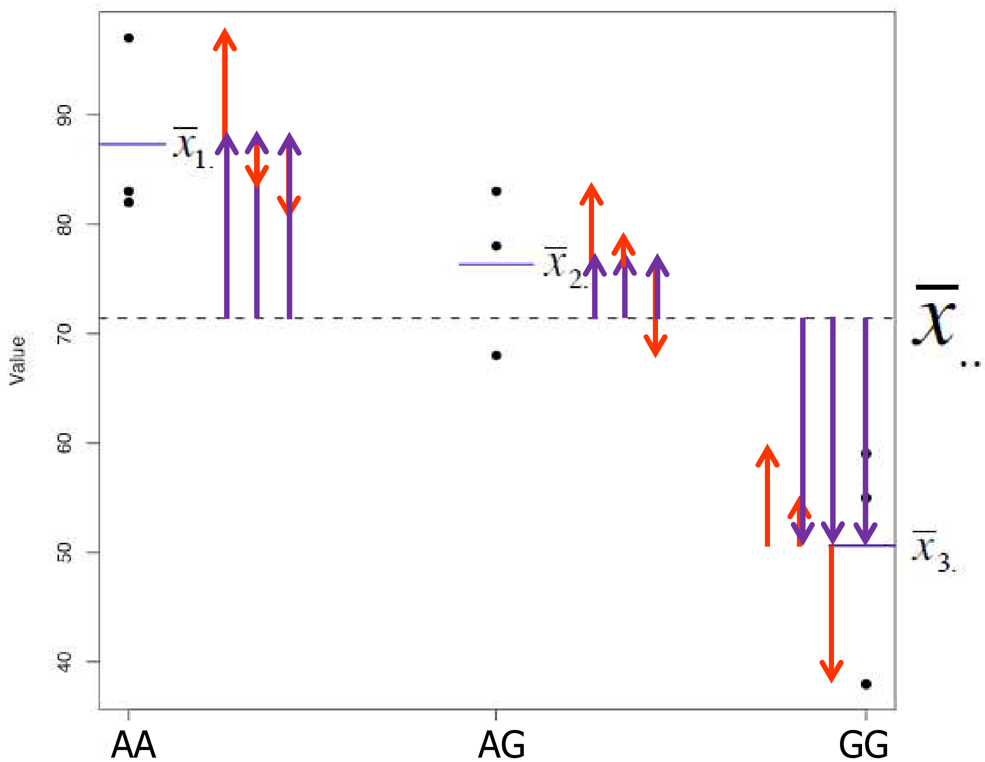
GG: 38, 59, 55 → *average* → $\bar{x}_{3.}$

Grand mean $\bar{x}_{..}$

ANOVA: comparing variances

- Compare between-group variation with within-group variation

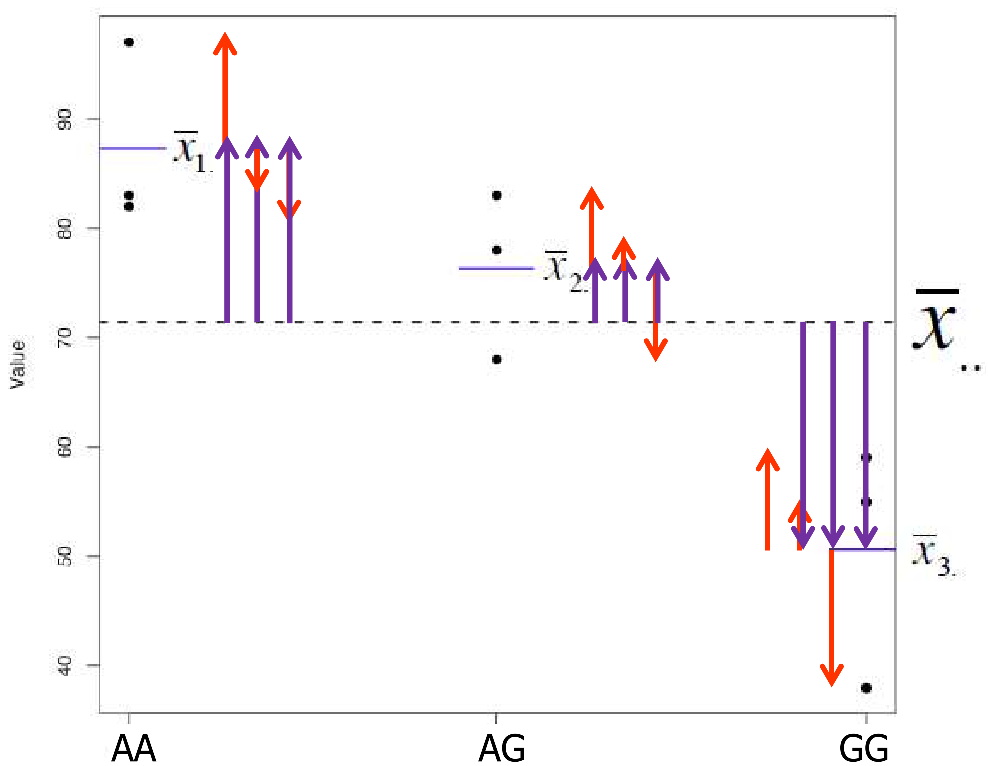
$$\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2}{\text{SST}} = \boxed{\underset{\sum_{i=1}^{K} n_i \bullet (\bar{x}_{i.}-\bar{x}_{..})^2}{\text{SST}_G}} + \boxed{\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2}{\text{SST}_E}}$$



Degrees of freedom in ANOVA

- Compare between-group variation with within-group variation

$$\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2}{\text{SST}} = \boxed{\underset{\sum_{i=1}^{K} n_i \bullet (\bar{x}_{i.}-\bar{x}_{..})^2}{\text{SST}_G}} + \boxed{\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2}{\text{SST}_E}}$$



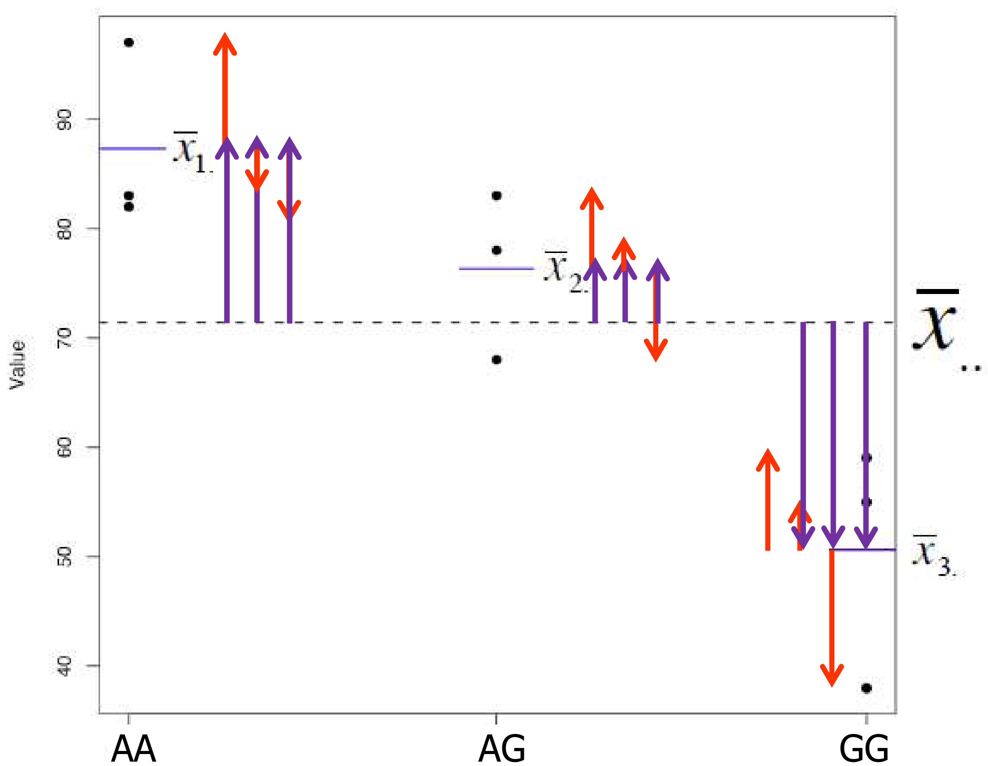
- There are N data points and K groups
- Df: (# independent scores) – (# intermediate scores)
- Between-group variance
  - Df: (K-1)
- Within-group variance
  - Df: (N-K)

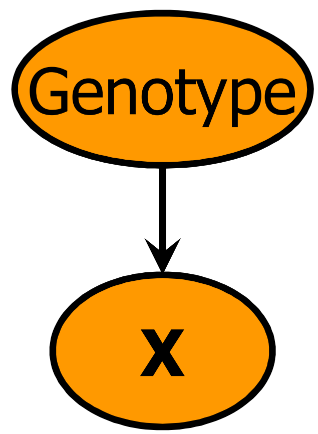
Degrees of freedom in ANOVA

- Compare **between-group variation** with within-group variation

$$\text{SST} = \boxed{\text{SST}_G} + \boxed{\text{SST}_E}$$

$$\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2 = \boxed{\sum_{i=1}^{K} n_i \bullet (\bar{x}_{i.}-\bar{x}_{..})^2} + \boxed{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2}$$

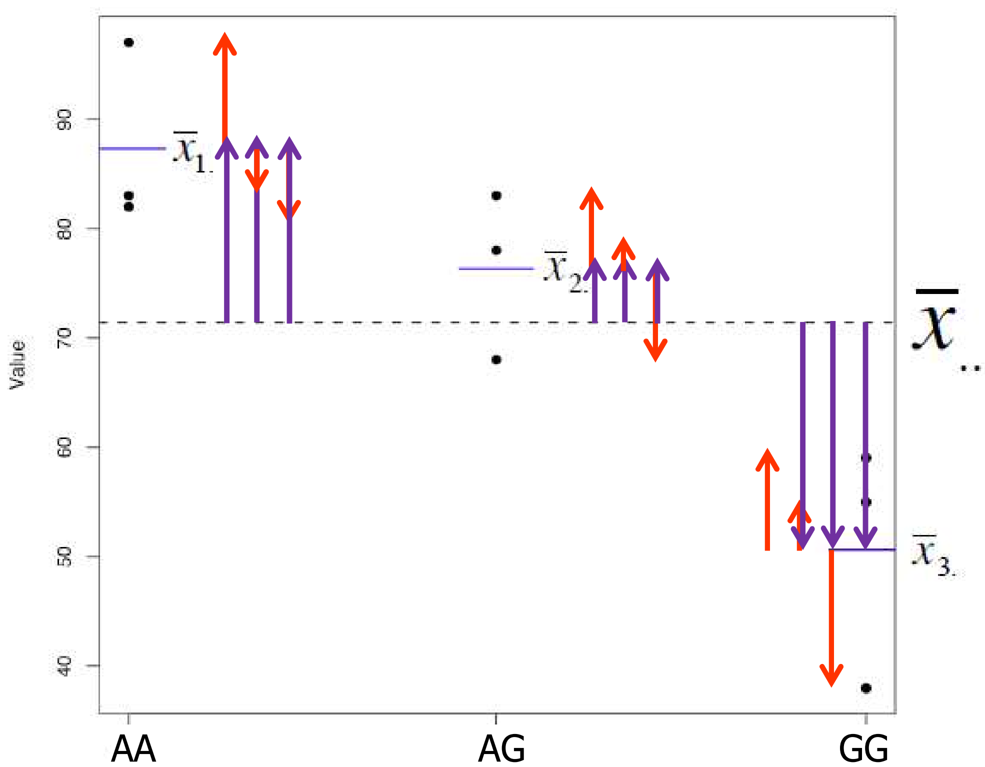


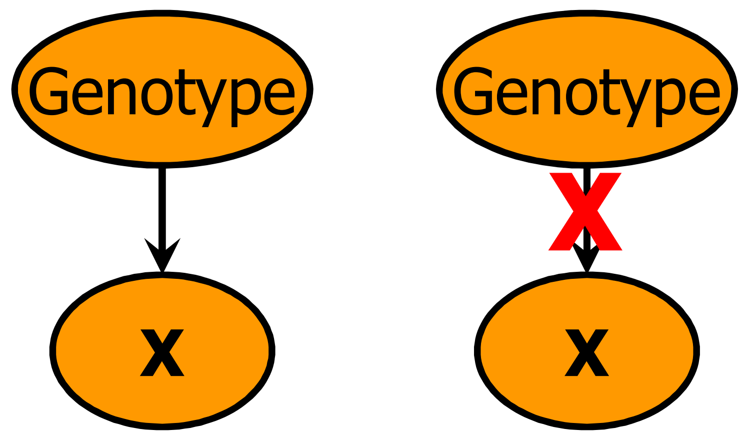


Degrees of freedom in ANOVA

- Compare between-group variation with **within-group variation**

$$\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{..})^2}{\text{SST}} = \boxed{\underset{\sum_{i=1}^{K} n_i \bullet (\bar{x}_{i.}-\bar{x}_{..})^2}{\text{SST}_G}} + \boxed{\underset{\sum_{i=1}^{K}\sum_{j=1}^{J}(x_{ij}-\bar{x}_{i.})^2}{\text{SST}_E}}$$





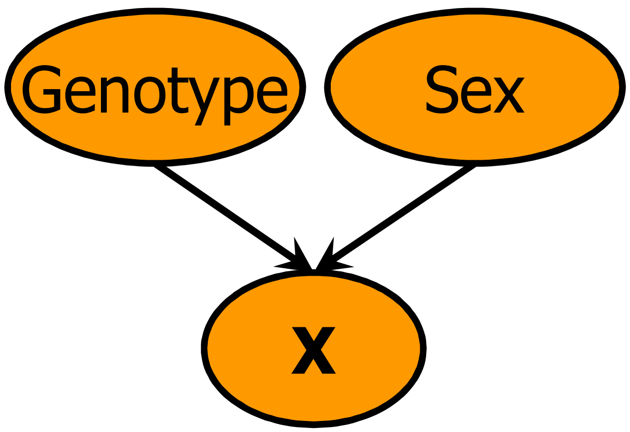
Outline

- Two-way ANOVA
  - ANOVA table
  - Decomposition of total variance
  - Measuring interaction between factors
  - Null hypothesis

- R exercise

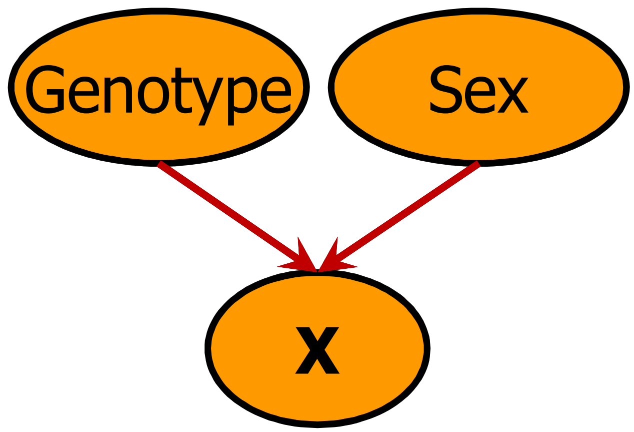
Two-Way ANOVA

- An extension of the one-way ANOVA test
- *Examines the influence of two different factors on one outcome variables*



Two-Way ANOVA

- The two-way ANOVA can do the followings:
  - Determining the *effect of contributions of each factor*
  - Identifying if there is a significant *interaction between the factors*



Two-Way ANOVA Data Table

Factor A	Factor B			
	1	2	…	b
1	y_{111} y_{112} :	y_{121} y_{122} :	…	y_{1b1} y_{1b2} :
2	y_{211} y_{212} :	y_{221} y_{222} :	…	y_{2b1} y_{2b2} :
:	:	:	:	:
a	y_{a11} y_{a12} :	y_{a21} y_{a22} :	…	y_{ab1} y_{ab2} :

$\boldsymbol{y_{ijk}}$ **:** *k*th observation in the *i*th level of factor A and *j*th level of factor B

Example with Two Factors

Gender
female/male

Factor A	Factor B			
	1	2	…	b
1	y_{111} y_{112} ⋮	y_{121} y_{122} ⋮	…	y_{1b1} y_{1b2} ⋮
2	y_{211} y_{212} ⋮	y_{221} y_{222} ⋮	…	y_{2b1} y_{2b2} ⋮
⋮	⋮	⋮	⋮	⋮
a	y_{a11} y_{a12} ⋮	y_{a21} y_{a22} ⋮	…	y_{ab1} y_{ab2} ⋮

Genotype of a certain SNP
AA/AG/GG

y_{ijk} **:** *k*th observation in the *i*th level of factor A and *j*th level of factor B

Some quantitative trait

Example with Two Factors

Gender
female/male

Factor A	Factor B		
	AA	AG	GG
female	4 5 6 5	7 9 8 12	10 12 11 9
male	6 6 4 4	13 15 12 12	12 13 10 13

a = 2

b = 3

Genotype of a certain SNP
AA/AG/GG

y_{ijk} **:** *k*th observation in the *i*th level of factor A and *j*th level of factor B

Some quantative trait

Example with Two Factors

Factor A	Factor B		
	AA	AG	GG
female	4 5 6 5	7 9 8 12	10 12 11 9
male	6 6 4 4	13 15 12 12	12 13 10 13

average $\rightarrow \bar{y}_{1..}$

average $\rightarrow \bar{y}_{2..}$

average $\downarrow$ $\bar{y}_{.1.}$ $\bar{y}_{.2.}$ $\bar{y}_{.3.}$

Grand mean $\bar{y}_{...}$

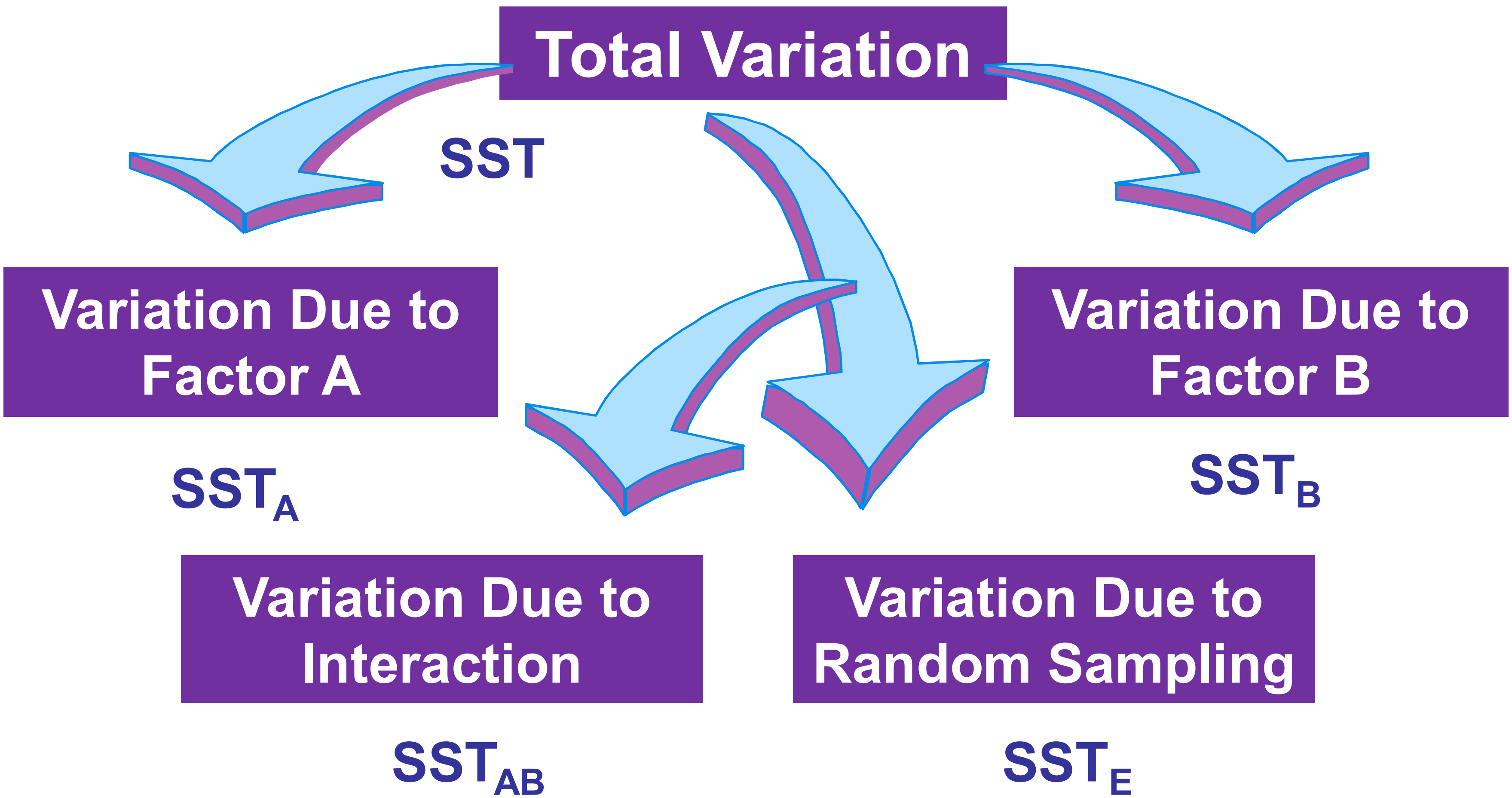
$\boldsymbol{y_{ijk}}$ **:** *k*th observation in the *i*th level of factor A and *j*th level of factor B

Outline

- Two-way ANOVA
  - ANOVA table
  - Decomposition of total variance
  - Measuring interaction between factors
  - Null hypothesis

- R exercise

Two-Way ANOVA Total Variation Partitioning



Partitioning Total Variation

- Error Decomposition

$$\mathrm{SST} = \mathrm{SST}_A + \mathrm{SST}_B + \mathrm{SST}_{AB} + \mathrm{SST}_E$$

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{r}\left(X_{ijk} - \bar{X}_{...}\right)^2$$

$$r \cdot b \cdot \sum_{i=1}^{a}(\bar{X}_{i..} - \bar{X}_{...})^2 \qquad r \cdot a \cdot \sum_{j=1}^{b}\left(\bar{X}_{.j.} - \bar{X}_{...}\right)^2$$

Sum of Squares for factor A:
Measures variation in the response due to the fact that different levels of factor A were used.

Sum of Squares for factor B:
Measures variation in the response due to the fact that different levels of factor B were used.

Partitioning Total Variation

- Error Decomposition

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{r}(X_{ijk} - \bar{X}_{...})^2$$

$$r \cdot \sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{X}_{ij.} - \bar{X}_{i..} - X_{.j.} + \bar{X}_{...})^2$$

$$\sum_{i=1}^{a}\sum_{j=1}^{b}\sum_{k=1}^{r}(X_{ijk} - \bar{X}_{ij.})^2$$

Interaction Sum of Squares:
Measures the variation in the response due to the *interaction between factors A and B*. If the interaction plot is perfectly parallel this will be 0.

Error or Residual Sum of Squares:
Measures the variation in the response within the a x b factor combinations.

Computing the group means

- There are 12 means…

Factor A	Factor B			
	AA	AG	GG	
female	4 5 6 5 $\bar{y}_{11.}$(5)	7 9 8 12 $\bar{y}_{12.}$(9)	10 12 11 9 $\bar{y}_{13.}$(10)	$\bar{y}_{1..}$ 8
male	6 6 4 4 $\bar{y}_{21.}$(5)	13 15 12 12 $\bar{y}_{22}$(13)	12 13 10 13 $\bar{y}_{23.}$(12)	$\bar{y}_{2..}$ 10
	$\bar{y}_{.1.}$ 5	$\bar{y}_{.2.}$ 11	$\bar{y}_{.3.}$ 11	$\bar{y}_{...}$ 9

Partitioning Total Variation

- Error Decomposition

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Factor A	Factor B			
	AA	AG	GG	
female	4 5 6 5 **(5)**	7 9 8 12 **(9)**	10 12 11 9 **(10)**	8
male	6 6 4 4 **(5)**	13 15 12 12 **(13)**	12 13 10 13 **(12)**	10
	5	11	11	9

$$\begin{aligned}
&\sum_{i=1}^{2}\sum_{j=1}^{3}\sum_{k=1}^{4}\left(Y_{ijk}-\bar{Y_{ij.}}\right)^2 \\
=\ & (4-5)^2+(5-5)^2+(6-5)^2+(5-5)^2 \\
& +(7-9)^2+(9-9)^2+(8-9)^2+(12-9)^2 \\
& \cdots\cdots \\
& +(12-12)^2+(13-12)^2+(10-12)^2+(13-12)^2 \\
=\ & 38
\end{aligned}$$

Partitioning Total Variation

- Error Decomposition

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Factor A	Factor B			
	AA	AG	GG	
female	4 5 6 5 (5)	7 9 8 12 (9)	10 12 11 9 (10)	**<u>8</u>**
male	6 6 4 4 (5)	13 15 12 12 (13)	12 13 10 13 (12)	**<u>10</u>**
	5	11	11	**<u>9</u>**

$$r \cdot b \cdot \sum_{i=1}^{2} \left(\bar{Y}_{i..} - \bar{Y}_{...} \right)^2$$

$$= \quad 4 \times 3 \times \left[(8-9)^2 + (10-9)^2 \right] = 24$$

Partitioning Total Variation

- Error Decomposition

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Factor A	Factor B			
	AA	AG	GG	
female	4 5 6 5 (5)	7 9 8 12 (9)	10 12 11 9 (10)	8
male	6 6 4 4 (5)	13 15 12 12 (13)	12 13 10 13 (12)	10
	5	**11**	**11**	**9**

$$r \cdot a \cdot \sum_{j=1}^{3} \left(\bar{Y_{.j.}} - \bar{Y_{...}} \right)^2$$

$$= \quad 4 \times 2 \times \left[(5-9)^2 + (11-9)^2 + (11-9)^2 \right] = 24$$

Partitioning Total Variation

- Error Decomposition

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Factor A	Factor B			
	AA	AG	GG	
female	4	7	10	
	5	9	12	
	6	8	11	
	5	12	9	
	(5)	(9)	(10)	8
male	6	13	12	
	6	15	13	
	4	12	10	
	4	12	13	
	(5)	(13)	(12)	10
	5	11	11	9

$$r \times \sum_{i=1}^{2}\sum_{j=1}^{3}\left(\bar{Y}_{ij\cdot} - \bar{Y}_{i\cdot\cdot} - \bar{Y}_{\cdot j\cdot} + \bar{Y}_{\cdot\cdot\cdot}\right)^2$$

$$= 4 \times \Big[(5-8-5+9)^2 + (9-8-11+9)^2$$

$$+ (110-8-11+9)^2 + \cdots + (12-11-10+9)^2\Big] = 12$$

What does SST_{AB} mean?

$$SST_{AB} = r \cdot \sum_{i=1}^{a}\sum_{j=1}^{b}(\bar{y}_{ij.} - \bar{y}_{i..} - \bar{y}_{.j.} + \bar{y}_{...})^2 = r \cdot \sum_{i=1}^{a}\sum_{j=1}^{b}\{\bar{y}_{ij.} - (\bar{y}_{i..} - \bar{y}_{...}) - (\bar{y}_{.j.} - \bar{y}_{...}) - \bar{y}_{...}\}^2$$

$$= r \cdot \sum_{i=1}^{a}\sum_{j=1}^{b}\{\bar{y}_{ij.} - ((\bar{y}_{i..} - \bar{y}_{...}) + (\bar{y}_{.j.} - \bar{y}_{...}) + \bar{y}_{...})\}^2$$

$$\{\bar{y}_{12.} - ((\bar{y}_{1..} - \bar{y}_{...}) + (\bar{y}_{.2.} - \bar{y}_{...}) + \bar{y}_{...})\}^2$$

Factor A	Factor B		
	AA	AG	GG
female	4	7	10
	5	9	12
	6	8	11
	5	12	9
male	6	13	12
	6	15	13
	4	12	10
	4	12	13

average $\rightarrow \bar{y}_{1..}$

average $\rightarrow \bar{y}_{12.}$

$\bar{y}_{.2.}$

Grand mean $\bar{y}_{...}$

Two-Way ANOVA Summary Table

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
A (Row)	*a-1*	SST_A	MST_A	MST_A/MST_E
B (Column)	*b-1*	SST_B	MST_B	MST_B/MST_E
AB (Interaction)	*(a-1) (b-1)*	SST_{AB}	MST_{AB}	MST_{AB}/MST_E
Error	*N-ab*	SST_E	MST_E	
Total	*N-1*	SST		

Two-Way ANOVA Null Hypotheses

1. No difference in means due to factor A (gender)
   - H_0: $\mu_{1.} = \mu_{2.} = \ldots = \mu_{a.}$

2. No difference in means due to factor B (genotype)
   - H_0: $\mu_{.1} = \mu_{.2} = \ldots = \mu_{.b}$

3. No interaction of factors A & B (gender & genotype)

Motivating Example: Capsule Dissolve Time

- Measured dissolve time of a capsule
  - of each of ***two capsule types (C or V)***
  - in each type of ***two digestive fluids (Gastric or Duodenal)***

factor A **factor B**

	Capsule Type		
Type of Digestive Juice	**C**	**V**	Juice Type Means
Gastric	39.5 45.7 49.8 50.2 63.8 $\overline{X}_{11} = 49.8$	47.4 43.5 39.8 36.1 41.2 $\overline{X}_{12} = 41.6$	$\overline{X}_{1\bullet} = 45.7$
Duodenal	33.5 36.7 42 38.1 31.2 $\overline{X}_{21} = 36.3$	44 41.2 47.3 45.3 42.7 $\overline{X}_{22} = 44.1$	$\overline{X}_{2\bullet} = 40.2$
Capsule Type Means	$\overline{X}_{\bullet 1} = 43.05$	$\overline{X}_{\bullet 2} = 42.85$	Grand Mean $\overline{X}_{\bullet\bullet} = 42.95$

***x_{ijk}* :** *k*th observation in the *i*th level of factor A and *j*th level of factor B

Questions of Interest

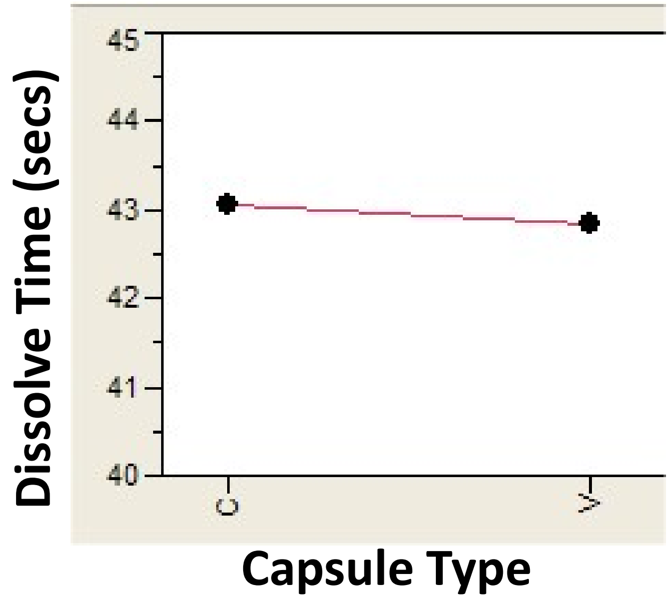
- What effect does each *capsule type* have on the dissolve time?

- What effect does each *fluid type* have on the dissolve time?

- Do both capsule types dissolve *in the same manner* in the two different fluid types?

Effect of each factor – Capsule Effect

Capsule Type			
Type of Digestive Juice	**C**	**V**	Juice Type Means
Gastric	39.5 45.7 49.8 50.2 63.8 $\bar{X}_{11} = 49.8$	47.4 43.5 39.8 36.1 41.2 $\bar{X}_{12} = 41.6$	$\bar{X}_{1\cdot} = 45.7$
Duodenal	33.5 36.7 42 38.1 31.2 $\bar{X}_{21} = 36.3$	44 41.2 47.3 45.3 42.7 $\bar{X}_{22} = 44.1$	$\bar{X}_{2\cdot} = 40.2$
Capsule Type Means	$\bar{X}_{\cdot 1} = 43.05$	$\bar{X}_{\cdot 2} = 42.85$	Grand Mean $\bar{X}_{\cdot\cdot} = 42.95$



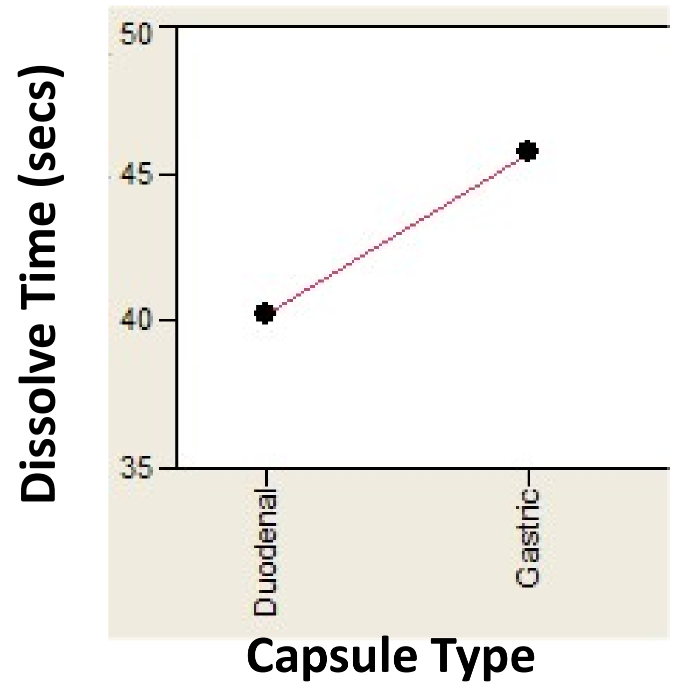
$\bar{x}_{.1.}$: mean dissolve time of type C capsules

$\bar{x}_{.2.}$: mean dissolve time of type V capsules

- There appears to be *very little difference between the capsule types* in terms of the time it takes to dissolve

Effect of each factor – Fluid Effect

	Capsule Type		
Type of Digestive Juice	**C**	**V**	Juice Type Means
Gastric	39.5 45.7 49.8 50.2 63.8 $\bar{X}_{11} = 49.8$	47.4 43.5 39.8 36.1 41.2 $\bar{X}_{12} = 41.6$	$\bar{X}_{1\bullet} = 45.7$
Duodenal	33.5 36.7 42 38.1 31.2 $\bar{X}_{21} = 36.3$	44 41.2 47.3 45.3 42.7 $\bar{X}_{22} = 44.1$	$\bar{X}_{2\bullet} = 40.2$
Capsule Type Means	$\bar{X}_{\bullet 1} = 43.05$	$\bar{X}_{\bullet 2} = 42.85$	Grand Mean $\bar{X}_{\bullet\bullet} = 42.95$



$\bar{x}_{1..}$: mean dissolve time in gastric juice
$\bar{x}_{2..}$: mean dissolve time in duodenal juice

- Capsules take ~5.5 seconds longer to dissolve in gastric juice than in duodenal juice.

Preliminary Conclusion

- There is very little difference between the capsule types in terms of the dissolve time.
- The average dissolve time of the capsules is ~5.5 seconds longer on average in gastric juice than in duodenal juice.
- These conclusions are wrong.

Capsule Effect Separately

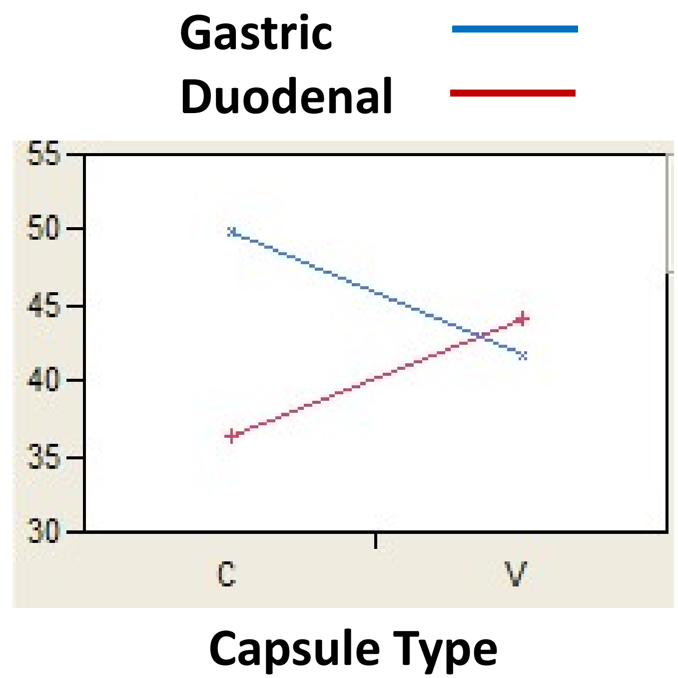
Capsule Type			
Type of Digestive Juice	**C**	**V**	Juice Type Means
Gastric	39.5 45.7 49.8 50.2 63.8 $\boxed{\bar{X}_{11} = 49.8}$	47.4 43.5 39.8 36.1 41.2 $\boxed{\bar{X}_{12} = 41.6}$	$\bar{X}_{1\bullet} = 45.7$
Duodenal	33.5 36.7 42 38.1 31.2 $\boxed{\bar{X}_{21} = 36.3}$	44 41.2 47.3 45.3 42.7 $\boxed{\bar{X}_{22} = 44.1}$	$\bar{X}_{2\bullet} = 40.2$
Capsule Type Means	$\bar{X}_{\bullet 1} = 43.05$	$\bar{X}_{\bullet 2} = 42.85$	Grand Mean $\bar{X}_{\bullet\bullet} = 42.95$

$\bar{x}_{11.}$: mean dissolve time of C capsules in gastric juice
$\bar{x}_{12.}$: mean dissolve time of V capsules in gastric juice

$\bar{x}_{21.}$: mean dissolve time of C capsules in duodenal juice
$\bar{x}_{22.}$: mean dissolve time of V capsules in duodenal juice

Capsule Effect Separately

	Capsule Type		
Type of Digestive Juice	**C**	**V**	Juice Type Means
Gastric	39.5 45.7 49.8 50.2 63.8	47.4 43.5 39.8 36.1 41.2	$\bar{X}_{1\bullet} = 45.7$
	$\boxed{\bar{X}_{11} = 49.8}$	$\boxed{\bar{X}_{12} = 41.6}$	
Duodenal	33.5 36.7 42 38.1 31.2	44 41.2 47.3 45.3 42.7	$\bar{X}_{2\bullet} = 40.2$
	$\boxed{\bar{X}_{21} = 36.3}$	$\boxed{\bar{X}_{22} = 44.1}$	
Capsule Type Means	$\bar{X}_{\bullet 1} = 43.05$	$\bar{X}_{\bullet 2} = 42.85$	Grand Mean $\bar{X}_{\bullet\bullet} = 42.95$

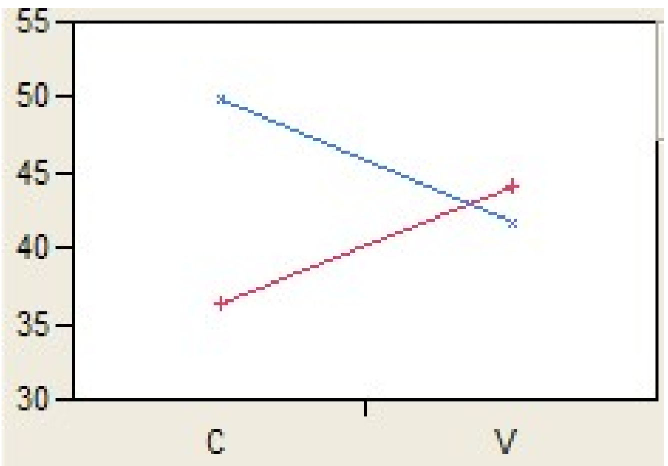


Type C capsules dissolve faster in duodenal juice than do type V capsules, where for gastric juice the opposite is true.

- The effect of capsule type on the dissolve time *depends on the juice type*

Interactions

- The capsule study is an example of situation where *there is an interaction between the two factors* being studied in terms of their effect on the numeric response.
- An interaction occurs when *the effect of one factor depends on the level of another factor*. Here the effect of capsule depends on the type of digestive juice used to dissolve it and vise versa.



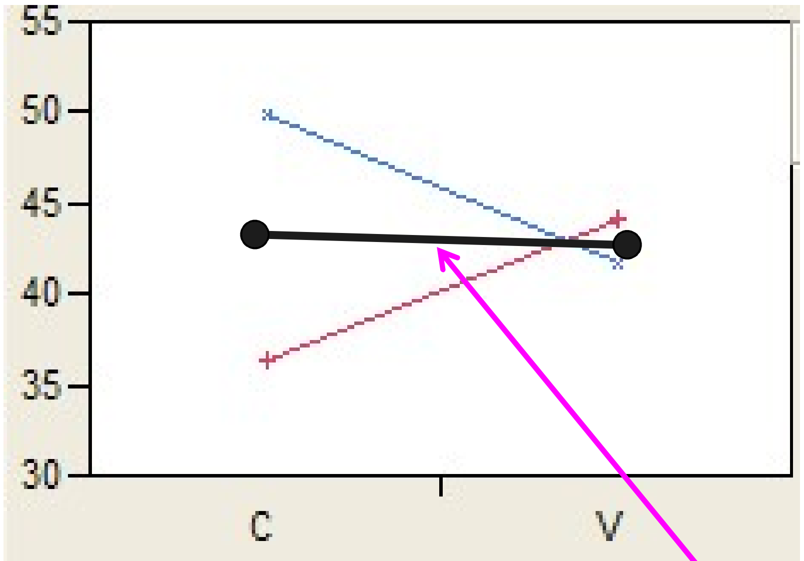
Capsule Type

Gastric

Duodenal

Type C capsules dissolve faster in duodenal juice than do type V capsules, where for gastric juice the opposite is true.

Interactions can mask *main* effects



Capsule Type

Gastric

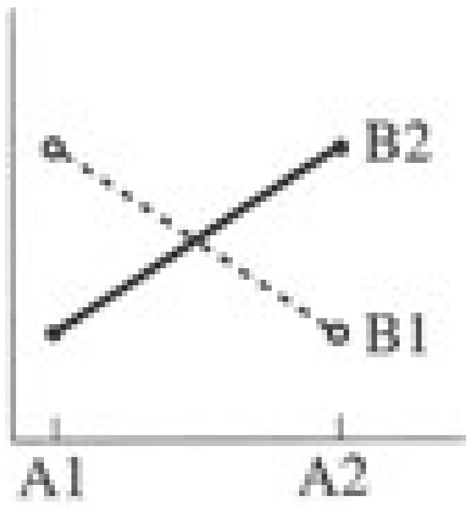
Duodenal

The apparent lack of a capsule effect is caused by the interaction of capsule type and fluid type.

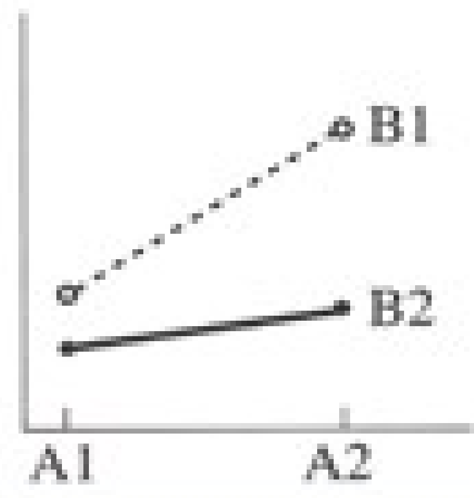
Types of Interactions

- There are two types of interactions

Differences in direction



Differences in magnitude



Questions of interest

- Do the effects that factors A and B have on the response variable interact, i.e. *is there a significant interaction between factors A and B* ?
- If we conclude there is a significant interaction then we conclude the effects of both factors are significant.
- If there is not a significant interaction effect then we can consider the main effects separately, i.e. we ask the following:
  - Question 2: Does *factor A alone* have a significant effect?
  - Question 3: Does *factor B alone* have a significant effect?

Two-Way ANOVA Null Hypotheses

1. No difference in means due to factor A (capsule type)
   - H_0: $\mu_{1.} = \mu_{2.} = \ldots = \mu_{a.}$

2. No difference in means due to factor B (juice type)
   - H_0: $\mu_{.1} = \mu_{.2} = \ldots = \mu_{.b}$

3. No interaction of factors A & B (capsule & juice)

Two-Way ANOVA Summary Table

$$SST = SST_A + SST_B + SST_{AB} + SST_E$$

Source of variation	Degrees of freedom	Sum of squares	Mean square	F ratio
A (Row)	*a-1*	SST_A	MST_A	MST_A/MST_E
B (Column)	*b-1*	SST_B	MST_B	MST_B/MST_E
AB (Interaction)	*(a-1) (b-1)*	SST_{AB}	MST_{AB}	MST_{AB}/MST_E
Error	*N-ab*	SST_E	MST_E	
Total	*N-1*	SST		

Tests of Hypotheses

- *If the interaction is not statistically significant (i.e. p-value > 0.05),* then we conclude the main effects (if present) are independent of one another.

- We can then test for significance of the main effects separately, again using an F-test.

- If a main effect is significant we can then use multiple comparison procedures to compare the mean response for different levels of the factor while holding the other factor fixed.

Tests of Hypotheses

- *If an interaction is significant (i.e., p-value < 0.05)*, we conclude the main effects are not independent of one another and that both effects are important.

- In this case (i.e. the interaction is significant) the tests for main effects in the Two-way ANOVA table are meaningless.

- *We must compare levels of factor A within each level of factor B (and vise versa).*

Outline

- Two-way ANOVA
  - ANOVA table
  - Decomposition of total variance
  - Measuring interaction between factors
  - Null hypothesis
- R exercise