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Two-way anova

VIB-UGENT CENTER FOR PLANT SYSTEMS BIOLOGY
UNIVERSITEIT GENT

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Aims

- Rationale of factorial ANOVA
- Partitioning variance
- Interaction effects
  - Interaction graphs
  - Interpretation

What is Two-Way Independent ANOVA?

- Two independent variables
  - Two-way = 2 Independent variables
  - Three-way = 3 Independent variables
- Different participants in *all* conditions
  - Independent = 'different participants'
- Several independent variables is known as a factorial design.

Benefit of Factorial Designs

- We can look at how variables *interact*.
- Interactions
  - Show how the effects of one var might depend on the effects of another
  - Are often more interesting than main effects.
- Examples
  - Interaction between stress and well-watered conditions on the growth of different plants.

An Example

- Field (2009): Testing the effects of alcohol and gender on 'the beer-goggles effect':
  - V 1 (**Alcohol**): none, 2 pints, 4 pints
  - V 2 (**Gender**): male, female
- Dependent variable (DV) was an objective measure of the attractiveness of the partner selected at the end of the evening.

Factorial anova as regression

- Subset the data
  - Gender: “Male” and “Female”, ref level=“Male”
  - For alcohol: keep the levels “None” and “4 pints”, ref level =“None”
  - We obtain 2 factors, each with two levels

Factorial anova as regression

$$Attractiveness_i = b_0 + b_1 * genderF_i + b_2 * alcohol_i + b_3 * genderF_i * alcohol_i + \varepsilon_i$$

E(A|M, none) = bo

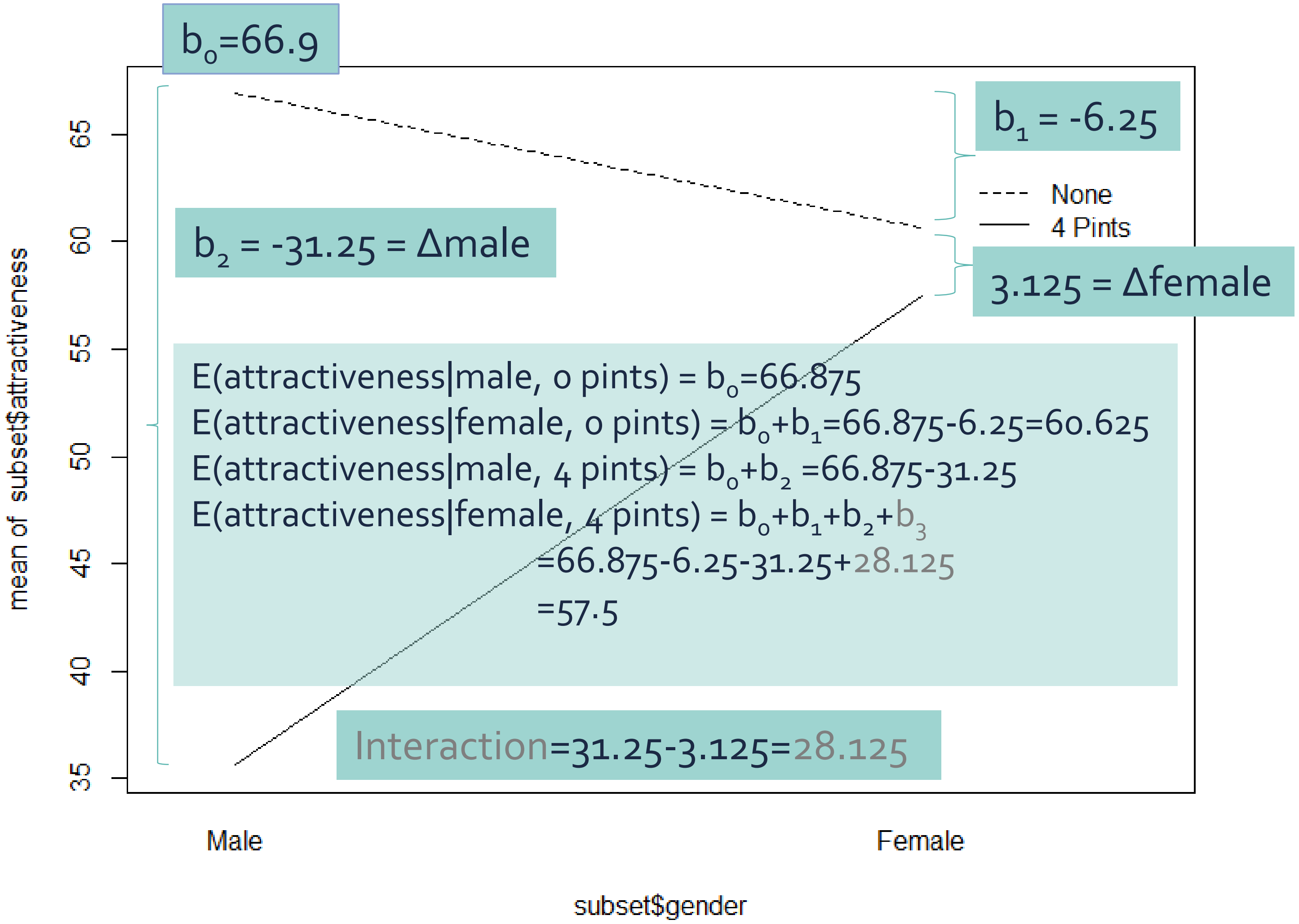
E(A|F, none) = bo + b1

E(A|M, 4pints) = bo + b2

E(A|F, 4pints) = bo + b1+ b2 + b3

Parameter		Estimate		Standard Error	t Value	Pr > |t|
Intercept	b_0	66.87500000	B	3.05502002	21.89	<.0001
gender Female	b_1	-6.25000000	B	4.32045075	-1.45	0.1591
gender Male		0.00000000	B	.	.	.
alcohol 4 Pints	b_2	-31.25000000	B	4.32045075	-7.23	<.0001
alcohol None		0.00000000	B	.	.	.
gender*alcohol Female 4 P	b_3	28.12500000	B	6.11004004	4.60	<.0001
gender*alcohol Female None		0.00000000	B	.	.	.
gender*alcohol Male 4 Pints		0.00000000	B	.	.	.
gender*alcohol Male None		0.00000000	B	.	.	.

What Is an Interaction?



Factorial anova as regression

E(A|M, none) = bo

E(A|F, none) = bo + b1

E(A|M, 4pints) = bo + b2

E(A|F, 4pints) = bo + b1+ b2 + b3

b_0 is the mean attractiveness for men that drunk no alcohol (ref=men, no alcohol)

b_1 is the difference in mean attractiveness between women that drunk no alcohol and men that drunk no alcohol

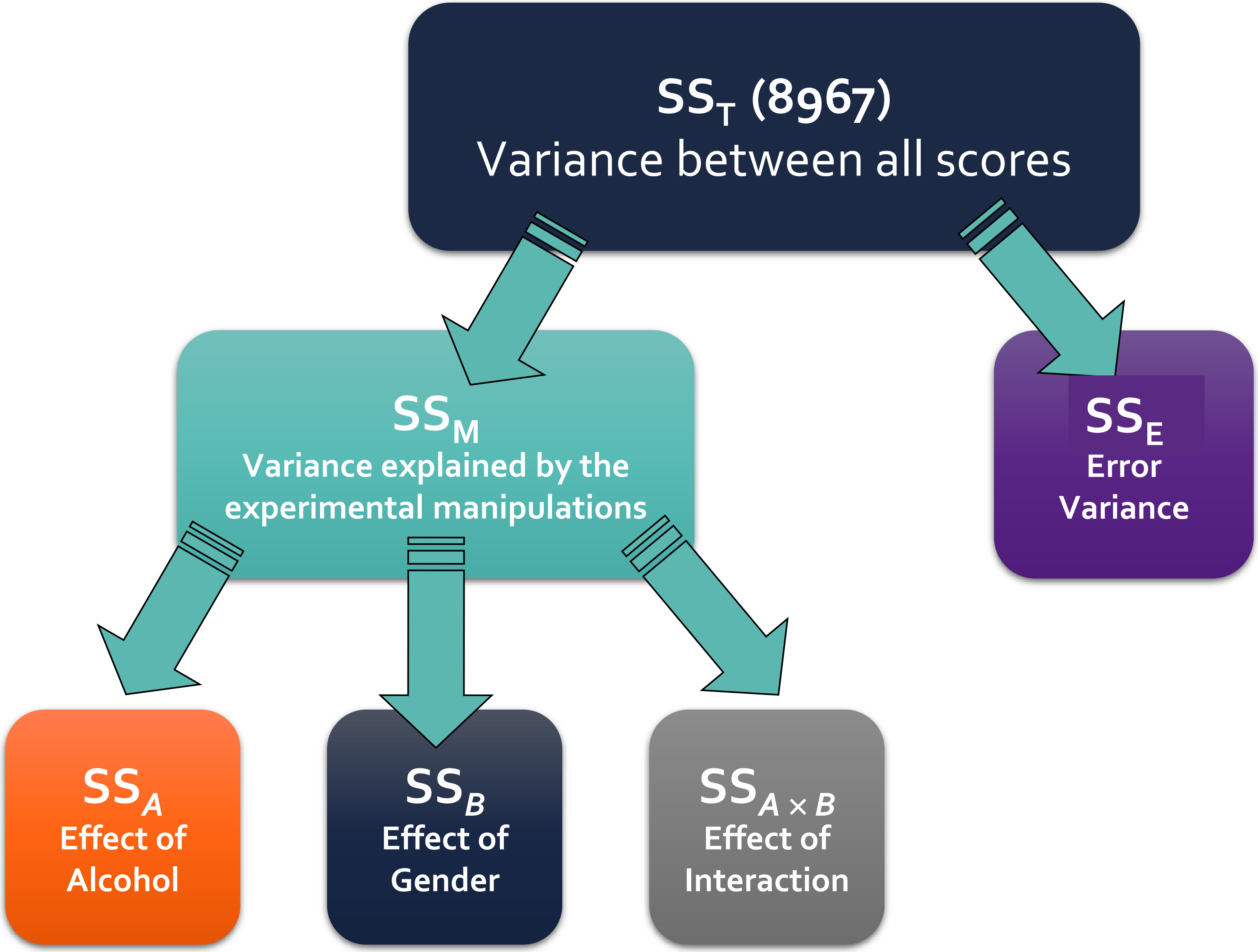
b_2 is the difference in mean attractiveness between men that drunk 4 pints vs none

b_3 compares the difference between men and women in the no alcohol condition to the difference between men and women in the 4 pints condition

Variance partitioning balanced data

Table 12.1: Data for the beer-goggles effect

Alcohol	**None** j=1		**2 Pints** j=2		**4 Pints** j=3	
Gender	**Female** k=1	**Male** k=2	**Female**	**Male**	**Female**	**Male**
	65	50	70	45	55	30
	70	55	65	60	65	30
	60	80	60	85	70	30
R=8	60	65	70	65	55	55
	60	70	65	70	55	35
	55	75	60	70	60	20
	60	75	60	80	50	45
	55	65	50	60	50	40
Total	485	535	500	535	460	285
Mean $\bar{x}_{.jk}$	60.625	66.875	62.50	66.875	57.50	35.625
Variance s^2_{jk}	24.55	106.70	42.86	156.70	50.00	117.41



SS_T (8967)
Variance between all scores
SS_M
Variance explained by the experimental manipulations
SS_E
Error Variance
SS_A
Effect of Alcohol
SS_B
Effect of Gender
$SS_{A \times B}$
Effect of Interaction

Step 1: Calculate SS_T

65	50	70	45	55	30
50	55	65	60	65	30
70	80	60	85	70	30
45	65	70	65	55	55
55	70	65	70	55	35
30	75	60	70	60	20
70	75	60	80	50	45
55	65	50	60	50	40

$\bar{x}_{...}$ = Grand Mean = 58.33

$$SS_T = s^2(N-1)$$
$$SS_T = 190.78(48-1)$$
$$SS_T = 8966.67$$

Step 2: Calculate SS_M

$$SS_M = \sum_{j=1}^{k} n_j(\bar{x}_{.j} - \bar{x}_{..})^2$$

$$SS_M = 8(60.625 - 58.33)^2 + 8(66.875 - 58.33)^2 + 8(62.5 - 58.33)^2$$
$$+8(66.875 - 58.33)^2 + 8(57.5 - 58.33)^2 + 8(35.625 - 58.33)^2$$
$$SS_M = 5479.167$$

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Step 2a: Calculate SS_G

Female		
65	70	55
70	65	65
60	60	70
60	70	55
60	65	55
55	60	60
60	60	50
55	50	50

Mean Female = 60.21

$\bar{x}_{..k}$

Male		
50	45	30
55	60	30
80	85	30
65	65	55
70	70	35
75	70	20
75	80	45
65	60	40

Mean Male = 56.46

$$SS_{Gender} = \sum_{k=1}^{K} n_k(\bar{x}_{..k} - \bar{x}_{...})^2$$

$$SS_{Gender} = 24(60.21 - 58.33)^2 + 24(56.46 - 58.33)^2 = 168.75$$

Step 2b: Calculate SS_A

None		2 Pints		4 Pints	
65	50	70	45	55	30
70	55	65	60	65	30
60	80	60	85	70	30
60	65	70	65	55	55
60	70	65	70	55	35
55	75	60	70	60	20
60	75	60	80	50	45
55	65	50	60	50	40

Mean None = 63.75

$\bar{x}_{.j.}$

Mean 2 Pints = 64.6875

Mean 4 Pints = 46.5625

$$SS_{Alcohol} = \sum_{j=1}^{J} n_j(\bar{x}_{.j.} - \bar{x}_{...})^2$$

$$SS_{Alcohol} = 16(63.75 - 58.33)^2 + 16(64.6875 - 58.33)^2 + 16(46.5625 - 58.33)^2$$
$$SS_{Alcohol} = 3332.292$$

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Step 2c: Calculate SS_{A*G}

$$SS_{A*G} = SS_M - SS_{Alcohol} - SS_{Gender}$$
$$SS_{A*G} = 5479.167 - 168.75 - 3332.292$$
$$SS_{A*G} = 1978.125$$

Note: True in balanced designs

Step 3: Calculate SS_E

$$SS_E = \sum_{j=1}^{J} \sum_{k=1}^{K} s_{jk}(n_{jk} - 1)$$

$$SS_E = s_{11}^2(n_{11} - 1) + s_{12}^2(n_{12} - 1)$$
$$+s_{21}^2(n_{21} - 1) + s_{22}^2(n_{22} - 1)$$
$$+s_{31}^2(n_{31} - 1) + s_{32}^2(n_{32} - 1)$$
$$SS_E = (24.55 * 7) + (106.7 * 7)$$
$$+(42.86 * 7) + (156.7 * 7)$$
$$+(50 * 7) + (117.41 * 7)$$
$$= 3487.52$$

Two-way anova table

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-ratio
Factor A	J-1	SS_A	$MS_A=SS_A/(J-1)$	$F_A=MS_A/MS_E$
Factor G	K-1	SS_G	$MS_G=SS_G/(K-1)$	$F_G=MS_G/MS_E$
Interaction	(J-1)(K-1)	SS_{AG}	$MS_{AG}=SS_{AG}/(J-1)(K-1)$	$F_{AG}=MS_{AG}/MS_E$
Error	JK(R-1)=N-JK	SS_E	$MS_E=SS_E/JK(R-1)$	

Sums of squares

- When data is unbalanced, there are different ways to calculate the sums of squares.
  Assume the model A + B + A*B
  - ***Type I SS***: Tests for the presence of an effect given that the previous one stated is already in the model
    - SS(A): reduction in residual SS attributable to A
    - SS(B|A): reduction in residual SS attributable to B when A is already in the model
    - SS(A*B|A,B): reduction in residual SS attributable to A*B when A and B are already in the model
  - ***Type II SS***: Tests for the presence of an effect, given that the others not containing this term are already in the model
    - SS(A|B), SS(B|A), SS(A*B|A,B)
  - ***Type III SS***: Tests for the presence of an effect, given that the others are in the model
    - SS(A|B, A*B), SS(B|A, A*B), SS(A*B|A, B)
- Do not interpret a main effect if interactions are present (generally speaking, if a significant interaction is present, the main effects should not be further analysed).
- When data is balanced, types I, II and III all give the same results.

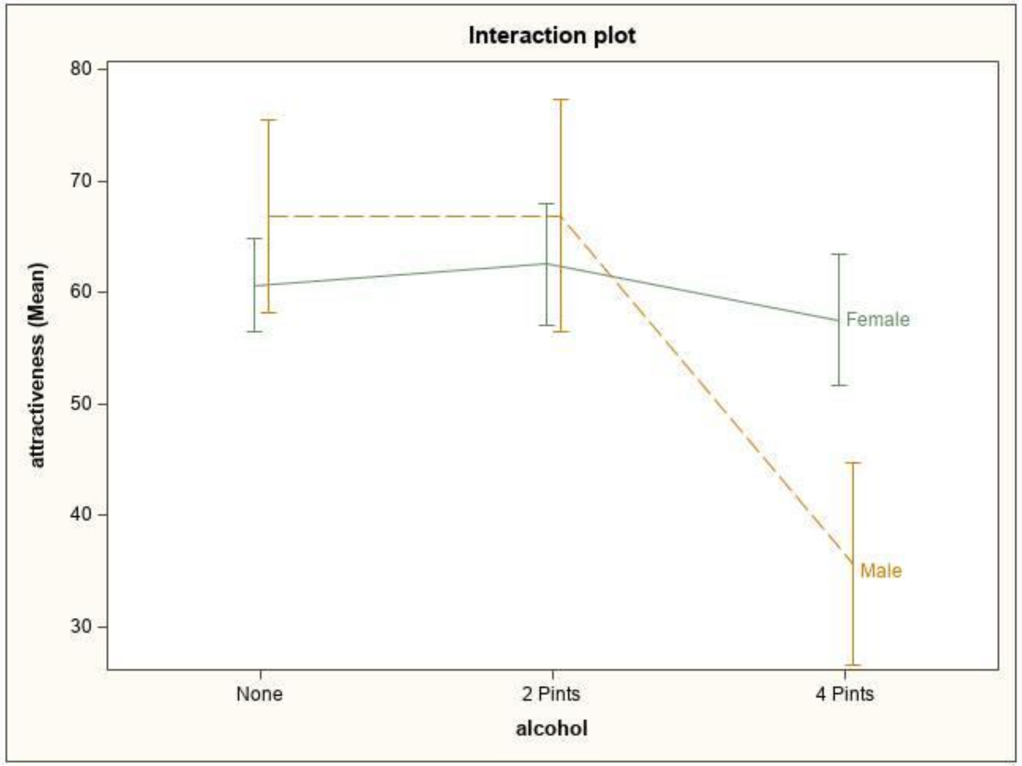
Interpreting Factorial ANOVA

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	4433.593750	1477.864583	19.79	<.0001
Error	28	2090.625000	74.665179		
Corrected Total	31	6524.218750			

R-Square	Coeff Var	Root MSE	attractiveness Mean
0.679559	15.66622	8.640901	55.15625

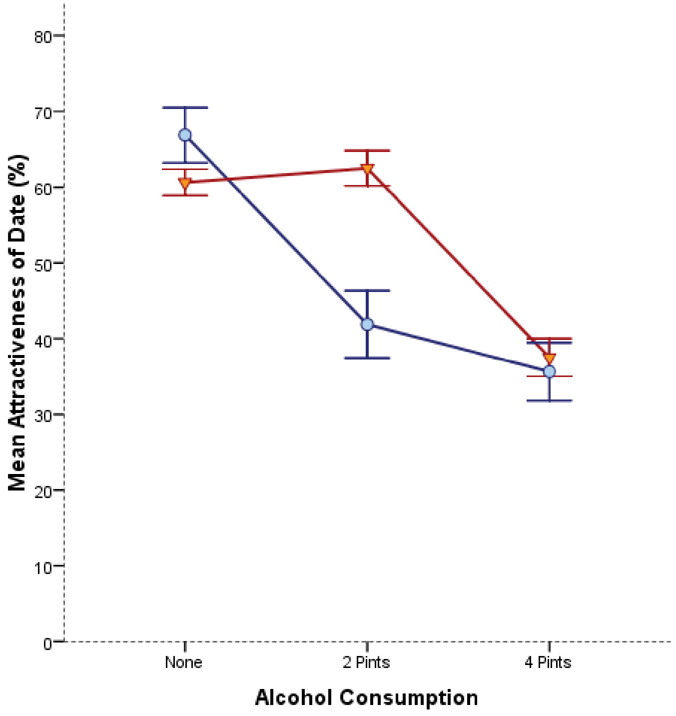
Source	DF	Type III SS	Mean Square	F Value	Pr > F
gender	1	488.281250	488.281250	6.54	0.0163
alcohol	1	2363.281250	2363.281250	31.65	<.0001
gender*alcohol	1	1582.031250	1582.031250	21.19	<.0001

Interpretation: Interaction

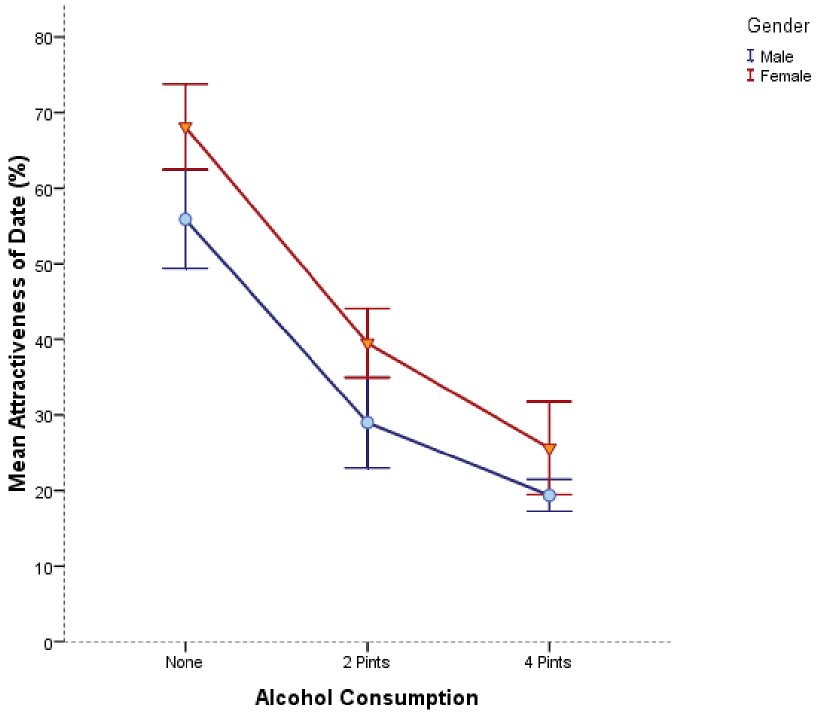


There was a significant interaction between the amount of alcohol consumed and the gender of the person selecting a mate, on the attractiveness of the partner selected (p=8e-5).

Is There Likely to Be a Significant Interaction Effect?



Yes



No

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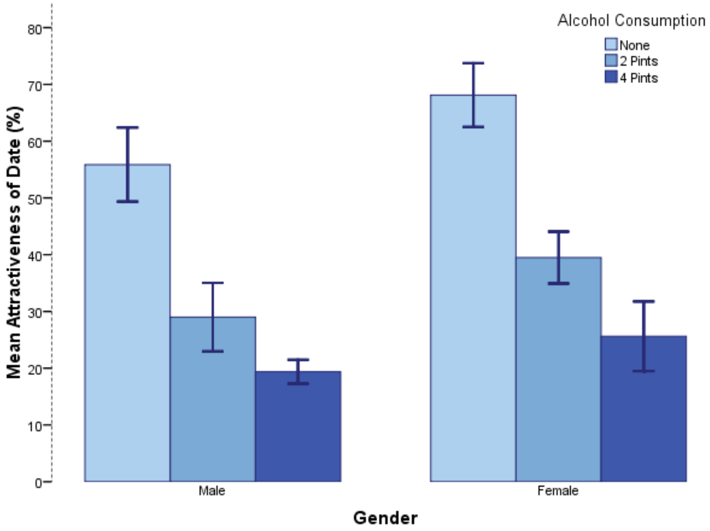
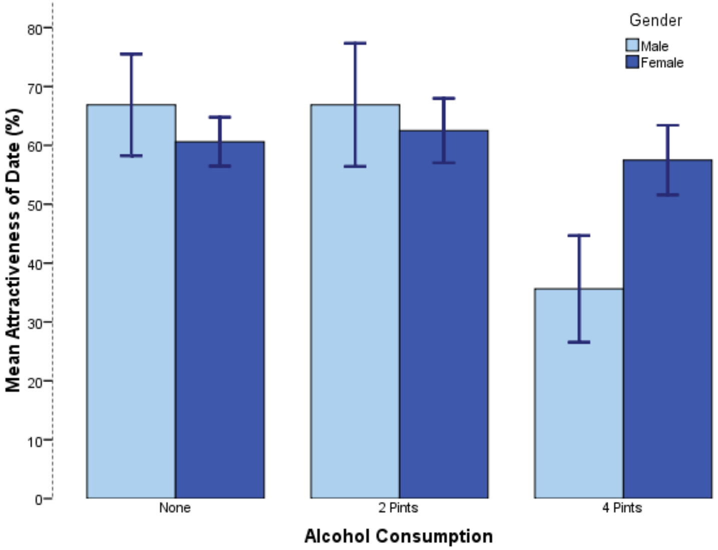
Is There Likely to Be a Significant Interaction Effect?





No

Yes

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Workflow two-way anova

1. Summary statistics
2. Graphs (box plots, bar charts, interaction plot)
3. Anova model
4. Testing assumptions
5. Post-hoc tests provided that a significant interaction was found

The anova table

glm model

The GLM Procedure

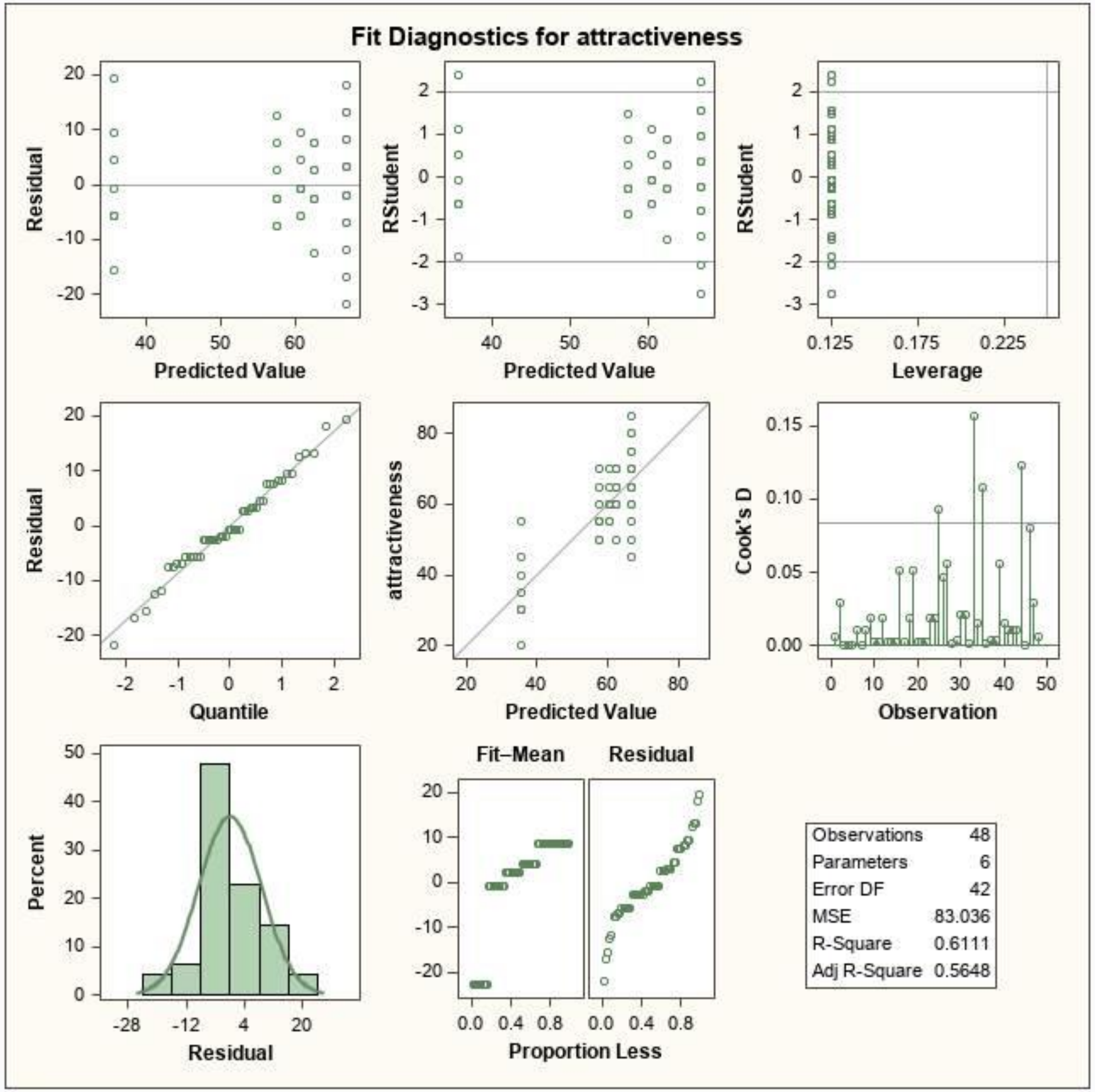
Dependent Variable: attractiveness

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	5	5479.166667	1095.833333	13.20	<.0001
Error	42	3487.500000	83.035714		
Corrected Total	47	8966.666667			

R-Square	Coeff Var	Root MSE	attractiveness Mean
0.611059	15.62125	9.112393	58.33333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
gender	1	168.750000	168.750000	2.03	0.1614
alcohol	2	3332.291667	1666.145833	20.07	<.0001
gender*alcohol	2	1978.125000	989.062500	11.91	<.0001

Testing the assumptions



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All-pairwise comparions

Differences of gender*alcohol Least Squares Means
Adjustment for Multiple Comparisons: Tukey

gender	alcohol	_gender	_alcohol	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
Female	2 Pints	Female	4 Pints	5.0000	4.5562	42	1.10	0.2787	0.8796	0.05	-4.1948	14.1948	-8.6013	18.6013
Female	2 Pints	Female	None	1.8750	4.5562	42	0.41	0.6828	0.9984	0.05	-7.3198	11.0698	-11.7263	15.4763
Female	2 Pints	Male	2 Pints	-4.3750	4.5562	42	-0.96	0.3424	0.9278	0.05	-13.5698	4.8198	-17.9763	9.2263
Female	2 Pints	Male	4 Pints	26.8750	4.5562	42	5.90	<.0001	<.0001	0.05	17.6802	36.0698	13.2737	40.4763
Female	2 Pints	Male	None	-4.3750	4.5562	42	-0.96	0.3424	0.9278	0.05	-13.5698	4.8198	-17.9763	9.2263
Female	4 Pints	Female	None	-3.1250	4.5562	42	-0.69	0.4966	0.9826	0.05	-12.3198	6.0698	-16.7263	10.4763
Female	4 Pints	Male	2 Pints	-9.3750	4.5562	42	-2.06	0.0459	0.3287	0.05	-18.5698	-0.1802	-22.9763	4.2263
Female	4 Pints	Male	4 Pints	21.8750	4.5562	42	4.80	<.0001	0.0003	0.05	12.6802	31.0698	8.2737	35.4763
Female	4 Pints	Male	None	-9.3750	4.5562	42	-2.06	0.0459	0.3287	0.05	-18.5698	-0.1802	-22.9763	4.2263
Female	None	Male	2 Pints	-6.2500	4.5562	42	-1.37	0.1774	0.7432	0.05	-15.4448	2.9448	-19.8513	7.3513
Female	None	Male	4 Pints	25.0000	4.5562	42	5.49	<.0001	<.0001	0.05	15.8052	34.1948	11.3987	38.6013
Female	None	Male	None	-6.2500	4.5562	42	-1.37	0.1774	0.7432	0.05	-15.4448	2.9448	-19.8513	7.3513
Male	2 Pints	Male	4 Pints	31.2500	4.5562	42	6.86	<.0001	<.0001	0.05	22.0552	40.4448	17.6487	44.8513
Male	2 Pints	Male	None	4.23E-15	4.5562	42	0.00	1.0000	1.0000	0.05	-9.1948	9.1948	-13.6013	13.6013
Male	4 Pints	Male	None	-31.2500	4.5562	42	-6.86	<.0001	<.0001	0.05	-40.4448	-22.0552	-44.8513	-17.6487

Simple tests of effect

Simple Differences of gender*alcohol Least Squares Means Adjustment for Multiple Comparisons: Bonferroni													
Slice	alcohol	_alcohol	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
gender Female	2 Pints	4 Pints	5.0000	4.5562	42	1.10	0.2787	0.8361	0.05	-4.1948	14.1948	-6.3616	16.3616
gender Female	2 Pints	None	1.8750	4.5562	42	0.41	0.6828	1.0000	0.05	-7.3198	11.0698	-9.4866	13.2366
gender Female	4 Pints	None	-3.1250	4.5562	42	-0.69	0.4966	1.0000	0.05	-12.3198	6.0698	-14.4866	8.2366

Simple Differences of gender*alcohol Least Squares Means Adjustment for Multiple Comparisons: Bonferroni													
Slice	alcohol	_alcohol	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
gender Male	2 Pints	4 Pints	31.2500	4.5562	42	6.86	<.0001	<.0001	0.05	22.0552	40.4448	19.8884	42.6116
gender Male	2 Pints	None	4.23E-15	4.5562	42	0.00	1.0000	1.0000	0.05	-9.1948	9.1948	-11.3616	11.3616
gender Male	4 Pints	None	-31.2500	4.5562	42	-6.86	<.0001	<.0001	0.05	-40.4448	-22.0552	-42.6116	-19.8884

Simple Differences of gender*alcohol Least Squares Means Adjustment for Multiple Comparisons: Bonferroni													
Slice	gender	_gender	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
alcohol 2 Pints	Female	Male	-4.3750	4.5562	42	-0.96	0.3424	0.3424	0.05	-13.5698	4.8198	-13.5698	4.8198

Simple Differences of gender*alcohol Least Squares Means Adjustment for Multiple Comparisons: Bonferroni													
Slice	gender	_gender	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
alcohol 4 Pints	Female	Male	21.8750	4.5562	42	4.80	<.0001	<.0001	0.05	12.6802	31.0698	12.6802	31.0698

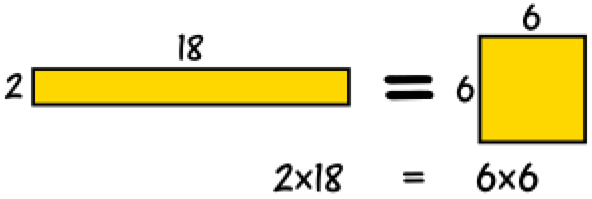
Simple Differences of gender*alcohol Least Squares Means Adjustment for Multiple Comparisons: Bonferroni													
Slice	gender	_gender	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
alcohol None	Female	Male	-6.2500	4.5562	42	-1.37	0.1774	0.1774	0.05	-15.4448	2.9448	-15.4448	2.9448

User defined contrasts

Least Squares Means Estimates Adjustment for Multiplicity: Sidak												
Effect	Label	Estimate	Standard Error	DF	t Value	Pr > |t|	Adj P	Alpha	Lower	Upper	Adj Lower	Adj Upper
gender*alcohol	none female - none male	-6.2500	4.5562	42	-1.37	0.1774	0.7904	0.05	-15.4448	2.9448	-19.3290	6.8290
gender*alcohol	2 pints female - 2 pints male	-4.3750	4.5562	42	-0.96	0.3424	0.9650	0.05	-13.5698	4.8198	-17.4540	8.7040
gender*alcohol	4 pints female - 4 pints male	21.8750	4.5562	42	4.80	<.0001	0.0002	0.05	12.6802	31.0698	8.7960	34.9540
gender*alcohol	d1:female 4 pints - female none	-3.1250	4.5562	42	-0.69	0.4966	0.9959	0.05	-12.3198	6.0698	-16.2040	9.9540
gender*alcohol	female 2 pints - female none	1.8750	4.5562	42	0.41	0.6828	0.9999	0.05	-7.3198	11.0698	-11.2040	14.9540
gender*alcohol	d2:male 4 pints - male none	-31.2500	4.5562	42	-6.86	<.0001	<.0001	0.05	-40.4448	-22.0552	-44.3290	-18.1710
gender*alcohol	male 2 pints - male none	4.23E-15	4.5562	42	0.00	1.0000	1.0000	0.05	-9.1948	9.1948	-13.0790	13.0790
gender*alcohol	d1 vs d2	28.1250	6.4434	42	4.36	<.0001	0.0006	0.05	15.1216	41.1284	9.6284	46.6216

To Transform … Or Not

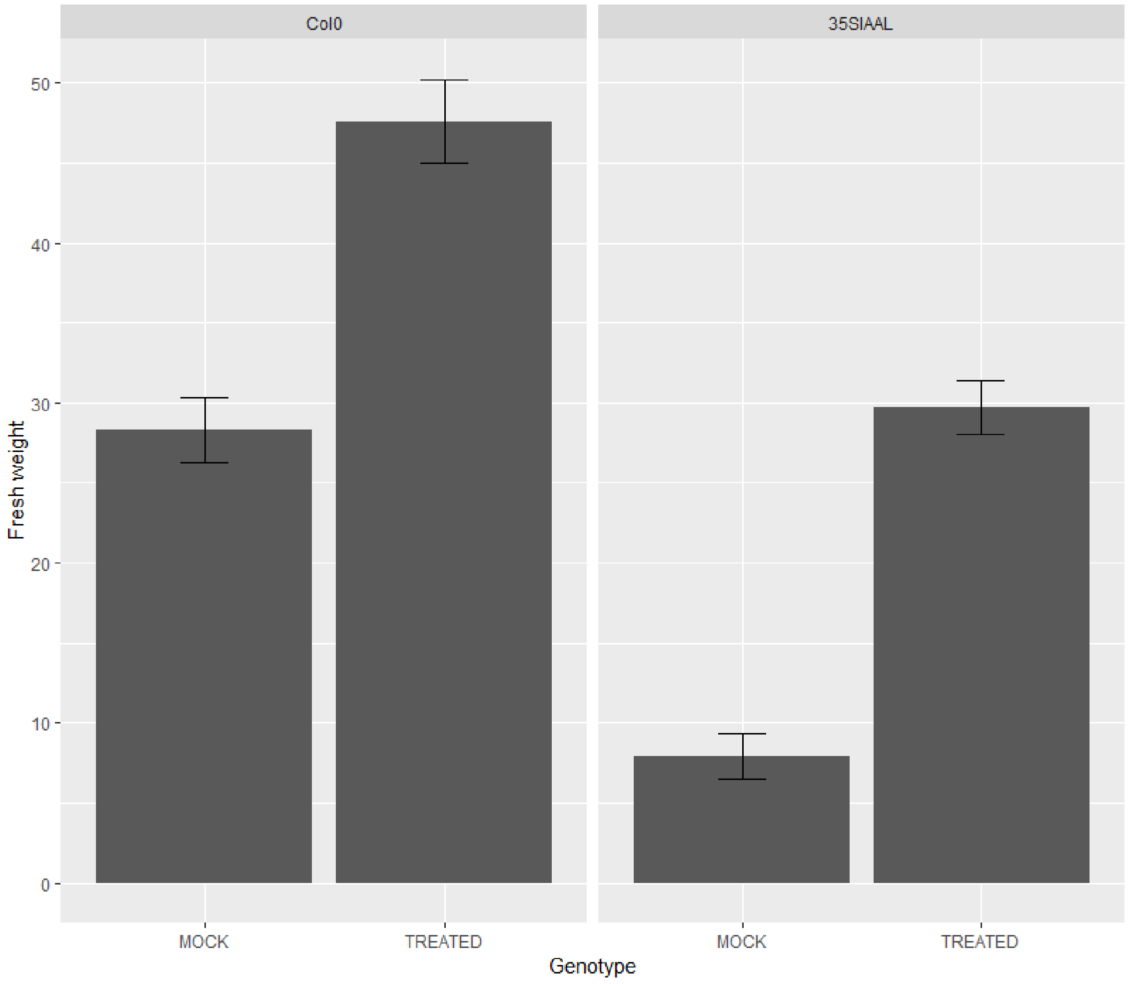
- Transforming the data helps as often as it hinders the accuracy of *F* (Games & Lucas, 1966).
- Games (1984):
  - The central limit theorem: sampling distribution of the mean will be normal in samples > 30 anyway.
  - Transforming the data changes the hypothesis being tested
    - E.g. when using a log transformation and comparing means, you change from comparing arithmetic means to comparing geometric means



$$\sqrt[n]{\prod_{i=1}^{n} x_i} = exp\left(\frac{\sum_{i=1}^{n} log_e(x_i)}{n}\right)$$

  - In small samples it is tricky to determine normality one way or another.
  - The consequences for the statistical model of applying the 'wrong' transformation could be worse than the consequences of analysing the untransformed scores.

Effect log transformation



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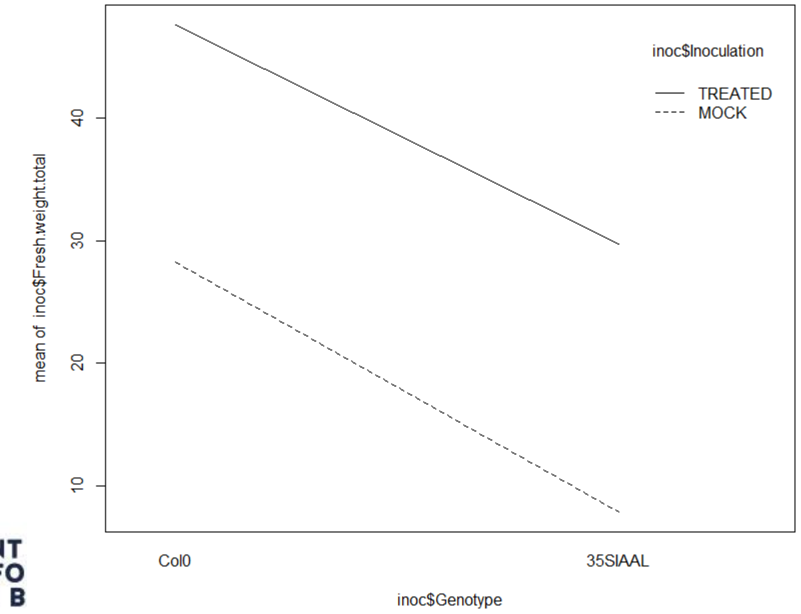
Effect log transformation

Original scale

Ho: Col T – Col M = 35S T – 35S M

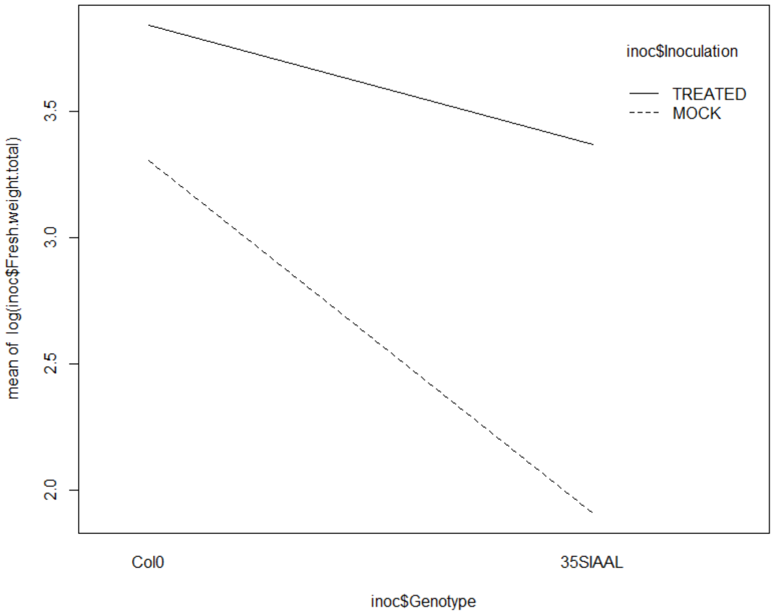


Log scale

Ho: log(Col T) – log(Col M)

= log(35S T) – log(35S M)

After backtransformation: Col T/Col M = 35S T/35S M



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